COMPARATIVE DIAGNOSTIC PHARMACOLOGY
Clinical and Research Applications in Living-System Models

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C.P. Coyne

Blackwell Publishing

Cody P. Coyne, DVM, PhD, DACVIM, is a Professor of Molecular Pharmacology and Immunology at the Veterinary Pharmacology Research Laboratory in the Department of Basic Sciences of the College of Veterinary Medicine, Mississippi State University, Mississippi State.

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2121 State Avenue, Ames, Iowa 50014, USA
Orders: 1-800-862-6657
Office: 1-515-292-0140
Fax: 1-515-292-3348
Web site: www.blackwellprofessional.com

Blackwell Publishing Ltd
9600 Garsington Road, Oxford OX4 2DQ, UK
Tel.: +44 (0)1865 776868

Blackwell Publishing Asia
550 Swanston Street, Carlton, Victoria 3053, Australia
Tel.: +61 (0)3 8359 1011

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First edition, 2006

Library of Congress Cataloging-in-Publication Data

Coyne, Cody Paul.
Comparative diagnostic pharmacology: applications in living-system models / C.P. Coyne.
p. cm.
Includes index.
ISBN-13: 978-0-8138-1753-8 (alk. paper)
ISBN-10: 0-8138-1753-6 (alk. paper)
1. Molecular probes—Diagnostic use. 2. Contrast media.
I. Title.
[DNLM: 1. Molecular Probes—diagnostic use—Laboratory Manuals. 2. Contrast Media—Laboratory Manuals. QY 25 C881c 2006]
RB43.7.C69 2006
616.07′56—dc22

2005009737

The last digit is the print number: 9 8 7 6 5 4 3 2 1

Contents

Preface ix
Journal Title Abbreviations xi

SECTION 1 **BODY SYSTEMS**

1 Cardiovascular System 5
Cardiac Arrhythmias and EKG-Pharmacology 5
Cell Physiology 16
Cardiac Disease and Physiology 21
Angina: Cardiac-Associated Thoracic Pain 21
Angina: Non-Cardiac-Associated Thoracic Pain 29
Cardiac Diseases without Angina 30
Cardiopulmonary Physiology 35
Peripheral Vascular Disease, Function and Physiology 36
Cardiovascular Response Tests 47

2 Central Nervous System 61
Autonomic Nervous System 61
Peripheral Nervous System and Neuromuscular Function 62
Cerebral Cortex and Cerebral Hemispheres 63
Brainstem, Spinal Cord, and Peripheral Nervous System 114
Special Senses 117
Special Senses: Non-Ophthalmic 126

3 Endocrine System 129
Gastrointestinal/Pancreatic Systems 129
Hypothalamus and Pituitary: General and Miscellaneous 130
Hypothalamus, Pituitary, and Adrenal Axis 134
Hypothalamus and Pituitary: Growth Hormone 150
Hypothalamus/Pituitary Function: Reproduction 164
Hypothalamus, Pituitary, and Thyroid Axis 170
Parathyroid 176

4 Gastrointestinal System 179
Dentistry, Oral Cavity, and Salivary Glands 179
Esophageal Function and Disorders 181
Gastric Function and Disorders 183
Pancreas 190
Intestinal Absorption Breath Tests 190
Intestinal Absorption and Permeability: Non-Breath Tests 191
Absorption and Transit Time: Non-Carbohydrate-Based Agents 199
Permeability and Ulceration 203
Intestinal Inflammation 209
Motility and Transit Time of Intestinal Tract 210
Pancreas Function and Disease 212
Neoplasia of the Intestine and Colon 219

5 Hematopoietic System 221
Hematopoiesis, Hematology, and Hemostasis 221
Immune System 228
Inflammation 244
Ex Vivo Living Systems 245
Cardiovascular: *Ex Vivo* 245
Hepatic: *Ex Vivo* 246
Hematopoietic: Lymphocytes (*Ex Vivo*) 247
Hematopoietic: Macrophages and Monocytes (*Ex Vivo*) 248
Hematopoietic: Neutrophils (*Ex Vivo*) 252
Hematopoietic: Platelets (*Ex Vivo*) 256
Hematopoietic: Red Blood Cells (*Ex Vivo*) 260
Musculoskeletal: *Ex Vivo* 260
Neoplastic Disease: *Ex Vivo* 263
Respiratory: *Ex Vivo* 264

6 Hepatic System 267
Biliary and Hepatic Diagnostics 267
Biliary 267
Hepatobiliary 268
Hepatic Blood/Plasma Flow and Perfusion 269
Hepatic Excretory Function 269
Hepatocyte Phase I Metabolism Pathways 273
Hepatocyte Phase II Biochemical Metabolism Pathways 278
Hepatic Pathology and Disease States 279

Extrahepatic Disease Caused by Liver Dysfunction 281
Pharmacogenetics 281
Pharmacogenetics Concepts and Disease Examples 281
Pharmacogenetics: General Hepatic Biochemical Metabolization 289
Pharmacogenetics: Phase I Metabolizing Enzyme Systems 290
Microsomal Enzyme Metabolizing Capacity (General) 290
Microsomal Cytochrome P450 Enzyme Systems (General) 292
Microsomal Cytochrome P450 1A1 and P450 1A2 294
Microsomal Cytochromes P450 2A6, 2C9, 2C19, 2D6, and 2E1 296
Microsomal Cytochromes P450 3A 305
Hepatic Cytochrome P450 4 Microsomal Enzymes 311
Microsomal Flavin-Based Metabolizing Systems 312
Microsomal Mixed Function Oxidases 313
Phase II Metabolizing Enzyme Systems 314
Conjugation Systems 314
Extrahepatic Pharmacogenetics 322
Extrahepatic—Cardiovascular System 322
Extrahepatic—Central Nervous System 325
Extrahepatic—Renal System 329
Extrahepatic—Respiratory System 330
Extrahepatic—Musculoskeletal System 330
Pharmacogenetics of Prognosis and Therapeutic Response 332
7 Integumentary System 337
8 Musculoskeletal System 341
Bone and Synovial Structures 341
Skeletal Muscle 343
9 Reproductive System 351
10 Renal and Urinary Systems 355
Urinary Bladder 370
Imaging Enhancement 372
11 Respiratory System 375
Respiratory Physiology 375
Respiratory Disease Conditions 380
Breath Tests 398

SECTION 2 **GENETIC DISORDERS, INFECTIOUS DISEASE, NEOPLASIA, AND METABOLIC CONDITIONS**

12 Cancer/Neoplastic Disease 407
General 407
Central Nervous System 409
Gastrointestinal Tract 410
Endocrine 415
Immune System 420
Integumentary System 421
Musculoskeletal System 423
Renal/Urinary Systems 423
Reproductive System 423
13 Genetics and Nucleic Acids 425
Gene Delivery and Targeting 428
Gene Expression 435
14 Inflammation, Infection, and Sepsis 439
General Abscess Formation, Sepsis, and Inflammation 439
Organ- and Tissue-Associated Sepsis and Inflammatory Responses 444
Parasitology 452
15 Metabolism, Electrolytes, and Hypoxia 455

SECTION 3 **CONTRAST AND IMAGE-ENHANCING AGENTS**

16 Fluorescent and Pigment Dye Reagents 473
Cardiovascular System 473
Cellular: General 481
Central Nervous System and Special Senses 482
Gastrointestinal System 487
Genetic 488
Hepatic System 488
Hematology 491
Immunology 492
Membrane Characterization 493
Musculoskeletal System 496
Neoplastic Disease States 497
Special Senses 507
Pathology: General 509
Pharmaceutical Delivery 509
Renal System 511
Reproductive System 514
Respiratory System 515
Sepsis 515
17 Magnetic Resonance Imaging (MRI) 517
MRI: General Concepts and Principles 517
Reactions to Contrast Agents 518
Contrast Agent Profiles and Properties 521
Cardiovascular System 523
Central Nervous System 532
Central Nervous System: Special Senses 544
Gastrointestinal System 544

Genetic 545
Hematology 545
Hepatic System 546
Ischemia and Infarction 552
Musculoskeletal System 552
Neoplasia 555
Pancreas 560
Renal System 562
Respiratory System 563
Experimental Imaging Contrast-Enhancing Agents 565
Stem Cells 566
18 Nuclear Planar Scintigraphy 569
General 569
Amyloid and Amyloidosis 569
Apoptosis 572
Gene Expression: *In Vivo* Detection 573
Cardiovascular: Cardiac 575
Central Nervous System: Neurology 595
Autonomic Nervous System 600
Endocrine System 603
Gastrointestinal System 606
Hematology 609
Immune System and Inflammatory (Septic/Non-Septic) Responses 611
Integumentary System 622
Metabolic and Metabolism 622
Musculoskeletal 622
Neoplastic Disease 626
Pancreas 666
Renal System 666
Respiratory System 669
Respiratory Breath Tests: Pulmonary and Hepatic Function Assessment 673
19 Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) 677
Cardiovascular System 677
Central Nervous System 685
Central Nervous System (CNS): Special Senses 705
Gene Expression 705
Hepatic System 706
Immune System 708
Inflammation and Sepsis 708
Metabolism: Hypoxia, Ischemia, Mitosis and Proliferation 709
Musculoskeletal System 712
Neoplasia 713
20 Radiographic Image-Enhancing Agents 731
Conventional Radiography 731
Cardiovascular System 742
Central Nervous System 747
Gastrointestinal System 747
Hepatobiliary System 748
Monocytes and Macrophages 749
Renal System 749
Reproductive System 750
Computed Tomography (CT) Radiography Image-Enhancing Agents 750
Cardiovascular System 752
Central Nervous System 758
Gastrointestinal System 759
Hepatic System 759
Inflammation and Sepsis 760
Lymph Nodes and Lymphatics 761
Neoplasia 762
Renal System 763
Respiratory System 763
Technique 764
Fluoroscopic Imaging 764
21 Ultrasonography 765
General Considerations and Concepts 765
Cardiovascular System 768
Central Nervous System 788
Gastrointestinal System 791
Hepatic System 792
Immune System 798
Musculoskeletal System 798
Neoplasia 800
Pancreas 804
Prostate 804
Renal System 806
Reproductive System 807

SECTION 4 **DELIVERY MODALITIES**

22 Biological and Synthetic Delivery Modalities 811
Macro-Biological Platforms 811
Cellular-Based Delivery Systems 811
Vector-Mediated Gene (Oligonucleotide) Delivery Systems 817
Macro-Synthetic Platforms 820
Liposomes 820
Microbubbles and Microspheres 845
Nanoparticles and Nanospheres 857
Molecular Platforms 869
Micro-Synthetic Agents 869
Peptides, Proteins and Immunoglobulins 883
Mechanical Platforms 904
Index 907

Preface

The composition of this text has been guided by a common need to document the wide spectrum and range of pharmaceutical and biopharmaceutical agents that have been, or potentially could be, utilized for diagnostic purposes. The sources and motivation for the development of such agents are quite varied in nature. In recent decades, an extensive body of investigative research has been devoted specifically to the design and evaluation of diagnostic pharmaceuticals. A significant portion of these efforts have been directed toward developing agents to meet the demands for enhancing the effectiveness of conventional imaging modalities and more advanced forms of diagnostic instrumentation, including contrast-enhanced helical computed tomography (CE-HCT), dynamic susceptibility contrast magnetic resonance imaging (DSC-MRI), and trans-cranial color-coded sonography (TCCS). Alternatively, the substantial amount of mechanistic-oriented research supported through federal agencies and private foundations has indirectly provided an invaluable experimental foundation for the development of a variety of biochemical, receptor, and gene-based diagnostic agents.

Written in an evidence-based format, the text is intended to serve as an information resource for identifying pharmaceutical and biopharmaceutical agents that can be applied in vivo or ex vivo in living systems relevant to both clinical medicine and investigative research. Organization of the contents has been structured on the assumption that in most instances, the specific organ system, physiological function, or disease process of interest is already known, but the identity of the appropriate molecular probe necessary to perform a specific diagnostic objective is uncertain. Given this perception, information in each chapter consists of an alphabetical listing of individual disease states or physiological processes of interest that have been entered in bold type above the text for each entry. On a secondary header line the name of the corresponding diagnostic pharmaceutical or biological agent(s) is provided in italics. In this manner, molecular probes can be located in the text based on their application or function without prior knowledge of their identity. Some entries also have additional clarifying statements contained within brackets to better indicate the contents of the descriptions provided for a particular diagnostic pharmaceutical.

Chapters in the text have been arranged or categorized into sections, including molecular agents that function as classical molecular probes, image-enhancing pharmaceutical or biopharmaceutical agents, and delivery modalities. The chapters contained within each of these major sections are themselves organized according to individual body systems. Diagnostic pharmaceuticals have also been included in chapters or subchapters that address broad topics related to endocrinology, genetic conditions, pharmacogenetics, neoplastic disease, metabolic function, inflammatory responses, infectious disease, immunity, ex vivo diagnostic models, and breath tests. Organization of the text in this fashion not only allows information about a diagnostic agent to be found in a more expedient manner, it also facilitates a more convenient grouping of pharmaceutical and biopharmaceutical agents that have similar diagnostic applications.

The majority of the information provided about diagnostic pharmaceuticals and delivery modalities can be applied to humans and other mammalian species, including laboratory animals, companion animals, domestic livestock, and wildlife, assuming some degree of preliminary optimization may be required. Although not specifically addressed, individual diagnostic pharmaceuticals and biopharmaceutical agents could also potentially be utilized for similar purposes in avian and reptilian species. Diagnostic agents could not be provided for the assessment of all known disease states or physiological processes. In part, this is because either some clinical conditions and biological functions cannot be appropriately characterized through the use of diagnostic pharmaceuticals and biological probes, or it is due to the fact that effective molecular agents still remain to be developed for such purposes. In accord with many published texts, there is a substantial amount of information that had to be excluded from this 1st edition due to various constraints. In the near future, it is hoped that an increased interest will be stimulated in the development of diagnostic pharmaceutical and biopharmaceutical agents in concert with their evaluation and application in clinical medicine and investigative research.

Journal Title Abbreviations

Abbreviation	Journal Title
Acad Radiol	Academic Radiology
Acta Anaesthesiol Scand	Acta Anaesthesiologica Scandinavica
Acta Cardiol	Acta Cardiologica
Acta Endocrinol (Copenh)	Acta Endocrinologica (Copenhagen)
Acta Hepatogastroenterol (Stuttg)	Acta Hepato-Gastroenterologica (Stuttgart)
Acta Neuropathol (Berl)	Acta Neuropathologica (Berlin)
Acta Orthop Scand	Acta Orthopaedica Scandinavica
Acta Otolaryngol	Acta Oto-Laryngologica
Acta Physiol Pharmacol Ther Latinoam	Acta Physiologica, Pharmacologica et Therapeutica Latinoamericana: Organo de la Asociacion Latinoamericana de Ciencias Fisiologicas y [de] la Asociacion Latinoamericana de Farmacologia
Acta Psychiatr Scand	Acta Psychiatrica Scandinavica
Acta Radiol	Acta Radiologica
Acta Oncol	Acta Oncologica
Acta Vet Hung	Acta Veterinaria Hungarica
AJNR Am J Neuroradiol	AJNR. American Journal of Neuroradiology
AJR Am J Roentgenol	AJR. American Journal of Roentgenology
Alcohol Clin Exp Res	Alcohol Clinical Experimental Research
Aliment Pharmacol Ther	Alimentary Pharmacology & Therapeutics
Am Heart J	American Heart Journal
Am J Cardiol	American Journal of Cardiology
Am J Chin Med	American Journal of Chinese Medicine
Am J Clin Nutr	American Journal of Clinical Nutrition
Am J Clin Pathol	American Journal of Clinical Pathology
Am J Dis Child	American Journal of Diseases of Children
Am J Gastroenterol	American Journal of Gastroenterology
Am J Health Syst Pharm	American Journal of Health-System and Pharmacy
Am J Hypertens	American Journal of Hypertension: Journal of the American Society of Hypertension
Am J Med Genet	American Journal of Medical Genetics
Am J Med	American Journal of Medicine
Am J Obstet Gynecol	American Journal of Obstetrics and Gynecology
Am J Pathol	American Journal of Pathology
Am J Pharmacogenomics	American Journal of Pharmacogenomics
Am J Physiol Endocrinol Metab	American Journal of Physiology, Endocrinology and Metabolism
Am J Physiol Heart Circ Physiol	American Journal of Physiology. Heart and Circulatory Physiology
Am J Physiol Renal Physiol	American Journal of Physiology. Renal Physiology
Am J Psychiatry	American Journal of Pharmacogenomics Psychiatry
Am J Respir Cell Mol Biol	American Journal of Respiratory Cell and Molecular Biology
Am J Respir Crit Care Med	American Journal of Respiratory and Critical Care Medicine
Am J Surg	American Journal of Surgery
Am J Ther	American Journal of Therapeutics
Am J Vet Res	American Journal of Veterinary Research
Anal Chem	Analytical Chemistry
Anesth Analg	Anesthesia and Analgesia
Angew Chem Int Ed Engl	Angewandte Chemie International Edition English
Ann Allergy Asthma Immunol	Annals of Allergy, Asthma & Immunology: Official Publication of the American College of Allergy, Asthma, & Immunology
Ann Allergy	Annals of Allergy
Ann Biol Clin (Paris)	Annales de Biologie Clinique
Ann Biomed Eng	Annals of Biomedical Engineering
Ann Hematol	Annals of Hematology
Ann Intern Med	Annals of Internal Medicine
Ann Nucl Med	Annals of Nuclear Medicine
Ann Oncol	Annals of Oncology
Ann Pharmacother	Annals of Pharmacotherapy
Ann Surg	Annals of Surgery
Annu Rev Med	Annual Review of Medicine
Anticancer Drug Des	Anti-Cancer Drug Design
Anticancer Res	Anticancer Research
Appl Opt	Applied Ophthamology
Arch Androl	Archives of Andrology
Arch Gen Psychiatry	Archieves of General Psychiatry
Arch Gynecol Obstet	Archives of Gynecology and Obstetrics

Arch Mal Coeur Vaiss	Archives Mal Coeur Vaiss
Arch Neurol	Archives of Neurology
Arch Ophthalmol	Archives of Ophthalmology
Arch Oral Biol	Archives of Oral Biology Arch Otolaryngol Archives of Otolaryngology
Arch Pharm (Weinheim)	Archives of Pharmacology
Arch Pharm Res	Archives of Pharmacal Research
Arch Phys Med Rehabil	Archives of Physical Medicine and Rehabilitation
Arq Bras Cardiol	Arquivos Brasileiros de Cardiologia
Arthritis Rheum	Arthritis and Rheumatism
ASAIO J	American Asociation of Artificial Internal Organs Journal
Aust N Z J Surg	Australian and New Zealand Journal of Surgery
Aust Vet J	Australian Veterinary Journal
Baillieres Clin Endocrinol Metab	Bailliere's Clinical Endocrinology and Metabolism
Biochem Biophys Res Commun	Biochemical Biophysical Research Communications
Biochem J	Biochemical Journal
Biochem Pharmacol	Biochemical Pharmacology
Biochim Biophys Acta	Biochimica et biophysica acta
Bioconjug Chem	Bioconjugate Chemistry
Biol Pharm Bull	Biological & Pharmaceutical Bulletin
Biol Psychiatry	Biological Psychiatry
Biomed Sci Instrum	Biomedical Sciences Instrumentation
Bioorg Med Chem Lett	Bioorganic and Medicinal Chemistry Letters
Bioorg Med Chem	Bioorganic and Medicinal Chemistry
BJU Int	British Journal of Urology International
Blood Press Monit	Blood Pressure Monit
Bone Marrow Transplant	Bone Marrow Transplantation
Br Heart J	British Heart Journal
Br J Anaesth	British Journal of Anaesthesia
Br J Clin Pharmacol Pharmacology	British Journal of Clinical
Br J Haematol	British Journal of Haematology
Br J Nutr	British Journal of Nutrition
Br J Pharmacol	British Journal of Pharmacology
Br J Radiol	British Journal of Radiology
Br J Surg	British Journal of Surgery
Brain Res	Brain Research
Brain	Brain; A Journal of Neurology
Braz J Med Biol Res	Brazilian Journal of Medical and Biological Research = Revista brasileira de pesquisas medicas e biologicas
Calcif Tissue Int	Calcified Tissue International
Can Anaesth Soc J	Canadian Anaesthetists' Society Journal
Can J Ophthalmol	Canadian Journal of Ophthalmology. Journal Canadien d'Ophtalmologie
Can J Vet Res	Canadian Journal of Veterinary Research = Revue canadienne de recherche veterinaire
Can Vet J	Canadian Veterinary Journal. La Revue Veterinaire Canadienne
Cancer Biother Radiopharm	Cancer biotherapy & radiopharmaceuticals
Cancer Chemother Pharmacol	Cancer Chemotherapy and Pharmacology
Cancer Epidemiol Biomarkers Prev	Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology
Cancer Gene Ther	Cancer Gene Therapy
Cancer J	Cancer Journal
Cancer Res	Cancer Research
Cardiovasc Intervent Radiol	Cardiovascular and Interventional Radiology
Cardiovasc Res	Cardiovascular Research
Chem Biol	Chemistry & Biology
Clin Cancer Res	Clinical Cancer Research: An Official Journal of the American Association for Cancer Research
Clin Cardiol	Clinical Cardiology
Clin Chem Lab Med	Clinical Chemistry and Laboratory Medicine: CCLM
Clin Chem	Clinical Chemistry
Clin Chim Acta	Clinica Chimica Acta; International Journal of Clinical Chemistry
Clin Electroencephalogr	Clinical EEG (Electroencephalography)
Clin Endocrinol (Oxf)	Clinical Endocrinology (Oxford)
Clin Exp Allergy	Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology
Clin Exp Obstet Gynecol	Clinical and Experimental Obstetrics and Gynecology
Clin Exp Rheumatol	Clinical and Experimental Rheumatology
Clin Genet	Clinical Genetics
Clin Nephrol	Clinical Nephrology
Clin Nucl Med	Clinical Nuclear Medicine
Clin Nutr	Clinical Nutrition: Official Journal of the European Society of Parenteral and Enteral Nutrition
Clin Pharm	Clinical Pharmacy
Clin Pharmacokinet	Clinical Pharmacokinetics
Clin Pharmacol Ther	Clinical Pharmacology and Therapeutics

Clin Physiol Funct Imaging	Clinical Physiology and Functional Imaging
Clin Physiol	Clinical Physiology
Cochrane Database Syst Rev	Cochrane Database of Systematic Reviews [electronic resource]
Crit Care Med	Critical Care Medicine
Crit Rev Ther Drug Carrier Syst	Critical Reviews in Therapeutic Drug Carrier Systems
Curr Atheroscler Rep	Current Atherosclerosis Reports
Curr Drug Metab	Current Drug Metabolism
Curr Gastroenterol Rep	Current Gastroenterology Reports
Curr Opin Crit Care	Current Opinion in Critical Care
Curr Opin Drug Discov Devel	Current Opinion in Drug Discovery & Development
Curr Opin Gen Surg	Current Opinion in General Surgery
Curr Opin Lipidol	Current Opinion in Lipidology
Curr Opin Mol Ther	Current Opinions in Molecular Therapeutics
Curr Opin Neurol	Current Opinion in Neurology
Diabetes Res Clin Pract	Diabetes Research and Clinical Practice
Diagn Mol Pathol	Diagnostic Molecular Pathology
Dig Dis Sci	Digestive Diseases and Sciences
Dig Liver Dis	Digestive and Liver Disease: Official Journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver
DNA Cell Biol	DNA Cell Biology
Domest Anim Endocrinol	Domestic Animal Endocrinology
Drug Des Deliv	Drug Design and Delivery
Drug Metab Dispos	Drug Metabolism and Disposition: The Biological Fate of Chemicals
Drug Saf	Drug Safety
Drugs Today (Barc)	Drugs Today (Barcelona)
Emerg Med Clin North Am	Emergency Medicine Clinics of North America
Endocr J	Endocrine Journal
Endocr Res	Endocrinology Research
Environ Health Perspect	Environmental Health Perspectives
Epilepsy Behav	Epilepsy and Behavior
Equine Vet J	Equine Veterinary Journal
Eur Heart J Suppl	European Heart Journal Supplements: Journal of the European Society of Cardiology
Eur Heart J	European Heart Journal
Eur J Clin Chem Clin Biochem	European Journal of Clinical Chemistry and Clinical Biochemistry
Eur J Clin Invest	European Journal of Clinical Investigation
Eur J Clin Pharmacol	European Journal of Clinical Pharmacology
Eur J Dent Educ	European Journal of Dental Education: Official Journal of the Association for Dental Education in Europe
Eur J Drug Metab Pharmacokinet	European Journal of Drug Metabolism and Pharmacokinetics
Eur J Endocrinol	European Journal of Endocrinology
Eur J Gastroenterol Hepatol	European Journal of Gastroenterology & Hepatology
Eur J Heart Fail	European Journal of Heart Failure
Eur J Neurosci	European Journal of Neuroscience
Eur J Nucl Med Mol Imaging	European Journal of Nuclear Medicine and Molecular Imaging
Eur J Nucl Med	European Journal of Nuclear Medicine
Eur J Pharmacol	European Journal of Pharmacology
Eur J Radiol	European Journal of Radiology
Eur J Vasc Endovasc Surg	European Journal of Vascular and Endovascular Surgery: The Official Journal of the European Society for Vascular Surgery
Eur Neuropsychopharmacol	European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology
Eur Radiol	European Radiology
Eur Respir J	European Respiratory Journal: Official Journal of the European Society for Clinical Respiratory Physiology
Eur Surg Res	European Surgical Research. Europaische Chirurgische Forschung. Recherches Chirurgicales Europeennes
Eur Urol	European Urology
Exp Hematol	Experimental Hematology
Exp Lung Res	Experimental Lung Research
Expert Rev Mol Diagn	Expert Review of Molecular Diagnosis
Fed Proc	Federation Proceedings
Fertil Steril	Fertility and Sterility
Food Nutr Bull	Food and Nutrition Bulletin
Fundam Appl Toxicol	Fundamental and Applied Toxicology: Official Journal of the Society of Toxicology
Gastroenterol Clin Biol	Gastroenterologie Clinique et Biologique
Gastroenterol Nurs	Gastroenterology Nursing
Gastrointest Endosc	Gastrointestinal Endoscopy
Gene Ther	Gene Therapy
Gynecol Endocrinol	Gynecological Endocrinology: The Official Journal of the International Society of Gynecological Endocrinology

Gynecol Obstet Invest	Gynocology and Obstetrics Investigations
Head Neck	Head & Neck
Heart Vessels	Heart and Vessels
Hematol J	Hematology Journal
Hepatology	Hepatology: Official Journal of the American Association for the Study of Liver Diseases
Horm Metab Res	Hormone Metabolism Research
Horm Res	Hormone Research
Hua Xi Yi Ke Da Xue Xue Bao	Journal of West China University of Medical Sciences = Huaxi yike daxue xuebao
Hum Mutat	Human Mutation
Hum Psychopharmacol	Human Psychopharmacology
Hum Reprod	Human Reproduction
Hypertens Res	Hypertension Research: Official Journal of the Japanese Society of Hypertension
IEEE Trans Biomed Eng	IEEE Transactions on Bio-medical Engineering
IEEE Trans Ultrason Ferroelectr Freq Control	IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control
Immunopharmacol Immunotoxicol	Immunopharmacology and Immunotoxicology
In Vitro Cell Dev Biol Anim	In Vitro Cellular Development and Biology: Animal
Indian Pediatr	Indian Pediatrics
Infect Immun	Infection and Immunity
Int J Cancer	International Journal of Cancer. Journal international du cancer
Int J Cardiol	International Journal of Cardiology
Int J Clin Pharmacol Ther Toxicol	International Journal of Clinical Pharmacology, Therapy, and Toxicology
Int J Clin Pract	International Journal of Clinical Practice
Int J Oncol	International Journal of Oncology
Int J Pediatr Otorhinolaryngol	International Journal of Pediatric Otorhino-laryngology
Int J Pharm	International Journal of Pharmaceutics
Intensive Care Med	Intensive Care Medicine
Intern Med	Internal Medicine
Invest Ophthalmol Vis Sci	Investigative Ophthalmology and Visual Science
Invest Radiol	Investigative Radiology
Isotopes Environ Health Stud	Isotopes in Environmental and Health Studies
Ital Heart J	Italian Heart Journal: Official Journal of the Italian Federation of Cardiology
Ital J Neurol Sci	Italian Journal of Neurological Sciences
J Affect Disord	Journal of Affective Disorders
J Allergy Clin Immunol	Journal of Allergy and Clinical Immunology
J Am Anim Hosp Assoc	Journal of the American Animal Hospital Association
J Am Coll Cardiol	Journal of the American College of Cardiology
J Am Coll Nutr	Journal of the American College of Nutrition
J Am Soc Echocardiogr	Journal of the American Society of Echocardiography: Official Publication of the American Society of Echocardiography
J Am Vet Med Assoc	Journal of the American Veterinary Medical Association
J Anesth	Journal of Anesthesia
J Anim Sci	Journal of Animal Science
J Appl Microbiol	Journal of Applied Microbiology
J Appl Physiol	Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology
J Asthma	Journal of Asthma: Official Journal of the Association for the Care of Asthma
J Autism Dev Disord	Journal of Autism and Developmental Disorders
J Cardiovasc Electrophysiol	Journal of Cardiovascular Electrophysiology
J Cardiovasc Pharmacol	Journal of Cardiovascular Pharmacology
J Cell Biochem Suppl	Journal of Cellular Biochemistry. Supplement
J Child Neurol	Journal of Child Neurology
J Chromatogr B Analyt Technol Biomed Life Sci	Journal of Chromatography. B, Analytical Technology and Biomedical Life Sciences
J Chromatogr B Biomed Sci Appl	Journal of Chromatography. B, Biomedical Sciences and Applications
J Clin Endocrinol Metab	Journal of Clinical Endocrinology and Metabolism
J Clin Gastroenterol	Journal of Clinical Gastroenterology
J Clin Invest	Journal of Clinical Investigation
J Clin Pharmacol	Journal of Clinical Pharmacology
J Clin Psychiatry	Journal of Clinical Psychiatry
J Control Release	Journal of Controlled Release: Official Journal of the Controlled Release Society
J Drug Target	Journal of Drug Targeting
J Electrocardiol	Journal of Electrocardiology
J Endocrinol Invest	Journal of Endocrinological Investigation
J Enzyme Inhib	Journal of Enzyme Inhibition
J Fam Pract	Journal of Family Practice

J Feline Med Surg	Journal of Feline Medicine and Surgery
J Gastroenterol Hepatol	Journal of Gastroenterology and Hepatology
J Gastroenterol	Journal of Gastroenterology
J Gastrointest Surg	Journal of Gastrointestinal Surgery
J Gerontol	Journal of Gerontology
J Heart Lung Transplant	Journal of Heart and Lung Transplantation: The Official Publication of the International Society for Heart Transplantation
J Helminthol	Journal of Helminthology
J Hepatol	Journal of Hepatology
J Immunol Methods	Journal of Immunological Methods
J Immunol	Journal of Immunology
J Invest Dermatol	Journal of Investigative Dermatology
J Investig Allergol Clin Immunol	Journal of Investigative Allergology Clinical Immunology
J Korean Med Sci	Journal of Korean Medical Science
J Leukoc Biol	Journal of Leukocyte Biology
J Liquid Chromatogr	Journal of Liquid Chromatography
J Magn Reson Imaging	Journal of Magnetic Resonance Imaging : JMRI
J Matern Fetal Neonatal Med	Journal of Maternal, Fetal and Neonatal Medicine
J Med Assoc Thai	Journal of the Medical Association of Thailand = Chotmaihet thangphaet
J Med Chem	Journal of Medicinal Chemistry
J Mol Endocrinol	Journal of Molecular Endocrinology
J Mol Recognit	Journal of Molecular Recognition: JMR
J Neural Transm	Journal of Neural Transmission
J Neuroimaging	Journal of Neuroimaging: Official Journal of the American Society of Neuroimaging
J Neurol	Journal of Neurology
J Neuropathol Exp Neurol	Journal of Neuropathology and Experimental Neurology
J Neurosci Methods	Journal of Neuroscience Methods
J Neurosci	Journal of Neuroscience
J Neurotrauma	Journal of Neurotrauma
J Nucl Cardiol	Journal of Nuclear Cardiology: Official Publication of the American Society of Nuclear Cardiology
J Nucl Med Allied Sci	Journal of Nuclear Medicine and Allied Sciences
J Nucl Med	Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine
J Nutr	Journal of Nutrition
J Parenter Enteral Nutr	Journal of Parenteral and Enteral Nutrition
J Pediatr Endocrinol Metab	Journal of Pediatric Endocrinology & Metabolism: JPEM
J Pediatr Gastroenterol Nutr	Journal of Pediatric Gastroenterology and Nutrition
J Pediatr Surg	Journal of Pediatric Surgery
J Pharm Biomed Anal	Journal of Pharmaceutical and Biomedical Analysis
J Pharm Pharm Sci	Journal of Pharmacology and Pharmacy Science
J Pharm Pharmacol	Journal of Pharmacy and Pharmacology
J Pharm Sci	Journal of Pharmaceutical Sciences
J Pharmacobiodyn	Journal of Pharmacobio-Dynamics
J Pharmacokinet Biopharm	Journal of Pharmacokinetics and Biopharmaceutics
J Pharmacol Exp Ther	Journal of Pharmacology and Experimental Therapeutics
J Pharmacol Sci	Journal of Pharmacological Sciences
J Pharmacol Toxicol Methods	Journal of Pharmacological and Toxicological Methods
J Physiol	Journal of Physiology
J Postgrad Med	Journal of Postgraduate Medicine
J Physio Pharm	Journal of Physiology and Pharmacology
J Rheumatol	Journal of Rheumatology
J Small Anim Pract	Journal of Small Animal Practice
J Smooth Muscle Res	Journal of Smooth Muscle Research = Nihon Heikatsukin Gakkai kikanshi
J Steroid Biochem Mol Biol	Journal of Steroid Biochemical and Molecular Biology J Trauma Journal of Trauma
J Urol	Journal of Urology
J Vasc Res	Journal of Vascular Research
J Vet Intern Med	Journal of Veterinary Internal Medicine
J Vet Med A Physiol Pathol Clin Med	Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine
J Vet Med B Infect Dis Vet Public Health	Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health
JPEN J Parenter Enteral Nutr	JPEN. Journal of Parenteral and Enteral Nutrition
Jpn Circ J	Japanese Circulation Journal
Jpn Heart J	Japanese Heart Journal
Jpn J Pharmacol	Japanese Journal of Pharmacology
Kaku Igaku	Kaku Igaku. The Japanese Journal of Nuclear Medicine
Kaohsiung J Med Sci	Kaohsiung Journal of Medical Sciences

Keio J Med	Keio Journal of Medicine
Kidney Int Suppl	Kidney International. Supplement
Kidney Int	Kidney International
Klin Monatsbl Augenheilkd	Klinische Monatsblatter fur Augenheilkunde
Laser Surg Med	Laser Surgery and Medicine
Leuk Lymphoma	Leukemia and Lymphoma
Life Sci	Life Sciences
Magn Reson Med	Magnetic Resonance in Medicine: Official Journal of the Society of Magnetic Resonance in Medicine
Mayo Clin Proc	Mayo Clinic Proceedings
Med Sci Monit	Medical Science Monitor: International Medical Journal of Experimental and Clinical Research
Methods Find Exp Clin Pharmacol	Methods and Findings in Experimental and Clinical Pharmacology
Minerva Med	Minerva Medica
Mol Cell Biochem	Molecular and Cellular Biochemistry
Mol Diagn	Molecular Diagnosis
Mol Genet Metab	Molecular Genetics and Metabolism Journal
Mol Immunol	Molecular Immunology
Mol Pharmacol	Molecular Pharmacology
Mol Psychiatry	Molecular Psychiatry
Mov Disord	Movement Disorders: Official Journal of the Movement Disorder Society
Mult Scler	Multiple Sclerosis Journal
N Engl J Med	New England Journal of Medicine
N Engl Reg Allergy Proc	New England and Regional Allergy Proceedings
N Z Med J	New Zealand Medical Journal
Nat Biotechnol	National Biotechnology
NCI Monogr	National Cancer Institute Monograph
Ned Tijdschr Geneeskd	Nederlands tijdschrift voor geneeskunde
Nephrol Dial Transplant	Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association-European Renal Association
Neurobiol Aging	Neurobiology of Aging
Neurogastroenterol Motil	Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society
Neurol Med Chir (Tokyo)	Neurologia Medico-Chirurgica (Tokyo)
Neurourol Urodyn	Neurourology and Urodynamics
NMR Biomed	NMR in Biomedicine
No To Shinkei	No To Shinkei. Brain and Nerve
Nucl Med Biol	Nuclear Medicine Biology
Nucl Med Commun	Nuclear Medicine Communications
Occup Environ Med	Occupational and Environmental Medicine
Oncology (Huntingt)	Oncology (Huntington, NY)
Oper Dent	Operative Dentistry
Opt Lett	Optics Letters
ORL J Otorhinolaryngol Relat Spec	Journal of Otorhinolaryngology and Its Relative Specialities
Orthopade	Der Orthopade
Orv Hetil	Orvosi hetilap
Pacing Clin Electrophysiol	Pacing and Clinical Electrophysiology: PACE
Panminerva Med	Panminerva Medica
Pediatr Cardiol	Pediatric Cardiology
Pediatr Nephrol	Pediatric Nephrology: Journal of the International Pediatric Nephrology Association
Pediatr Neurol	Pediatric Neurology
Pediatr Res	Pediatric Research
Pharmacogenomics J	Pharmacogenetics Journal
Pharmacol Biochem Behav	Pharmacology, Biochemistry, and Behavior
Pharmacol Res	Pharmacology Research
Photochem Photobiol	Photochemistry and Photobiology
Proc Natl Acad Sci U S A	Proceedings of the National Academy of Sciences of the United States of America
Prog Neuropsychopharmacol Biol Psychiatry	Progress in Neuro-Psychopharmacology & Biological Psychiatry
Prostaglandins Leukot Essent Fatty Acids	Prostaglandins, Leukotrienes, and Essential Fatty Acids
Psychopharmacology (Berl)	Psychopharmacology (Berlin)
Radiat Med	Radiation Medicine
Radiol Med (Torino)	La Radiologia medica (Torino)
Recent Results Cancer Res	Recent Results in Cancer Research
Ren Fail	Renal Failure
Res Vet Sci	Research in Veterinary Science
Respir Care	Respiratory Care
Respir Med	Respiratory Medicine
Respir Physiol Neurobiol	Respiratory Physiology and Neurobiology
Respiration	Respiration; International Review of Thoracic Diseases
Rev Environ Health	Review of Environmental Health
Scand J Clin Lab Invest	Scandinavian Journal of Clinical and Laboratory Investigation
Scand J Gastroenterol	Scandinavian Journal of Gastroenterology
Scand J Thorac Cardiovasc Surg	Scandinavian Journal of Thoracic and Cardiovascular Surgery
Semin Neurol	Seminars in Neurology
Skin Res Technol	Skin Research and Technology: Official Journal of International Society for Bioengineering and the Skin (ISBS)

	[and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI)
Stroke	Stroke; A Journal of Cerebral Circulation
Surg Neurol	Surgical Neurology
Surg Today	Surgery Today
Swiss Med Wkly	Swiss Medical Weekly: Official Journal of the Swiss Society of Infectious Diseases, the Swiss Society of Internal Medicine, the Swiss Society of Pneumology
Ther Drug Monit	Therapeutic Drug Monitoring
Ther Hung	Therapia Hungarica: Hungarian Medical Journal
Thromb Haemost	Thrombosis and Haemostasis
Thromb Res	Thrombosis Research
Thyroid	Thyroid: Official Journal of the American Thyroid Association
Tierarztl Prax	Tierarztliche Praxis
Toxicol Appl Pharmacol	Toxicology and Applied Pharmacology
Toxicol Sci	Toxicological Sciences: An Official Journal of the Society of Toxicology
Trends Mol Med	Trends in Molecular Medicine
Trends Neurosci	Trends in Neuroscience
Trop Doct	Tropical Doctor
Ultrasound Med Biol	Ultrasound in Medicine & Biology
Ultrasound Obstet Gynecol	Ultrasound Obstetrics and Gynecology
Urology	Juan D Urology
Vasc Med	Vascular Medicine
Vet Clin North Am Small Anim Pract	Veterinary Clinics of North America. Small Animal Practice
Vet J	Veterinary Journal
Vet Radiol Ultrasound	Veterinary Radiology & Ultrasound: The Official Journal of the American College of Veterinary Radiology and the International Veterinary Radiology Association
Vet Rec	Veterinary Record
Wien Klin Wochenschr	Wiener klinische Wochenschrift
World J Gastroenterol	World Journal of Gastroenterology: WJG
Yonsei Med J	Yonsei Medical Journal
Z Gastroenterol	Z Gastroenterology
Z Kardiol	Zeitschrift fur Kardiologie
Zentralbl Gynakol	Zentralblatt fur Gynakologie
Zhonghua Yi Xue Za Zhi (Taipei)	Zhonghua yi xue za zhi = Chinese Medical Journal; Free China ed (Taipei)

COMPARATIVE DIAGNOSTIC PHARMACOLOGY
Clinical and Research Applications in Living-System Models

1 BODY SYSTEMS

1 Cardiovascular System

CARDIAC ARRYTHMIAS AND EKG-PHARMACOLOGY (ELECTROPHARMACOLOGY)

Arrythmias: Differential Diagnosis
Adenosine Triphosphate (ATP)

Background Information: The agent adenosine triphosphate has been applied for decades as a major medication for the termination of supraventricular tachycardia crisis (reentry mechanism implicating the atrioventricular node).
Application: Differential diagnosis of cardiac arrythmias including narrow or wide QRS tachycardia; latent accessory pathway, intranodal duality (non-invasive); evaluation of slow pathway ablation (non-invasive); and obscure tachycardia mechanism with palpitations or tachycardia.

Atrial Flutter: Fetal/Pregestation
Adenosine

Physiological Mechanism of Action: Negative chronotropic effect on the heart.
Application: Differentiate between atrioventricular nodal reentrant supraventricular tachyarrhythmia and atrial flutter.
Route of Administration: Cordocentesis (fetus).
Implementation: Evaluation of a cardiac arrythmia with 1:1 atrioventricular conduction applying prenatal echocardiography. Adenosine is infused via cordocentesis for the purpose of differentiating between atrioventricular nodal reentrant supraventricular tachyarrhythmia and atrial flutter.
Human Model: Fetal tachyarrhythmia with 1:1 atrioventricular conduction in a fetus at 25 weeks gestation.
Interpretation and Analysis: After infusion, transient 2:1 atrioventricular dissociation may be observed in conditions corresponding to a diagnosis of atrial flutter. Transplacental therapy with digoxin and amiodarone can then be employed for successful conversion.

Fetal tachyarrhythmia with 1:1 atrioventricular conduction. Adenosine infusion in the umbilical vein as a diagnostic test. Leiria TL, Lima GG, Dillenburg RF, Zielinsky P. Arq Bras Cardiol 2000 Jul; 75 (1), pp. 65–8.

Atrioventricular (A-V) Accessory Conduction Pathway/(Wolff-Parkinson-White Syndrome)
Flecainide

Cellular/Molecular Mechanism of Action: Flecainide inhibits membrane sodium fast channels.
Application: Flecainide is utilized as a Class Ic antiarrhythmic agent.
Route of Administration: Flecainide (intravenous). Flecainide can also be given orally since it is not as extensively removed from the portal circulation as the other pharmaceutical agents in this class of antiarrhythmic agents.
Dosage: Flecainide (2 mg/kg over 5-minute period).
Implementation: Conversion of accessory A-V conduction pathway arrythmias. Cases of accessory A-V conduction pathway can present in various classification types (e.g., total n = 21; apparent n = 16/21; latent = 1/21; concealed/blocked in the anterograde direction = 4/21) and clinical presentations (e.g., spontaneous and inducible n = 17/21; supraventricular tachycardias due to orthodromic reentry including the accessory A-V pathway n = 13/21; and atrial arrhythmias n = 4/21).
Interpretation and Analysis: Intravenous flecainide can successfully terminate supraventricular tachycardia over a time span averaging 3 minutes (e.g., n = 13/13). Such effects are achieved through depression and then blocking of retrograde conduction within the accessory pathway. Similary, approximately 75% (e.g. n = 3 of 4) of cases with atrial arrhythmia can be converted. Conduction in the accessory pathway is blocked in the anterograde direction in 75% of affected cases and depressed in the rest; it is blocked in the retrograde direction in about half the cases and depressed in the remaining cases. Intravenous flecainide completely prevents the induction of arrhythmias in approximately 76% of affected cases (e.g. n = 13/17). Oral flecainide blocks the accessory pathway in the anterograde and retrograde direction in 68.7% and 62% of these cases respectively, while preventing arrhythmias during provocative testing in approximately 82% of cases (n = 14/17). With an average follow-up of 20.7 ± 2.6 months for oral doses adapted to body weight and to the response to flecainide IV, the observed rate of recurrence for atrial fibrillation is very low (e.g., n = 1/21). Conversely, a high level of prevention for spontaneous supraventricular can be appreciated (e.g., 100%), as well as a high level of prevention for all arrhythmias (e.g., n = 16/17 or 94%). The predictive value for the response to oral therapy of the tests of regularization of SVT by IV flecainide and of the tests of non-provocation of SVT with oral or IV flecainide is excellent (100%), and cardiac tolerance is very good. A moderate percentage of cases will experience general intolerance to oral therapy in a manner that is not dose related and very few of these cases actually require interruption of therapy.

Effects of oral and injectable flecainide in patients with an accessory atrioventricular pathway. Fauchier JP, Cosnay P, Rouesnel P, Moquet B, Bonnet P, Scala PJ, Demeyer JF. Arch Mal Coeur Vaiss 1985 Oct; 78 Spec No:81–90.

Atrioventricular (A-V) Block/Intermittent High-Degree A-V Block
Procainamide

Application: Characterization of atrioventricular block.
Route of Administration: Intravenous.
Dosage: Procainamide, ≤ 10 mg/kg.
Implementation: Evaluation of cases with resting 1:1 A-V conduction but have bifascicular block and a history of syncope, with high-degree A-V block not diagnostically documented prior to performing the procainamide challenge test.

Before procainamide administration, the HV interval can be greater than 60 msec in a relative large number of cases (n = 17/42

or 41%). Results can be compared to negative reference controls (e.g., n = 3).

Human Model: Cases of suspected atrioventricular block (n = 42). Note: Absence of the development of infra-Hisian block with fixed rate atrial pacing or following programmed atrial extrastimuli in cases with > 60 msec HV intervals.

Interpretation and Analysis: Procainamide administration lengthens the mean HV interval by 11.9 msec, and in approximately 14% of cases (e.g., n = 7/42) the HV increment can be marked (e.g., between 15 and 75 msec change). Some cases may develop second or third degree infra-Hisian block (e.g., n = 4/42 or 9.5%), while a subpopulation of these cases (e.g., n = 2/4), their HV prior to procainamide administration is normal or only mildly prolonged (less than 60 msec). Among cases with bifascicular block with previously documented transient A-V block but 1:1 A-V conduction at the time of study (e.g., n = 4 cases), a relatively large percent may develop high-degree A-V block following procainamide (e.g., n = 3/4 or 75%). In conditions of bifascicular block but without syncope or documented high-degree A-V block (e.g., n = 5 cases), the mean HV interval may lengthen by 18.8 msec or more (e.g., n = 3/5 HV increment 24–30 msec). In conditions without syncope or an intraventricular conduction defect (e.g., n = 38 cases), the mean HV interval may lengthen by 5.3 msec (e.g., n = 2/38 cases with increases of 20 to 25 msec). Cases in the latter 2 groups frequently do not experience high-degree A-V block (e.g., n = 0). During follow-up periods of up to 10 years (mean 46 months), approximately 43% of cases in which procainamide provokes high-degree A-V block will subsequently progress to fixed complete A-V block (e.g., n = 3/7 cases). Although the incidence of provocation of A-V block is relatively low, it can be concluded that in conditions with possible intermittent A-V block, administration of procainamide as a test of distal conduction has limited value but is still useful and may provide information additional to that obtained from mere assessment of the HV interval.

Procainamide administration during electrophysiology study—Utility as a provocative test for intermittent atrioventricular block. Twidale N, Heddle WF, Tonkin AM. Pacing Clin Electrophysiol 1988 Oct; 11 (10), pp. 1388–97.

Atrioventricular (A-V) Block/Intermittent High-Degree Atrioventricular (A-V) Block/ Dose Infusion Optimization
Procainamide

Cellular/Molecular Mechanism of Action: Procainamide inhibits Na^+/K^+ pump function and promotes weak parasympatholytic activity similar to quinidine (quinidine has a stronger influence on cardiac parasympathetic input).

Background Information: No previous studies have determined the pharmaco-dynamics of intravenous procainamide when administered in a dose of 15 mg/kg and at a rate of 50 mg/min, as is common practice during electropharmacologic testing.

Application: Characterization of A-V block and intermittent high-degree A-V block.

Route of Administration: Procainamide (intravenous).

Dosage: Procainamide (4 mg/min to 8 mg/min).

Implementation: Evaluation of cardiovascular conditions using procainamide during electropharmacological testing procedures. In this fashion, the right ventricular effective refractory period and the QRS duration at a ventricular pacing rate of 120/min can be determined every minute for 20 minutes. Plasma procainamide concentrations are measured at 1, 5, 10, 15, and 20 minutes following loading-dose administration.

Human Model: n = 30; n = 10 cases given no maintenance infusion; n = 10 cases @ 4 mg/min maintenance infusion; n = 10 cases @ 8 mg/min maintenance infusion; n = 10 reference control cases given no procainamide.

Interpretation and Analysis: A stable plasma procainamide concentration may not be present in cases receiving procainamide administration until 15 minutes after loading-dose infusion. The effective refractory period and QRS duration increase compared with baseline at 1 minute, decrease between 1 and 10 minutes, and then remain essentially unchanged between 10 and 20 minutes (all cases groups receiving procainamide). Concentration-effect relationships are linear in cases receiving multiple procainamide administration schedules. The plasma procainamide concentrations in cases receiving infusions at 8 mg/min are significantly greater than in cases receiving 4 mg/min infusion rates; however, the effects on refractoriness and QRS duration are similar in both groups. These findings indicate that with a procainamide dosing method commonly used during electropharmacologic testing, the plasma procainamide concentration decreases significantly during the first 15 minutes after the loading dose is administered.

Pharmacodynamics of intravenous procainamide as used during acute electropharmacologic testing. Morady F, Kou WH, Schmaltz S, Annesley T, De Buitleir M, Nelson SD, Kushner JA. Am J Cardiol 1988 Jan 1; 61 (1), pp. 93–8.

Atrioventricular Nodal Reentrant Tachycardia/ Atrioventricular Nodal Block Induction
Adenosine

Physiological Mechanism of Action: Negative chronotropic effect on cardiac function.

Background Information: Adenosine at low doses preferentially blocks fast over slow pathway conduction in conditions of dual atrioventricular (A-V) nodal physiology and typical A-V nodal reentrant tachycardia (A-VNRT). During atrial pacing, this effect is manifested as an abrupt increase in the AH interval with low doses of adenosine. The demonstration of dual A-V nodal physiology may be useful as a diagnostic tool during electrophysiologic studies in cases with supraventricular tachycardia that are not easily inducible, as clear demonstration of dual A-V nodal pathways may indicate that A-VNRT is a likely diagnosis and that further attempts at arrhythmia induction should be tailored with that objective in mind. However, to be a useful test, adenosine should not cause an abrupt increase in AH interval in conditions without dual A-V nodal physiology.

Application: Incremental adenosine infusion during electrophysiologic studies can be used as a highly specific diagnostic tool for cases with dual A-V nodal pathways.

Route of Administration: Adenosine (intravenous).

Dosage: Adenosine (incrementally larger doses administered until development of A-V nodal block).

Implementation: Baseline Wenckebach cycle length (WCL) and A-V nodal effective refractory periods are measured at atrial pacing cycle lengths of 400 and 600 msec. The atrium is then paced at WCL +50 ms, and WCL +100 ms, while incrementally larger doses of intravenous adenosine are administered until induction of A-V nodal block.

Human Model: Electrophysiology cases (n = 37 without prior history of A-VNRT).

Interpretation and Analysis: The mean (± standard deviation) doses of adenosine required to cause A-V nodal block while pacing at WCL +50 msec and WCL +100 msec is approximately 7.1 ± 3.9 and 7.4 ± 4.5 mg, respectively. In a small number of cases (e.g., n = 1/37 = 2.7%, 95% confidence interval 0–8%), an abrupt prolongation of the AH interval can be appreciated with the administration of adenosine during atrial pacing as well as during the atrial refractory period determination. In the majority of cases, no dual A-V nodal

physiology can be appreciated during the refractory period determination, and there are only gradual changes in the AH interval with atrial pacing during adenosine administration. In cases without a history suggestive of A-V nodal reentrant tachycardia, only 2.7% have clinically silent dual A-V nodal pathways using the described method. Incremental adenosine infusion during electrophysiologic study can be used as a highly specific diagnostic tool for cases with dual A-V nodal pathways.

Use of adenosine as a diagnostic tool for dual atrioventricular nodal pathways: Response of control patients to incremental doses of adenosine. Burkart TA, Scozzaro MJ, Angella FR, Jayaram KN, Gonzalez MD, Conti JB, Curtis AB. Clin Cardiol 2002 Jun; 25 (6), pp. 263–6.

Brugada Syndrome/Genetic Locus: SCN5A Gene Mutation
Flecainide

Cellular Mechanism of Action: Flecainide suppresses fast sodium channels resulting in declines in Vmax and action potential overshoot within the atria, ventricles, and Purkinje fibers.
Physiological Mechanism of Action: Flecainide and the other agents in this class of local anesthetics including encainide and lorcainide exert cellular membrane electrophysiological responses that are distinctly different from those created by lidocaine. A slowing of conduction is most prominent within the His-Purkinje system, but there is minimal effect on action potential repolarization duration. On EKG tracings a noticeable widening of the QRS complex can be appreciated.
Background Information: The flecainide test is widely used to evaluate conditions of Brugada syndrome. However, its reproducibility and safety remain ill-defined.
Route of Administration: Flecainide test (intravenous bolus).
Dosage: Flecainide test (2 mg/kg over 10 minutes).
Implementation: (i) Evaluation of the frequency of mutations in the SCN5A gene in cases of Brugada syndrome that are candidates for flecainide response testing; (ii) determination of the degree of correlation that exists between SCN5A expression and results from flecainide response testing; (iii) determination of the repeatability of flecainide test response. The flecainide response is performed at baseline (e.g., n = 21/22 cases) and a second time within 2 months.

Human Model: Cases of Brugada syndrome (n = 22; n = 18 men; age = 34 years; n = 2 cases of aborted sudden cardiac death; n = 8 cases with syncope/presyncope; n = 12 asymptomatic cases).
Interpretation and Analysis: Mutations in the SCN5A gene can be appreciated in a relatively large percentage of cases of Brugada syndrome (e.g., n = 8/22). Analyses of ECG tracing may be (i) diagnostic in 86% (e.g. n = 19/22); and (ii) suggestive in 14% (e.g. n = 3/22). The flecainide test can be performed by infusion of a 2 mg/kg IV bolus over 10 minutes (e.g. n = 21/22 test subjects). In a very high percentage of cases the flecainide test is diagnostic or amplifies the typical ECG pattern (e.g., n = 21/21 or 100%). At the end of drug infusion, sustained VT that lasts 7–10 minutes may ultimately develop (e.g., n = 2/21 cases).

A second flecainide test performed within 2 months may be diagnostic in a high percentage of cases (e.g., n = 20/20 or 100%; note n = 2 cases with prior development of ventricular arrythmias; n = 1/2 with sustained VT; n = 1/2 recurrent VF). The reproducibility of the flecainide test is relatively high (e.g. 100%). Approximately 18% of cases (e.g., n = 4/22) develop ventricular arrythmias documented after the end of flecainide infusion. Ventricular arrythmias occur in 43% (e.g. n = 3/7) of cases with SCN5A gene mutation compared to 7% (e.g. n = 1/15) without an SCN5A gene mutation ($p < 0.05$). Diagnostic ECG changes or arrhythmias are usually not appreciated in reference controls (e.g., n = 25). The reproducibility of the flecainide response test is very high for supporting a diagnosis of Brugada syndrome. However, the occurrence of major VA, (significantly higher in cases with documented SCN5A gene mutation, including in asymptomatic conditions) mandates performance of the procedures under appropriate medical supervision. Whether a slower rate of drug infusion can lower the risk of VA induction while maintaining the sensitivity of the test needs to be explored.

Flecainide test in Brugada syndrome: A reproducible but risky tool. Gasparini M, Priori SG, Mantica M, Napolitano C, Galimberti P, Ceriotti C, Simonini S. Pacing Clin Electrophysiol 2003 Jan; 26 (1 Pt 2), pp. 338–41.

Brugada Syndrome/Genetic Locus: Distinct from SCN5A (Low Procainamide Sensitivity)
Procainamide

Background Information: Brugada syndrome is a form of idiopathic ventricular fibrillation characterized by a right bundle-branch block pattern and S-T segment elevation (S-TE) in the right ECG precordial leads. Sodium channel blockers increase S-TE. Mutations of the cardiac sodium channel SCN5A cause the disorder, and an implantable cardioverter-defibrillator is often recommended for the management of such conditions. Mutations in other genes have not been identified, and it is not known if the efficacy of drug testing or the malignancy of arrhythmias correlates closely with the frequency of the genetic defect.
Application: Procainamide infusions do not identify additional affected cases of Brugada syndrome beyond those already identified through the interpretation of other clinically-relevant parameters.
Implementation: Collective evaluation of histories, physical examinations, ECGs, and drug testing in cases with Brugada syndrome. Samples of DNA isolated from blood samples, polymorphic genomic markers, and polymorphisms within sodium channels can be used for a genome-wide screen, fine mapping, and linkage analysis.

Human Model: Families with multigenerations with Brugada syndrome.
Interpretation and Analysis: Some affected cases (n = 12 right bundle-branch block, ≥ 1 mm S-TE) with an autosomal dominant inheritance profile display patterns of incomplete penetrance that appears to be dependent on age and gender. Some cases (e.g. n = 4/12) also display signs of syncope, while others (e.g., n = 2/12) have documented evidence of ventricular arrhythmias. In some scenarios, there is minimal sudden death in the family history. Procainamide infusions do not identify additional affected individuals. Linkage is present to an approximately equal 15 cM region on chromosome 3p22-25 (maximum LOD score = 4.00). The sodium channel genes SCN5A, SCN10A, and SCN12A on chromosome 3 have been excluded as candidates (LOD scores ≤ −2). A Brugada syndrome locus distinct from SCN5A is associated with progressive conduction disease, a low sensitivity to procainamide testing, and a relatively good prognosis in a single large pedigree.

Clinical and molecular heterogeneity in the Brugada syndrome: A novel gene locus on chromosome 3. Weiss R, Barmada MM, Nguyen T, Seibel JS, Cavlovich D, Kornblit CA, Angelilli A, Villanueva F, McNamara DM, London B. Circulation 2002 Feb 12; 105 (6), pp. 707–13.

Brugada Syndrome and Long Q-T Interval Syndromes
Flecainide

Cellular Mechanism of Action: Flecainide suppresses fast sodium channels resulting in declines in Vmax and action potential overshoot within the atria, ventricles, and Purkinje fibers.

Physiological Mechanism of Action: In this context, flecainide and the other agents in this class of local anesthetics including encainide and lorcainide exert cellular membrane electrophysiological responses that are distinctly different than those produced by lidocaine. A slowing of conduction is most prominent within the His-Purkinje system, but there is minimal effect on action potential repolarization duration. On EKG tracings a noticeable widening of the QRS complex can be appreciated.
Application: Evaluation of SCN5A gene-related cardiac sodium channel disfunction associated with both [i]long Q-T syndrome (Q-T interval prolongation) with S-T segment elevations (leads V1 through V3); and [ii] Brugada syndrome. Intravenous flecainide is the standard provocative test used to unmask Brugada syndrome in individuals with concealed forms of the disease, and oral flecainide has been proposed as a treatment option for LQ-T3 patients because it may shorten their Q-T interval.
Route of Administration: Intravenous.
Dosage: Brugada syndrome protocol.
Considerations: The typical manifestations of Q-T syndrome (Q-T interval prolongation) and Brugada syndrome (S-T segment elevation in leads V1 through V3) may coexist in the same patients, which raises questions about the actual differences between these 2 conditions. Theoretically, differentiation between them can be achieved with the intravenous administration of flecainide based on the hypothesis that it might not only shorten the Q-T interval but may also produce elevations of the S-T segment in cases of long Q-T syndrome (Q-T interval prolongation) with S-T segment elevations (leads V1 through V3).
Interpretation and Analysis: Administration of intravenous flecainide according to Brugada syndrome protocols in conditions of long Q-T syndrome (Q-T interval prolongation) with S-T segment elevations (leads V1 through V3) can produce (i) shortening of the Q-T, Q-Tc, JT, and JTc intervals in a high percentage of cases (e.g., n = 12/13 or 92%); and (ii) S-T segment elevation in leads V1 through V3 ($\geq$ 2 mm) may also sometimes be appreciated (e.g., n = 6/13 or 46%). In this context, flecainide may induce S-T segment elevation in conditions of long Q-T syndrome (Q-T interval prolongation) accompanied by S-T segment elevations (leads V1 through V3), thereby raising concerns about the safety of flecainide therapy and demonstrates the intriguing consideration that an overlap may exist between this condition and Brugada syndrome.

The elusive link between LQ-T3 and Brugada syndrome: The role of flecainide challenge. Priori SG, Napolitano C, Schwartz PJ, Bloise R, Crotti L, Ronchetti E. Circulation 2000 Aug 29; 102 (9), pp. 945–7.

Brugada Syndrome/Tachyarrythmias: Long Q-T Syndrome
Catecholamine/Isoproterenol

Route of Administration: Isoproterenol (intravenous infusion).
Dosage: Isoproterenol.
Considerations: Catecholamines have long been used as a provocative test in some forms of tachyarrhythmias including long Q-T syndrome. In addition, catecholamines are also reported to decrease S-T segment elevation in leads V1-V3 in some conditions of Brugada syndrome.
Implementation: Differential effects of catecholamines on Q-T interval, action potential duration, transmural dispersion of repolarization, and torsade de pointes between LQ-T1, LQ-T2, and LQ-T3 forms of long Q-T syndrome can be evaluated in experimental models utilizing (i) arterially-perfused wedge preparations; and (ii) cases with congenital long Q-T syndrome, including children.
Interpretation and Analysis: Preliminary results in adult and prejuvenile forms of Brugada syndrome receiving isoproterenol infusion reveals that it is effective in decreasing the S-T segment elevation in leads V1-V2 and in suppressing the electrical storm of ventricular fibrillation.

Catecholamines in children with congenital long Q-T syndrome and Brugada syndrome. Shimizu W, Kamakura S. J Electrocardiol 2001; 34 Suppl, pp. 173–5.

Brugada Syndrome/Vagal Stimulation Sensitivity/ Angina: Atypical Thoracic Pain (Coronary Vasospastic)/Ischemic-Related Sudden Death Risk
Acetylcholine (ACh)/Ergonovine Maleate (EM)

Physiological Mechanism of Action: Coronary artery spasm can be induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the endothelium is intact by releasing nitric oxide (NO). In conditions of coronary artery spasm, acetylcholine induces a spasmodic response that can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. Such findings imply that some form of abnormality in endothelial integrity exists in conditions of coronary spasm that can be accentuated by acetylcholine and histamine, and to a lesser by ergonovine and serotonin. In general, all ergot alkaloids exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptor complexes. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentrations. Similarly, the contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.
Background Information: Experimental studies suggest that a prominent transient outward current (I_{to})-mediated action potential notch and a subsequent loss of the action potential dome in the epicardium, but not in the endocardium, give rise to S-T segment elevation and subsequent ventricular fibrillation (VF).
Application: Characterization of the sensitivity of Brugada syndrome to vagal stimulation and possible prediction of the risk of developing ischemic-related sudden death.
Route of Administration: Acetylcholine (ACh).
Implementation: Evaluation of the frequency of coronary spasm, augmentation ($\geq$ 0.1 mV) of S-T segment elevation in leads V1 to V3, and induction of VF by intracoronary injection of ACh and/or EM.

Human Model: Cases of symptomatic Brugada syndrome (n = 27 with n = 30 control).
Interpretation and Analysis: Coronary spasm can be induced in a relatively small percentage of cases affected by Brugada syndrome (e.g., n = 3/27 or 11%) compared to approximately 43% of healthy/normal subjects (e.g., n = 13/30). S-T segment elevation is augmented in approximately 33% (e.g., n = 11/33) of right coronary injections (e.g., ACh: n = 6/11 or 55%; EM: n = 5/22 or 23%), without coronary spasm, but in essentially no or a very low percentage of left coronary injections in cases with Brugada syndrome (e.g., n = 0 or 0%). Ventricular fibrillation can be induced in approximately 9% of right coronary injections (e.g., n = 3/33; ACh: 2/11 or 18%; EM: 1/22 or 5%), but in no or only a very low percentage of left coronary injections (e.g., n = 0 or 0%). In contrast, neither S-T segment elevation nor VF can be commonly observed in control subjects (e.g., n = 0 or 0%). Such observations support the hypothesis that mild ischemia and vagal influences act additively or synergistically with the substrate responsible for Brugada syndrome elevated

S-T segments and precipitation of VF. These observations suggest that Brugada conditions may be at a higher risk for ischemia-related sudden death.

S-T segment elevation and ventricular fibrillation without coronary spasm by intracoronary injection of acetylcholine and/or ergonovine maleate in patients with Brugada syndrome. Noda T, Shimizu W, Taguchi A, Satomi K, Suyama K, Kurita T, Aihara N, Kamakura S. J Am Coll Cardiol 2002 Nov 20; 40 (10), pp. 1841–7.

Brugada Syndrome: Unmasking Silent ECG Pattern/ [Syncope of Unknown Origin]
Ajmaline/Pro-Arrhythmic Effect

Cellular/Molecular Mechanism of Action: Ajmaline is a a blocking agent for sodium channels.
Background Information: The diagnostic ECG pattern in Brugada syndrome (BS) can transiently normalize and may be unmasked by sodium channel blockers. Proarrhythmic effects of the drug have been well documented in the literature. A detailed protocol for the ajmaline challenge in Brugada syndrome has not been thoroughly described.
Application: Standardized ajmaline test for unmasking ECG evidence of Brugada syndrome.
Route of Administration: Ajmaline (intravenous).
Dosage: Ajmaline (10 mg fractions every 2 minutes up to a target dose of 1 mg/kg).
Implementation: Evaluation of ajmaline as a "test" agent during ECG evaluation of Brugada syndrome.

Human Model: n = 158 cases subjected to a ajmaline test over a 60-month period.
Interpretation and Analysis: In approximately one-fourth of all cases of Brugada syndrome, the typical coved-type ECG pattern of the condition is unmasked with ajmaline administration (e.g., n = 37/158 or 23%). During test evaluations, symptomatic VT can occasionally be recognized (e.g., n = 2/158 or 1.3%). In other cases of Brugada syndrome, ajmaline challenge does not induce VT if the target dose; QRS prolongation > 30%, presence/appearance of the typical ECG; or the occurrence of premature ventricular ectopy are considered as criteria end points. A positive response to a ajmaline can be induced in a small number of cases (e.g., n = 2/94 or 2%) that have a normal baseline ECG but are subjected to evaluation solely for syncope of unknown origin.

The ajmaline challenge using a fractionated drug administration protocol is a safe method to diagnose BS. Because of the potential induction of VT, it should be performed under continuous medical surveillance with advanced life-support facilities. Due to the prognostic importance all cases with aborted sudden death or unexplained syncope without demonstrable structural heart disease and family members of affected individuals should presently undergo drug testing for unmasking Brugada syndrome.

The ajmaline challenge in Brugada syndrome: Diagnostic impact, safety, and recommended protocol. Rolf S, Bruns HJ, Wichter T, Kirchhof P, Ribbing M, Wasmer K, Paul M, Breithardt G, Haverkamp W, Eckardt L. Eur Heart J 2003 Jun; 24 (12), pp. 1104–12.

Brugada Syndrome (Case Report)/Vasospastic Angina: Associated Syncope
Acetylcholine/Class I Antiarrhythmic Agents/Procainamide/[Diltiazam]/[Flicanide]

Cellular/Molecular Mechanism of Action: Acetylcholine promotes vascular smooth muscle contraction by acting as a neurotransmitter for the parasympathetic segment of the autonomic nervous system. Procainamide is a Class I antiarrhythmic sodium channel blocker.
Physiological Mechanism of Action: Coronary artery spasm can be induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the endothelium is intact by releasing nitric oxide (NO). In conditions of coronary artery spasm, acetylcholine induces a spasmodic response that can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. Such findings imply that some form of abnormality in endothelial integrity exists in conditions of coronary spasm that can be accentuated by acetylcholine and histamine, and to a lesser by ergonovine and serotonin. In general, all ergot alkaloids exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptor complexes. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentrations. Similarly, the contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.

Procainamide exerts both a direct effect on the heart and an indirect effect mediated through the autonomic nervous system. The overall effect of procainamide on cardiac automaticity, excitability, responsiveness and conduction are virtually identical to that reported for quinidine.
Application: Administered as a provocative test for inducing spasms of coronary arteries applied in combination with electrocardiography monitoring.
Route of Administration: Acetylcholine (selective intracoronary injection).
Dosage: Acetylcholine.
Implementation: Evaluation of conditions associated with coronary artery spasm.

Human Model: Cases of coronary artery spasm accompanied by syncopal attacks and chest pain (e.g. n = 1/68-year-old male)with evidence of Brugada syndrome on electrocardiogram tracings. Ventricular fibrillation (VF) can be induced by programmed electrical stimulation.
Interpretation and Analysis: S-T segment elevations can be exaggerated by procainamide, which may not prevent the induction of VF. Coronary angiography may reveal no stenotic lesions, while spasm in the left coronary artery can be induced by intracoronary administration of acetylcholine resulting in the creation of similar chest pain similar to previous natural experiences. Under treatment with diltiazem and flecainide, which can suppress the induction of VF, patients may experience no recurrence of symptoms despite persistent S-T segment elevations. No previous reports have described coronary spasm associated with Brugada-type ECG abnormalities, and patients with syncope should be evaluated carefully.

Related reports have described conditions of vasospastic angina and Brugada-type ECG abnormalities. Cases may present with signs of chest pain and evidence of transient S-T segment elevations on ECG profiles. Coronary angiogram shows no organic stenosis. Syncopal episodes may follow anginal chest pain, and the same symptoms can often be created by intracoronary acetylcholine-induced vasospasm. The ECG profile at rest in many cases shows S-T segment elevation in leads V1 and V2 and a right bundle branch block pattern, which can be accentuated by a Class I antiarrhythmic drug. Ventricular fibrillation also can be induced by programmed electrical stimulation. Susceptibility to ventricular fibrillation can be modulated by the interaction of coronary vasospasm with Brugada syndrome or vice versa; therefore, it is important to study the clinical implications of the coexistence of the 2 diseases in such conditions.

A case of vasospastic angina presenting Brugada-type ECG abnormalities. Itoh E, Suzuki K, Tanabe Y. Jpn Circ J 1999 Jun; 63 (6), pp. 493–5.

Vasospastic angina accompanied by Brugada-type electrocardiographic abnormalities. Chinushi M, Kuroe Y, Ito E, Tagawa M, Aizawa Y. J Cardiovasc Electrophysiol 2001 Jan; 12 (1), pp. 108–11.

Long Q-T Syndrome: Genetic *Implications*

Background Information: The molecular genetic background of inherited cardiac arrhythmias has only recently been delineated. Delays in establishing comparisons to other inherited cardiac disorders has partly been due to the high mortality and early disease onset of these arrhythmias resulting in mostly small nucleus families. Thus, traditional genetic linkage studies, which are based on the genetic information obtained from large multigeneration families, have been difficult to conduct. Inherited arrhythmogenic disorders can be divided into primary electrical disorders (e.g., long Q-T [LQ-T] syndrome), in which evidence of organic heart disease can not be detected, and inherited diseases affecting myocardial structures (e.g., hypertrophic cardiomyopathies), in which arrhythmias occur combined with structural (functional anatomical) alterations. To date, all inherited arrhythmogenic disorders in which the causative genes have been identified can be classified as channelopathies, since the genes encode channel subunits that regulate important ion currents that "tune" the cardiac action potential. The discovery of the genetic bases of the LQ-T syndrome became a new methodologic paradigm because with the use of "classical" genetic linkage strategies (named [positional] candidate strategies), not only the causative genes have been found, but moreover, functional components with a previously unknown but fundamental role for a normal repolarization process have been discovered. Disease mutations have been determined to be not only a family-specific event with a distinct phenotype and the potential of an additional diagnostic tool, but also, when expressed in heterologous expression systems, characterize the defective ion channel in a topological way and lead to a more specific understanding of ion channel function. Most, if not all, primary electrical cardiac disorders show a high level of genetic diversity. For the LQ-T syndromes, sixth disease loci and the responsible gene have been recently discovered (so-called locus or genetic heterogeneity). Within all disease genes, the mutations are spread over the entire gene (allelic heterogeneity); in addition, more than 1 disease mutation may be present. This complexity requires, at the least, complete mutation analysis of all LQ-T genes before medical advice should be given. In addition, genotype-phenotype correlations in large families have been used to evaluate intergene, interfamilial, and intrafamilial differences in the clinical phenotype, reflecting gene specific, gene-site specific, and individual consequences of a given mutation. A widespread phenotypic heterogeneity even within mutation carriers in the same family illustrates the importance of modifying factors and genes that are mostly unknown to date. The reduced penetrance and variable expressivity associated with the LQ-T mutations remains unexplained. First insights into the complex actions of mutations are being extracted from expression data; these preliminary results may lead to potential implications for a specific (gene-site directed) therapy. The referenced paper discusses data relevant to molecular genetics and genotype-phenotype correlations in LQ-T syndrome and related disorders and the potential implications for diagnosis and treatment.

Molecular genetics of arrhythmias—A new paradigm. Schulze-Bahr E, Haverkamp W, Borggrefe M, Wedekind H, Mönnig G, Mergenthaler J, Assmann G, Funke H, Breithardt G. Z Kardiol 2000; 89 Suppl 4, pp. IV12–22.

Long Q-T Syndrome (LQ-TS) *Dobutamine*

Cellular/Molecular Mechanism of Action: Directly binds to and activates β_1 adrenergic receptor complexes.
Physiological Mechanism of Action: Dobutamine, to a lesser degree than isoproterenol, increases sinus node automaticity. Conduction velocity through the A-V node is accelerated. Atrial and ventricular impulse conductions are not influenced by dobutamine. Dobutamine has a greater effect on myocardial contraction and exerts relatively less pronounced chronotropic properties. In conditions of prolonged Q-T intervals (Q-T syndrome), dobutamine shortens the Q-T interval on EKG tracings.
Application: Low-dose dobutamine along with isoproterenol has been applied for the diagnosis and evaluation of prolonged Q-T intervals (Q-T syndrome or LQ-TS) associated with syncope and abnormal TU complexes. Diagnostically, dobutamine can be helpful in determining if syncope is due to a prolongation in Q-T intervals or if episodes are precipitated by non-cardiac related abnormalities.
Route of Administration: Continuous intravenous infusion can be implemented due to a brief plasma half-life of approximately $T_{1/2} = 2$ minutes. Dobutamine cannot be effectively administered orally.
Dosage: Dobutamine (low dose 10 μg/kg/min). In domestic animals, dobutamine administration rates between 2.5 to 15 μg/kg/min elevate myocardial contractility and cardiac output. In instances where the administration rate exceeds 20 μg/kg/min, increases in heart rate become much more pronounced.
Considerations: Dobutamine is rapidly metabolized by the liver to an inactive glucuronic conjugate. In contrast to dopamine, dobutamine does not promote renal vasculature vasodilation.
Interpretation and Analysis: When dobutamine is infused at a rate of 10 μg/kg/min, the Q-T interval corrected for heart rate (Q-Tc) is prolonged from 460 msec to 620 msec with the abnormal development of TU complexes and torsades de pointes profiles. In contrast, the Q-Tc does not appreciably change in normal unaffected patients ($n = 5$) during low-dose dobutamine infusion. Low-dose dobutamine, in addition to isoproterenol, may be useful for evaluating Q-T interval prolongation and abnormal TU complexes when LQ-TS is a consideration.

Diagnostically, dobutamine can be helpful in determining if syncope in affected cases is due to a prolongation in Q-T intervals or if episodes originate from non-cardiac related abnormalities.
Toxicity: Essentially the same as observed with isoproterenol including arrythmias and should be avoided or used with care in conditions of atrial fibrillation.

Induction of torsades de pointes by dobutamine infusion in a patient with idiopathic long Q-T syndrome. Fujikawa H, Sato Y, Arakawa H, Mitsuhashi T, Minezaki K, Kuroki S, Sekiguchi H, Nakayama T, Ikeda U, Shimada K. Intern Med 1998 Feb; 37 (2), pp. 149–52.

Norepinephrine, Epinephrine, and the Sympathomimetic Smines. Weiner N (Chapter 8: pp. 145–80). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Long Q-T Syndrome (LQ-T1): Congenital/Silent LQ-T1 Mutation Carriers *Epinephrine*

Cellular/Molecular Mechanism of Action: Epinephrine is an endenous neurotransmitter that functions as a positive adrenergic agonist/sympathomimetic agent at α-adrenergic and β-adrenergic postsynaptic receptor complexes.

Background Information: The LQ-T1 form of congenital long Q-T syndrome is associated with a high vulnerability to sympathetic stimulation and clinically presents with incomplete penetrance.
Application: Detection of silent LQ-T1 mutation carriers.
Route of Administration: Epinephrine (infusion).
Dosage: Epinephrine (0.1 μg/kg/min).
Implementation: Evaluation of the LQ-T1 form of congenital long Q-T syndrome using epinephrine infusion. Electrocardiographic (12-lead) parameters are recorded before and after epinephrine infusion.

Human Model: Congenital LQ-T1 (n = 19 mutation carriers with a baseline corrected Q-T interval (Q-Tc) of ≥ 460 msec (Group I); n = 15 mutation carriers with a Q-Tc of < 460 msec (Group II); n = 12 non-mutation carriers (Group III), and 15 controls (Group IV).
Interpretation and Analysis: The mean corrected Q-Tend (Q-Tce), Q-Tpeak (Q-Tcp), and Tpeak-end (Tcp-e) intervals among 12-leads prior to epinephrine are significantly larger in Group I cases than in other cases groups. Epinephrine significantly increases the mean Q-Tce, Q-Tcp, and Tcp-e and the dispersion of Q-Tcp in Groups I and II, but not in Groups III and IV. Sensitivity and specificity of Q-Tce measurements to identify mutation carriers is approximately 59% (e.g. n = 20/34) and 100% (e.g. n = 27/27), respectively, before epinephrine, and the sensitivity is substantially improved to 91% (e.g. n = 31/34) without the expense of specificity (100%, or n = 27/27) after epinephrine. The mean Q-Tce, Q-Tcp, and Tcp-e before and after epinephrine is significantly larger in symptomatic (e.g., n = 15) than in asymptomatic (e.g. n = 19) mutation carriers in case Groups I and II, and the prolongation of the mean Q-Tce with epinephrine is significantly larger in symptomatic cases. Epinephrine challenge can function as a powerful test to establish electrocardiographic diagnosis in silent LQ-T1 mutation carriers, thus allowing implementation of prophylactic measures aimed at reducing sudden cardiac death.

Epinephrine unmasks latent mutation carriers with LQ-T1 form of congenital long Q-T syndrome. Shimizu W, Noda T, Takaki H, Kurita T, Nagaya N, Satomi K, Suyama K, Aihara N, Kamakura S, Sunagawa K, Echigo S, Nakamura K, Ohe T, Towbin JA, Napolitano C, Priori SG. J Am Coll Cardiol 2003 Feb 19; 41 (4), pp. 633–42.

Long Q-T Syndrome/Ventricular Tachycardias/ Wolff-Parkinson-White Syndrome
Therapeutic Response/[Encainide]/[Flecainide]/ [Lidocaine]/[Lorcainide]

Cellular/Molecular Mechanism of Action: Flecainide suppresses fast sodium channels resulting in declines in Vmax and action potential overshoot within the atria, ventricles, and Purkinje fibers.
Physiological Mechanism of Action: Flecainide and the other agents in this class of local anesthetics, including encainide and lorcainide, exert cellular membrane electrophysiological responses that are distinctly different from those associated with lidocaine. A slowing of conduction is most prominent within the His-Purkinje system but there is minimal effect on action potential repolarization duration. On EKG tracings a noticeable widening of the QRS complex can be appreciated.
Application: The local anesthetics encainide, flecainide, and lorcainide as a group possess advantages in inhibiting premature ventricular contractions and potentially lethal ventricular tachycardias. Encainide and flecainide have been applied in the conversion of Wolff-Parkinson-White syndrome.
Dosage: The estimated half-life of flecainide has not been extensively studied, but in the few reports that have described these values in humans reveal a $T_{1/2}$ between 7 and 24 hours.
Considerations: Similar to many local anesthetics (e.g., lidocaine, encainide, lorcainide), flecainide is absorbed following oral administration. Flecainide differs from most of these agents by the fact that it is removed from the peripheral intravascular compartment to a much lesser degree on first pass through the hepatic circulation.

Antiarrythmic Drugs. Bigger JT, Hoffman BF (Chapter 31: pp. 748–83). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Purkinje Fiber Action Potential Duration/ (Bladder Contractions)
Potassium ATP Channel Opener/[^{125}I]A-312110

Cellular/Molecular Mechanism of Action: [^{125}I]A-312110 or [(9R)-9-(4-fluoro-3-^{125}iodophenyl)-2,3,5,9-tetrahydro-4H-pyrano[3,4-b]thieno[2,3-e]pyridin-8(7H)-one-1,1-dioxide] functions as a potassium ATP (KATP) channel opener in cardiac tissues.
Physiological Mechanism of Action: In the *in vivo* application of [^{125}I]A-312110 or [(9R)-9-(4-fluoro-3-^{125}iodophenyl)-2,3,5,9-tetrahydro-4H-pyrano[3,4-b]thieno[2,3-e]pyridin-8(7H)-one-1,1-dioxide], can either suppress action potential duration (APD) in Purkinje fibers and can relax electrical field-stimulated bladder contractions.
Application: [^{125}I]A-312110 or [(9R)-9-(4-fluoro-3-125iodophenyl)-2,3,5,9-tetrahydro-4H-pyrano[3,4-b]thieno[2,3-e]pyridin-8(7H)-one-1,1-dioxide] can be applied for detecting and characterizing potassium ATP channels in addition to functioning as a potential imaging agent for delineating the distribution and expression density of potassium ATP channels. In this context, [^{125}I]A-312110 can be used to delineate the molecular and functional properties of the KATP channel complex and the identification of new channel blockers and openers that interact with cardiac/smooth muscle-type KATP channels.
Route of Administration: [^{125}I]A-312110 (intravenous).
Considerations: Although ATP-sensitive K+ channels continue to be explored for their therapeutic potential, developments in high-affinity radioligands to investigate native and recombinant KATP channels have been less forthcoming.
Interpretation and Analysis: The agent [^{125}I]A-312110 binds with high affinity to a single class of binding sites in guinea pig cardiac membranes (KD = 5.8 nM) and urinary bladder membranes (KD = 4.9 nM) in a saturable manner. Displacement of [^{125}I]A-312110 by structurally diverse potassium channel openers (KCOs) indicates a similar rank order of potency in both guinea pig cardiac and bladder membranes (Ki, heart): A-312110 (4.3 nM) > N-cyano-N′-(1,1-dimethylpropyl)-N′′-3-pyridylguanidine (P1075) > (-)-N-(2-ethoxyphenyl)-N′-(1,2,3-trimethylpropyl)-2-nitroethene-1,1-diamine (Bay X 9228) > pinacidil > (-)-cromakalim > N-(4-benzoyl phenyl)-3,3,3-trifluro-2-hydroxy-2-methylpropionamine (ZD6169) > 9-(3-cyanophenyl)-3,4,6,7,9,10-hexahydro-1,8-(2H,5H)-acridinedione (ZM244085) >> diazoxide (16.7 μM). Displacement by KATP channel blockers, the sulfonylurea glyburide, and the cyanoguanidine N-[1-(3-chlorophenyl)cyclobutyl]-N′-cyano-N′′-3-pyridinyl-guanidine (PNU-99963) are biphasic in the heart but monophasic in the bladder with about a 100- to 500-fold difference in Ki values between high- and low-affinity sites. Good correlations exist between cardiac or bladder-binding affinities of potassium channel openers with functional activation as assessed by their

respective potencies to either suppress APD in Purkinje fibers or to relax electrical field-stimulated bladder contractions. Collectively, these results demonstrate that [^{125}I]A-312110 binds with high affinity and has an improved activity profile compared with other radiolabeled potassium channel opener agents. [^{125}I]A-312110 is a useful tool for investigating the molecular and functional properties of the KATP channel complex and for the identification, in a high throughput manner, of both novel channel blockers and openers that interact with cardiac/smooth muscle-type KATP channels.

[^{125}I]A-312110, a novel high-affinity 1,4-dihydropyridine ATP-sensitive K+ channel opener: Characterization and pharmacology of binding. Davis-Taber R, Molinari EJ, Altenbach RJ, Whiteaker KL, Shieh CC, Rotert G, Buckner SA, Malysz J, Milicic I, McDermott JS, Gintant GA, Coghlan MJ, Carroll WA, Scott VE, Gopalakrishnan M. Mol Pharmacol 2003 Jul; 64 (1), pp. 143–53.

Sick Sinus Syndrome: Sinus Node and S-A Node Function

Atropine/Neostigmine/Isoprenaline (Isoproterenol)

Cellular/Molecular Mechanism of Action:

Atropine: Binds to muscarinic cholinertic receptor complexes in a manner that competitively inhibits the biological activity of acetylcholine.

Neostigmine (Quaterinary Amine): Reversibly binds to and biochemically inactivates acetylcholine esterase in a manner that promotes the postsynaptic accumulation of acetylcholine neurotransmitter. The carbamyl ester configuation of neostigmine is hydrolyzed by acetylcholine esterase. Under physiological conditions, neostigmine becomes highly cationic in character and this feature encourages its direct interaction with the biochemically active site of acetylcholine esterase. In this manner, neostigmine functions as a competitive substrate resulting in disruption of the hydroxyl serine group of acetylcholine and covalent bonding of neostigmine through the formation of a reversible carbamate bond structure.

Isoproterenol: Binds to and positively activates both β_1 and β_2 adrenergic receptor complexes.

Physiological Mechanism of Action:

Atropine: Suppresses the function of exocrine gland secretion, smooth muscle, and cardiac muscle. Postganglionic cholinergic stimulation is also depressed in function. The primary influence of atropine on the heart is a transient negative chronotropic effect with mid-range doses presumably due to increased central vagal tone just before the occurance of peripheral muscarinic cholinergic blockage. Changes in heart rate are usually minor and not accompanied by changes in peripheral blood pressure or cardiac output. Incremental increases in dose subsequently produce proportional increases in heart rate achieved through inhibition of vagal tone influences on the S-A node pacemaker. Maximal heart rate during strenuous exercise is not altered. Neonates and geriatric patients can often be refractory to the effect of atropine on heart rate. Although atropine can cause cardiac arrythmias (e.g., atrial arrhythmias and ventricular dissociation), it often does so without causing cardiovascular symptoms.

The vascular effects of atropine when administered alone are not profound, which is in part due to the belief that vascular beds are not extensively innervated by cholinergic axons. Furthermore, cholinergic sympathetic vasodilatory axons synapsing on vessels with skeletal muscle tissues seem not to be important in maintaining normal vascular tone.

Neostigmine: Interaction of neostigmine with the biochemically active site of acetylcholine esterase competitively inhibits the ability of this enzyme to hydrolyze acetylcholine neurotransmitter. As a result, acetylcholine begins to accumulate, resulting in excessive stimulation of postsynaptic membrane acetylcholine receptor complexes. The impact of acetylcholine esterase inhibition includes activation of muscarinic receptors expressed by autonomic responsive tissues; activation of autonomic ganglia; activation of nicotinic skeletal muscle; and stimulation of cholinergic receptors within the central nervous system (CNS) if they are able to pass across the blood-brain barrier. Both autonomic ganglia and skeletal muscle receptors can become over-stimulated to the point of paralysis or depressed function.

Neostigmine impact on cardiac function involves ganglionic and postganglionic interactions. Elevations in peripheral acetylcholine concentrations primarily have the effect of slowing the heart rate, in contrast to blood pressure changes that only become altered at relatively higher dosages since this is due to greater CNS penetration of neostigmine. The cardiac refractive period becomes shorter due to neostigmine's influence, resulting in a lengthening of the refractory period and prolonged conduction.

At the level of the vasculature, neostigmine promotes vasodilation. Interesingly, the opposite vasoactive response is observed in coronary and pulmonary vascular structures. Hypotension is not a profound feature, though, because ganglionic acetylcholine first activates but then exerts a suppressive effect at higher dosages. The stimulatory effect on parasympathetic ganglia enhances decreases in cardiac output, in contrast to the influence of elevated acetylcholine levels on sympathetic ganglia and cardiac output. The influence of acetylecholine on medullary vasomotor and cardiac brainstem centers is initially excitatory and subsequently diminished stimulation. Furthermore, increases in secondary sympathetic tone are complemented by acetylcholine-mediated adrenal medulla epinephrine release. An appreciation of these concepts is therefore responsible for the wide spectrum of cardiovascular responses observed in response to neostigmine administration and related anticholinesterase pharmaceuticals.

Since neostigmine is a quaterinary ammonium compound, it is poorly absorbed from the gastrointestinal tract and almost none of the compound penetrates across the CNS blood-brain barrier. Consequently, neostigmine is active primarily at skeletal muscle neuromuscular junctions and functions both as an anticholinergic agent and as a direct acting agonist. Other important sites of action include the eye and gastrointestinal tract when administered at clinically relevant dosages.

Isoprenaline (Isoproterenol): Due to relatively minimal influence on α-adrenergic receptor complexes, changes in cardiac function, bronchial smooth muscle, skeletal muscle vasculature, and the gastrointestinal tract are the primary organ systems influenced by the physiological properties exerted by isoproterenol. . . . Cardiovascular influences of isoproterenol include diminished peripheral vascular resistance, especially within vascular networks perfusing musculoskeletal structures. The increases in heart rate and contractility induced by isoproterenol promote declines in diastolic pressure and elevations in cardiac output sufficient to maintain or elevate systolic pressures (mean blood pressure is lowered).

Application: Characterization of sick sinus syndrome.

Route of Administration: Isoprenaline, atropine, *and* neostigmine can be injected intravenously. Neostigmine is poorly absorbed from the gastrointestinal tract since it is a quaterinary ammonium compound.

Considerations: Atropine can be applied in the conversion of parasympathetic (cholinergic) mediated atrial block arrythmias.

Implementation: Evaluation of sick sinus syndrome conditions using various physical (postural reflex tests, Valsalva maneuver, carotid sinus massage), pharmacological challenges (intravenous iso-

prenaline, atropine, neostigmine, and total autonomic blockade), and electrophysiological tests in order to identify simple non-invasive markers of intrinsic sick sinus syndrome.

Human Model: Cases of sick sinus syndrome (n = 13).

Interpretation and Analysis: Following autonomic blockade, nearly 50% of cases may develop a normal heart rate (e.g., n = 6/13), while slightly greater than 50% (e.g., n = 7/13) may have an abnormal intrinsic heart rate. Electrophysiological testing can reveal abnormal sinus node parameters in approximately two-thirds of cases in the basal state (e.g., n = 8/13), and 85% (e.g., n = 11/13) after autonomic blockade. Carotid sinus massage is abnormal in most cases with an abnormal intrinsic heart rate (e.g., n = 13/13, 100%), compared to 33% of cases with a normal intrinsic heart rate (e.g., n = 2/6 and $p < 0.05$). The heart rate response to isoprenaline (isoproterenol) is abnormal in approximately 83% of cases (e.g., n = 5/6) with normal as compared to only about 14% (e.g., n = 1/7) with abnormal intrinsic heart rate. Isoprenaline (isoproterenol) yields a significantly ($p < 0.05$) higher increase in heart rate in conditions with abnormal as compared to those with normal intrinsic heart rate. Other physical and drug tests appear not to be helpful for the purpose of differentiating between intrinsic and extrinsic mechanisms. Thus, carotid sinus massage and, to some extent, isoprenaline (isoproterenol) administration appear to be simple bedside tests that may be helpful in identifying the underlying mechanism of sick sinus syndrome.

Instrumentation: Electrocardiogram diagnostic system (EKG).

Anticholinesterase Agents. Taylor P (Chapter 6: pp. 113–29). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Atropine, Scopolamine, and Related Anti-Muscarinic Drugs. Weiner N (Chapter 7: pp. 130–44) Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Norepinephrine, Epinephrine, and the Sympathomimetic Amines. Weiner N (Chapter 8: pp. 145–80). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Value of physical and pharmacological tests in predicting intrinsic and extrinsic sick sinus syndrome. Bhandari S, Talwar KK, Kaul U, Bhatia ML. Int J Cardiol 1986 Aug; 12 (2), pp. 203–12.

Sinus Node Disease: Case Report
Flecainide Acetate

Cellular/Molecular Mechanism of Action: Flecainide exerts a suppressive effect on fast sodium channels.

Physiological Mechanism of Action: Flecainide promotes a decrease in the Vmax and and "overshoot" of the action potentials within the atrial, ventricles, and Purkinje fibers, thereby slowing conduction velocities within these cardiac structures.

Application: Flecaininde is used as a Class IC antiarrhythmic agent.

Route of Administration: Flecainide (intravascular injection).

Interpretation and Analysis: There can be dramatic increases in sinus node recovery times and in sinoatrial conduction times, and the magnitude of the response can only be witnessed because of the emergence of a subsidiary junctional pacemaker without retrograde conduction to the atria.

Response of an abnormal sinus node to intravenous flecainide acetate. Hellestrand KJ, Nathan AW, Bexton RS, Camm AJ. Pacing Clin Electrophysiol 1984 May; 7 (3 Pt 1), pp. 436–9.

T Wave Alternans/Coronary Artery Disease/ Ventricular Arrhythmias: Life-Threatening Risk Stratification
Esmolol

Physiological Mechanism of Action: Esmolol promotes sympathetic blockade, while atropine exerts parasympathetic blockade.

Background Information: T wave alternans (TWA) is an important non-invasive measure of ventricular arrhythmia vulnerability.

Implementation: Evaluation of the influence of the autonomc nervous system on T wave alternans (TWA) measurements in high-risk conditions of coronary artery disease, left ventricular dysfunction, and inducible sustained ventricular tachycardia during electrophysiological studies. Parameters for TWA are measured at baseline with atrial pacing at 100 bpm (600 msec), 109 bpm (550 msec), and 120 bpm (500 msec). After a 10-minute recovery period, TWA is measured again after sympathetic blockade (esmolol, n = 20), parasympathetic blockade (atropine, n = 20), or no intervention (control subjects, n = 20).

Human Model: Coronary artery disease (n = 60 cases).

Interpretation and Analysis: The prevalence of significant TWA is unchanged compared with baseline profiles after atropine infusion and in controls. Conversely, the amplitude of TWA in the vector magnitude lead is significantly reduced after esmolol infusion ($p < 0.001$), and the number of positive TWA tests is reduced by 50% (70 vs 35%, $p < 0.05$). Such observations have important implications for the administration of TWA to risk-stratify cases for life-threatening ventricular arrhythmias and provide a new potential mechanism for the reduction in sudden cardiac death conferred by beta-blockers among cases with coronary artery disease and congestive heart failure.

Effects of selective autonomic blockade on T wave alternans in humans. Rashba EJ, Cooklin M, MacMurdy K, Kavesh N, Kirk M, Sarang S, Peters RW, Shorofsky SR, Gold MR. Circulation 2002 Feb 19; 105 (7), pp. 837–42.

T Wave Lability (Non-Alternating)/Genotype LQ-T1, LQ-T2, and LQ-T3
Phenylephrine/Dobutamine

Cellular/Molecular Mechanism of Action: Phenylephrine and dobutamine function as catecholamine neurotransmitter agonists.

Application: Electropharmacology challenge tests with phenylephrine and dobutamine for identifying alterations in repolarization lability in conditions with genotyped long Q-T syndrome (LQ-TS). Quantification of such phenomenon may assist in identifying LQ-TS cases with an increased risk of sudden cardiac death.

Route of Administration: Intravenous injection.

Implementation: Determination of the effects of phenylephrine and dobutamine on repolarization lability in cases with genotyped long Q-T syndrome (LQ-TS). Electrocardiographic stress testing involving aperiodic repolarization lability can be quantified from digitized electrocardiograms recorded during catecholamine challenge with phenylephrine and dobutamine. T wave lability is quantified as a root-mean-square of the differences between corresponding signal values of subsequent beats. The magnitude of aperiodic T wave lability is quantified by using a newly derived T wave lability index (TWLI).

Human Model: Cases with the genotyped LQ-TS (n = 23 total; n = 13 LQ-T1; n = 7 LQ-T2; n = 3 LQ-T3; n = 16 reference controls).

Interpretation and Analysis: The TWLI is significantly greater in LQ-TS genotypes relative to controls (0.0945 ± 0.0517 vs 0.0445 ± 0.0123; $p < 0.003$). Marked T wave lability (TWLI ≥ 0.095) may be detected in all 3 LQ-TS genotypes (e.g. n = 10/23) but not in reference controls ($p < 0.003$). There is no correlation between TWLI and baseline corrected Q-T interval. High-risk cases that have either a history of out-of-hospital cardiac arrest or syncope almost invariably have a TWLI of 0.095 or greater. Beat-to-beat non-alternating T wave lability occurs in conditions of LQ-T1, LQ-T2, and LQ-T3 during catecholamine provocation and is associated with a history of prior cardiac events. Quantification of this novel phenomenon may assist in identifying LQ-TS cases with an increased risk of sudden cardiac death.

Instrumentation: Electrocardiography system.

Catecholamine-induced T wave lability in congenital long Q-T syndrome: A novel phenomenon associated with syncope and cardiac arrest. Nemec J, Hejlik JB, Shen WK, Ackerman MJ. Mayo Clin Proc 2003 Jan; 78 (1), pp. 40–50.

Tachycardia: Inappropriate Sinus Tachycardia (IST)
Adenosine

Physiological Mechanism of Action: Adenosine exerts a negative chronotropic effect on the heart.

Background Information: Adenosine is an endogenous nucleoside that has an important role in the diagnosis and treatment of several cardiac arrhythmias. However, its effects on IST have not been well established.

Application: Potential diagnostic agent for detecting and characterizing IST.

Dosage: Adenosine (0.1 to 0.15 mg/kg).

Implementation: Determine the response to intravenous adenosine (0.1 to 0.15 mg/kg) and evaluate its direct effect during pharmacologic β-adrenergic and cholinergic blockade. Atrial cycle length (ACL) is measured before adenosine injection, at the time of the greatest cycle length prolongation, and during the maximum rebound acceleration of heart rate.

Human Model: Cases (n = 18, age 46 ± 15 years) given adenosine alone, compared to cases (n = 5) to determine the direct effects of adenosine during pharmacologic β-adrenergic and cholinergic blockade. Reference controls (n = 18, age 46 ± 11 years) with normal sinus rhythm undergoing clinically indicated electrophysiologic study.

Interpretation and Analysis: Adenosine does not terminate IST in the vast majority of cases. The maximum dose of adenosine prolongs the sinus interval significantly, from 780 ± 128 msec to 985 ± 225 msec ($p < 0.001$) in the control subjects. Conversely, adenosine causes no significant lengthening of atrial cycle length (527 ± 69 msec vs 590 ± 148 msec; p = ns) in the patients with IST. Similar differences in the response to adenosine can be appreciated during the pharmacologic autonomic blockade. Reflex increase of the sinus rate (rebound effect) is greater in control subjects than in IST cases (21.2% ± 9.7 vs 8.5% ± 8.8%; $p < 0.001$). The usual negative chronotropic effect of adenosine is impaired in IST cases. This may have important diagnostic implications and provide new insight into the mechanism(s) of IST.

Impaired negative chronotropic response to adenosine in patients with inappropriate sinus tachycardia. Still AM, Huikuri HV, Airaksinen KE, Koistinen MJ, Kettunen R, Hartikainen J, Mitrani RD, Castellanos A, Myerburg RJ, Raatikainen MJ. J Cardiovasc Electrophysiol 2002 Jun; 13 (6), pp. 557–62.

Tachycardia: Regular Broad Complex Type/ Atrioventricular Function in Wolff-Parkinson-White Syndrome (Accessory Atrioventricular Connections)
Adenosine/Adinosine Triphosphate/Propanolol (IV) Reversal

Cellular/Molecular Mechanism of Action:

Adenosine: Shortens antegrade refractoriness of accessory A-V connections, and in some patients this action is mediated by β-adrenergic stimulation. Adenosine may cause acceleration of pre-excited atrial arrhythmias, but these effects are transient and should not discourage the use of adenosine as a diagnostic agent in broad complex, regular tachycardias of uncertain origin.

Propanolol: Binds to and blocks sympathetic/β-adrenergic receptor complexes.

Physiological Mechanism of Action:

Adenosine: shortens antegrade refractoriness of accessory A-V connections, and in some cases this action is mediated by β-adrenergic stimulation. Adenosine may cause acceleration of pre-excited atrial arrhythmias, but these effects are transient and should not discourage the use of adenosine as a diagnostic agent in broad complex, regular tachycardias of uncertain origin.

Propanolol: In the atria, propanolol inhibits spontaneous frequency, maximal driving frequency, and contractility but increases the electrical threshold and A-V conduction time. Spontaneous depolarization rate of ectopic pacemakers is decreased but resting potentials and repolarization are minimally altered since propanolol reduces intracellular sodium currents, resulting in decreases in the height and rate of action potential elevations.

In the normal heart at rest, propanolol has less of an effect in contrast to a heart subjected to elevations in adrenergic tone as occurs in certain heart conditions and exercise. The most obvious effect of β-adrenergic blockade on cardiac physiology is a negative chronotropic effect, declines in cardiac output, increased duration of systole, and subtle declines in blood pressure at rest. Sodium secretion influences β-adrenergic blockade properties exerted by propanolol; in this context, in situations where there are elevations in sodium retention and extracellular fluid volume, especially in conditions of cardiac dysfunction.

Route of Administration: Adenosine and propanolol (intravenous).

Dosage: Adenosine (12 mg, rapid bolus) and propranolol (0.2 mg/kg).

Considerations: Several groups of clinicians have suggested the use of intravenous adenosine or adenosine triphosphate for the diagnosis of regular broad complex tachycardias. However, the short half-life of these agents has precluded assessment of their effects on refractoriness of accessory connections, and their safety in pre-excited arrhythmias has not been demonstrated.

Implementation: Evaluation of the effects of intravenous adenosine on accessory atrioventricular (A-V) connections in Wolff-Parkinson-White syndrome.

Human Model: Cases of accessory atrioventricular (A-V) connections in Wolff-Parkinson-White syndrome (n = 30).

Interpretation and Analysis: Adenosine administration (n = 14) during continuous atrial pacing at a cycle length 20 msec below that required to cause 2:1 conduction block in the accessory connection (mean pacing cycle length 261 ± 41 msec) creates a transient 1:1 conduction (n = 12/14) via the accessory connection (shortening

of antegrade refractoriness). Such effects can be abolished (n = 3/7 of affected subjects) following intravenous propranolol (0.2 mg/kg). Adenosine administration in conditions of Wolff-Parkinson-White syndrome (n = 19 during induced, pre-excited atrial arrhythmias) results in observed minimum RR interval during pre-excited atrial fibrillation that subsequently transiently decreases (252 ± 44 msec to 224 ± 35 msec, $p < 0.01$) after adenosine, but no change in average RR interval can be observed (360 ± 59 msec to 357 ± 60 msec, ns). The pre-excited ventricular response to atrial flutter is transiently accelerated (n = 5/8: 415 ± 21 msec to 360 ± 49 msec, $p < 0.05$) due to shortening of flutter cycle length (207 ± 10 msec to 180 ± 24 msec, $p < 0.05$). However, 2:1 accessory connection conduction is maintained (n = 8/8). All effects are short lived, with the decrease in RR interval during atrial fibrillation ocurring for a maximum of 2 RR intervals only. Ventricular arrhythmias or hemodynamic deterioration are usually not appreciated.

Toxicity: Increased sodium retention in conditions of pre-existing severe cardiac disease.

Effects of intravenous adenosine on antegrade refractoriness of accessory atrioventricular connections. Garratt CJ, Griffith MJ, O'Nunain S, Ward DE, Camm AJ. Circulation 1991 Nov; 84 (5), pp. 1962–8.

Tachycardia: Regular Narrow Complex (Unknown Origin)/Sinus Node Reentrant Tachycardia
Adenosine

Cellular/Molecular Mechanism of Action: A minimum of 2 adenosine receptors exist based on their response to adenosine analogs and whether activation increases or decreases cAMP production.

Physiological Mechanism of Action: Adenosine shortens antegrade refractoriness of accessory A-V connections, and in some cases this action is mediated by β-adrenergic stimulation. Adenosine may cause acceleration of pre-excited atrial arrhythmias, but these effects are transient and should not discourage the use of adenosine as a diagnostic agent in broad complex, regular tachycardias of uncertain origin.

Application: Sinus node reentrant tachycardia.

Route of Administration: Intravenous bolus of 0.1 mg/kg and 0.15 mg/kg.

Dosage: Adenosine (0.1 mg/kg and 0.15 mg/kg).

Considerations: Its effects are usually transient owing to its extremely short plasma half-life and, as a consequence, it is thought to be safer than other drugs used in the acute treatment of such arrhythmias. However, adenosine may created a proarrhythmic effect when administered in attempts to confirm a diagnosis of atrial flutter.

Interpretation and Analysis: For narrow complexes, as expected, a transient increase in atrioventricular block can be seen, but this is followed by a doubling of the ventricular rate and hemodynamic compromise requiring immediate DC cardioversion. It is postulated that the secondary catecholamine-mediated effects of adenosine are responsible for this phenomenon.

Adenosine may slow the atrial rate and actually terminate tachycardia profiles. These effects of adenosine on sinus node reentrant tachycardia suggest that sinus nodal tissue (as opposed to perinodal atrial tissue) is involved in the tachycardia circuit and that these effects should be considered when adenosine is used as a diagnostic agent in narrow complex tachycardia.

Instrumentation: Electrocardiogram.

The effects of adenosine on sinus node reentrant tachycardia. Griffith MJ, Garratt CJ, Ward DE, Camm AJ. Clin Cardiol 1989 Jul; 12 (7), pp. 409–11.

Tachycardia: Ventricular Dysrhythmias/ Differentiation
Therapeutic Response: [Adenosine]/[Magnesium Sulfate]/[Lidocaine]/[Procainamide]

Physiological Mechanism of Action: Adenosine shortens antegrade refractoriness of accessory A-V connections, and in some conditions this action is mediated by β-adrenergic stimulation. Adenosine may cause acceleration of pre-excited atrial arrhythmias, but these effects are transient and should not discourage the use of adenosine as a diagnostic agent in broad complex, regular tachycardias of uncertain origin.

Background Information: Wide complex tachycardias present diagnostic challenges for emergency physicians. The history, physical examination, and ECG provide information required to arrive at a correct diagnosis. When a previous history of heart disease exists, VT should be suspected; however, no single clinical feature is sufficiently reliable for distinguishing VT from SVT.

Application: Conditions of ventricular tachycardia (dysrhythmia differentiation).

Considerations: Reports of complications with the use of adenosine as a diagnostic agent have not yet been recognized, but may occur after sufficient numbers of cases have accumulated. Magnesium sulfate may be useful in refractory cases of VT and torsades de pointes. Chronic treatment of patients prone to VT may include complex pharmacotherapy and AICDs.

Implementation: Cases with VT may tolerate their dysrhythmias for several hours and maintain hemodynamic stability. ECG analysis is the most useful process in differentiating SVT and VT. Characteristics suggestive of VT include evidence of A-V dissociation, QRS duration of longer than 0.16 seconds, and QRS axis between −90 degrees ± 180 degrees. Predictive QRS morphologic criteria also have been established for VT. A 4-step approach to ECG analysis has been reported to accurately identify cases with VT, but prospective validation in an ED setting is lacking. The initial approach to treating patients with wide QRS tachycardias depends on hemodynamic stability. Until the identity of a dysrhythmia is certain, consider all cases to be suffering from VT. Unstable conditions require immediate cardioversion. Acute treatment of stable cases includes lidocaine or procainamide. Adenosine is appropriate when wide QRS SVT is the diagnosis, and it also has been used as a diagnostic aid to identify dysrhythmias.

Interpretation and Analysis: Development of new class III agents and enhancement of AICD technology may result in improved patient outcomes and the availability of more choices for emergent therapy of wide QRS tachycardias.

Instrumentation: Electrocardiogram.

Wide complex tachycardia. Delbridge TR, Yealy DM. Emerg Med Clin North Am 1995 Nov; 13 (4), pp. 903–24.

Torsade de Pointes (TdP)/U Wave to T Wave Amplitude (UTA) Ratio Identification Electrocardiographic Index for Predicting Torsade de Pointes
Calmodulin Inhibitor (W7)

Cellular/Molecular Mechanism of Action: The agent W7 inhibits the biological function of calmodulin.

Application: Selective suppression of TdP inducibility by W7, without shortening the duration of cardiac repolarization, allows identification of the UTA ratio, an electrocardiographic index for predicting TdP onset.

Implementation: Analysis of ECGs acquired from a well-characterized animal model of TdP to identify more reliable predictors of life-threatening ventricular arrhythmia.

Animal Model: Rabbit (n = 14; n = 12 TdP induced with methoxamine and clofilium pretreated with vehicle control).

Interpretation and Analysis: Methoxamine and clofilium can induce TdP in rabbits pretreated with vehicle control (n = 12/14). Pretreatment with W7 (50 μM/kg), an inhibitor of the intracellular Ca^{2+}-binding protein calmodulin, significantly suppresses TdP induction (n = 1/11 with TdP, $p < 0.001$). W7 does not affect heart rate, increases in Q-T intervals, or dispersion compared with measurements in vehicle-treated controls. However, a progressive and significant increase in the ratio of UTA occurs before TdP onset in control animals, and this can be prevented by W7.

The calmodulin inhibitor W7 suppresses TdP without shortening the Q-T interval, which is consistent with other findings that Q-T prolongation, per se, is insufficient to generate TdP. Selective suppression of TdP inducibility by W7, without shortening the duration of cardiac repolarization, allows identification of the UTA ratio as a new electrocardiographic index for predicting TdP onset. These findings are consistent with the idea that prolonged repolarization is not the proximate cause of arrhythmia initiation, and they suggest that an increased UTA ratio reflects activation of intracellular Ca^{2+}/calmodulin-dependent processes that are required for triggering TdP in this model.

Calmodulin inhibitor W7 unmasks a novel electrocardiographic parameter that predicts initiation of torsade de pointes. Gbadebo TD, Trimble RW, Khoo MS, Temple J, Roden DM, Anderson ME. Circulation 2002 Feb 12; 105 (6), pp. 770–4.

Ventricular Arrythmias
Lidocaine

Cellular/Molecular Mechanism of Action: Inhibits membrane-associated Na^+/K^+ pump activity. Cells within the ventricular Purkinje system are relatively more sensitive to the inhibition of membrane-associated Na^+/K^+ pump activity compared to cells within the epicardium and myocardium. Influence of lidocaine on atrial membrane action potentials is minimal to non-existent.

Diagnostic Application: Lidocaine is utilized for the correction of arrythmias originating from biochemical and/or organic lesions residing within the ventricular myocardium. Examples include premature ventricular contractions, ventricular fibrillation, and ventricular tachycardia.

Route of Administration and Dosage: Intravenous infusion is the only preferred administration route for lidocaine when applied for the purpose of correcting ventricular arrhythmias. Lidocaine must be administered by continuous infusion due to the very high rate of "first-pass" extraction following perfusion through the hepatic sinousoid network.

Considerations: Lidocaine is ineffective for the correction of arrythmias originating from within the atrial myocardium.

Interpretation and Analysis: Correction (cardioconversion) of the ventricular arrhythmia based upon interpretation of ECG analysis. Although constant lidocaine infusion may correct a ventricular arrhythmia, this effect may be temporary and the abnormality may subsequently return in the absence of the pharmaceutical. Such occurrences are common in situations where a permanent organic lesion resides within the ventricular myocardium that is responsible for generation of the arrhythmia condition.

CELL PHYSIOLOGY

Endothelial Adhesion/Platelet Interactions/[Retinal Microvascular Pathology]
Platelets (Fluorescent Labeled)/Carboxyfluorescein Diacetate Succinimidyl Ester

Cellular/Molecular Mechanism of Action: Carboxyfluorescein diacetate succinimidyl ester interacts with light and functions as a fluorescent marker molecule suitable for "tracing" the *in vivo* migration of different cell types.

Application: Visualization of *in vivo* platelet behavior within the retinal microcirculation. The methodology allows for quantitative evaluation of platelet dynamics and platelet-endothelial interactions in pathologic conditions affecting the retina.

Route of Administration: Intravascular.

Considerations: Represents the development of a new method for evaluating the *in vivo* dynamics of platelets within the retinal microcirculation and the quantitative investigation of platelet-endothelial interactions.

Implementation: Isolated platelet samples are labeled with carboxyfluorescein diacetate succinimidyl ester. After intravenous administration, platelet behavior in the retinal microcirculation can be evaluated with a scanning laser ophthalmoscope. Images are recorded on S-VHS videotape and analyzed with a computer-assisted image analysis system. Platelet-endothelial interactions in the retinal microcirculation can also be investigated employing lipopolysaccharide-stimulated endothelium or platelets activated with thrombin.

Animal Model: Rat (retina).

Interpretation and Analysis: Fluorescent platelets are recognized as distinct dots within the retinal microcirculation that can be traced frame by frame. The velocity of platelets in the retinal arteries, capillaries, and veins is approximately 26.1 ± 6.4, 1.6 ± 0.4, and 19.9 ± 8.2 mm/sec, respectively. In control rats, even activated platelets show minimal interaction with retinal endothelial cells. Conversely, stimulated retinal endothelium show active platelet-endothelial interactions, with many platelets observed rolling and adhering along the major retinal veins. The interactions between platelets and stimulated endothelial cells are substantially inhibited with the injection of P-selectin monoclonal antibody.

In vivo evaluation of platelet-endothelial interactions in retinal microcirculation of rats. Tsujikawa A, Kiryu J, Nonaka A, Yamashiro K, Nishiwaki H, Tojo SJ, Ogura Y, Honda Y. Invest Ophthalmol Vis Sci 1999 Nov; 40 (12), pp. 2918–24.

Endothelial Adhesion/Cellular Interactions: Colon Carcinoma/Intravital Observation of Cancer Cell/Microcirculation Interactions
CalceinAM

Background Information: Organ-specific tumor cell adhesion within the microcirculation of host organs is an important step within the metastatic cascade. Circulating tumor cells have to adhere within the microcirculatory vessels, quickly stabilize their adhesion to endothelial surfaces, and then probably need to leave the circulation to avoid the toxic effects of hydrodynamic shear forces of circulating blood.

Application: Intravital observation of colon carcinoma cell adhesion within the hepatic microcirculation.

Implementation: CalceinAM can be used to label colon carcinoma cell populations (e.g., HT-29 [human] and CC531 [rat]) that are then injected intra-arterially as single-cell suspensions. Intravital fluorescence microscopy is then applied to detect adhesive interactions between circulating tumor cells and endothelial surfaces within the hepatic microcirculation. These interactions are then analyzed regarding their time course and site localization within the vascular tree.

Animal Model: Rats (Sprague-Dawley).

Interpretation and Analysis: Autofluorescence of liver parenchyma is sufficient to facilitate distinction of hepatic sinusoids. Intravital microscopy is capable of differentiating early events in adhesion formation within hepatic sinusoids, adhesion stabilization, and extravasation of the tumor cells into the liver parenchyma. Tumor cell adhesion occurs almost exclusively within sinusoidal capillaries; however, the diameter of these vessels is usually larger than that of the tumor cells leaving remaining perfused lumen of the capillaries. Colon carcinoma cells rapidly migrated into the liver parenchyma after successful adhesion within the sinusoids. In contrast to common endpoint assays of the metastatic cascade, this *in vivo* model allows the investigation of metastatic colon carcinoma cell adhesion within the liver microcirculation as specific steps during the formation of hematogenous metastasis and their underlying mechanisms.

Instrumentation: Intravital fluorescence microscopy.

An intravital model to monitor steps of metastatic tumor cell adhesion within the hepatic microcirculation. Haier J, Korb T, Hotz B, Spiegel HU, Senninger N. J Gastrointest Surg 2003 May–Jun; 7 (4), pp. 507–14; discussion 514–5.

Endothelial Integrity/Endothelial Injury and Viability
Evans Blue Dye Staining

Cellular/Molecular Mechanism of Action: Evans blue dye is a pigment-based reagent that becomes differentially associated with viable vs compromised cell populations (e.g., vascular endothelium).

Physiological Mechanism of Action: Tumor necrosis factor-alpha (TNF-α) soluble receptor (TNFsr) has been shown *in vitro* and *in vivo* to neutralize TNF-mediated biological activity.

Background Information: Tumor necrosis factor-α is expressed locally in arteries at sites of balloon injury. In vitro studies have shown that TNF inhibits cell cycle progression and induces apoptosis in endothelial cells. Accordingly, we performed a series of experiments to test the hypothesis that inhibiting TNF could accelerate endothelial recovery after angioplasty.

Application: Evans blue dye staining can be applied to evaluate viable *ex vivo* preparations of vascular structures for the purpose of establishing the integrity of endothelial surfaces.

Implementation: Evaluation of vascular endothelial integrity using Evans Blue dye as a indicator of cellular injury. In this context, Evans Blue dye can be used to evaluate the efficacy of agents designed to enhance the integrity of vascular endothelial structure.

A relevant application example includes the evaluation of TNFsr vs control IgG administered by intraperitoneal injection following De-endothelializing created by balloon injury. The resulting endothelial lesions created can then be assessed at 1 week post-injury for evidence of reendothelialization (Evans blue dye staining); and then again after 2 weeks in order to evaluate reendothelialization and endothelial function.

Animal Model: Rat (Sprague-Dawling strain).

Interpretation and Analysis: At both time points, blockade of TNF biological activity with TNFsr results in increased reendothelialization measured as absolute area and percent area reendothelialized. TNFsr also accelerates functional endothelial recovery that manifests as an increase in nitric oxide production. Neointimal thickening can also be appreciated as being inhibited. Blockade of TNF *in vivo* accelerates functional endothelial recovery after barotraumatic de-endothelializing injury. Locally expressed TNF therefore acts to inhibit functional endothelial recovery after angioplasty and transient blockade of TNF may improve the long-term success of angioplasty.

Evans Blue Staining: The reagent facilitates detection of a demarcation between viable endothelial cell populations and subendothelial collagen.

In vivo blockade of tumor necrosis factor-alpha accelerates functional endothelial recovery after balloon angioplasty. Krasinski K, Spyridopoulos I, Kearney M, Losordo DW. Circulation 2001 Oct 9; 104 (15), pp. 1754–6.

Endothelial Integrity/Vascular Permeability/ Extravasation
Evans Blue Dye

Cellular/Molecular Mechanism of Action: Evans blue dye is a pigment-based reagent that can be detected visually within the visible light range.

Background Information: Oxidative stress appears to be relevant to asthma pathogenesis.

Application: Evans blue dye is a pigment-based reagent that can be used as a visual marker or indicator molecule and has the advantage of being compatible with biological systems. In this context, it is often applied as a dilution marker system within the intravascular compartment for measuring total intravascular compartment size and extravasation of plasma proteins and hematologial cell types into the extravascular tissue space.

Implementation: Applicaton of Evans blue dye to evaluate extravasation into bronchoalveolar lavage fluid following antigen-induced pulmonary responses in sensitized *in vivo* models and the effectiveness of the antioxidant N-acetylcysteine (oral, 1 mmol/kg per day for 7 days before challenge).

Animal Model: Rat (Brown Norway strain).

Interpretation and Analysis: N-acetylcysteine does not reduce the immediate bronchospasm that follows aerosol antigen exposure but does prevent airway hyperreactivity to 5-hydroxytryptamine at 24 hours after antigen challenge, and reduces eosinophil numbers (from 0.178 ± 0.038 in the absence to $0.064 \pm 0.020 \times 10^6$ cells/ml in the presence of N-acetylcysteine; $p < 0.05$), and Evans blue dye extravasation into bronchoalveolar lavage fluid. Taurine levels in bronchoalveolar lavage fluid from antigen-challenged *in vivo* models are higher than control values but treatment with N-acetylcysteine fails to further increase these augmented levels. N-acetylcysteine appears to have a beneficial effects *in vivo* in models of experimental asthma and serves to validate related findings pertaining to other models of lung injury.

Effectiveness of oral N-acetylcysteine in a rat experimental model of asthma. Blesa S, Cortijo J, Martinez-Losa M, Mata M, Seda E, Santangelo F, Morcillo EJ. Pharmacol Res 2002 Feb; 45 (2), pp. 135–40.

Endothelial Integrity (Retinal)/Minimally-Invasive Diagnostic Strategy [Diabetes]
Sodium Fluorescein

Background Information: Diabetic retinopathy is the leading cause of blindness in working-age individuals in the United States. Breakdown of the blood-retinal barrier is one of the earliest events in the progression of diabetic retinopathy. Ideally, therapeutic measures would be directed at this early stage, but there are few sensitive, quantitative methods to assess the retinal vascular barrier *in vivo*.
Application: Quantitative assessment of vascular barrier such as those associated with the retina.
Implementation: Utilization of 2 fluorescent tracers in combination with fluorescence microangiography to quantitatively assess retinal vascular barrier integrity (± infusion of a hyperosmolar solution of 1.6 M mannitol for 5 minutes).
Animal Model: Rat (Long-Evans strain).
Interpretation and Analysis: Permeability × surface area/flow (PS/F) describing the retinal vasculature is 0.086 ± 0.031 (n = 13, avg. ± SD). Based on estimates of flow and surface area, estimated permeability of sodium fluorescein is approximately 1.2 x 10^{-5} cm/s. Infusion of a hyperosmolar mannitol solution significantly increases PS/F within individual veins and significantly increases flow-weighted PS/F from 0.073 ± 0.028 to 0.16 ± 0.034 (n = 3). Quantitative *in vivo* assessment of retinal vasculature can be delineated applying an adapted indicator dilution technique. Dual-tracer fluorescence angiography appears to be a sensitive indicator for detecting increases in the blood-retinal barrier produced by hyperosmolar mannitol. The methodology may be a promising new minimally-invasive strategy that can be adapted to quantitatively track retinal vascular permeability.
Instrumentation: Fluorescence microangiography.

Retinal vascular permeability determined by dual-tracer fluorescence angiography. Russ PK, Gaylord GM, Haselton FR. Ann Biomed Eng 2001 Aug; 29 (8), pp. 638–47.

Gene Expression/ Imaging the Location, Magnitude, and Time Course of Gene Expression
Adenovirus-Expressing Firefly Luciferase/D-Luciferin

Cellular/Molecular Mechanism of Action: D-luciferin is a fluorescent substrate for firefly luciferase enzyme fractions.
Background Information: Studies of cardiac gene transfer rely on postmortem analysis using histologic staining or enzyme assays. Non-invasive imaging of the temporal and spatial characteristics of cardiac gene expression in the same subject offers significant advantages.
Application: Fluorescent marker system for validating the positive detection of gene-specific transcription processes.
Route of Administration: D-luciferin can be injected intraperitoneally following adenovirus-facilitated firefly luciferase gene expression.
Implementation: Direct myocardial injection via left thoracotomy with adenovirus-expressing firefly luciferase (Ad-CMV-Fluc; n = 30). The reporter substrate D-luciferin can be injected intraperitoneally. Serial images are acquired by use of a cooled charge-coupled detector (CCD) camera. Results are expressed as relative light unit per minute (RLU/min). To assess the detection sensitivity, serially diluted titers of Ad-CMV-Fluc can be injected: 1×10^9 (195, 393 ± 14,896), 1×10^8 (33,777 ± 18,179), 1×10^7 (417 ± 91), 1×10^6 (185 ± 64), 1×1^5 (53 ± 1), and control (54 ± 1) ($p < 0.05$ for 1×10^9, 1×10^8, and 1×10^7 plaque-forming units vs control adenovirus-expressing mutant thymidine kinase [Ad-CMV-HSV1-sr39tk]; n = 3).
Animal Model: Rat.
Interpretation and Analysis: Rats transduced with 1×10^9 plaque-forming units show decremental cardiac luciferase activity over time: 152,070 ± 21,170 (day 2), 195,806 ± 62,630 (day 5), 7250 ± 2941 (day 8), and 2040 ± 971 RLU/min (day 14). Rats are sacrificed, and *in vitro* luciferase activity correlated with *in vivo* CCD signals ($r^2 = 0.92$). Such observations demonstrate the feasibility of imaging the location, magnitude, and time course of cardiac reporter gene expression in living (*in vivo*) systems. Cardiac gene therapy studies could be aided with wider application of this approach.

Optical imaging of cardiac reporter gene expression in living rats. Wu JC, Inubushi M, Sundaresan G, Schelbert HR, Gambhir SS. Circulation 2002 Apr 9; 105 (14), pp. 1631–4.

Glucose Metabolization: Myocardial
$H_2[^{15}O]$/1-[^{11}C]-Glucose/Phenylephrine/[Positron Emission Tomography]

Cellular/Molecular Mechanism of Action: The agent $H_2[^{15}O]$ is an analog that mimics the behaviors of natural water molecules within biological systems. Similarly, 1-[^{11}C]-glucose is transported across cellular membranes and perceived biochemically/metabolically in a manner analogous to natural/native glucose molecules. Procainamide inhibits Na^+/K^+ pump function and promotes weak parasympatholytic activity similar to quinidine (quinidine has a stronger influence on cardiac parasympathetic input).
Background Information: Measurements of the rate of myocardial glucose utilization (rMGU) play a key role in the assessment of alterations in myocardial substrate metabolism in normal and abnormal cardiac states. In this context, rMGU can be quantified *in vivo* using 1-[^{11}C]glucose in combination with positron emission tomography (PET) imaging analysis.
Application: 1-[^{11}C]Glucose can function as a probe for assessing alterations in myocardial glucose metabolism in both normal and abnormal myocardium.
Implementation: Measurements of myocardial blood flow and rMGU applying PET methodologies facilitated by the use of $H_2[^{15}O]$ and 1-[^{11}C]-glucose, respectively. Arterial-coronary sinus sampling is performed to measure rMGU according to the Fick method.
Animal Model: Canine (n = 20; n = 5 fasting; n = 6 hyperinsulinemia-euglycemic clamp at rest; n = 5 clamp and phenylephrine; n = 4 clamp and dobutamine).
Interpretation and Analysis: Values for rMGU range from 50 to 2436 nmol/g/min. Myocardial 1-[^{11}C]-glucose images of high quality can be obtained. There is a close and direct correlation between values for rMGU measured by PET and those measured directly ($y = 0.86x + 112$, $r = 0.98$, $p < 0.0001$). The coefficient of variation for the regional estimates of rMGU ranges from between 11.3% ± 7.4% during clamp at rest to 16.3% ± 8.4% during clamp with phenylephrine.

It now appears possible to quantify myocardial glucose utilization by PET with 1-[^{11}C]-glucose.

Quantification of myocardial glucose utilization by PET and 1-carbon-11-glucose. Herrero P, Weinheimer CJ, Dence C, Oellerich WF, Gropler RJ. J Nucl Cardiol 2002 Jan-Feb; 9 (1), pp. 5–14.

Ischemia: Myocardial
Dipyridamole/Leukocyte Chemotaxis

Cellular/Molecular Mechanism of Action: Dipyridamole influences the activity of phosphodiesterase in a manner that promotes elevations in intracellular (platelet) cAMP concentrations and potentiates the vasoactive and antiplatelet aggregatory properties mediated by PGI_2. Dipyridamole is classified as a adenosine nucleotide trasport blocker whereby it prevents adenosine influx into myocardial cells.
Application: Detection parameter for identifying conditions of myocardial ischemia.
Implementation: Evaluation of myocardial ischemia conditions as a function of neutrophil chemotactic responses with and without the administration of dipyridamole.
Interpretation and Analysis: Chemotaxis of control PMNs toward plasma isolated from cases without signs of myocardial ischemia 7 minutes after dipyridamole administration is significantly diminished compared to baseline values ($p = 0.003$). Plasma obtained 7 minutes after dipyridamole infusion from patients manifesting signs of myocardial ischemia by single photon emission computed tomography (SPECT) attract control PMNs more intensively compared to plasma isolated at time = 0 (significant at $p = 0.0005$).
Interpretation and Analysis: Transient myocardial ischemia induced by dipyridamole challenge leads to the generation of chemotactic factors detectable within peripheral blood plasma.
Instrumentation: Boyden chemotactic chamber.

Plasma chemotactic activity during dipyridamole-induced myocardial ischemia. Wysocki H, Kazmierczak M, Wykretowicz A, Szczepanik A, Minczykowski A. Int J Cardiol 1998 Jan 5; 63 (1), pp. 15–9.

Reactive Oxygen Species Production/Myocardial
Dihydroethidium

Background Information: Protein kinase C (PKC) and reactive oxygen species (ROS) are known to have a role in anesthetic preconditioning (APC). Cardiac preconditioning by triggers other than volatile anesthetics, such as opioids or brief ischemia, is known to be isoform selective, but the isoform required for APC is not known.
Application: Detection and characterization of intracellular reactive oxygen species production. Examples include the identification of PKC isoform involved in APC and elucidation of the relative positions of PKC activation and ROS formation within the APC signaling cascade.
Implementation: Evaluation of the influence of sevoflurane APC in heart tissue prior to the initiation of ischemic reperfusion injury in the absence or presence of a non-specific PKC inhibitor(chelerythrine), a PKC-delta inhibitor (PP101), or a PKC-epsilon inhibitor (PP149). Spectrofluorometry and the fluorescent probe dihydroethidium is used to measure intracellular ROS, while assay of effluent dityrosine is used to measure extracellular ROS release.
Animal Model: Isolated guinea pig hearts subjected to 30 minutes of ischemia and 120 minutes of reperfusion.
Interpretation and Analysis: Previous sevoflurane exposure protects heart tissues against ischemia-reperfusion injury. Chelerythrine or PP149 abolishes such protection, but PP101 does not. Reactive oxygen species formation can be observed during sevoflurane exposure but it is not altered by any of the PKC inhibitors. Anesthetic preconditioning is mediated by PKC-epsilon but not by PKC-delta. Furthermore, PKC activation probably occurs downstream of ROS generation in the APC signaling cascade.

Reactive oxygen species precede the epsilon isoform of protein kinase C in the anesthetic preconditioning signaling cascade. Novalija E, Kevin LG, Camara AK, Bosnjak ZJ, Kampine JP, Stowe DF. Anesthesiology 2003 Aug; 99 (2), pp. 421–8.

Permeation Properties: Myocardial Cells
Ethidium Bromide (EB)

Cellular/Molecular Mechanism of Action: Ethidium bromide selectively binds to nucleic acids and can also function as a passive biological marker molecule. Ethidium bromide also possesses fluorescent properties when exposed to ultraviolet light energy.
Background Information: Strong electric pulses produce reversible or irreversible membrane breakdown (electroporation).
Application: Detection of alteration in membrane pore size.
Implementation: Analyze the permeation properties of minute pores caused by hyperpolarization or lysophosphatidylcholine (LPC) by comparing the amount of charge carried by irregular inward currents (I^{hi}) with changes in EB fluorescence in isolated rabbit ventricular myocytes.
Technique: Forty-second negative pulses from a holding potential of -20 mV induces I^{hi} whose conductance increases with hyperpolarization.
Interpretation and Analysis: Mean conductance (G^{hi}) is 63.6 ± 9.9 pS/pF (mean ± S.E.M., n = 9) at −160 mV. Ethidium bromide fluorescence increases during voltage pulses in parallel with the time integral of I^{hi} (Q^{hi}), with the magnitude of increases in nuclear EB fluorescence being 5.3 times greater than in the cytoplasm at −160 mV. Similar hyperpolarization-induced parallel increases in I^{hi} and EB fluorescence is also obtained in Na^+-free, N-methyl-D-glucamine (NMDG) solution. LPC (10 μM) induced large (101.2 ± 21.2 pS/pF, n = 16), rapid (rise times, 1–10 msec) I^{hi} with slow relaxation rates at −80 mV that reflect increases in G^{hi} to 94.3 ± 24.8 pS/pF (n = 8) at 6 minutes. Plots of ethidium bromide fluorescence vs Q^{hi} can be well fitted by a common Hill's equation with a Hill coefficient of 0.97. Taken together, such findings indicate that hyperpolarization and LPC produce pores that have the same filter properties for the permeation of small ions, including ethidium(+), and that I^{hi} (carried in part by Ca^{2+} generated by membrane breakdown) is capable of supplying sufficient ions to evoke abnormal excitation and contraction in cardiac myocytes.

Hyperpolarization and lysophosphatidylcholine induce inward currents and ethidium fluorescence in rabbit ventricular myocytes. Song YM, Ochi R. J Physiol 2002 Dec 1; 545 (Pt 2), pp. 463–73.

Platelet Function in Arterial Disease/Peripheral Arterial Disease (PAD) of the Lower Limb/Carotid Artery Disease/Abdominal Aorta Aneurysm (AAA)
Adenosine Diphosphate/Collagen

Cellular/Molecular Mechanism of Action: Adenosine diphosphate is released by platelets during activation phenomenon which in turn promotes activation/aggregation of nearby platelets. Soluble collagen binds membrane-associated receptors expressed by platelets and promotes aggregatory responses.
Background Information: Conditions of PAD have a 3-fold increase in cardiovascular mortality. Standard antiplatelet treatment

may not confer uniform benefit in different patient groups. Studies have described the comparison of platelet function in cases of lower limb PAD, carotid disease, and AAA with age- and sex-matched healthy controls.

Application: Detection and chacterization of arterial disease related to alterations in platelet function.

Implementation: Evaluation of PAD as a function of spontaneous platelet aggregatory (SPA) properties can be assessed in whole-blood samples applying adenosine diphosphate (ADP) and collagen to induce aggregation responses. Flow cytometry analyses can also be used to detect platelet P-selectin and the PAC-1 antigen as markers of platelet activation and aggregation.

Human Model: Cases of PAD of the lower limbs (n = 20 PAD); carotid artery disease (n = 40); abdominal aortic aneurysm (n = 13 AAA); and age-/sex-matched healthy controls (n = 20).

Interpretation and Analysis: Conditions of lower limb PAD or AAA have higher baseline SPA compared to normal controls ($p < 0.01$). There is significantly higher collagen-induced aggregation in IC patients compared to normal controls ($p < 0.01$). However, there is no difference in ADP-induced aggregation between lower limb PAD and reference controls. There is no difference in PAC-1 binding between control patients and the patients with lower limb PAD, carotid disease, or AAA. Conditions of carotid artery disease express higher levels of P-selectin compared to normal controls ($p < 0.05$).

Therefore, evidence exists that suggests platelet hyperactivity is present in conditions of PAD despite the use of antiplatelet therapy. Further antiplatelet strategies may be indicated for the therapeutic management of such disease states.

Instrumentation: Flow cytometry instrumentation.

Increased platelet aggregation and activation in peripheral arterial disease. Robless PA, Okonko D, Lintott P, Mansfield AO, Mikhailidis DP, Stansby GP. Eur J Vasc Endovasc Surg 2003 Jan; 25 (1), pp. 16–22.

Reperfusion Injury/Leukocyte Rolling Phenomenon Following Ischemic Insult

Acridine Orange

Cellular/Molecular Mechanism of Action: Acridine orange emits a fluorescent color when exposed to certain wavelengths of light energy.

Background Information: Recent reports have shown that ischemic preconditioning induces substantial protection against retinal damage by subsequent prolonged ischemia and that this protection is mediated by mechanisms involving the adenosine A1 receptor.

Application: Evaluate quantitatively the effects of ischemic preconditioning on leukocyte-mediated reperfusion injury after transient retinal ischemia and to define the role of the adenosine A1 receptor in these effects.

Implementation: The adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) is administered intramuscularly immediately after ischemic preconditioning. Leukocyte behavior in the retina after 60 minutes of ischemia is evaluated *in vivo* with acridine orange digital fluorography.

Animal Model: Rats/male (transient retinal ischemia is induced by temporary ligation of the optic nerve. Ischemic preconditioning [5 minutes of ischemia] is induced 24 hours before 60 minutes of ischemia).

Interpretation and Analysis: Ischemic preconditioning inhibits leukocyte rolling. The maximum number of rolling leukocytes is reduced to 3% at 12 hours after reperfusion ($p < 0.01$). Subsequent leukocyte accumulation is also decreased with ischemic preconditioning. The maximum number of accumulated leukocytes is reduced to 22.6% at 24 hours after reperfusion ($p < 0.01$). These inhibitory effects are suppressed by the administration of DPCPX ($p < 0.0001$). The numbers of rolling leukocytes at 12 hours after reperfusion and accumulated leukocytes at 24 hours after reperfusion are approximately 102.7% (ns) and 83.4% ($p < 0.01$), respectively, compared with numbers without ischemic preconditioning. Ischemic preconditioning, therefore, has an inhibitory effect on leukocyte rolling and subsequent leukocyte accumulation during retinal ischemia-reperfusion injury. Furthermore, the adenosine A1 receptor may play an important role in these inhibitory effects.

Instrumentation: Digital fluorography.

Inhibitory effect of ischemic preconditioning on leukocyte participation in retinal ischemia-reperfusion injury. Nonaka A, Kiryu J, Tsujikawa A, Yamashiro K, Nishijima K, Miyamoto K, Nishiwaki H, Honda Y, Ogura Y. Invest Ophthalmol Vis Sci 2001 Sep; 42 (10), pp. 2380–5.

Stem Cells: Mesenchymal/Retention, Engraftment, and Migration

Iron Fluorophore Particle (IFP)/Mesenchymal Stem Cells

Cellular/Molecular Mechanism of Action: Stem cells have the potential to differentiate into a multitude of different cell types within tissue/organ environments.

Background Information: Delivery and tracking of endomyocardial stem cells is limited by the inability to image transplanted cells noninvasively in the beating heart.

Application: Mesenchymal stem cells (MSCs) can be labeled with iron fluorophore particle (IFP) to provide MRI contrast for *in vivo* assessment of immediate and long-term localization in order to establish parameters pertaining to retention, engraftment, and migration within tissues/organs.

Route of Administration: Mesenchymal stem cells (variable including percutaneous).

Implementation: Short-term incubation of mesenchymal stem cells with IFP results in efficient dose-dependent labeling. Postlabeling, cells remain viable for multiple passages and retain *in vitro* proliferation and differentiation capacity. Labeled mesenchymal stem cells (10^4 to 10^6 cells/150 μL) are then injected percutaneously into normal and freshly infarcted myocardial tissues. Serial cardiac MRI (1.5 T) are then performed at 4 (e.g. n = 1 *in vivo* preparations), 8 (e.g. n = 3 *in vivo* preparations), and 21 days (e.g. n = 1 *in vivo* preparation). MRI contrast properties are then measured both *in vivo* and *in vitro* for cells embedded in agar. Injection sites containing as few as 10^5 MSCs can be detected and contain intact IFP-bearing mesenchymal stem cells on histology.

Animal Model: Swine (mesenchymal stem cells and porcine myocardium [normal & infracted]).

Interpretation and Analysis: IFP labeling of mesenchymal stem cells imparts useful MRI contrast, enabling ready detection in the beating heart on a conventional cardiac MR scanner after transplantation into normal and infarcted myocardium. The dual-labeled MSCs can be identified at locations corresponding to injection sites, both *ex vivo* using fluorescence microscopy and *in vivo* using susceptibility contrast on MRI. This technology may permit effective *in vivo* study of stem cell retention, engraftment, and migration.

Serial cardiac magnetic resonance imaging of injected mesenchymal stem cells. Hill JM, Dick AJ, Raman VK, Thompson RB, Yu ZX, Hinds KA, Pessanha BS, Guttman MA, Varney TR, Martin BJ, Dunbar CE, McVeigh ER, Lederman RJ. Circulation. 2003 Aug 26; 108 (8), pp. 1009–14. Epub 2003 Aug 11.

CARDIAC DISEASE AND PHYSIOLOGY

ANGINA: CARDIAC-ASSOCIATED THORACIC PAIN

Angina Pectoris/Atherosclerosis: Coronary Artery/ Hydroxyapatite Deposition/Musculoskeletal: Osteoblastic Activity/Neoplastic Disease: Osteosarcoma
Near-Infrared Light Emitting Probes/Fluorescent Bisphosphonate Derivatives/[Near-Infrared]

Background Information: In vertebrates, the development and integrity of the skeleton requires hydroxyapatite (HA) deposition by osteoblasts. Sites of osteoblastic activity have most commonly been imaged *in vivo* using gamma-emitting radioisotopes. The scan times required are long, and the resultant radioscintigraphic images suffer from relatively low resolution.
Application: Hydroxyapatite deposition is a marker of, or a participant in, processes as diverse as cancer and atherosclerosis.
Semi-Synthetic Methods: Production of a near-infrared (NIR) fluorescent bisphosphonate derivative.
Implementation: Evaluation of the ability for NIR fluorescent bisphosphonate derivatives to physically bind to hydroxyapatite deposits.
Interpretation and Analysis: Near-infrared light-based probes like fluorescent bisphosphonate derivatives possess an ability to bind to hydroxyapatite under *in vitro* conditions, in addition to recognizing osteoblastic activity in living animals. Such biotechnology can be applied to study skeletal development, osteoblastic metastasis, coronary atherosclerosis, and other disease states.

In vivo near-infrared fluorescence imaging of osteoblastic activity. Zaheer A, Lenkinski RE, Mahmood A, Jones AG, Cantley LC, Frangioni JV. Nat Biotechnol 2001 Dec; 19 (12), pp. 1148–54.

Angina Pectoris/Coronary Artery Spasms/ Vasospastic Disease
Acetylcholine

Cellular/Molecular Mechanism of Action: Acetylcholine binds to and activates both nicotinic and muscarinic acetylcholine receptor complexes.
Physiological Mechanism of Action: Coronary artery spasm can be induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the endothelium is intact by releasing nitric oxide (NO). In conditions of coronary artery spasm, acetylcholine induces a spasmodic response that can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. Such finding imply that some form of abnormally in endothelial integrity exists in conditions of coronary spasm that can be accentuated by acetylcholine and histamine, and to a lesser by ergonovine and serotonin. In general, all ergot alkaloids exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptor complexes. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentrations. Similarly, the contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.
Application: Determine the sensitivity and specificity of coronary artery spasm induced by intracoronary artery injection of acetylcholine in conditions of vasospastic angina.
Considerations: Intracoronary injection of acetylcholine is a sensitive and reliable method for the induction of coronary spasm in patients with vasospastic angina as well as in those with variant angina.
Route of Administration: Direct intracoronary artery injection.
Dosage: Incremental doses of acetylcholine (20, 30, and 50 μg).
Dosage: Evaluation of coronary artery function/response to incremental doses of intracoronary artery injected acetylcholine.
Human Model: Cases with variant angina (Group A; n = 21); cases with other types of vasospastic angina (Group B; n = 28), and cases without any significant coronary artery disease (Group C; n = 20).
Interpretation and Analysis: Coronary artery spasm is defined as severe vasoconstriction (greater than or equal to 90% of reduction in luminal diameter) with chest pain and/or ischemic changes appreciated on electrocardiogram tracings. Intracoronary injection of acetylcholine induces spasm of at least 1 coronary artery in 95% of Group A cases (e.g. n = 20/21), in 96% of Group B cases (e.g. n = 27/28); and in only a relatively low percentage of Group C cases (e.g. n = 2/20 or 10%).Low-dose acetylcholine regimens (20 μg) induces coronary spasm more frequently in Group A cases (81%) than in Group B patients (43%) ($p < 0.05$). S-T segment elevations associated with anginal attacks are significantly ($p < 0.05$) more frequent in Group A (71%) than in Group B (39%) cases. When acetylcholine is injected separately into the left and right coronary arteries, spasm of both coronary arteries can be observed in approximately 50% of Group A type individuals (e.g. n = 7/14); in 36% of Group B type individuals (e.g. n = 8/22), and in a very low percentage of 20 Group C type individuals (e.g. n = 0/20 or 0%).

Usefulness of intracoronary injection of acetylcholine as a provocative test for coronary artery spasm in patients with vasospastic angina. Miwa K, Fujita M, Ejiri M, Sasayama S. Heart Vessels 1991; 6 (2), pp. 96–101.

Angina Pectoris: Coronary Artery Spasm
Acetylcholine/Ergonovine/[Electrocardiography]

Cellular/Molecular Mechanism of Action: Acetylcholine binds directly to and positively activates acetylcholine receptor complexes located on the surface of postsynaptic membranes. Ergonovine directly binds to and positively (submaximally) activates tryptaminergic and α-adrenergic receptor complexes to a degree that is less potent than ergotamine. At dopaminergic receptor complexes, ergonovine functions as a weak antagonist. At both tryptaminergic and dopaminergic sites, ergonovine can exert both partial agonist and antagonist properties within certain regions of the central nervous system (CNS). In various smooth muscle tissues, the compound exerts antagonist properties.
Physiological Mechanism of Action: Coronary artery spasm can be induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the endothelium is intact by releasing nitric oxide (NO). In conditions of coronary artery spasm, acetylcholine induces a spasmodic response that can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. Such finding imply that some form of abnormally in endothelial integrity exists in conditions of coronary spasm that can be accentuated by acetylcholine and histamine, and to a lesser by ergonovine and serotonin. In general, all ergot alkaloids exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptor complexes. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentrations.

Similarly, the contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.

Application: Acetylcholine and ergonovine can be used to identify (detect) and characterize cases suffering from spasms of the coronary artery. Speculation suggests that there may be some potential applications related to other vascular disorders including patent ductus arteriosus and extrahepatic portosystemic shunts.

Implementation: Delineation of mechanisms involved in the pathogenesis of coronary artery spasms can be facilitated by comparing the action of acetylcholine with that of ergonovine. Coronary arteriography is performed immediately after intracoronary injections of titrated incremental doses of acetylcholine or ergonovine.

Human Model: Cases of vasospastic angina (n = 11 Group 1), and chest pain cases (n = 15 Group 2).

Interpretation and Analysis: Occlusive or near-occlusive (99% luminal narrowing) coronary spasm associated with angina and ischemic electrocardiographic S-T changes can be appreciated in a large percentage of Group 1 type cases (e.g., n = 9/11 with acetylcholine and n = 11/11 patients with ergonovine). The region and degree of the most severe coronary spasm on coronary arteriograms evoked by the 2 agents are the same in majority of Group 1 type cases (e.g., n = 9/11). In the remaining Group 1 type cases (e.g., n = 2/11), spontaneous focal coronary spastic stenosis in the baseline coronary arteriogram can be relieved by the intracoronary injection of acetylcholine, and a focal coronary occlusive spasm in the same region can be induced repeatedly by the subsequent intracoronary injection of ergonovine (paradoxic phenomenon). Conversely, occlusive or near-occlusive coronary spasm cannot be induced by either agent in the vast majority of Group 1 type cases (e.g., n = 0/15). Such findings suggest that the 2 provocative tests for coronary spasm employing acetylcholine and ergonovine are clinically useful in the diagnosis of vasospastic angina, but testing with intracoronary ergonovine is needed when a spontaneous focal coronary spasm is relieved by intracoronary injection of acetylcholine. The results also indicate that in many conditions with vasospastic angina, non-specific hypersensitivity to acetylcholine or ergonovine in a definite region of the coronary arteries generally plays an important role in the induction of coronary spasm.

Induction of coronary artery spasm by intracoronary acetylcholine: Comparison with intracoronary ergonovine. Suzuki Y, Tokunaga S, Ikeguchi S, Miki S, Iwase T, Tomita T, Murakami T, Kawai C. Am Heart J 1992 Jul; 124 (1), pp. 39–47.

Angina Pectoris: Differential Diagnosis with Esophogeal Spasm
Ergonovine Maleate

Application: Differential diagnosis of chest pain symptoms. Intravenous ergonovine may be useful in identifying esophageal spasm in selected cases with chest pain who have normal coronary arteries or in whom coronary artery disease is insufficient to explain the severity of symptoms.

Implementation: Evaluation of cases of chest pain resembling angina pectoris employing intravenous administration of ergonovine maleate.

Interpretation and Analysis: Administration of intravenous ergonovine maleate in cases of chest pain resembling angina pectoris (e.g., n = 14) can induce the typical signs of discomfort previously experienced by affected cases (e.g., n = 5/14) while manometric signs of esophageal spasm may also develop. A certain percentage of affected cases (e.g., n = 9/14) and negative reference control subjects will not experience signs of chest pain. However, the vast majority of affected cases will experience some form of symptomatic response to ergonovine, including chest warmth or heaviness, headache, mild choking sensation, facial numbness, flushing, or nausea. Manometric signs of esophageal spasm after ergonovine can be detected in some patients that experience no signs of chest pain (e.g., n = 2/9) on a level similar to negative reference controls. In this context, the potential risks of ergonovine do not justify its routine use as a provocative agent for esophageal spasm.

Use of ergonovine to identify esophageal spasm in patients with chest pain. Eastwood GL, Weiner BH, Dickerson WJ 2nd, White EM, Ockene IS, Haffajee CI, Alpert JS. Ann Intern Med 1981 Jun; 94 (6), pp. 768–71.

Angina Pectoris/Coronary Artery Spasm/ Vasospastic Disease/S-T Segment Depression
Ergonovine/Angiography

Cellular/Molecular Mechanism of Action: Directly binds and positively, but submaximally activates tryptaminergic and α-adrenergic receptor complexes at a level that is less than produced by ergotamine. At dopaminergic receptor complexes, ergonovine functions as a weak antagonist. At both tryptaminergic and dopaminergic sites, ergonovine can exert both partial agonist and antagonist properties at certain sites within the central nervous system. In various smooth muscle tissues, the compound exerts antagonist properties.

Physiological Mechanism of Action: Promotes vasoconstrictive responses within both venous and arterial structures through direct stimulation of vascular smooth muscle. Systemic administration can produce elevations in blood pressure due to peripheral vasoconstriction. Ergonovine can produce coronary artery vascular responses that are detected as ischemic patterns on EKG tracings and development of angina pain in cases with coronary artery disease. Bradycardia is often induced by ergot alkaloids even when no elevations in blood pressure are evident, due in part to increased vagal activity, and to a lesser extent, by diminished CND sympathetic tone and direct-acting myocardial depression.

Coronary artery spasm can be induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the vascular endothelial cell layer is intact by releasing nitric oxide (NO). Vascular responses of this nature can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. Such findings imply that some form of abnormality in endothelial integrity exists in conditions of coronary spasm that can be accentuated by acetylcholine and histamine, and to a lesser by ergonovine and serotonin.

In general, all ergot alkaloids exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptor complexes. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentrations. Similarly, the contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.

Application: Induction and characterization of coronary artery vasocontrictive responses.

Route of Administration: Intravascular.

Implementation: Evaluation of conditions of angina pectoris suspected of originating from spasms of the coronary artery.

Interpretation and Analysis: Reoccurrence of angina pectoris upon selective infusion of ergonovine suggests that natural episodes of angina pectoris are due to vasoactive episodes affecting the coronary artery and may reflect the presence of coronary artery disease.

Oxytocin, Prostaglandin, Ergot Alkaloids, and Tocolytic Agents. Rall TW, Schleifer LS (Chapter 39: pp. 926–45). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW,

Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Angina Pectoris/Coronary Artery Spasm/ Vasospastic Disease/Inhibition of Spasm Activity
Molsidomine

Application: Molsidomine has been applied as an anticoronary artery spasm agent following alkaline buffer infusion.
Route of Administration: Molsidomine (intravascular); alkaline buffer (intravascular).
Dosage: The rapid infusion of an alkaline buffer followed by maximal hyperventilation and molsidomine (4 mg) in cases of angina pectoris.
Considerations: Evaluation of the appearance of anginal pain and developmet of significant, transient ischemic changes in the S-T segment resulting from alkalosis-induced coronary spasm.
Interpretation and Analysis: Reversal of pro-coronary artery spasm effect induced by alkaline buffer infusion utilizing molsidomine. A 2nd provocative test can be performed under the same conditions, 24 hours later, after the administration of 4 mg of molsidomine. Molsidomine prevents the development of coronary artery spasm in a high percentage of cases (approximately 80%). These preliminary results justify further clinical evaluation of molsidomine in the characterization and treatment of vasospastic angina.

Angina Pectoris: Vasospastic Coronary Artery
Systemic Alkalosis/Nitroglycerin Reversal

Physiological Mechanism of Action: Induced systemic alkalosis promotes vasoconstriction or spasms of the coronary artery.
Application: Detection of coronary spasms by creating systemic alkalosis in cases with infrequent, angina-compatible chest pain.
Considerations: Previous reports have applied the provocative test without a previous coronary arteriography procedure but only in cases with negative submaximal exercise test responses.

Hyperventilation appears to be a safe and specific diagnostic procedure in a subset of cases in whom the probability of coronary artery disease may not be judged sufficient to warrant coronary arteriography as a primary diagnostic procedure.
Interpretation and Analysis: Rapid infusion of alkaline solution followed by maximal hyperventilation raises arterial pH above the 7.65 value necessary for diagnostic significance in approximately 83% of cases (e.g., n = 196). In approximately 12% of cases, (e.g., n = 24), the provocative test induces significant ischemic S-T segment changes. In all cases with a positive response, coronary artery disease that is predominantly vasospastic (e.g., n = 19 cases) or atheromatous with a vasospastic contribution (e.g., n = 5 cases) can be demonstrated by coronary arteriography followed, if necessary, by diagnostic injection of a ergot derivative.

Chest pain and ECG changes can essentially always be reversed within 5 minutes by intravenous nitroglycerin. In instances where coronary arteriography is not performed in cases with a negative response, the sensitivity of the procedure cannot be accurately assessed. However, in cases with a negative response to hyperventilation that undergo coronary arteriography, approximately 92% (e.g., n = 33) have normal arteriograms and a negative response to ergot derivatives.

Systemic alkalosis as a provocative test for coronary artery spasm in patients with infrequent resting chest pain. Weber S, Cabanes L, Simon JC, Letrait M, Fouchard J, Guerin F, Degeorges M. Am Heart J 1988 Jan; 115 (1 Pt 1), pp. 54–9.

Angina Pectoris/Coronary Artery Spasm/ Vascular Response Testing/Intractable Coronary Artery Spasm: Prinzmental's Variant Angina
Ergonovine Maleate/Nifedipine

Physiological Mechanism of Action: Coronary artery spasm can be induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the endothelium is intact by releasing nitric oxide (NO). In conditions of coronary artery spasm, acetylcholine induces a spasmodic response that can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. Such findings imply that some form of abnormality in endothelial integrity exists in conditions of coronary spasm that can be accentuated by acetylcholine and histamine, and to a lesser by ergonovine and serotonin. In general, all ergot alkaloids exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptor complexes. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentrations. Similarly, the contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.
Application: Detection and characterization of angina pectoris conditions.
Implementation: Evaluation of the management of cases suffering from intractable coronary arterial spasms that have Prinzmetal's variant angina, normal or mildly abnormal coronary arteriograms, and a positive ergonovine maleate provocative test. Characterization angina pectoris conditions can involve delineation of functional responses to ergonovine maleate and nifedipine reversal.

Human Model: Cases with anginal attacks occurring at least 3 times a week during isosorbide dinitrate therapeutic regimens.
Interpretation and Analysis: Majority of affected cases demonstrate a decrease in the frequency of ischemic attacks with nifedipine. Holter monitor evaluation can serve to confirm the absence of ischemic changes in response to nifedipine (e.g., n = 7 cases). When nifedipine dosage is decreased or therapy is discontinued, a very large percentage of cases will demonstrate a recurrence of anginal attacks (e.g., n = 6/6). Some cases will experience minor side effects that require a decrease in nifedipine dosage (e.g., n = 2). Nifedipine in general is well tolerated, and no major complications are usually appreciated. Nifedipine appears to be effective in the management of cases with symptomatic coronary arterial spasm and normal or mildly abnormal coronary arteriograms. Investigations conducted to date imply that further investigation of nifedipine for the characterization and treatment of such patients is justified.

Nifedipine therapy for refractory coronary arterial spasm. Heupler FA Jr, Proudfit WL. Am J Cardiol 1979 Oct 22; 44 (5), pp. 798–803.

Angina Pectoris/Coronary Artery Spasm/ Vasospastic Disease/Angiography Classification/ Coronary Artery Diameter/M-Mode Characterization of Coronary Artery Flow Dynamics/Vascular Response Testing: Differentiation of Variants/Vasospastic Angina without Coronary Stenosis/Vasospastic Angina with Coronary Stenosis/Coronary Stenosis without Vasospastic Angina
Ergonovine/Acetylcholine/Nitroglycerin Reversal

Physiological Mechanism of Action: Coronary artery spasm can be induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the endothelium is intact

by releasing nitric oxide (NO). In conditions of coronary artery spasm, acetylcholine induces a spasmodic response that can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. Such finding imply that some form of abnormally in endothelial integrity exists in conditions of coronary spasm that can be accentuated by acetylcholine and histamine, and to a lesser by ergonovine and serotonin. In general, all ergot alkaloids exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptor complexes. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentrations. Similarly, the contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.

Background Information: The definite diagnosis of vasospastic angina can be established by performing a coronary spasm provocative test using ergonovine maleate or acetylcholine facilitated by selective cardiac catheterization. Current non-invasive, non-pharmacologic diagnostic methods are not sensitive enough for the diagnosis of vasospastic angina.

Application: Diagnosis of angina associated with cardiovascular function (ergonovine).

Route of Administration: Nitroglycerin (sublingual).

Dosage: Nitroglycerin (0.3 mg).

Considerations: Induced episodes of cardiovascular angina are reversible through the administration of nitroglycerin.

Implementation: Evaluation of normal left main coronary artery branch applying angiography imaging procedures for the purpose of classification into 4 groups based on the presence or absence of more than 50% stenosis in the coronary arteries except for the left main trunk and results observed with acetylcholine or ergonovine provocative testing. The left main trunk diameter can be measured by M-mode echocardiography before and after sublingual administration of nitroglycerin (0.3 mg), and alterations in dilation calculated to assess coronary artery tone.

Human Model: Cases with angiographically normal left main trunk (n = 38).

Interpretation and Analysis: The percent dilation of the left main trunk diameter induced by sublingual nitroglycerin at 7 A.M. and at noon is 22.4 ± 4.7% (mean ± SD) and 18.1 ± 4% in conditions of vasospastic angina and without coronary stenosis (e.g., n = 11); 14.9 ± 7.1% and 11.2 ± 6.9% in conditions of vasospastic angina and coronary stenosis (e.g., n = 9); 6.1 ± 3.5% and 7.0 ± 5.1% in conditions without vasospastic angina but with coronary stenosis (e.g., n = 8); and 8.1 ± 5.6% and 7.8 ± 5.7% in normal reference controls (e.g., n = 10). The percent dilation at 7 A.M. is significantly greater in conditions with vasospastic angina without coronary stenosis than in conditions of vasospastic angina and coronary stenosis or coronary stenosis without vasospastic angina. In case groups with vasospastic angina, the percent dilation at 7 A.M. is significantly greater than values observed at noon. Establishing a 7 A.M. percent dilation exceeding 15% as being defined as positive for the diagnosis of vasospastic angina, the sensitivity and specificity of the procedure can be improved to 80% and 94%, respectively. Therefore, it appears that basal tone of the left main trunk is elevated in the early morning in vasospastic angina. Dilation of the left main trunk diameter exceeding 15% induced by sublingual nitroglycerin in the early morning as measured by M-mode echocardiography is a highly sensitive and specific criterion for the diagnosis of vasospastic angina.

Instrumentation: M-mode echocardiogram capabilities (left main coronary artery trunk).

A new noninvasive method of diagnosing vasospastic angina based on dilation response of the left main coronary artery to nitroglycerin as measured by echocardiography. Morita H, Ohmori K, Matsuyama T, Mizushige K, Matsuo H. J Am Coll Cardiol 1996 May; 27 (6), pp. 1450–7.

Angina Pectoris: Prinzmetal's Variant Angina *Ergonovine/Hyperventilation*

Physiological Mechanism of Action: Ergonvine promotes vasoconstriction of both venous and arterial structures through direct stimulation of vascular smooth muscle. Systemic administration in this manner can produce elevations in blood pressure due to peripheral vasoconstriction. Ergonovine can produce coronary artery vascular responses that can be detected as ischemic patterns on EKG tracings and angina pain in patients with coronary artery disease. Bradycardia is often induced by ergot alkaloids even when no elevations in blood pressure are evident, due in part to increased vagal activity, and to a lesser extent, diminished CND sympathetic tone and direct-acting myocardial depression.

Coronary spasm is induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the endothelium is intact, and is promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. In general, all ergot alkaloid exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptors. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentration. The contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.

Background Information: Prinzmetal's variant angina is a classification for a subtype of angina pectoris cases in which attacks are experienced at rest, while exercise capacity is well preserved and attacks are associated with electrocardiographic evidence of S-T segment elevations.

Application: Detection and characterization of spontaneous variant angina response.

Route of Administration: Ergonovine (intravenous).

Considerations: The incidence of chest pain and ventricular arrhythmias is similar during both the hyperventilation and ergonovine tests. However, spontaneous remission of ischemia is more frequent after hyperventilation than following ergonovine administration (48 vs 14%).

Interpretation and Analysis: Hyperventilation produces a positive response in a high percentage of cases (n = 25/30 or 83%) and ergonovine (n = 28/30 or 93%). Hyperventilation can also induce S-T segment elevation (n = 22/25 or 88%), S-T segment depression (n = 2/25 or 8%), or T wave pseudonormalization (n = 1/25 or 4%). Similarly, ergonovine can promote S-T segment elevation (n = 23/25 or 92%), S-T segment depression (n = 4/25), or T wave pseudonormalization (n = 1/25). In the vast majority of cases, the electrocardiographic changes occur in the same leads as during episodes of spontaneous attacks.

Acute ischemia develops at a mean of 218 ± 112 seconds after the end of hyperventilation in majority of positive tests (e.g., n = 19/25); and at that time the double product is not significantly different from basal values. The sensitivity of hyperventilation is similar to ergonovine in cases with greater than or equal to 1 daily attack (95% -vs- 100%), while in those with less than 1 daily attack the sensitivity of hyperventilation decreased to 55% compared to 77% for ergonovine.

In conditions of variant angina the sensitivity of both the hyperventilation and ergonovine tests closely correlate with disease activ-

ity. Hyperventilation is a safe provocative test with a level of sensitivity similar to ergonovine in conditions of active disease; however, in patients with sporadic attacks hyperventilation has a lower sensitivity than ergonovine and therefore a limited diagnostic value.

Hyperventilation and ergonovine tests in Prinzmetal's variant angina pectoris in men. Previtali M, Ardissino D, Barberis P, Panciroli C, Chimienti M, Salerno JA. Am J Cardiol 1989 Jan 1; 63 (1), pp. 17–20.

Oxytocin, Prostaglandin, Ergot Alkaloids, and Tocolytic Agents. Rall TW, Schleifer LS (Chapter 39: pp. 926–45). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Angina Pectoris: Prinzmetal's Variant Angina
Nifedipine

Cellular/Molecular Mechanism of Action: Nifedipine functions as a calcium-blocking agent.
Application: Characterization of the cause of thoracic pain (e.g., angina) and the responsiveness of coronary vessels to vasodilatory intervention.
Background Information: Prinzmetal's variant angina is a classification for a subtype of angina pectoris characterized by the onset of attacks during periods of rest, while exercise capacity is well preserved and attacks are associated with electrocardiographic evidence of S-T segment elevations.
Implementation: Evaluation of angina pectoris conditions using responses to nifedipine administration as a diagnostic parameter.

Human Model: Cases with variant angina pectoris (e.g., n = 12; 44 to 67 years of age [e.g. n = 5/12 with vasospasm of the left anterior descending coronary artery; n = 7/12 with spasm of a dominant right coronary artery). The frequency of anginal attacks per 24 hours can vary stubstantially (e.g., n = 1 to 12/24 hrs) with ventricular tachycardia episodes being accompanied by (i) ischemic episodes (e.g., n = 7/12 cases) and (ii) high grade atrioventricular block (e.g., n = 2/12 cases).
Interpretation and Analysis: Nifedipine administration results in a high percentage of cases demonstrating profound improvement and relief of clinical signs (e.g., n = 11/12 patients), many of which experience a long-term benefit (e.g., n = 7/11). Withdrawal of nifedipine frequently leads to recurrence of angina (e.g., n = 6 episodes per 4 cases).

Provocative testing with the aid of cardiac catheterization procedures in concert with the cold pressor test or ergonovine maleate before and after nifedipine administration demonstrates that this agent is capable of blocking both α-adrenergic and ergonovine-induced vasospasms. Nifedipine may have a significant role in the diagnosis and therapy of angina caused by coronary spasm.

Nifedipine in the treatment of Prinzmetal's (variant) angina. Goldberg S, Reichek N, Wilson J, Hirshfeld JW Jr, Muller J, Kastor JA. Am J Cardiol 1979 Oct 22; 44 (5), pp. 804–10.

Angina Pectoris: Prinzmetal's Variant Angina (PVA)
Ergonovine Maleate

Physiological Mechanism of Action: Coronary artery spasm can be induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the endothelium is intact by releasing nitric oxide (NO). In conditions of coronary artery spasm, acetylcholine induces a spasmodic response that can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. Such finding imply that some form of abnormally in endothelial integrity exists in conditions of coronary spasm that can be accentuated by acetylcholine and histamine, and to a lesser by ergonovine and serotonin. In general, all ergot alkaloids exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptor complexes. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentrations. Similarly, the contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.
Application: Ergonovine maleate (IV) is sometimes used as a diagnostic aid for detecting/characterizing PVA conditions. The cold pressor test is also used in a similar diagnostic manner.
Background Information: Prinzmetal's variant angina is a classification scheme for a subtype of angina pectoris in which attacks are experienced at rest, while exercise capacity is well preserved and attacks are associated with electrocardiographic evidence of S-T segment elevations.
Considerations: Prinzmetal's variant angina is a form of angina caused by coronary-artery vasospasm (CAS) that is not associated with exertion. Nitrates, adrenergic-blocking agents, and calcium-channel blocking agents can be used in the treatment of PVA conditions. Nitroglycerin and isosorbide dinitrate effectively relieve CAS. However, long-term prospective studies on the use of these drugs for PVA are lacking in the literature. Studies on treating PVA with adrenergic-blocking agents have been equivocal, with some studies reporting improvement and some reporting worsening. Calcium-channel blocking agents are promising drugs for PVA. Nifedipine is generally considered the prototype of this class for antianginal activity. It is effective when administered orally in PVA conditions. Side effects are mild and do not usually require termination of therapy. Verapamil hydrochloride, the prototype calcium-channel blocking agent for arrhythmias, is effective for PVA, but only 10–20% of an orally administered dose reaches systemic circulation because of the hepatic first-pass effect. Other calcium-channel blockers, including perhexilene maleate, diltiazem hydrochloride, prenylamine, and lidoflazine, have been tested in a few CAS cases with some success; adverse effects and toxicities limit the use of some of these agents, especially perhexilene. Therapy, using combinations of nitrates, adrenergic-blocking agents, and calcium-channel blocking agents, is needed in some patients. Dosing guidelines for all drugs have been described (see citation). Treatment of PVA should begin with oral nitrates. Calcium-channel blocking agents are indicated in cases that fail to respond or that are intolerant to maximum doses of nitrates given in various forms.
Interpretation and Analysis: Prinzmetal's variant angina is diagnosed by history, electrocardiogram, or coronary-artery angiography.

Diagnosis and treatment of Prinzmetal's variant angina. McMahon MT, McPherson MA, Talbert RL, Greenberg B, Sheaffer SL. Clin Pharm 1982 Jan-Feb; 1 (1), pp. 34–42.

Angina Pectoris: Prinzmetal's Variant Angina (PVA)/ Vascular Response Testing
Methylergonovine Maleate/Nifedipine

Cellular/Molecular Mechanism of Action: Methylergonovine maleate (methergine) directly binds and positively activates at a submaximal level tryptaminergic, and α-adrenergic receptor complexes but to a lesser extent than does ergotamine. At dopaminergic receptor complexes, ergonovine functions as a weak antagonist. At both tryptaminergic and dopaminergic sites, ergonovine

can exert both partial agonist and antagonist properties at certain CNS sites. In various smooth muscle tissues, the compound exerts antagonist properties. Nifedipine functions as a calcium inhibitor.

Physiological Mechanism of Action: Promotes vasoconstriction of both venous and arterial structures through direct vascular smooth muscle stimulation. Systemic administration can produce elevations in blood pressure due to peripheral vasoconstriction. Ergonovine produces coronary artery vascular responses that can be detected as ischemic patterns on EKG tracings and angina pain in cases with coronary artery disease. Bradycardia is often induced by ergot alkaloids even when no elevations in blood pressure are evident, due in part to increased vagal activity, and to a lesser extent, diminished CND sympathetic tone and direct-acting myocardial depression.

Coronary spasm is induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when vascular endothelial cell layers are intact. The response can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. In general, all ergot alkaloid exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptors. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentration. The contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.

Application: Detection of PVA cases applying 3 challenge schedules.

Route of Administration: Intravascularly infused (injected) directly into the coronary artery.

Dosage: Nifedipine (0.2 mg).

Considerations: Coronary spasm, frequently recognized during coronary arteriography procedures in cases with PVA, can be treated with intravenous or intracoronary injections of nitroglycerin or nifedipine.

Implementation: Evaluation of vascular spasm responses and the capacity of nifedipine administration to suppress vasoconstrictive responses.

Interpretation and Analysis: Nifedipine (0.2 mg) injected directly into the involved artery suppresses vascular spasms in approximately 75% (e.g., n = 9/12 cases). In the residual 25% of cases (e.g., n = 3/12) nifedipine increases coronary sinus flow that had been decreased by spasm responses in 1 of the branches of the left coronary artery. Patients with PVA that have spasm superimposed on atherosclerotic lesions can benefit from coronary arterial bypass grafting combined with partial denervation of the heart. This combination yields acceptable results (83.4% favorable outcome); recurrence of attacks occurs in only 6.7% of cases of this profile type. In those forms of angina in which spasm occurs in angiographically-normal coronary arteries, medical rather than surgical intervention is preferred for therapeutic management. In conditions treated with oral nifedipine (30–40 mg/day), suppression of attacks can be achieved in a relatively large percentage of cases (e.g., n = 11/13). During periods of treatment, the methergine provocative test, which is consistently positive prior to treatment subsequently converts to negative (e.g. n = 12/12 cases). Transient withdrawal of nifedipine may cause recurrence of pain in some cases (e.g. n = 2).

Oxytocin, Prostaglandin, Ergot Alkaloids, and Tocolytic Agents. Rall TW, Schleifer LS (Chapter 39: pp. 926–45). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Myocardial Infarction
[Antimyosin]-PEG/Liposomes/PEG and Selective Antibody Content/[Nuclear Scintigraphy]

Cellular/Molecular Mechanism of Action: Liposomes function as a physical barrier to dilution of pharmaceutical agents into biological fluids, prevent accelerated excretion or metabolism, and serve as a potential modality for selective delivery at "high" concentrations.

Background Information: Experimental myocardial infarction in rabbits can be detected as a function of their biodistribution patterns and relative size of infarct accumulations utilizing different liposome preparations.

Application: Liposomes formulated with optimized concentrations of poly(ethylene glycol) (PEG) and antimyosin immunoglobulin can function as a delivery format for the detection and characterization of experimental lesions of myocardial infarction.

Route of Administration: Intravenous.

Implementation: Delineation of the influence of parameters such as liposome size and presence or absence of PEG and infarct-specific antimyosin antibody (AM) on *in vivo* liposome biodistribution.

Interpretation and Analysis: The variables of liposome size, PEG content, and infarct-specific antimyosin antibody all affect patterns of liposome biodistribution. Liposome size is the least significant variable. Statistical analysis reveals that of all variables, PEG coating expresses the strongest influence on liposome blood clearance, significantly ($p = 0.0001$) increasing the mean level of blood radioactivity in all conditions. Infarct accumulation depends upon the presence of both PEG ($p = 0.0013$) and antimyosin monoclonal antibody content ($p = 0.005$). The infarct-to-normal accumulation ratio is affected by the presence of antimyosin monoclonal antibody ($p = 0.0002$), but the extent of the effect depends also on the presence of PEG ($p = 0.01$). The 2 different mechanisms of slow accumulation via the impaired filtration (accumulation of PEG-liposomes), and specific binding of immunoliposomes with the exposed antigen (AM-liposomes), collectively contribute to the development of relatively high target-to-background ratios. Both mechanisms are supplementary (complementary) in scenarios of liposomes carrying PEG and AM simultaneously. Multiple strategies exist for futher optimizing PEC/AM liposome preparations as a selective delivery modality.

Poly(ethylene glycol)-coated anticardiac myosin immunoliposomes: Factors influencing targeted accumulation in the infarcted myocardium. Torchilin VP, Narula J, Halpern E, Khaw BA. Biochim Biophys Acta 1996 Feb 21; 1279 (1), pp. 75–83.

Myocardial Infarction
Antimyosin Monoclonal Antibody/[Nuclear Scintigraphy]

Application: Non-invasive *in vivo* detection of acute myocardial infarction.

Route of Administration: Antimyosin monoclonal antibody (intravenous).

Design and Validation: Antimyosin antibody preparations have been development as a diagnostic imaging platform for the *in vivo* detection of acute myocardial infarction. Through its application in this capacity, it has subsequently became apparent that antimyosin antibody can detect the presence of an assortment of other disease states affecting the heart where the hallmark of the pathogenesis of the condition includes myocyte necrosis. Consistent with

this concept, antimyosin antibody has been used as a non-invasive imaging agent for the diagnosis of acute myocarditis, heart transplant rejection, drug-induced cardiotoxicity, and other cardiomyopathies.

Clinical experience gained with first-generation antimyosin monoclonal antibody preparations has served as a foundation for the design of second-generation monoclonal antibodies such as antifibrin and antiplatelet immunoglobulins used in the *in vivo* diagnosis of deep venous thrombosis of the upper limbs, pulmonary embolism, and antiatherosclerotic lesion-specific antibody for diagnosis of metabolically active lesions. Potential candidates for the class of third-generation monoclonal antibodies includes ultrasmall antigen-binding immunoglobulin fragments or synthetic peptides and negative charge-modified antibodies.

Interpretation and Analysis: Positive accumulation of antimyosin monoclonal antibody within a region of the myocardium is an indication of the presence of some type of cellular injury within the heart (e.g., ischemia/ischemic necrosis). Antimyosin preparations have also been used clinically for non-invasive diagnosis of acute myocarditis, heart transplant rejection, drug-induced cardiotoxicity, and other cardiomyopathies.

Antibody imaging in the evaluation of cardiovascular diseases. Khaw BA, Narula J. J Nucl Cardiol 1994 Sep-Oct; 1 (5 Pt 1), pp. 457–76.

Myocardial Infarction (MI)/Innervation: Heterogeneous Cardiac Sympathetic Innervation *[^{131}I]-Meta-Iodobenzylguanidine (MIBG)/[^{125}I]-Iodocyanopindolol (ICYP)/[Nuclear Scintigraphy]*

Cellular/Molecular Mechanism of Action: Meta-iodobenzylguanidine is a norepinephrine analog. Iodocyanopindolol is a β-adrenoreceptor ligand.

Application: Detection and characterization of MI lesions.

Route of Administration: [^{131}I]-meta-iodobenzylguanidine (MIBG) and [^{125}I]-iodocyanopindolol (ICYP) are both administered intravenously.

Implementation: Evaluation of cardiac neuronal function and beta-receptor expression with a dual-tracer method utilizing [^{131}I]-meta-iodobenzylguanidine (MIBG) and [^{125}I]-iodocyanopindolol (ICYP) in rat heart failure after MI.

Animal Model: Rat (MI).

Interpretation and Analysis: In rats with MI, left ventricular (LV) systolic function is decreased, and LV dimension and right ventricular (RV) mass increase gradually. Accumulations of [^{131}I]-meta-iodobenzylguanidine (MIBG) within non-infarcted LV (remote region) and RV are decreased by 15% at 1 week compared with sham-operated *in vivo* models. These same accumulations are restored by 71% and 56%, respectively, at 24 weeks compared with age-matched sham *in vivo* models despite sustained depletion of myocardial norepinephrine contents within these anatomical regions. Accumulations of [^{125}I]-iodocyanopindolol (ICYP) in the remote region and in the RV do not decrease during any stage. Myocardial MIBG distribution is heterogeneous at 1 week and lower in the peri-infarcted region than in the remote region, associated with reduced ICYP accumulation in the peri-infarcted region. At week 12, the heterogeneous distribution of both isotopes disappears. Thus cardiac sympathetic neuronal alteration appears to be coupled with a down-regulation of beta-receptors in heart failure after myocardial infarction. The abnormal adrenergic signaling occurs heterogeneously in terms of ventricular distribution and time course after myocardial infarction.

Heterogeneous cardiac sympathetic innervation in heart failure after myocardial infarction of rats. Igawa A, Nozawa T, Yoshida N, Fujii N, Inoue M, Tazawa S, Asanoi H, Inoue H. Am J Physiol Heart Circ Physiol 2000 Apr; 278 (4), pp. H1134–41.

Myocardial Infarction: Myocardial Potassium (K^+) *Glibenclaminde/Pinacidil/Bumetanide/Triphenyl Tetrazolium Chloride/Evans Blue Dye/[^{87}Rb]/[Nuclear Scintrigraphy]*

Cellular/Molecular Mechanism of Action: (i) Rubidium ion (Rb^+) is a K^+ tracer element; (ii) glibenclamide is a blocker of ATP-sensitive K^+ channels; (iii) pinacidil is a K^+(ATP) opener; (iv) bumetanide is a $K^+/Na^+/2Cl^-$ co-transporter inhibitor.

Application: [^{87}Rb] can be applied in MRI procedures to identify and characterize myocardial infarcts. Evans blue and triphenyl tetrazolium chloride staining can be applied to delineate areas at risk (AAR) and areas of necrosis, respectively.

Dosage: Glibenclamide (3 μM); pinacidil (10 μM); bumetanide (10 μM).

Implementation: Delineation of the effect of drugs that can modulate passive permeability of K^+ into cardiomyocytes in normal and reperfusion-damaged cardiac muscle. Rubidium ion (Rb^+) is used as a K^+ tracer and [^{87}Rb] MRI as a detection method. Preparations are incubated in buffer containing either (i) glibenclamide, (ii) pinacidil, (iii) bumetanide, or (iv) no drug (control). Upon reperfusion, 3-dimensional [^{87}Rb] MR images are acquired to obtain kinetics profiles of [Rb^+] uptake and its distribution. Areas at risk and areas of necrosis are determined by Evans blue dye and triphenyl tetrazolium chloride staining techniques, respectively.

Animal Model: Swine (left anterior descending artery [LAD] of isolated pig hearts perfused with Krebs-Henseleit buffer [KHB] occluded for 2 hours and subsequently reperfused for 2 hours with KHB containing 4.7 mM RbCl instead of KCl).

Interpretation and Analysis: The kinetics remain monoexponential in the affected anterior wall and the uptake rates are approximately 32%, 36%, 37%, and 21% of that in the posterior wall in the control, glib, pin, and bum groups, respectively. Infarct sizes determined histologically as a percentage of total ventricular (left + right) mass (14%–22%) correspond to the size of areas with 20% to 40% of maximal Rb image intensity [I_{Rb}^{Max} 15%–22%], except for values observed with pinacidil (12.5% vs 21%). The sizes of areas with 20% to 50% of I_{Rb}^{Max} (30%–36%) closely correlate with those of AAR determined histologically (31–33%). Lactate dehydrogenase release does not differ between any of the preparations. Therefore, (i) reperfusion damage quickly inhibits [Rb^+]uptake; (ii) [Rb^+] uptake in normal and reperfused tissue does not significantly depend on K^+(ATP) or the $K^+/Na^+/2Cl^-$ co-transporter; and (iii) areas with 20%–40% of maximal image intensity correspond to areas of infarct lesions.

The effects of drugs modulating K(+) transport on Rb(+) uptake and distribution in pig hearts following regional ischemia: (87)Rb MRI study. Kupriyanov VV, Xiang B, Sun J, Jilkina O. NMR Biomed 2002 Aug; 15 (5), pp. 348–55.

Myocardial Infarction (Acute)/Prognosis: Mortality Prediction *Streptokinase (SK) Response*

Cellular/Molecular Mechanism of Action: Streptokinase promotes fibrinolytic activity.

Application: Promotion of biochemical fibrinolysis activity in conditions of acute stroke or myocardial infarction.
Route of Administration: Streptokinase (intravascular).
Implementation: Determination of the relationship between response to SK and troponinT (TnT) concentrations in cases of acute myocardial infarction.

Human Model: Acute myocardial infarction (AMI) (e.g., n = 76 AMI cases) assessed follow hospital admission.
Interpretation and Analysis: Approximately 37% of TnT positive cases display a response to SK (e.g., n = 10/27) as determined by non-invasive criterion for reperfusion. Conversely, approximately 49% of TnT negative cases respond to SK (n = 24/49). There appears to be a trend toward a better response to SK in TnT cases. The mean time interval between the onset of symptoms and thrombolytic treatment among TnT positive "non-responders" is significantly ($p < 0.005$) higher than with TnT negative "non-responders" (5.23 + 3.42 hours vs 2.38 ± 1.37 hours). During reevaluations at 18 months, a higher mortality rate (33%) can be appreciated with TnT positive cases compared to TnT negative cases (10%). Mortality among TnT positive "non-responders" is significantly higher ($p = 0.0494$) than mortality among TnT negative "non-responders" (43 vs 9%), indicating that TnT positive cases , non-responsive to SK, are at a greater risk of cardiac death. Such findings suggest that TnT levels at admission can be of value in risk stratification of AMI conditions.

Admission troponin T as a prognostic marker and its relationship to streptokinase treatment patients with acute myocardial infarction. Kazmi KA, Iqbal MP, Rahbar A, Mehboobali N. Trop Doct 2003 Jan; 33 (1), pp. 18–22.

Myocardial Infarction (MI)/Salvage of Myocardium as a Function of Infarct Location/Anterior Wall -vs- Posterior Wall MI Size Reduction
AMP579/[^{99m}Tc]-SestaMIBI/[Nuclear Scintigraphy]

Cellular/Molecular Mechanism of Action: AMP579 is a mixed adenosine agonist with both A1 and A2 effects.
Background Information: Evidence suggests that myocardial ischemic preconditioning and reperfusion injury may be mediated by adenosine A1 and A2 receptors. In animal models of acute MI, AMP579 reduces infarct size at serum levels of 15 to 24 ng/mL.
Application: Establishing predictive indicators for myocardial salvage as a function of infarct location.
Route of Administration: AMP579 (6-hour continuous infusion).
Dosage: AMP579 (multiple including 60 μg/kg).
Considerations: Placebo-controlled studies can be employed.
Implementation: Evaluation of the influence of 3 different AMP579 doses infused continuously over a 6-hour period. The primary end point is final MI size measured by technetium [^{99m}Tc]-sestaMIBI scanning at 120 to 216 hours following percutaneous transluminal coronary angioplasty (PTCA). Secondary end points include (i) myocardial salvage and salvage index at the same time interval (case subset subjected to baseline technetium [^{99m}Tc]-sestaMIBI scan); (ii) left ventricular ejection fraction and heart failure at 4 to 6 weeks; (iii) duration of hospitalization, and (iv) cardiac events at 4 weeks and 6 months.

Human Model: Cases undergoing primary PTCA after acute S-T segment elevation myocardial infarction (n = 311).
Interpretation and Analysis: Final infarct size does not differ among case groups receiving either AMP579 or placebo pertaining to either anterior myocardial infarction or non-anterior myocardial infarction. For cases with anterior myocardial infarction, median myocardial salvage is increasingly higher in groups receiving ascending dosages of AMP579 plus PTCA. Serum levels approaching levels shown to reduce infarct size in animal models can be achieved only with 60 μg/kg dose levels. AMP579 is safe at the doses tested, but it does not reduce infarct size. There is a trend toward greater myocardial salvage in treated patients with anterior MI.

A randomized, double blinded, placebo-controlled, dose-ranging study measuring the effect of an adenosine agonist on infarct size reduction in patients undergoing primary percutaneous transluminal coronary angioplasty: The ADMIRE (AmP579 Delivery for Myocardial Infarction REduction) study. Kopecky SL, Aviles RJ, Bell MR, Lobl JK, Tipping D, Frommell G, Ramsey K, Holland AE, Midei M, Jain A, Kellett M, Gibbons RJ. Am Heart J 2003 Jul; 146 (1), pp. 146–52.

Myocardial Infarction/Superoxide Anion Production/Mitochondrial [K^+/ATP] Channels
Fluorescent Probe/[K^+/ATP] Channel Blocker/Vitality Stain/Triphenyltetrazolium (Myocardial Infarct)/Dihydroethidium (Fluorescent Probe)/5-Hydroxydecanoate (5-HD = K^+/ATP Channel Blocker)

Cellular/Molecular Mechanism of Action: Dihydroethidium emits fluorescent color when exposed to certain wavelengths of light energy. 5-Hydroxydecanoate inhibits the cellular function of membrane-associated K^+/ATP channels.
Background Information: It is unknown if the opening of mitochondrial adenosine triphosphate-regulated potassium (K^+/ATP) channels is a trigger or an end effector of anesthetic-induced preconditioning.
Application: Assess superoxide anion production.
Implementation: Determination if opening of mitochondrial K^+/ATP channels *in vivo* triggers isoflurane-induced preconditioning by generating reactive oxygen species (ROS). To achieve this purpose, the fluorescent probe dihydroethidium can be used to assess superoxide anion production during the administration of 5-HD or the ROS scavengers N-acetylcysteine or N-2-mercaptopropionyl glycine (2-MPG) in the presence or absence of 1.0 MAC isoflurane. Myocardial infarct size and superoxide anion production can be measured using triphenyltetrazolium staining and confocal fluorescence microscopy, respectively.

Animal Model: Rabbit (pentobarbital anesthetized) subjected to a 30-minute coronary artery occlusion followed by a 3-hour period of reperfusion. Rabbits can be randomly assigned to receive a vehicle (0.9% saline) or the selective mitochondrial K^+/ATP channel blocker, 5-hydroxydecanoate (5-HD) alone 10 minutes before or immediately after a 30-minute exposure to 1.0 minimum alveolar concentration (MAC) isoflurane.
Interpretation and Analysis: Isoflurane ($p < 0.05$) decreases infarct size to 19 ± 3% (mean ± SEM) of the left ventricular area at risk as compared to the control (38 ± 4%). 5-HD administered before but not after isoflurane abolishes this beneficial effect (37 ± 4% as compared to 24 ± 3%). 5-HD alone has no effect on infarct size (42 ± 3%). Isoflurane increases fluorescence intensity. Pretreatment with N-acetylcysteine, 2-MPG, or 5-HD before isoflurane abolishes increases fluorescence, but administration of 5-HD after isoflurane only partially attenuates increases in fluorescence produced by volatile anesthetic agents (e.g., isoflurane). Therefore, mitochondrial K^+/ATP channel opening acts as a trigger for isoflurane-induced preconditioning by generating ROS *in vivo*.
Instrumentation: Confocal fluorescence microscopy.

Mitochondrial adenosine triphosphate-regulated potassium channel opening acts as a trigger for isoflurane-induced preconditioning by generating reactive oxygen species. Tanaka K, Weihrauch D, Ludwig LM, Kersten JR, Pagel PS, Warltier DC. Anesthesiology 2003 Apr; 98 (4), pp. 935–43.

ANGINA: NON-CARDIAC-ASSOCIATED THORACIC PAIN

Angina: Thoracic Pain of Non-Cardiac Origin
Atropine

Cellular/Molecular Mechanism of Action: Atropine blocks muscarinic parasympathetic (cholinergic) receptor complexes.
Physiological Mechanism of Action: Parasympatholytic effect.
Background Information: Intraesophageal balloon distension has been introduced recently as a provocative test for the assessment of non-cardiac chest pain.
Application: Differentiation between angina of cardiovascular origin and non-cardiovascular associated chest pain.
Route of Administration: Atropine (intravenous)
Dosage: Atropine (10 μg/kg)
Implementation: Evaluation of the effect of balloon location and muscarinic blockade on distension-induced pain in, asymptomatic normal controls studied on 2 separate days using a low-compliance perfused manometry system incorporating a silicone rubber balloon. Five-second-duration balloon distensions using balloon volumes of 2.5, 5, 7.5, and 10 ml of air are performed with the balloon located both 16 cm (proximal site) and 6 cm (distal site) above the lower esophageal sphincter (LES) before and after administration of atropine or placebo in a randomized double blind fashion. A standardized scoring system can be used to assess balloon distension-induced pain.

Human Model: Cases classified as asymptomatic male volunteers (e.g. n = 10).
Interpretation and Analysis: Pain scores vary directly with balloon volume but are consistently higher with the balloon located at the proximal site vs the distal site. This is usually not associated with any differences in intraballoon pressures between the 2 sites; however, contraction amplitude orad to the balloon is greater with balloon distension at the proximal site. Atropine significantly decreases pain sensation scores with the balloon located distally but not proximally. Such attenuation is not associated with significant changes in intraballoon pressures; however, contractions orad to the balloon are markedly inhibited by atropine with distal but not with proximal distension. Such findings indicate that balloon distension-induced pain varies depending on the location of distension. This difference can not be explained by differences in esophageal wall tension at the site of distension.

Effect of intraesophageal location and muscarinic blockade on balloon distension-induced chest pain. Paterson WG, Selucky M, Hynna-Liepert TT. Dig Dis Sci 1991 Mar; 36 (3), pp. 282–8.

Angina Pectoris: Thoracic Pain of Non-Cardiac Origin
Edrophonium Chloride

Cellular/Molecular Mechanism of Action: Edrophonium binds to and directly inactivates the biochemical activity of acetylcholine esterase. As a consequence of acetylcholine esterase inhibition, acetylcholine neurotransmitter concentrations accumulate within the synaptic cleft, thereby increasing the (over) stimulation of cholinergic receptor complexes.
Physiological Mechanism of Action: Promotes increases in parasympathetic tone (parasympathomimetic).
Application: Assessment of non-cardiac chest pain (NCCP).
Route of Administration: Intravenous.
Dosage: Alternating doses of edrophonium (80 μg/kg bolus compared to a 10 mg bolus).
Implementation: Distal esophageal pressures can be measured before and after pharmaceutical injection in response to 10 5 cc wet swallows.

Human Model: Study of 150 consecutive NCCP patients and 50 age-matched controls that alternatively receive either 80 μg/kg or 10 mg intravenous bolus doses of edrophonium preceded by saline placebo injections.
Interpretation and Analysis: Side effects are similar between doses, but there is a significant ($p = 0.02$) linear relationship between intensity of side effects and the edrophonium dose per kilogram of body weight. Edrophonium (10 mg) produces chest pain sensations (e.g., 33% of cases) and control subjects (e.g., 4%) while 29% of cases and no controls receiving an 80 μg/kg dose experience chest pain sensations. Amplitude changes after either dose are not significantly different for all comparisons, but the duration of response does distinguish the 2 doses in cases with chest pain. A significantly greater ($p = 0.01$) increase in distal contraction duration occurs after 10 mg (74 ± 12% SE) compared to 80 μg/kg doses (43 ± 6%). However, individual response to the 2 doses overlaps considerably. If a positive test is redefined to include both chest pain and manometric changes that are significantly different from controls, the positivity rate changes drastically: 33% to 9% in the 10 mg group and 30% to 3% in the 80 μg/kg group.

Anticholinesterase Agents. Taylor P (Chapter 6: pp. 113–29). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Edrophonium provocative test in non-cardiac chest pain. Evaluation of testing techniques. Dalton CB, Hewson EG, Castell DO, Richter JE. Dig Dis Sci 1990 Dec; 35 (12), pp. 1445–51.

Angina Pectoris/Thoracic Pain of Non-Cardiac Origin/Esophogeal Motor Activity Differential Diagnosis
Ergonovine Maleate

Physiological Mechanism of Action: Coronary artery spasm can be induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the endothelium is intact by releasing nitric oxide (NO). In conditions of coronary artery spasm, acetylcholine induces a spasmodic response that can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. Such finding imply that some form of abnormally in endothelial integrity exists in conditions of coronary spasm that can be accentuated by acetylcholine and histamine, and to a lesser by ergonovine and serotonin. In general, all ergot alkaloids exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptor complexes. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentrations. Similarly, the contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.
Implementation: Evaluation of angina pectoris–like chest pain cases that have been excluded from having significant coronary heart disease by cardiac catheterization procedures utilizing ergonovine maleate (EM) to determine if esophageal motor activity is the cause of clinical signs.

Human Model: Angina pectoris–like chest pain cases that have been excluded from having significant coronary heart disease by cardiac catheterization procedures (e.g., n = 18),

Interpretation and Analysis: Over half of such cases (e.g., n = 12/18 or 66%) can demonstrate abnormal baseline levels of esophageal motility. Following the injection of EM, a number of cases will experience a typical form of chest pain (e.g., n = 10/18) at the onset of repetitive contractions. Such provocative tests aid in delineating a direct association between chest pain and esophageal dysfunction. Compared with saline injection, only the repetitive contractions are significantly increased after AM in such cases ($p \leq 0.01$). Amplitude and duration of contractions are increased after EM, but not significantly. Due to potentially serious adverse effects, however, EM cannot be recommended as a routine provocative agent for establishing a differential diagnosis of angina pectoris–like chest pain.

Ergonovine-induced esophageal spasm in patients with chest pain resembling angina pectoris. Koch KL, Curry RC, Feldman RL, Pepine CJ, Long A, Mathias JR. Dig Dis Sci 1982 Dec; 27 (12), pp. 1073–80.

Angina Pectoris/Thoracic Pain of Non-Cardiac Origin/Esophagitis/Gastroesophogeal Reflux Disease (GERD)/MIBI Negative NCCP
High-Dose Omeprazole/[^{99m}Tc-SestaMIBI]

Cellular/Molecular Mechanism of Action: Omeprazole inhibits H^+/K^+-ATPase that exchanges hydrogen ion for potassium ion within parietal cell populations. In this fashion, less hydrochloric acid is available for secretion into the gastric lumen. [^{123}I]-meta-iodobenzylguanidine functions as an analog of norepinephrine.
Background Information: Non-cardiac chest pain (NCCP) presents as a frequent diagnostic challenge, with affected cases tending to use a disproportionate level of health care resources. Gastroesophageal reflux disease is the most frequent cause of NCCP.
Application: Detection of NCCP etiologies due to esophagitis and gastric esophageal reflux disease in order to avoid nuclear scintigraphy evaluation of cardiac function/physiology.
Route of Administration: Omeprazole (oral).
Dosage: Omeprazole (40 mg twice daily).
Considerations: Gastroesophageal reflux disease is the most frequent cause of NCCP rest/stress perfusion.
Implementation: Evaluation of recurrent chest pain conditions utilizing a prospective, double blinded, placebo-controlled, crossover trial using high-dose omeprazole. Findings are then compared with those of endoscopy, manometry, and ambulatory 24-hour 2-channel esophageal pH monitoring. Initial diagnostic upper endoscopy, esophageal manometry, and 24-hour pH monitoring can also applied to establish evaluation parameters. Cases are then randomly assigned to either placebo or omeprazole for 14 days, washed out for 21 days, and then crossed over. Clinical symptoms are determined using a Visual Analogue Scale to measure the severity of chest pain before and after each period.

Human Model: Cases (n = 18 men; n = 24 women; age 22–77 years) with recurrent complaints of chest pain of a non-cardiac etiology, as determined by rest/stress perfusion imaging with technetium [^{99m}Tc]-sestaMIBI.
Interpretation and Analysis: Improvement in perceived chest pain can be detected in approximately 71% of cases receiving omeprazole compared to only 18% for placebo reference controls. Abnormal results on manometry (20%), 24-hour pH monitoring (42%), or endoscopy with visual evidence of esophagitis (26%) can be appreciated less frequently. Combination of the 3 tests does not significantly increase their usefulness. In NCCP patients with GERD, as defined by positive results on a 24-hour pH test or presence of esophagitis on endoscopy, omeprazole treatment results in a response in 95% of cases, whereas 90% of GERD-positive patients treated with placebo do not respond. Of NCCP patients determined to be GERD negative, approximately 39% will respond to omeprazole. Therefore, omeprazole as a first diagnostic tool in the evaluation of MIBI-negative NCCP is sensitive and specific for determining the cause of NCCP. Endoscopy, manometry, and 24-hour pH monitoring are not only less sensitive in diagnosing NCCP, but they were significantly more expensive.

Short course of omeprazole: A better first diagnostic approach to non-cardiac chest pain than endoscopy, manometry, or 24-hour esophageal pH monitoring. Pandak WM, Arezo S, Everett S, Jesse R, DeCosta G, Crofts T, Gennings C, Siuta M, Zfass A. J Clin Gastroenterol 2002 Oct; 35 (4), pp. 307–14.

Angina Pectoris/Thoracic Pain of Non-Cardiac Origin/Gastric Reflux -vs- Cardiac-Associated Pain
Lansoprazole

Physiological Mechanism of Action: Lansoprazole reduces gastric acid production.
Application: Potential detection of gastric function/acid production as a cause for non-cardiac chest pain. The agent could possibly be used as a screening diagnostic agent prior to performing cardiac angiography and possibly gastric endoscopy studies.
Route of Administration: Lansoprazole (oral).
Dosage: Lansoprazole (30 mg).
Implementation: Evaluation of the influence of lansoprazole on non-cardiac thoracic pain applying 24-hour ambulatory esophageal pH monitoring (daily for 4 weeks duration). Chest pain symptoms are then recorded before and 1 month after treatment validated by the use of a questionnaire. The symptom score is calculated by multiplying the severity and frequency of the symptom and symptom improvement defined as > 50% reduction in the symptom score.

Human Model: Cases suffering from chest pain that have normal angiography imaging profiles and are without gastric or esophageal lesions (n = 68; n = 36 lansoprazole, n = 32 placebo).
Interpretation and Analysis: Symptom scores are reduced significantly in both groups ($p < 0.001$). In cases receiving lansoprazole, more individuals with vs without abnormal reflux show symptomatic improvement (92 vs 33%; odds ratio = 22; 95% confidence interval, 2.3–201.8; $chi^2 = 10.9$; $p = 0.001$), giving a sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of 92%, 67%, 58%, 94%, and 75%, respectively. Alternatively, cases receiving a placebo display rates of symptom improvement that are similar between those with and without abnormal reflux (33 vs 35%, p = ns). Treatment with lansoprazole is a useful test in diagnosing endoscopy-negative gastroesophageal reflux disease in cases with non-cardiac chest pain.

Symptomatic response to lansoprazole predicts abnormal acid reflux in endoscopy-negative patients with non-cardiac chest pain. Xia HH, Lai KC, Lam SK, Hu WH, Wong NY, Hui WM, Lau CP, Chen WH, Chan CK, Wong WM, Wong BC. Aliment Pharmacol Ther 2003 Feb; 17 (3), pp. 369–77.

CARDIAC DISEASE WITHOUT ANGINA

Aortic Valve/Left Ventricular Systolic Dysfunction and Aortic Stenosis/Low Output and Low Mean Gradient/Surgical Candidate Identification
Dobutamine

Background Information: Although aortic valve replacement can be performed at an acceptable risk level in selected cases with left ventricular systolic dysfunction and low-output low-gradient

aortic stenosis, not all cases presenting with these hemodynamics will benefit from surgical intervention. Some cases may have only mild aortic stenosis, despite a small calculated valve area. Some reports have described the clinical utility of diagnostic dobutamine stimulation during cardiac catheterization in these diagnostically challenging conditions.

Application: In cases with left ventricular systolic dysfunction and aortic stenosis with a low output and a low mean gradient, dobutamine challenge may aid in selecting those who would benefit from an aortic valve operation.

Route of Administration: Intravascular.

Implementation: Evaluation of the influence of dobutamine infusion in cases of low-output, low-gradient aortic stenosis

Human Model: Cases of low-output, low-gradient aortic stenosis and an ejection fraction $< 40\%$ ($n = 32$).

Interpretation and Analysis: On the basis of the results of the dobutamine test, approximately 66% of cases may meet the criteria necessary to justify performing aortic valve replacement (e.g. $n = 21/32$). A high percentage of cases with a final aortic valve area ≤ 1.2 cm^2 at peak dobutamine infusion and a mean gradient of > 30 mm Hg have severe calcific aortic stenosis that can be appreciated the time of surgical intervention (e.g., 100% in 1 study). In cases with contractile reserve identified during dobutamine challenge based on observed increases in stroke volume ($> 20\%$), complications that can occur include perioperative death ($n = 1/15$ or 7%); but the majority are still alive ($n = 12/15$ New York Heart Association class I or II status) at the time of case follow-up.

Low-output, low-gradient aortic stenosis in patients with depressed left ventricular systolic function: The clinical utility of the dobutamine challenge in the catheterization laboratory. Nishimura RA, Grantham JA, Connolly HM, Schaff HV, Higano ST, Holmes DR Jr. Circulation 2002 Aug 13; 106 (7), pp. 809–13.

Aortic Valve/Subaortic Stenosis: Aortic Valve/ Idiopathic Hypertrophic
Phentolamine

Cellular/Molecular Mechanism of Action: Competitive α-adrenergic blockade (transient) and vasodilation is due to a direct action on vascular smooth muscle.

Physiological Mechanism of Action: Inhibition of responses to 5-HT, gastrointestinal tract stimulation (blocked by atropine), vasodilation, decreased blood pressure, and cardiac stimulation.

Considerations: The clinical use of phentolamine has increased since its introduction as an antihypertensive agent. The vasodilatory action of phentolamine, as well as its positive inotropic effect has led to its use in the treatment of congestive heart failure. Recently, phentolamine has been used by several clinical groups to improve left ventricular function in acute myocardial infarction. There appears to be great promise for the use of phentolamine in such clinical settings. The drug given intravenously or orally can suppress ventricular premature beats and supraventricular premature beats. However, experience with phentolamine as an antiarrhythmic agent is still limited. Similarly, the relief of angina pectoris by phentolamine requires confirmation by additional clinical studies.

Interpretation and Analysis: Phentolamine can be used as a provocative test for characterizing idiopathic hypertrophic subaortic stenosis. Since it does not produce cardiac arrhythmias, it may be safer than isoproterenol for such purposes. The comparative (relative) effectiveness of phentolamine and isoproterenol in diagnosing idiopathic hypertrophic subaortic stenosis is unknown. Phentolamine has been advocated for several years as an effective agent for the treatment of shock. The experience is still limited to a few groups that have reported favorable results. Phentolamine has also been used as a bronchodilator and a pulmonary artery dilator. Preliminary results suggest that phenotolamine is effective for this purpose but further investigation is necessary to validate these early impressions. A sensitive measurement of the blood levels of phentolamine is not available. When this is accomplished, further insight into the metabolism of this drug will be forthcoming.

Phentolamine. Gould L, Reddy CV. Am Heart J 1976 Sep; 92 (3), pp. 397–402.

Left Ventricular Aortic Outflow Obstruction
Phentolamine/(Isoproterenol Inhalation Alternative)/(Amyl Nitrite Alternative)/Valsalva's Maneuver Alternative

Cellular/Molecular Mechanism of Action: Phentolamine transiently binds directly to α-adrenergic receptor complexes in a manner that competitively inhibits the biological activity of adrenergic neurotransmitter agents. Interestingly, the biological activity of 5-HT is also inhibited, and its vasodilatory effect is due to a direct effect of phentolamine on vascular smooth muscle.

Physiological Mechanism of Action: Cardiac and smooth muscle structures are the most prominent tissues that have their function altered by phentolamine. Effects include or result in cardiac stimulation, increased gastrointestinal motility and secretion, and peripheral vascular vasodilation accompanied by declines in blood pressure. In effect, decreases in peripheral/pulmonary vascular resistance and increases in venous compacitance are produced. The influence of phentolamine on peripheral vascular resistance is not solely responsible for its effect on cardiac function.

Application: Enhances myocardial contractility and decreased afterload with induction of only slight tachycardia. Beneficial in the diagnosis of cases suffering from idiopathic hypertrophic subaortic stenosis and valvular dysfunction.

Route of Administration: Phentolamine (intravenous).

Dosage: Phentolamine (5 mg).

Considerations: Intravenous injection of phentolamine potentially offers a better provocative test for aortic left ventricular outflow tract obstruction than does the Valsalva's maneuver, or the inhalation of either isoproterenol or amyl nitrite.

Interpretation and Analysis: Applying echocardiographic and phonocardiographic recordings, systolic pressures can fall an average of 20 mm Hg while remaining above 90 mm Hg and accompanied by no notable increases in heart rate. Phentolamine appears to be a safe, simple, and specific diagnostic agent for eliciting dynamic obstruction in IHSS.

Basis for Characterizing Alternative: In typical idiopathic hypertrophic subaortic stenosis, amyl nitrite increases the obstructive index (39.6 ± 12 to 51 ± 18.9: $p < 0.05$) compared to phentolamine (increased obstructive index 69.8 ± 25.6: $p < 0.015$). After a 2-week course of oral administration of 80 mg of propranolol daily, and then either inhalation of amyl nitrite or injection of phentolamine, there is usually no change from the mean resting obstructive index. Phentolamine is more potent than amyl nitrite in eliciting dynamic obstruction in IHSS; phentolamine and amyl nitrite do not affect the obstructive index in conditions with beta blockade.

Toxicity: Creation of cardiac arrythmias (tachycardias), hyperperistaltic-induced diarrhea with abdominal discomfort.

Evaluation of phentolamine as a provocative test for idiopathic hypertrophic subaortic stenosis. Kerin NZ, Mori I, Edelstein J, Blonder R, Rubenfire M. Am Heart J 1979 Feb; 97 (2), pp. 204–10.

Phentolamine. Gould L, Reddy CV. Am Heart J 1976 Sep; 92 (3), pp. 397–402.

Left Ventricular Hypertrophy (LVH)/Functional Response: Dynamic
Acetylcholine/Adenosine/Sodium Nitroprusside

Physiological Mechanism of Action: Acetylcholine (parasympathetic cholinergic agonist); adenosine (negative chronotropic response); sodium nitroprusside (lowers systemic blood pressure).
Application: Dectection and characterization of alterations in cardiovascular function.
Implementation: Evaluation of changes in coronary morphology and hemodynamic function during Q regression in established conditions of LVH following surgical unloading. Alterations delineated can include degree of LVH, coronary hemodynamic function, and contemporaneous vessel morphology 42 days postoperation. Parameter can then be reassessed in an identically treated *in vivo* models debanded and the same parameters measured after 1, 3, and 6 weeks to assess hemodynamic and morphological changes that occur with hypertrophy regression.

Animal Model: Guinea pig (induced LVH created by aortic banding while sham operated animals serve as controls).
Interpretation and Analysis: Banding results in an aortic pressure gradient of 41 $\pm$ 9 mm Hg and increases in heart/body weight ratio (46%), myocyte size (26%), and a doubling of arteriolar wall thickness (e.g. all at $p < 0.01$). These changes are accompanied by a reduction in coronary reserve (38%) and significant ($p < 0.01$) decreases in maximal response to acetylcholine (70%), sodium nitroprusside (87%), adenosine (70%), and reactive hyperemia (52%). Surgical debanding normalizes the systemic hemodynamics and removes the aortic gradient after 7 days. There is some limited improvement in coronary structure and, to a lesser extent, function despite continued presence of significant LVH. Such developments completely regress to normal levels 23 days after debanding and are accompanied by a normalization of coronary structure and function, although systolic impedance to flow remains significantly increased. After 44 days, debanding results in complete cardiac morphological and functional recovery. Left ventricular hemodynamic unloading can result in complete normalization of LVH, coronary morphology, and hemodynamic function. Although morphological and functional recovery is closely correlated, recovery of coronary morphology and function slightly precedes that of the myocardium in this aortic banded/debanded model.

Recovery of coronary function and morphology during regression of left ventricular hypertrophy. Kingsbury M, Mahnke A, Turner M, Sheridan D. Cardiovasc Res 2002 Jul; 55 (1), pp. 83–96.

Murmurs: Cardiac Murmurs
Isobutyl Nitrite (ISBN)/(Analytical Assay)

Application: Delineation of cardiac murmurs and for the therapeutic management of angina pectoris.
Considerations: Compounds are highly volatile vasodilators and sometimes abused as inhalant agents.
Interpretation and Analysis: A mean recovery of ISBN from the blood is approximately 86% over a concentration range of 1.0 to 400 ng/ml (rat). The interday coefficient of variation is below 15% at the lowest quantifiable blood concentration of 1 ng/ml ISBN (rat). Applying the newly developed gas chromatography assay, new pharmacokinetic information about ISBN has been acquired. Results discovered with the new analysis method have shown that rats inhaling 900 ppm ISBN for 45 minutes produce steady-state blood concentrations of about 290 ng/ml and a rapid elimination half-life of 1.4 minutes.
Instrumentation: A gas chromatographic assay has been developed using electron capture detection for the analysis of the representative nitrite inhalant, ISBN, in rat and human whole blood. Unconventional sampling and processing techniques are required because of the high volatility and chemical instability of nitrites in biological fluids.

Analysis of isobutyl nitrite inhalant in rat and human blood: Application for pharmacokinetic investigations. Kielbasa WB, Bauer JA, Fung HL. J Chromatogr B Biomed Sci Appl 1999 Oct 29; 734 (1), pp. 83–9.

Syncope Differentiation Diagnosis
ATP/Isoproterenol (Tilt Test)

Cellular/Molecular Mechanism of Action: Isoproterenol is a non-selective β-adrenergic receptor agonist.
Background Information: In patients with vasovagal syndrome, head-up tilt testing may reproduce symptoms generally associated with vasodepression. Recent research suggests ATP testing can identify cases with abnormal vagal cardiac inhibition.
Application: The tilt and ATP tests individually and jointly can be utilized to determine the underlying mechanisms of vasovagal symptoms in most cases of syncope.
Route of Administration: ATP (20 mg).
Dosage: ATP (intravenous).
Implementation: Evaluation of isoproterenol and ATP provocative tests performed in random order during 1 session and outside of predominant sympathetic periods in cases related to syncope. In passive and isoproterenol-provocative tilt testing by standard protocol, reproduction of symptoms is defined as a positive test. The ATP test consists of administering injectable ATP (at bedside) while continuously monitoring ECG and blood pressure with a vagal cardiac pause > 10 seconds defined a positive test result. For most cases (64%), a ≥ 1 test will be positive.

Human Model: Cases suffering from syncope ($n = 56$); cases of presyncope ($n = 56$) for whom no cardiac or extracardiac etiologies can be identified.
Interpretation and Analysis: In 57% of cases (e.g., $n = 41/72$) with a positive tilt test (either passive or provoked by isoproterenol), approximately 32% have cardiac disease, while a very low percentage have significant bradycardia (e.g., $n = 0$; < 50 bpm). In cases with positive ATP test findings (e.g., $n = 8$ or 11%), approximately two-thirds or 62% have cardiac disease. The probability of a positive result increases with age ($p = 0.015$). Both tests are positive in a small number of cases (e.g., $n = 3$) while a relatively larger number have a negative responses to both evaluation procedures (e.g., $n = 26$). Results from tilt and ATP test procedures appear to be uncorrelated ($p = 0.28$). Therefore, tilt and ATP tests individually and jointly can assist in determining the mechanism of vasovagal symptoms in most cases of syncope. Vagal cardiac inhibition appears to increase with age.

Contribution of head-up tilt testing and ATP testing in assessing the mechanisms of vasovagal syndrome: Preliminary results and potential therapeutic implications. Flammang D, Erickson M, McCarville S, Church T, Hamani D, Donal E. Circulation 1999 May 11; 99 (18), pp. 2427–33.

Syncope/Hypoxia (Sudden)/Bradycardia (Sudden)/ Vasovagal Syncope (Unexplained)/ [Vascular Graft Patency] *Nitroglycerin/Isoproterenol*

Cellular/Molecular Mechanism of Action: Isoproterenol recognizes, physically binds to, and activates sympathetic/adrenergic receptor complexes.

Physiological Mechanism of Action: Nitroglycerin induces both venous and arterial vasodilation. Relatively selective venous vasodilation can be achieved at the lower dosage ranges of nitroglycerin, thereby promoting declines in end-diastolic left and right ventricular pressures. Such changes are more pronounced than are changes in peripheral arterial pressure. Cardiac output is decreased in normal subjects and patients with coronary artery disease when nitroglycerin is given by the sublingual route of administration. High-dose nitroglycerin administered rapidly causes declines in both systolic and diastolic pressures accompanied by lower blood pressure levels, pallor, weakness, dizziness, and initiation of reflex sympathetic-mediated responses (tachycardia, arteriolar vasoconstriction). Transient increases in coronary blood flow (coronary vasodilation) and declines in arterial blood pressure contribute to decreases in cardiac output. Sublingual administration can therefore occasionally produce a significant hypotensive episode.

Application: Diagnosis of vasovagal syncope and can be utilized in combination with quantitative angiography for the evaluation of vascular blood flow integrity (vasodilation vs vasoconstriction) and patency of vascular grafts. Injectable nitroglycerin (TNG) is employed during cardiac catheterizations primarily in cases undergoing provocative tests for coronary spasm with ergonovine maleate where it has demonstrated considerable efficacy.

Route of Administration: Nitroglycerin (intravascular).

Dosage: Nitroglycerin given at progressively increasing infusion rates (increments of 0.86 μg/kg/hr at each stage).

Implementation: Evaluation of unexplained syncope conditions through analysis of provoked changes in coronary artery vascular blood flow. A maximum of 5 successive stages consisting of 5 minutes in the supine position are performed plus 10 minutes of 80-degree upright tilt at progressively increasing infusion rates (increments of 0.86 μg/kg/hr every stage).

Human Model: Cases of unexplained syncope (n = 40; n = 23/40 women, mean age 47 years; n = 25 asymptomatic controls with negative baseline head-up tilt undergoing 2 other tilting tests, 1 during nitroglycerin infusion and 1 during isoproterenol infusion).

Interpretation and Analysis: During the nitroglycerin test a positive response (syncope in association with sudden hypotension and bradycardia) can occur in approximately 53% of cases (e.g., n = 21/40) with unexplained syncope; an exaggerated response (minor symptoms in association with slowly increasing hypotension alone) occurs in about 25% (e.g., n = 10/40); a negative response in 22% of cases (e.g., n = 9/40); and drug intolerance in a very small percentage (e.g., n = 0 or 0%). During the isoproterenol test these percentages are 25%, 25%, 32%, and 18%, respectively. Only a small percentage of controls have a positive response to the nitroglycerin test and to the isoproterenol test (e.g., n = 2 or 8% for each test). Thus the nitroglycerin test seems to be a useful alternative tool for diagnosis of vasovagal syncope; it is equally specific but more sensitive and feasible than the isoproterenol test.

Nitroglycerin infusion during upright tilt: a new test for the diagnosis of vasovagal syncope. Raviele A; Gasparini G; Di Pede F; Menozzi C; Brignole M; Dinelli M; Alboni P; Piccolo E. Am Heart J] 1994 Jan; Vol. 127 (1), pp. 103–11.

Syncope: Neurocardiogenic *Isosorbide Dinitrate/Isoproterenol*

Cellular/Molecular Mechanism of Action: Isosorbide dinitrate (active metabolite: isosorbide-2-mononitrate; isosorbide-5-mononitrate) is believed to activate guanylate cyclase and increase the synthesis of guanosine 3′,5′ monophophate (cGMP) in smooth muscle and other tissues. Formation of reactive free radical nitric oxide (NO) occurs, which interacts with and activates guanylate cyclase. Isoproterenol binds to and potently activates β-adrenergic receptor complexes (β-adrenergic receptor agonist).

Physiological Mechanism of Action: Isosorbide dinitrate is an arterial and venous dilating agent. The (sympathomimetic) effect of isoproterenol is mainly directed at the heart and smooth muscle tissues of the bronchioles, skeletal muscle vasculature, and alimentary tract. Physiologically isoproterenol promotes declines in peripheral vascular resistance primarily at the level of the skeletal muscle vasculature.

Application: Isosorbide dinitrate and isoproterenol can be applied to identify and characterize cases of suspected cardiogenic syncope.

Route of Administration: Isosorbide dinitrate (sublingual), isoproterenol (intravenous).

Dosage: Isosorbide dinitrate (5 mg), isoproterenol (4 μg/min).

Implementation: In cases of suspected neurocardiogenic syncope (e.g., n = 120; 15–77 years), the response to a head-up tilt test (HUT) can be divided into subjects that either do or do not develop syncope/presyncope during a passive HUT test for 30 minutes. Cases that do not develop symptoms are subjected to repeated HUT tests with isoproterenol influsions (4 μg/min; 10 minutes) and then tilted after a sublingual administration of isosorbide dinitrate (another 12 minutes). The HUT/isosorbide dinitrate test is usually performed after the HUT/isoproterenol test.

Interpretation and Analysis: Sensitivity and specificity of passive HUT is 41% (95% C.I. 32.9% to 51%) and 100%, respectively. Sensitivity of isoproterenol HUT is 51.4% (95% C.I. 39.2% to 63.6%) and specificity 70% (95% C.I. 55.4% to 82.1%), and for isosorbide dinitrate HUT is 70% (95% C.I. 57.9% to 80.4%) and 88% (95% C.I. 75.7% to 95.5%), respectively. The accuracy of the isosorbide HUT is significantly higher than the accuracy of the isoproterenol HUT 77.5% (95% C.I. 68.9% to 84.6%). Sublingual isosorbide dinitrate is at least as sensitive as isoproterenol for the assessment of suspected cases of neurocardiogenic syncope that display a negative tilt test without provocation. The low rate of side effects and the higher accuracy of the idosrobide dinitrate HUT, along with the simplicity of this challenge compared to the isoproterenol HUT test, suggest that sublingual isosorbide dinitrate should be preferred as a provocative agent to evaluate neurocardiogenic syncope after a negative passive tilt test.

Tilt testing in neurocardiogenic syncope: Isosorbide vs isoproterenol. Hermosillo AG, Marquez MF, Jauregui-Renaud K, Falcon JC, Casanova JM, Guevara M, Cardenas M. Acta Cardiol 2000 Dec; 55 (6), pp. 351–5.

Syncope: Neurocardiogenic *Glyceryl Trinitrate (Nitroglycerin)/Isoprenaline (Isoproterenol)*

Cellular/Molecular Mechanism of Action: Glyceryl trinitrate (nitroglycerin) activates guanylate cyclase and increases the synthesis of guanosine 3′,5′ monophophate (cGMP) in smooth muscle and other tissues. Formation of reactive free radical nitric oxide (NO) occurs, which interacts with and activates guanylate cyclase.

Isoprenaline (isoproterenol) binds to and potently activates β-adrenergic receptor complexes (β-adrenergic receptor agonist).

Physiological Mechanism of Action: Glyceryl trinitrate (nitroglyerin) is an arterial and venous dilating agent. Isoprenaline (isoproterenol: sympathomimetic) exerts effects mainly directed at the heart and smooth muscle tissues of the bronchioles, skeletal muscle vasculature, and alimentary tract. Physiologically isoprenaline (isoproterenol) promotes declines in peripheral vascular resistance primarily at the level of the skeletal muscle vasculature.

Application: Glyceryl trinitrate (nitroglycerin) and isoprenaline (isoproterenol) can be used to detect and characterize conditions of neurocardiogenic syncope.

Implementation: In unexplained cases of syncope with a negative passive head-up tilt at 70 degrees (40 minutes), the glyceryl trinitrate head-up tilt and isoprenaline head-up tilt tests can be of diagnostic value (performed 1 week apart). Diagnostic criteria include induced symptoms (syncope, presyncope) in concert with the development of hypotension.

Interpretation and Analysis: In cases with negative passive head-up tilt, the sensitivity of glyceryl trinitrate in diagnosing vasovagal syncope is approximately 48% with a 71% level of specificity. Isoprenaline sensitivity is 21% with a 64% level of specificity. There is a rather drastic difference in the incidence of undesirable side effects observed with glyceryl trinitrate compared to isoprenaline. Glyceryl trinitrate is well tolerated. In contrast, a large percentage of cases (68%) display side effects with isoprenaline that are severe enough to require termination of testing procedures. The most common adverse effects are the development of hypertension or tachycardia and intolerable "flushing" or nausea. Based on comparison evaluations, glyceryl trinitrate (nitroglycerin) head-up tilt is as effective as isoprenaline (isoproterenol) head-up tilt as a provocative agent for vasovagal syncope and has a lower incidence of adverse events.

Comparison of provocative tests for unexplained syncope: Isoprenaline and glyceryl trinitrate for diagnosing vasovagal syncope. Graham LA, Gray JC, Kenny RA. Eur Heart J 2001 Mar; 22 (6), pp. 497–503.

Syncope: Vasovagal
Edrophonium

Cellular/Molecular Mechanism of Action: Edrophonium functions as an inhibitor of acetylcholinesterase biochemical activity.

Physiological Mechanism of Action: Edrophonium through the inhibition of acetycholine esterase promotes accumulation of acetylecholine neurotransmitter at cholinergic receptor complexes associated with postsynaptic membranes.

Background Information: Vasovagal syncope after head-up tilting is thought to be secondary to a complex, neurally-mediated reflex with both vasodepressor and cardioinhibitory efferent components.

Application: Edrophonium has been utilized as a provocative agent for triggering syncope during head-up tilt testing protocols of benefit in detecting cases of vasovagal mediated syncope. The edrophonium/head-up tilt test (i) is currently considered to significantly increase the sensitivity of detecting cases of vasovagal syncope; (ii) may be particularly useful when provocation with isoproterenol is undesirable; and (iii) may be an effective method to help differentiate cases with a significant reflex cardioinhibitory component from those with a predominantly reflex vasodepressor component.

Route of Administration: Edrophonium (intravenous).

Dosage: Edrophonium (10 mg).

Implementation: Evaluation of the efficacy of edrophonium as a provocative diagnostic agent for the purpose of "triggering" syncope during head-up tilt testing procedures. In cases suffering from recurrent unexplained syncope, edrophonium is administered after 30 minutes of 60 degrees head-up tilting alone.

Human Model: Cases suffering from recurrent unexplained syncope (e.g., n = 45 affected and n = 20 negative reference controls).

Interpretation and Analysis: Syncope can be induced in a little less than 50% of cases (e.g., n = 19/45), with some cases (e.g. n = 9/19) observed to develope syncope with head-up tilt with the predominant hemodynamic alteration recognized during such episodes being marked vasodepression. Conversely, other cases will develop syncope only after head-up tilting in combination with edrophonium (e.g., n = 10/19), with the predominant hemodynamic findings being marked vasodepression and bradycardia. Syncope can occasionally be induced in normal reference controls (e.g., n = 1/20) following head-up tilting and edrophonium. There are no long-term complications associated with edrophonium in diagnostic procedures.

Evaluation of edrophonium as a provocative agent for vasovagal syncope during head-up tilt-table testing. Lurie KG, Dutton J, Mangat R, Newman D, Eisenberg S, Scheinman M. Am J Cardiol 1993 Dec 1; 72 (17), pp. 1286–90.

Syncope: Vasovagal
Nitroglycerin/[Tilt-Table Test]

Cellular/Molecular Mechanism of Action: Nitroglycerin has a direct action on vascular smooth muscle and its effects are not directly mediated through modifications of the autonomic nervous system.

Physiological Mechanism of Action: Induces both venous and arterial vasodilation. Relatively selective venous vasodilation can be achieved at the lower dosage ranges of nitroglycerin, thereby promoting declines in end-diastolic left and right ventricular pressures. Such changes are more pronounced than are the changes in peripheral arterial pressure. Cardiac output is decreased in normal subjects and patients with coronary artery disease when nitroglycerin is given by the sublingual route of administration. High-dose nitroglycerin administered rapidly causes declines in both systolic and diastolic pressures accompanied by lower blood pressure levels, pallor, weakness, dizziness, and initiation of reflex sympathetic-mediated responses (tachycardia, arteriolar vasoconstriction). Transient increases in coronary blood flow (coronary vasodilation) and declines in arterial blood pressure contribute to decreases in cardiac output. Sublingual administration can therefore occasionally produce significant episodes of hypotension.

Background Information: Vasovagal syncope can occur in any individual, given sufficient provocation, and probably half the population suffers at least 1 episode during life. Often it occurs in youth and may occur in clusters. Usually there is a history of a previous episode. Prodromal symptoms include nausea, sweatiness, and a sensation of warmth.

Diagnostic Application: Patients suffering from syncope of unknown origin that may be due to vasovagal syncope.

Considerations: A classical history and a positive tilt test obviate the need for further investigation in clinical practice, but in the context of aviation (pilots), it is wise to determine if there is a small possibility of intermittent rhythm and/or conduction disturbance as an alternative explanation for the episode.

Implementation: Diagnostic detection of vasovagal syncope can be achieved by obtaining a careful history and performing a tilt test in concert with nitroglycerin administration. In instances where intermittent rhythm and/or conduction disturbance are thought to be important as an alternative explanation for episodes, it then becomes reasonable to carry out a Holter recording and exercise electrocardiogram, perhaps also echocardiography.

Interpretation and Analysis: Patients suffering from syncope of unknown origin display a 53% rate of positive response (hypotension and bradycardia) when subjected to a tilt-table test and nitroglycerin administration. An exaggerated response (hypotension only) is observed in 20% of cases presented for diagnosis (suffering from vasovagal syncope). Other reports have described a 1.3% false positive rate for passive tilt test, and the true positive rate being about 70% when a nitroglycerin challenge is included. No treatment is of much benefit, although many agents, including beta-blocking drugs, have been used. Some cases have undergone permanent dual chamber pacing with some favorable results. Explanation and reassurance is important. From a licensing point of view (e.g. pilots), following investigation after an attack, consideration may be given to providing restricted certification with regular follow-up examinations. Case review with diagnostic investigations after an event-free interval, (arbitrarily set for 2 years), may permit full certification. Malignant vasovagal syncope with no warning of impending attack should warrant disbarment.

Drugs Used for the Treatment of Angina: Organic Nitrates, Calcium Channel Blockers, and β-Adrenergic Antagonists. Needleman P, Corr PB, Johnson EM (Chapter 33: pp. 806–26). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Vasovagal syncope: Prevalence and presentation. An algorithm of management in the aviation environment. Sutton R. Eur Heart J Suppl 1999 Apr; 1 Suppl D, pp. D109–13.

Syncope of Unknown Origin
Isoproterenol/[Tilt-Table Test]

Cellular/Molecular Mechanism of Action: Isoproterenol binds to and positively activates both β_1 and β_2 adrenergic membrane-associated receptor complexes.

Due to a relatively minimal influence on α-adrenergic receptor complexes, cardiac function, bronchial smooth muscle, skeletal muscle vasculature, and the gastrointestinal tract are the primary organ systems influenced by the physiological properties exerted by isoproterenol administration. Cardiovascular influences of isoproterenol include diminished peripheral vascular resistance, especially within the vascular networks that perfuse musculoskeletal structures. The increases in heart rate and contractility induced by isoproterenol promote declines in diastolic pressure and elevations in cardiac output sufficient to maintain or elevate systolic pressures (mean blood pressure is lowered).

Background Information: Isoproterenol is widely used as a provocative medium for vasovagal responses during tilt test procedures.

Diagnostic Application: Multi-state isoproterenol tilt-table (MSITT) test.

Route of Administration: Intravenous.

Dosage: Isoproterenol given as constant infusion (3 μg/min). Dose of isoproterenol infusion is generally titrated empirically based on increases in resting heart rate before tilt up test. Administration can involved the use of a 2-stage isoproterenol-tilt protocol, with doses of 0.01 and 0.02 μg/kg per minute for 10 minutes each, or 1-stage isoproterenol-tilt protocol, with a dose of 1 or 2 μg/min for 10 minutes.

Implementation: Evaluation of the optimal increase in resting heart rate with isoproterenol for tilt-induced vasovagal responses. After the end of a negative baseline tilt (80 degrees for 30 minutes), the isoproterenol tilt is performed using 1 of 2 protocols: 2-stage isoproterenol-tilt protocol, or 1-stage isoproterenol-tilt protocol. Resting heart rate increases are defined as a percentage increase in the resting heart rate after isoproterenol infusion, compared to baseline heart rates before initiation of the tilt test.

Human Model: Cases of unexplained syncope (n = 97 consecutive patients).

Interpretation and Analysis: Combining the use of a tilt-table and isoproterenol diagnostic challenge increases the sensitivity of detecting cases suffering from vasovagal mediated syncope (95% by 5 minutes). Conversely, the application of the MSITT alone without the use of isoproterenol results in an 80% positive response rate. Cases that are suffering from bradycardia show a lower rate of positive response rate compared to the MSITT test (10%).

Ninety-three percent (93%) of positive tilt-table procedures (e.g., n = 28/30) occur with a resting heart rate increases of ≥ 21%. With a resting heart rate increase of 60% and 100%, 18 (60%) and 27 (90%) positive responses can be observed, respectively. In conclusion, a minimum resting heart rate increase of ≥ 21% is required to provoke a vasovagal response during a subsequent isoproterenol tilt (80 degrees for 10 minutes). Preferably, heart rate should be increased to 60–100% by isoproterenol titration before tilting.

Norepinephrine, Epinephrine, and the Sympathomimetic Amines. Weiner N (Chapter 8: pp. 145–80). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

What is the optimal increase in resting heart rate with low-dose isoproterenol infusion for tilt-induced vasovagal response? Sumiyoshi M, Abe H, Mineda Y, Tokano T, Yasuda M, Nakazato K, Nakazato Y, Nakata Y, Daida H. J Cardiovasc Pharmacol 2003 Dec; 42 Suppl 1, pp. S19–22.

CARDIOPULMONARY PHYSIOLOGY

Cardiac Filling and Output/Intravascular Dynamics/ Cardiography
Lithium Dilution Cardiac Output (LiDCO)

Cellular/Molecular Mechanism of Action: Lithium ion can be applied as a passive elemental tracer/indicator agent.

Background Information: Lithium dilution cardiac output has primarily been developed as a simple calibration technique for establishing continuous arterial waveform analysis (PulseCO trademark, LiDCO, London, UK).

Application: Minimally invasive passive indicator dilution analysis technique for the measurement of cardiac output.

Route of Administration: Lithium chloride (intravenous).

Dosage: Lithium chloride.

Implementation: Evaluation of cardiac output employing a small dose of lithium chloride injected as an intravenous bolus. Cardiac output is derived from the dilution curve generated by the measurement of lithium using a lithium-sensitive electrode attached to an arterial line.

Interpretation and Analysis: The technique is quick, simple, and requires only an arterial line and central or peripheral venous access. Studies in humans and animals have shown good agreement compared with results obtained with other techniques, and the efficacy in pediatric cases has also been established. Compared with thermodilution, lithium dilution shows closer agreement in clinical studies with electromagnetic flow measurement. The PulseCO trademark is a beat-to-beat cardiac output monitor that calculates stroke volume from the arterial pressure waveform using an autocorrelation algorithm. The algorithm is not dependent on waveform morphology; rather, it calculates nominal stroke volume from a pressure-volume transform of the entire waveform. The nominal stroke volume is converted to actual stroke volume by calibration of the algorithm with lithium tracer agents. Initial studies indicate good fidelity, and results

from centers in the United States and the United Kingdom have validated the effectiveness of the methodology. The PulseCO trademark monitor incorporates software for interpretation of the hemodynamic data generated and provides real-time analysis of arterial pressure variations (i.e., stroke volume variation, pulse pressure variation, and systolic pressure variation) as theoretical guides to intravascular and cardiac filling.

Instrumentation: Lithium-sensitive electrode attached to the arterial line.

Lithium dilution measurement of cardiac output and arterial pulse waveform analysis: An indicator dilution calibrated beat-by-beat system for continuous estimation of cardiac output. Jonas MM, Tanser SJ. Curr Opin Crit Care 2002 Jun; 8 (3), pp. 257–61.

Cardiopulmonary Function/Influence of Altitude
Propanolol/Glycopyrolate

Physiological Mechanism of Action: Propanolol functions as a β-adrenergic antagonist (sympatholytic agent), while glycopyrrolate functions as a muscarinic/parasympatholytic agent.

Background Information: After acclimatization to high altitude, maximal exercise cardiac output (Q-T) is reduced. Possible contributing factors include (i) blood volume depletion, (ii) increased blood viscosity, (iii) myocardial hypoxia, (iv) altered autonomic nervous system (ANS) function affecting maximal heart rate (HR), and (v) reduced flow demand from reduced muscle work capability.

Application: Potential agents for characterizing cardiopulmonary parameters associated with alterations in elevation/altitude and physical conditioning.

Route of Administration: Propranolol (intravenous), glycopyrrolate (intravenous).

Dosage: Propanolol (8.0 mg), glycopyrrolate (0.8 mg).

Implementation: Evaluation of the role of ANS-mediated reductions of HR in have in the phenomenon of altitude acclamation. One approach to addressing this objective can entail separate pharmacological "blocking" of the sympathetic and parasympathetic arms of the ANS during maximal exercise following a 2-week period of acclimatization at 3,800 m in concert with maximal HR measurment. Examples of pharmaceutical agents that can be employed for such purposes include propranolol and glycopyrrolate.

Human Model: Normal healthy subjects (n = 5)

Interpretation and Analysis: At altitude, peak HR is approximately 170 ± 6 beats/min and is reduced from 186 ± 3 beats/min ($p = 0.012$) at sea level. Propranolol further reduces peak HR to 139 ± 2 beats/min ($p = 0.001$), whereas glycopyrrolate increases peak HR to sea level values, 184 ± 3 beats/min, confirming adequate dosing with each drug. Conversely, peak O_2 consumption, work rate, and Q-T are similar at altitude under all drug treatments (peak Q-T = 16.2 ± 1.2 [control], 15.5 ± 1.3 [propranolol], and 16.2 ± 1.1 l/min [glycopyrrolate]). All Q-T results at altitude are lower than those at sea level (20.0 ± 1.8 l/min in air). Such findings suggest that, whereas the ANS may affect HR at altitude, peak Q-T is unaffected by ANS blockade. The effect of altered ANS function on HR is not the cause of the reduced maximal Q-T at altitude.

Role of the autonomic nervous system in the reduced maximal cardiac output at altitude. Bogaard HJ, Hopkins SR, Yamaya Y, Niizeki K, Ziegler MG, Wagner PD. J Appl Physiol 2002 Jul; 93 (1), pp. 271–9.

Left Ventricular Volume
Saline/Trans-Cardiac Conductance (TCC)

Background Information: The TCC method provides on-line left ventricular (LV) volume signals by determining the electrical conductance of blood in the LV by means of central venous and epithoracic electrodes. Conductive structures outside the LV blood pool cause a "parallel conductance" offset term (Vp) that can be determined by bolus injections of hypertonic saline in the pulmonary artery (Vp_{saline}), which cause a transient increase in blood conductivity.

Application: Measurement of left ventricular volume. The saline method can be applied to determine Vp for TCC. IA-V is substantial, so that Vp must be determined in each individual *in vivo* subject, but within individual subject variability is relatively small.

Implementation: Evaluation of the accuracy of the saline calibration method and intersubject variabilities of Vp; variations in hemodynamic conditions and during the cardiac cycle. The conventional intracardiac conductance catheter method is used to obtain independent estimates of Vp by the zero-volume method ($Vp_{zero\ volume}$).

Animal Model: Sheep (anesthetized).

Interpretation and Analysis: Mean baseline Vp_{saline} and $Vp_{zero\ volume}$ are 104 ± 6 ml and 106 ± 6 ml, respectively. Bland-Altman analysis reveals a small non-significant bias (-2.5 ml) and narrow limits of agreement (4.6 ml). Vp are not significantly different between hemodynamic conditions (baseline, dobutamine, volume load, propranolol), but have a substantial interanimal variability (IA-V) (38%). Average variations during the cardiac cycle are < 10% of mean Vp.

The trans-cardiac conductance method for on-line measurement of left ventricular volume: Assessment of parallel conductance offset volume. Staal EM, Steendijk P, Baan J. IEEE Trans Biomed Eng 2003 Feb; 50 (2), pp. 234–40.

PERIPHERAL VASCULAR DISEASE, FUNCTION AND PHYSIOLOGY

Angiogenesis: Neoplastic Disease/Tumor Dimethylarginine Dimethylaminohydrolase Overexpression
NC100150/Hoechst 33342/[Fluorescent Imaging]

Cellular/Molecular Mechanism of Action: The orientation of NC100150 becomes altered within a magnetic field. Alternatively, Hoechst 33342 emits fluorescent light energy.

Background Information: Intracellular factors that regulate nitric oxide (NO) synthesis represent important targets in tumor progression. Overexpression of dimethylarginine dimethylaminohydrolase (DDAH), which metabolizes the endogenous inhibitors of NO synthesis asymmetric dimethylarginine and N-monomethyl-L-arginine, results in C6 gliomas that display enhanced growth rates compared with wild type.

Application: NC100150 can be utilized as an intravascular blood pool contrast agent that is detectable by magnetic resonance imaging (MRI) methodologies. Hoechst 33342 can be used as a fluorescent perfusion-marker dye that can be measured by flourescent detection systems.

Route of Administration: Utilization of NC100150 and Hoechst 33342 for neoplastic lesion angiogenesis requires that these be delivered intravascularly.

Implementation: Delineate the effects of DDAH on tumor vascular morphogenesis *in vivo*, through the measurement of the transverse relaxation rates R_2* and R_2 in (i) clone D27 gliomas overexpressing DDAH; and (ii) C6 wild-type gliomas utilizing susceptibility contrast-enhanced MRI aided by the use of NC100150 (intravascular blood pool contrast agent). As a point of reference, findings can be compared with results from fluorescence microscopy for assessing tumor uptake of the perfusion marker Hoechst 33342,

and/or intrinsic susceptibility MRI, sensitive to changes in endogenous [deoxyhemoglobin].

Interpretation and Analysis: The baseline R_2* is significantly faster in D27 tumors, consistent with greater vascular development ($p < 0.02$, ANOVA). There is no significant difference between the response of the 2 tumor types to hypercapnia (5% CO_2/95% air), used as a probe for vascular maturation, or hyperoxia (5% CO_2/95% O_2), used as a probe for vascular function. NC100150 increases the R_2* and R_2 rates of both tumor types and demonstrates a significantly larger blood volume in the D27 tumors ($p < 0.02$, ANOVA). This correlates with a significantly greater uptake of Hoechst 33342 in D27 tumors compared to C6 wild-type tumors ($p < 0.02$, ANOVA). Despite increased tumor blood volume, the Delta R_2*/Delta R_2 ratio, an index of microvessel size, shows that the capillaries in the 2 tumor types are of a similar caliber. Such observations demonstrate the potential of susceptibility MRI-derived quantitative end points to non-invasively assess tumor angiogenesis. Application of intravascular blood pool contrast agents such as NC100150 for such purposes appears very promising. Overexpression of DDAH results in increased *in vivo* neovascularization of C6 gliomas. The lack of significant difference in hypercapnic/hyperoxic response between the C6 and D27 tumors and the similar vessel caliber are also consistent with a role for DDAH in the initial stages of vasculogenesis.

Instrumentation: MRI and fluorescence microscopy instrumentation.

Effects of overexpression of dimethylarginine dimethylaminohydrolase on tumor angiogenesis assessed by susceptibility magnetic resonance imaging. Kostourou V, Robinson SP, Whitley GS, Griffiths JR. Cancer Res 2003 Aug 15; 63 (16), pp. 4960–6.

Angiogenesis/Vascular Perfusion Density Changes/(Prostatic Carcinoma: PC-3m)/Evaluation of Angiogenesis Inhibitory Agents
Protamine/Contrast-Enhanced Doppler Ultrasound

Cellular/Molecular Mechanism of Action: Contrast media is can be utilized to enhance Doppler ultrasound image quality and sensitivity based on the interaction of ultrasound energy with the physical properties of media preparations (echogenic properties).

Application: Detection of altrations in tumor vascularity.

Route of Administration: Intravenous.

Implementation: Evaluation of vascularity and blood flow in neoplastic lesions. Contrast agent is administered and color flow signal-pixel rate (SPR) of images calculated using the number of pixels showing color Doppler signals as a ratio of the total number of pixels covering the lesion.

Animal Model: Murine. (BALB/C nude mice; $n = 48$) first received subcutaneous transplants of human prostate carcinoma PC-3m tumor followed by protamine injections (SQ) as an angiogenesis inhibitor. Subjects are randomly divided into 3 groups according to protamine dose.

Interpretation and Analysis: The SPR of the high- and low-dose groups is significantly lower than that of control groups ($p < 0.01$). Mean SPR $\pm$ SD is 0.09 ± 0.05, 0.11 ± 0.05, and 0.22 ± 0.10 for different treatment groups, respectively. The SPR parameters correlate significantly with the density of microvessel structures ($r = 0.86$ to 0.94, $p < 0.01$). Therefore, contrast-agent enhanced Doppler ultrasound imaging can effectively reveal changes in vascularity in neoplastic lesions (e.g., $\pm$ protamine). In addition to microvessel density, contrast-agent enhanced Doppler ultrasound imaging may become one of several independent prognostic indexes of angiogenesis inhibitor therapy.

Evaluation of the effect of protamine on human prostate carcinoma PC-3m using contrast enhanced Doppler ultrasound. Tang J, Li S, Li J, Zhang Y, Li X, Dong B, Shi H, Zhang G. J Urol 2003 Aug; 170 (2 Pt 1), pp. 611–4.

Biomarker Selective Targeting/Vascular Endothelium/Inflammation/Ischemia/Neoplastic Disease
Polymerized Nanoparticle

Background Information: In order to be successful in personalizing treatment protocols, methods for effective case selection as well as development of good surrogate biomarkers for monitoring the effects of treatment in concert with the development of an efficacious targeted therapy is required.

Application: Selective detection and targeting of disease-specific biomarker systems.

Route of Administration: Intravascular.

Implementation: Development of a polymerized nanoparticle platform technology that will allow the application of different targeting moieties on the surface of the particles in addition to loading particles with different contrast and therapeutic agents.

Interpretation and Analysis: Nanoparticles can be targeted to endothelial receptors and different payloads of contrast and therapeutic agents can be delivered to target cells with high target-to-background ratios. Using this combined vascular targeting approach for imaging and therapy, it is highly possible that personalize/individualize treatment regimens can be developed for different disease processes such as cancer, inflammatory responses, and ischemia.

Combined vascular targeted imaging and therapy: A paradigm for personalized treatment. Li KC, Guccione S, Bednarski MD. J Cell Biochem Suppl 2002; 39, pp. 65–71.

Capillary Bed Blood Flow/Multiphoton In Vivo Imaging
Semiconductor Nanocrystal Fluorescent Markers/Cadmium Selenide-Zinc Sulfide Quantum Dots/[Fluorescent Imaging]

Cellular/Molecular Mechanism of Action: Cadmium selenide-zinc sulfide quantum dot modalities emit fluorescent light energy.

Application: Cadmium selenide-zinc sulfide quantum dots can be applied as a fluorescent tracer modality for the evaluation of various physiological parameters.

Fluorescent labels for multiphoton microscopy that enable multicolor imaging in demanding biological environments such as living tissue.

Implementation: Semiconductor nanocrystals (quantum dots) can be applied as fluorescent tracer agents for *in vivo* multiphoton microscopy, enabling the acquisition of multicolor image analyses. In this context, water-soluble cadmium selenide-zinc sulfide quantum dots can be used for multiphoton *in vivo* imaging. The fluorescent probes have 2-photon action cross sections as high as 47,000 Goeppert-Mayer units, by far the largest of any label used in multiphoton microscopy.

Animal Model: Mouse.

Interpretation and Analysis: It is possible to visualize quantum dots dynamically through the skin of living mice, within capillaries hundreds of micrometers deep. Evidence of blinking (fluorescence intermittency) cannot be appreciated in solution on nanosecond to millisecond time scales.

Instrumentation: Multiphoton microscopy.

Water-soluble quantum dots for multiphoton fluorescence imaging *in vivo*. Larson DR, Zipfel WR, Williams RM, Clark SW, Bruchez MP, Wise FW, Webb WW. Science 2003 May 30; 300 (5624), pp. 1434–6.

Capillary Bed Tolerance of Small Particle or Liquid Plastic Occlusion
Lidocaine

Cellular/Molecular Mechanism of Action: Lidocaine creates alterations in Na^+/K^+ pump function resulting in increased K^+ ion conductance.
Physiological Mechanism of Action: Promotes vasodilation.
Physiological Mechanism of Action: Exerts a direct effect on the heart independent of any influences imposed by the autonomic system. Declines in the pacemaker current and increases in time-independent outward current are induced by lidocaine which can be recognized as decreases in the slope of the normal Purkinje phase-4 depolarization phase. Depolarized Purkinje fibers under tension display depressed levels of automaticity, and the diastolic threshold in cardiac Purkinje fibers is increased (increased K^+ conductance). Parameters for Vm or threshold voltage are not affected. In the Purkinje system and ventricles, the chronological duration of action potentials is lengthened slightly (disruption of small Na^+ current flow during action potential plateau), but non-specialized or atrial tissues are not affected. Minimal changes are usually appreciated on EKG tracings except for a shortening of the Q-T interval. Refractoriness of the A-V node is most often unaffected. One of the most important influences of lidocaine is its ability to promote pronounced decreases in the refractory period of the His-Purkinje system.
Application: Evaluation of capillary bed tolerance for small particle or liquid plastic occlusion.
Route of Administration: Intra-arterial (affected arterial structures).
Considerations: Endovascular obliteration of hypervascular lesions of the head and neck has become clinically accepted, but it may cause stroke and peripheral cranial nerve palsy. By using a flow-controlled technique to deliver the materials and by knowing the vascular anatomy of the cranial nerves, these problems are less likely to occur. Occasionally, though, vascular anatomy is distorted by the lesion or is anomalous in its distribution.
Implementation: Evaluation of various branches of the external carotid arteries utilizing a lidocaine challenge protocol.

Human Model: Cases with vascular abnormalities (e.g. n = 26)
Interpretation and Analysis: Case may developed transient palsies (e.g. n = 3), thereby requiring modification of treatment regimens. Cases usually do not develop complications of embolization (e.g. n = 0/26).

Lidocaine injection into external carotid branches: Provocative test to preserve cranial nerve function in therapeutic embolization. Horton JA, Kerber CW. AJNR Am J Neuroradiol 1986 Jan–Feb; 7 (1), pp. 105–8.

Extracellular Tissue Space/Volume Estimation
$TmDOTP^{-5}$ and $CoEDTA^-$

Application: Extracellular tissue space volume estimation.
Dosage: $TmDOTP^{-5}$ (0.16 mmol/rat in washout studies).
Implementation: Evaluation and comparison of the distribution of $TmDOTP^{-5}$ and $CoEDTA^-$ (anionic complex previously used as a marker system for establishing extracellular space). Heart, liver, muscle, blood, and urine are collected after infusion of either complex and quantitatively analyzed by atomic absorption spectroscopy. Experiments can also be performed to evaluate the washout kinetics of $TmDOTP^{-5}$ washout kinetics.

Animal Model: Rat (anesthetized in washout studies).
Interpretation and Analysis: Although total $TmDOTP^{-5}$ in blood and tissue is consistently lower (0.88 ± 0.04; n = 6) than $CoEDTA^-$ after an identical infusion protocol (presumably because of some association of the phosphonate complex with bone), a comparison of blood and tissue contents indicates that the 2 anionic complexes distribute into identical extracellular spaces. Relative extracellular space in the liver (evaluated *in vivo*), as determined by $TmDOTP^{-5}$ and $CoEDTA^-$, is 0.18 ± 0.02 and 0.15 ± 0.01, respectively. The corresponding relative *in vivo* extracellular space values for the heart calculated using the 2 agents are identical (0.11 ± 0.02). In washout kinetic studies with $TmDOTP^{-5}$ (0.16 mmol total dose), 81% appears in urine by 180 minutes, and $< 2\%$ is found in all remaining soft tissue, leaving approximately 18% undetected. The rate of Tm appearance in urine can be fitted to a standard pharmacokinetic model that includes 4 tissue compartments: plasma, 1 fast equilibrating space, 1 slow equilibrating space, and 1 very slow equilibrating space (presumably bone). The best fit result suggests that the highly charged $TmDOTP^{-5}$ complex is cleared from plasma more rapidly than are the typical lower charged Gd-based contrast agents and that release from bone is slow compared with renal clearance.

Distribution of $TmDOTP^{-5}$ in rat tissues: $TmDOTP^{-5}$ vs $CoEDTA^-$ as markers of extracellular tissue space. Makos JD, Malloy CR, Sherry AD. J Appl Physiol 1998 Nov; 85 (5), pp. 1800–5.

Extracellular Fluid Space Distribution Parameters/ Interstitial Fluid Compartment/Water Diffusion Coefficients
Gallamine/Inulin

Cellular/Molecular Mechanism of Action: Gallamine and inulin per se do not exert any biochemical or physiological mechanism of action. Instead they function as diagnostic tracer agents based in part due to their molecular weight which directly influences their relative permeability across capillary membranes and facilitate their detection in biological fluids.
Application: Gallamine and inulin can be detected in diluted form within biological fluids where they can function as passive marker/indicator molecules.
Route of Administration: Gallamine and inulin (simultaneous intravenous injection).
Implementation: Simultaneous evaluation of the kinetics of inulin and gallamine. The distribution of both compounds in extracellular fluid space can be characterized by a 3-compartment model in which the mean central compartment blood volume of 1.37 liters is identical with expected values.

Animal Model: Canine (anesthetized).
Interpretation and Analysis: The 2 peripheral compartments of the model appear to represent rapid and slow equilibrating interstitial fluid compartments. A mammillary model structure can be selected in which intercompartmental clearance corresponds to transcapillary exchange. Previous studies indicate that inulin and smaller hydrophyllic molecules diffuse across capillary walls at rates proportional to their respective free water diffusion coefficients. For the ratio of the transcapillary permeability coefficients of inulin and gallamine to equal their free water diffusion coefficient ratio of 5.34 ± 0.02 ($\pm$ SD), it appears that the sum of blood flow to the fast and slow interstitial fluid compartments is less than cardiac output. When this assumption is made, blood flow to fast equilibrating interstitial

fluid is estimated to be 39% of cardiac output, in agreement with previous measurements of splanchnic blood flow. This supports the hypothesis that the fast equilibrating interstitial fluid space is supplied by porous splanchnic capillaries that lack a continuous investment of basement membrane.

Heterogeneity of interstitial fluid space demonstrated by simultaneous kinetic analysis of the distribution and elimination of inulin and gallamine. Henthorn TK, Avram MJ, Frederiksen MC, Atkinson AJ Jr. J Pharmacol Exp Ther 1982 Aug; 222 (2), pp. 389–94.

Hemorrhage: Intracranial
Hirudin

Cellular/Molecular Mechanism of Action: Hirudin has a relatively strong binding avidity for thrombin and functions as an inhibitor of most of the biological properties associated with this enzyme fraction.

Background Information: In humans, intracerebral hemorrhage (ICH) causes marked perihematomal edema formation and neurological deficits. A rat ICH model, involving infusion of autologous blood into the caudate, has been used extensively to study mechanisms of edema formation. However, examination of the behavioral outcome would improve its preclinical utility and provide a more rigorous assessment of the pathological cascade of events that occur over time. Batteries of sensorimotor function tests have been applied (e.g. rat) to examine the neurological effects of ICH and to examine which components of the hematoma are involved in generating those effects.

Application: Detection and characterization of central nervous system hemorrhagic conditions.

Route of Administration: Intravenous.

Implementation: Evaluation neurologic function from day 1 to day 28 postinjection of (i) autologous whole blood; (ii) injection of blood plus hirudin (thrombin inhibitor); (iii) packed red blood cells; (iv) thrombin; (v) saline; (vi) or needle placement only.

Animal Model: Rat (behavioral tests employing forelimb placing, preference for forelimb use for weight shifts during vertical exploration of a cylindrical enclosure, and a corner turn test).

Interpretation and Analysis: Radioisotope-labeled hirudin accumulates at sites of intravascular thrombosis. Behavioral reaction tests detect the presence of marked neurological deficits by day 1 after ICH, with progressive recovery of function over 4 weeks. The forelimb placing score parallels changes in edema. Injection of thrombin causes (promotes), while injected hirudin reduces, ICH-induced neurological deficits. Injection of packed red blood cells (promotes delayed edema formation) induces delayed neurological deficits. Such tests allow continuous monitoring of neurological deficits after rat ICH and assessment of therapeutic interventions. The time course of the neurological deficit closely matches the time course of cerebral edema for both ICH and injection of blood components. There is marked recovery of function after ICH, which may be amenable to therapeutic manipulation.

Behavioral tests after intracerebral hemorrhage in the rat. Hua Y, Schallert T, Keep RF, Wu J, Hoff JT, Xi G. Stroke 2002 Oct; 33 (10), pp. 2478–84.

Intravascular Blood Volume Estimation/ Extravascular Volume
Indocyanine Green (COLD System)/Methylene Blue/Dual Thermal-Dye Double Indicator Technique

Cellular/Molecular Mechanism of Action: Indocyanine green and methylene blue can function as indicator/tracer molecules that passively diffuse throughout the intravascular and potentially other body fluid compartments.

Physiological Mechanism of Action: Methylene blue can induce acute vasoconstrictive and positive inotropic effects during episodes of septic shock.

Application: Indocyanine green and methylene blue can be used as detectable molecular markers in biological systems.

Route of Administration: Methylene blue (intravenous).

Dosage: Bolus dose of methylene blue (3 mg/kg over 10 minutes).

Implementation: Assess the effects of inhibiting guanylate cyclase, an enzyme involved in sepsis-related vascular and myocardial dysfunctions, on hemodynamic variables including blood volume and pulmonary vascular permeability during conditions of septic shock.

A fiber-optic catheter is inserted to determine blood and extravascular volumes applying the thermal-dye double indicator technique, using indocyanine green (COLD system) in combination with methylene blue. COLD-derived variables are recorded before methylene blue and 20 minutes, 1 hour, and 2 hours after the end of methylene blue infusion.

Human Model: Septic shock associated with persisting hypotension despite conventional treatment including fluid loading, vasopressors, and inotropes ($n = 15$ cases). Standard hemodynamic and oxygen-derived variables; total, intrathoracic, systolic, and diastolic cardiac blood volumes; extravascular lung water; plasma osmolarity; and lactate and protein concentrations are recorded.

Interpretation and Analysis: Mean arterial and pulmonary artery pressures, systemic and pulmonary vascular resistances, and left ventricular stroke work index increase, and blood lactate transiently decreases after methylene blue ($p < 0.05$). Other variables recorded are unchanged during a 2-hour period following methylene blue infusion. Such findings demonstrate the acute vasoconstrictive and positive inotropic effects of methylene blue during septic shock. These effects are not associated with changes in blood volume, myocardial diastolic function, or pulmonary vascular permeability assessed by extravascular lung water.

Does methylene blue administration to septic shock patients affect vascular permeability and blood volume? Donati A, Conti G, Loggi S, Münch C, Coltrinari R, Pelaia P, Pietropaoli P, Preiser JC. Crit Care Med 2002 Oct; 30 (10), pp. 2271–7.

Intravascular Compartment Volume Estimation
Labeled Albumin (Non-Radioactive)

Cellular/Molecular Mechanism of Action: Functions as a detectable biological "marker" system that is largely incapable of simple diffusion across biological membranes. The relatively large molecular weight of albumin fractions compared to pharmaceutical agents is the foundation for the application of this particular protein fraction for such purposes.

Diagnostic Application: Non-radioactive labeled albumin has been applied to estimate systemic intravascular fluid volume and detect extravascular hemorrhage or transcapillary "weeping." It has been of particular benefit in the estimation of intravascular fluid volume in the field of exercise physiology.

Route of Administration and Dosage: Intravenous injection only.

Considerations: Disease abnormalities such as protein-losing enteropathy or nephropathy will promote an underestimation of the intravascular fluid volume. Usually the blood sample utilized to estimate the degree of dilution of labeled albumin within the intravascular compartment is collected within a brief period of time following

intravenous injection. This makes underestimation of intravascular fluid volume in the presence of disease conditions less probable.

Interpretation and Analysis: Labeled albumin concentrations within blood (serum) samples are measured and values are ultimately utilized to estimate approximate intravascular fluid volume based upon the known total dose administered.

Instrumentation: Spectrophotometric assay techniques can be applied for the detection and measurement of certain non-radioactive "marker" systems. Examples include *p*-nitroanalide (pNA), fluorescent covalent cross-linking reagents, and biotin:strepavidin-HRPO detection systems.

Intravascular Compartment Volume Estimation
Evans Blue Dye

Cellular/Molecular Mechanism of Action: Functions as a detectable biological dye marker system that can be quantitated in biological fluid samples (whole blood).

Clinical Application: Utilized for calculating the approximate systemic intravascular fluid volume. This is calculated based upon the known initial amount (total dose) of Evans blue dye initially administered and its final concentration following complete dispersal within the intravascular compartment.

Route of Administration: Evans blue (intravenous).

Dosage: Evans blue (1 mg/kg single intravenous bolus).

Considerations: In instances of lowered systemic pH levels (running excercise, metabolic acidosis), the Evans blue dye/albumin complex may dissociate to some degree. As a result, excessively high estimates of intravascular compartment volume may be observed (calculated) since the lower pH level displaces the Evans blue dye from its protein binding site. Ultimately, the increased amount of "free" Evans Blue dye (displaced from albumin by hydrogen ion) is eliminated from the intravascular compartment at a more accelerated rate than is the protein-bound Evans Blue dye. Although Evans blue dye has a different binding constant, this phenomenon is analogous to hydrogen ion displacement of penicillins and non-steroidal anti-inflammatory drugs (NSAIDS) from their respective protein binding sites on albumin.

Interpretation and Analysis: Blood concentration is approximately 0.027 mg/ml. Estimated intravascular volume is calculated using the mathematical formula 1000/0.027 = 37 L (total intravascular blood volume).

Laboratory Instrumentation: Measurement of spectrophotometric absorbance at 570 nm.

Intravascular Compartment Volume Estimation/ Intravascular Compartment
Fluorescein Isothiocyanate Labeled Hydroxyethyl Starch/Radiolabeled Red Blood Cells/Radiolabeled Serum Albumin

Cellular/Molecular Mechanism of Action: Labeled albumin, labeled red blood cells, and labeled hydroxyethyl starch can be applied to measure approximate intravascular blood volume.

Application: Estimation of approximate blood volume.

Route of Administration: Intravascular, usually by intravenous injection

Semi-Synthetic Methods: A proprietary brand of hydroxyethyl starch (Elohaes) covalently labeled with fluorescein isothiocyanate.

Implementation: Evaluation of blood volume measurements in suspected cases of polycythemia utilizing labeled albumin, red blood cells, and hydroxyethyl starch (Elohaes). Dilution of labeled hydroxyethyl starch (Elohaes) *in vivo* can be used to calculate approximate measurements of blood volume. Reference methodologies can employ the use of radiolabeled albumin and radiolabeled red cells.

Human Model: Cases of suspected polycythemia (e.g., n = 17; n = 8 undergoing major surgery and/or receiving intensive postoperative care).

Interpretation and Analysis: Elimination of the labeled starch occurs in a manner essentially the same as the "parent" unlabeled compound. Such findings indicate that *in vivo*, the fluorescent tag is stable and does not significantly alter the molecular characteristics of the carrier molecule. The volume of distribution for labeled starch is 2.5 mL/kg lower than that for labeled albumin ($p = 0.05$). Blood volume, measured from the dilution of fluorescent starch, is lower (4.9 mL/kg) than that measured with albumin ($p = 0.048$) but higher (6.61 mL/kg) than that measured with red blood cells ($p = 0.0007$). The latter difference may be even smaller at marginally higher doses of the fluorescent starch. Hydroxyethyl starch appears to provide a valid alternative to labeled red cells as a means of calculating blood volume. Labeling of starch with a fluorescent marker makes the assay procedure more sensitive and infinitely easier to perform. The dose required is not high enough to affect hemodynamic parameters.

Measuring blood volume with fluorescent-labeled hydroxyethyl starch. Thomas E, Jones G, de Souza P, Wardrop C, Wusteman F. Crit Care Med 2000 Mar; 28 (3), pp. 627–31.

Thrombosis: Intravascular
Platelet-Specific Monoclonal Antibody (P256)/[Nuclear Scintigraphy]

Cellular/Molecular Mechanism of Action: Radiolabeled platelet-specific monoclonal antibody (P256) selectively detects antigens expressed on the surface membrane of aggregated platelets.

Application: Detection of vascular thrombosis through diagnostic nuclear imaging.

Experimental Interpretation: Intact (whole) immunoglobulin promotes platelet aggregation at concentration ranging between 10^{-9} to 10^{-7} M with a maximum of 70% aggregation. The F(ab′)2 fragment is less potent in promoting platelet aggregation and displays a maximum response of only 45%. In contrast, monovalent Fab′ fragment causes no aggregation at concentrations up to 10^{-7} M. Binding studies with [^{111}In]-labelled monovalent Fab′ fragment demonstrate saturable displaceable binding ($Kd = 9.1 \times 10^{-9}$ M). Note: aggregation induced by the divalent forms of the antibody is partially dependent upon the addition of fibrinogen.

Interpretation and Analysis: Monovalent Fab′ fragment of P256 may be used in platelet-labelling studies without risk of inducing aggregation irrespective of concentration. The whole antibody, or its divalent F(ab′)2 fragment, may also be used provided their concentrations are kept below 10^{-9} M.

Instrumentation: Computer-integrated nuclear medicine scintigraphy camera.

In vitro studies with an antiplatelet monoclonal antibody; P256. Stuttle AW, Ritter JM, Peters AM, Lavender JP. Nucl Med Commun 1988 Oct; 9 (10), pp. 813–5.

Vascular Blood Flow and Patency/Ultrasound Contrast-Enhancing Agents
C_3F_8 Microbubbles/N_2 Microbubbles/SF_6 Microbubbles/[Ultrasonography]

Cellular/Molecular Mechanism of Action: Use of fluorocarbon vapor extends (prolongs) the *in vivo* persistence of microbubbles from seconds to minutes, thereby facilitating the application of contrast ultrasonography in clinical medicine.

Application: Imaging techniques and reagents that selectively suppress the intensity of signal from tissue structures, but not injected microbubbles, serve to further enhance image contrast. Approved products consist of C_3F_8 or SF_6 microbubbles, and N_2 microbubbles osmotically stabilized with C_6F_{14}. These agents allow the detection and characterization of cardiovascular abnormalities and solid organ lesions, such as tumors. By providing higher quality images, they improve the accuracy and confidence of disease diagnosis and can play a decisive role in clinical decision making. Future development objectives include the design of agents that target specific cells for the molecular imaging of disease, and drug and gene delivery, including ultrasound-triggered delivery.
Route of Administration: Preparations of C_3F_8 microbubbles, SF_6 microbubbles, and N_2 microbubbles osmotically stabilized with C_6F_{14} are administered intravenously.
Dosage: C_3F_8 microbubbles, SF_6 microbubbles, and N_2 microbubbles osmotically stabilized with C_6F_{14} are administered at dosages commensurate with the diagnostic procedure being performed.
Considerations: Ultrasonography has, until recently, lacked effective contrast-enhancing agents. Micrometer-sized gas bubbles that resonate at diagnostic frequencies are ideal reflectors for ultrasound. However, simple air bubbles, when injected into the blood stream, disappear within seconds through the combined effects of Laplace pressure, blood pressure, and exposure to ultrasound energy.
Interpretation and Analysis: These agents allow the detection and characterization of cardiovascular abnormalities and solid organ lesions, such as tumors. By providing higher quality images, they improve the accuracy and confidence for diagnosing disease conditions and can play a decisive role in clinical decision making. New objectives include the design of agents that target specific cells for the molecular imaging of disease, and drug and gene delivery, including ultrasound-triggered delivery.

Injectable microbubbles as contrast agents for diagnostic ultrasound imaging: The key role of perfluorochemicals. Schutt EG, Klein DH, Mattrey RM, Riess JG. Angew Chem Int Ed Engl 2003 Jul 21; 42 (28), pp. 3218–35.

Vascular Blood Flow and Perfusion/Capillary Circulation/Perfusion
Fluorescein Isothiocyanate-Labeled Dextran

Cellular/Molecular Mechanism of Action: Fluorescein isothiocyanate can be used as a light-emitting molecular marker system.
Application: Fluorescein isothiocyanate can be used as a light-emitting tracer/marker molecule in biological systems. Dextran labeled with fluorescein isothiocyanate can be utilized as an *in vivo* molecular tracer system for evaluating the acute microhemodynamic effects of various stimuli (e.g., static and alternating magnetic fields delivered at threshold levels in muscle capillary mirocirculation beds).
Route of Administration: FITC-dextran solution (intravascular: e.g., vena cava).
Implementation: The skin in a tibialis anterior is circularly removed with 1.5 mm diameter sufficient to faciliate intravital-microscopic recording of capillary blood velocity in the tibialis anterior muscle. Fluorescein isothiocyanate (FITC)-labeled dextran (MW 150 kDa) can be used as an *in vivo* fluorescent plasma marker system applicable for evaluating muscle capillaries. Following a bolus injection of FITC-dextran solution into the caudal vein, the peak blood velocity in the muscle capillaries is measured prior to, during, and following exposure to static magnetic fields (SMF) or 50 Hz electromagnetic fields (EMF). The whole body of each experimental animal is then placed on an observation stage of a fluorescence microscope and exposed to static magnetic fields (SMF = 0.3, 1, and 10 mT) or 50 Hz EMF (0.3 and 1 mT) for 10 minutes with the electromagnet not energized during sham exposures.
Animal Model: Mice anesthetized with pentobarbital and exposed to static and alternating magnetic fields (threshold levels).
Interpretation and Analysis: During periods of exposure and postexposure to SMF of 10 mT, the peak blood velocity significantly increases compared to sham exposure preparations. After the withdrawal of SMF and 50 Hz EMF of 1 mT, significant similar effects on the blood velocity can be recognized (e.g. equivalent or enhanced/greater responses). Such findings suggest that field intensity of 1 mT might be considered as a threshold level for enhancing muscle microcirculation under pentobarbital-induced hypnosis.
Instrumentation: Utilization of an (i) electromagnet specifically designed to deliver static magnetic fields (SMF = 0.3, 1, and 10 mT); (ii) 50 Hz EMF (0.3 and 1 mT) instrumentation (fluorescence epi-illumination system); and (iii) a fluorescence microscope.

Acute effects of whole-body exposure to static magnetic fields and 50 Hz electromagnetic fields on muscle microcirculation in anesthetized mice. Xu S, Okano H, Ohkubo C. Bioelectrochemistry 2001 Jan; 53 (1), pp. 127–35.

Vascular Blood Flow Characteristics
Fluorescent-Labeled Red Blood Cells

Application: Evluation of blood flow characteristics.
Considerations: Whole organ studies in cat skeletal muscle have shown that increases in venous resistance associated with reduced arterial pressures are nearly abolished when muscle is perfused with a non-aggregated red blood cell suspension.
Implementation: Delineation of the influence red blood cell aggregation has on *in vivo* vascular flow patterns and determination of whether it leads to blunted red blood cell velocity profiles at reduced shear rates. In this context, fluorescent-labeled red blood cells can be applied in tracer quantities to obtain velocity profiles in venous microvessels.
Interpretation and Analysis: Velocity profiles in venous microvessels (45–75 μm) of rat spinotrapezius muscle have centerline velocities between 0.3 and 14 mm/sec (pseudoshear rates 3-120 mm/sec under normal (non-aggregating) conditions, and following induction of red blood cell aggregation with Dextran 500. Profiles are nearly parabolic (Poiseuille flow) over this flow rate range in the absence of aggregation. When aggregation is present, profiles are parabolic at high shear rates and become significantly blunted at pseudoshear rates of 40 mm/sec and below. These results indicate a possible mechanism for increased venous resistance at reduced flows.
Instrumentation: Video system equipped with a gated image intensifier.

Effect of erythrocyte aggregation on velocity profiles in venules. Bishop JJ, Nance PR, Popel AS, Intaglietta M, Johnson PC. Am J Physiol Heart Circ Physiol 2001 Jan; 280 (1), pp. H222–36.

Vascular Blood Flow Characterization/Aorta/Renal Artery and Vein/Portal Vein/Vena Cava
Indocyanine Green/SH U 508 A Microbubbles (Levovist)/[Doppler Ultrasound]

Cellular/Molecular Mechanism of Action: Indocyanine green and microbubbles can be infused into the intravascular compartment where they passively disperse throughout the blood volume whereby they function as a tracer agents.

Application: Indocyanine green and microbubbles can be applied to characterize blood flow through vascular structures.
Route of Administration: Indocyanine green and microbubbles are both administered intravascularly.
Dosage: Indocyanine green (1.25 mg/mL at 0.1 mL/kg); SH U 508 A (300 mg/mL at 0.1 mL/kg).
Considerations: SH U 508 A microbubbles (Levovist) can be detected by Doppler ultrasound.
Implementation: Evaluation of blood flow within the vena cava, aorta, renal artery, renal vein, and portal vein. The kinetics of indocyanine green can be evaluated by measuring absorbance values using a photodiode, while SH U 508 A microbubbles (Levovist) are detectable using Doppler ultrasound.
Animal Model: Rabbit (n = 5).
Interpretation and Analysis: Peak signal intensity is observed immediately after injection of SH U 508 A, followed by a biphasic decay pattern. The rates of biphasic decay are similar in all vessels. A second peak of the signal that represents microbubble recirculation is observed within the vena cava. The circulation and recirculation times of the microbubbles after injection of SH U 508 A are similar to that of indocyanine green. These findings suggest that majority of the SH U 508 A microbubbles circulate through the body in a manner similarly to blood flow and without retention in microvasculature structures.

In vivo kinetics of microbubbles of SH U 508 A (Levovist): Comparison with indocyanine green in rabbits. Uchimoto R, Niwa K, Eguchi H, Kamiyama N, Mine Y, Miyazawa T, Brautigam M. Ultrasound Med Biol 1999 Nov; 25 (9), pp. 1365–70.

Vascular Malformation (Cardiac): Patent Ductus Arteriosus
Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Cellular/Molecular Mechanism of Action: Permanently inhibits cyclooxygenase biochemical activity through direct acetylation of the enzyme, thereby preventing the conversion of arachidonic acid (generated by phospholipase A_2) to the prostaglandin intermediates, PGG and PGH.
Diagnostic Application: In cases of systolic murmurs in neonatal infants, NSAIDs can be applied to diagnose and therapeutically manage congenital patent ductus arteriosus disorders. If it has already been established through auscultation and echocardiography that a neonatal case has a patent ductus arteriosus, then NSAIDs can assist in determining if the PDA is physiological or functional. The proposed mechanism behind the response to NSAIDs in physiological PDA is that the endothelium within the PDA persistently synthesizes elevated concentrations of PGI_2, which is one of the most potent vasodilators identified to date. Indomethocin is an example of one non-steroidal agent used in human neonatal internal medicine that can facilitate the closure of physiological PDA through inhibition of PDA endothelial PGI_2 synthesis.
Route of Administration and Dosage: Intravenous or oral administration.
Considerations: Prolonged administration promotes development of several types of pathological lesions including gastric/intestinal ulceration and renal papillary necrosis. Such responses have been theorized as being associated with vasoconstriction due to decreases in the synthesis of vasodilatory prostacyclin (PGI_2).
Laboratory Instrumentation: Inhibition of platelet PGI_2 synthesis by NSAIDs can be detected by direct RIA or ELISA based assay methodologies. Alternatively, inhibition of platelet PGI_2 synthesis can be indirectly detected by measurement of malondialdehyde production, applying thiobarbituric acid in a low pH buffer environment. The chemical reaction is very sensitive but its specificity, even with improvement of preanalytical (sampling, preservatives) and analytical stages (fluorescence, HPLC), is still a matter of debate. At present, the concept of "thiobarbituric acid reactive substances" (TBARS) has merged and progressively replaced the initial malondialdehyde assay.

Vascular Malformation (Central Nervous System): Arteriovenous
Methohexital/[Angiography]

Physiological Mechanism of Action: Methohexital is a barbiturate that acts as a central nervous system depressant.
Application: Existing reports suggest that methohexital is a safe provocative agent for use prior to embolotherapy for brain arteriovenous malformations. However, the full effects of methohexital within the cerebral circulation remain to be elucidated.
Route of Administration: Methohexital (intra-arterial).
Dosage: Methohexital (1% concentration with individual doses of 1–6 mg/injection).
Interpretation and Analysis: Arterovenous malformation can initially be characterized with the aid of digital vascular imaging methods performed just prior to and just after intra-arterial methohexital injection.
Human Model: Cases without angiographic or clinically detectable evidence of vasospasms (e.g. n = 66 vascular pedicle injections in n = 30 cases).
Interpretation and Analysis: Perfusion of functional tissue with methohexital can often immediately precipitate evidence of altered neurologic status that fortunately almost invariably resolves within 2 minutes of the initial onset. No signs of either prolongation of the induced clinical symptoms or seizures are evident to suggest any adverse effects related to either methohexital crystallization or the effects of injecting an alkaline solution into the cerebral vascular circulation.

Though the full effects of methohexital in the cerebral circulation remain to be elucidated, existing reports suggest it is a safe provocative agent for use prior to embolotherapy for brain arteriovenous malformations.

Intra-arterial use of sodium methohexital for provocative testing during brain embolotherapy. Peters KR, Quisling RG, Gilmore R, Mickle P, Kuperus JH. AJNR Am J Neuroradiol 1993 Jan–Feb; 14 (1), pp. 171–4.

Vascular Malformation (Central Nervous System)/ Arteriovenous Embolism (Cerebral)/Vascular Blood Flow
Sodium Amobarbital/N-butyl Cyanoacrylate Glue/[Angiography]

Application: Determine whether a method of targeted neurologic and cognitive testing procedures performed as a guide during arteriovenous malformation (AVM) embolization techniques can reduce the incidence of focal cognitive and other neurologic deficits associated with performing such procedure.
Route of Administration: In each feeder-vessel initially being considered for embolization procedures, sodium amobarbital is superselectively delivered applying a microcatheter system. N-butyl cyanoacrylate glue is sequentially delivered by the same route of administration if the proposed site of embolization is deemed to be without undesirable side effects.
Dosage: Sodium amobarbital (50 mg); N-butyl cyanoacrylate glue (dose appropriate for the diameter of the cerebral AVM).

Considerations: Transarterial embolization of cerebral AVMs has been associated with postprocedural neurologic complications in 7 to 39% of affected cases.
Implementation: Prior to AVM embolization, cases are subjected to cognitive neurologic examinations (n = 12 cases) involving a battery of tests tailored for their specific ability using stimuli selected from standard and experimental cognitive tests to probe specific regions of the brain directly related to the location of the cerebral arteriovenous malformations. Immediately following the administration of sodium amobarbital into each feeder vessel to be embolized, neurologic and cognitive testing is initiated utilizing a tailored battery of stimuli and then the position of the microcatheter tip validated by fluoroscopy. If the provocative test results were negative, the evaluated feeder vessel is then embolized with an injection of N-butyl cyanoacrylate glue.
Interpretation and Analysis: Provocative amobarbital injections often produce negative results (27/29), which implies that subsequent embolization of cerebral AVMs will not produce an undesirable neurologic deficit. A small percentage of cases (e.g. n = 2/29) will experience cognitive deficits during tailored provocative testing with amobarbital injections with the evoked deficits that subsequently resolve when the pharmaceutical dissipates. In such instances, the feeder vessels are usually not embolized. Neurologic and cognitive evaluations are performed after permanent embolization with N-butyl cyanoacrylate glue.

Tailored cognitive testing with provocative amobarbital injection preceding A-VM embolization. Moo LR, Murphy KJ, Gailloud P, Tesoro M, Hart J. AJNR Am J Neuroradiol 2002 Mar; 23 (3), pp. 416–21.

Vascular Malformation (Central Nervous System)/Autonomic Nervous System Dynamic Time-Frequency Analysis
Sodium Amobarbitol/Wada Test

Background Information: Recently, time-frequency analysis has become very popular for examining non-stationary time series and for researching fast-changing phenomena.
Application: Utilization in Wada test techniques involving the injection of sodium amobarbitol into the internal carotid artery, resulting in the inactivation of cerebral structures supplied by the ipsilateral anterior and middle cerebral arteries. The intracarotid instillation of amobarbital can be combined with electrocardiographic analyses to monitor regions and extent of localized CNS anesthesia (electrocardiopharmacology test).
Route of Administration: Amobarbitol (catheter instillation into the internal carotid artery).
Implementation: Utilization of a smoothed-pseudo Wigner-Ville distribution to model the underlying dynamic autonomic nervous system changes induced during intracarotid sodium amobarbital administration (ISA Wada test). Electrocardiogram (EKG) tracing are recorded during the entire procedure and 10 minutes prior to each injection to establish baseline values for a given hemisphere. Interbeat-interval time series are created from such data and then further analyzed (smoothed-pseudo Wigner-Ville distribution to model).

Human Model: Case affected by a vascular malformation within the central nervous system (e.g. n = 1 that is 13-years of age).
Interpretation and Analysis: Review of data generated in the fashion described reveals that an injection of sodium amobarbitol into either side (e.g. cerebral hemisphere) produces decreased power in the vagally mediated high-frequency band (0.14–0.40 Hz). Importantly, the decrease is greater when the right hemisphere is inactivated in contrast to the left side. Such results are consistent with the known lateralized innervation of the heart such that right-sided autonomic input has a greater influence on cardiac chronotropy. Very rapid changes in autonomic control occur that characterize the inactivation and subsequent recovery of the cerebral hemispheres and serve to confirm the utility of time-frequency analysis in the investigation of cardiac time series.

Time frequency analysis of the cardiovascular response during the intracarotid amobarbital test. Sollers JJ 3rd, Ahern GL, Merritt MM, Thayer JF. Biomed Sci Instrum 2002; 38, pp. 267–71.

Vascular Malformation: Hepatic Portosystemic Shunt/Non-Invasive Detection of Portosystemic Shunt Patency/Postoperative Shunt Anastomois Occlusion
D-Xylose/[Urinary Excretion]

Physiological Mechanism of Action: D-xylose is normally assimilated by hepatocytes. In circumstances where the plasma concentration of D-xylose exceeds the renal threshold, significant amounts are excreted into the urinary filtrate.
Application: The D-xylose excretion test appears to be a useful method of monitoring shunt patency after a portasystemic venous anastomsis for portal hypertension.
Implementation: Evaluation of cases of hepatic portosystemic shunt using a D-xylose 0.5-hour urinary excretion test.

Human Model: Cases of hepatic portosystemic shunt.
Interpretation and Analysis: A 5-hour D-xylose urinary excretion test yielding a total estimate greater than 6 grams appears to be an acceptable indicator that a shunt is patent. A second criterion for patency is an increase of 4 grams or more over results observed during preoperative tests. If the excretion rate falls in the postoperative period or remains less than 4–2 grams/5 hours, the anastomosis may be assumed to be occluded.

Assessment of portasystemic shunt patency by estimation of D-xylose excretion. Smith RC, Macpherson AI. Br J Surg 1976 Jun; 63 (6), pp. 435–7.

Vascular Malformation/Recirculation
Glucose

Application: Vascular access recirculation is an important cause of diminished dialysis efficiency. A screening test has been developed based on glucose infusion as a tracer for assessing recirculation.
Implementation: The glucose infusion test (GIT) protocol involves collection of a basal blood sample (A) from the arterial port, followed by injection of a 5 mL bolus of 20% glucose into the venous chamber (time 0). A second sample (B) is collected in 4 seconds (from 13 to 17 s with QB 300 mL/min) from the same port. Blood glucose levels can be determined at the bedside with a reflectance photometer (CV 1.8%).

Human Model: Cases subjected to hemodialysis (e.g. n = 39).
Interpretation and Analysis: Interpretation of the test is straightforward: If B = A, there is no recirculation, whereas if B > A, recirculation can be calculated from the regression equation, $0.046 \times (B - A) + 0.07$, obtained from *in vitro* tests reproducing artificial recirculation at 0, 5, and 10%. To validate the method *in vivo*, comparisons can be made between GIT and the urea test in cases managed by hemodialysis. The degree of correlation is relatively high (r = 0.93). The 2 tests are considered positive (presence of recirculation) when the lower 95% confidence intervals are more than 0. Cases can be divided into 2 groups: those with evidence of recirculation (e.g. n = 22/39 with 11.8% mean recirculation) or

without evidence of recirculation (e.g. n = 17/39; mean 0.06%). The urea test does not recognize approximately 30% of cases (e.g., n = 7/22) because they have a small degree of recirculation below the urea test limit of detection. GIT is more sensitive (detection limit 0.3%), simpler, and immediate in the generation of meaningful results compared to the urea test. It is an accurate and low-cost technique for screening and the follow-up of vascular access in a dialysis unit.

Glucose infusion test: A new screening test for vascular access recirculation. Magnasco A, Alloatti S, Bonfant G, Copello F, Solari P. Kidney Int 2000 May; 57 (5), pp. 2123–8.

Vascular Patency (Vascular Grafts)/Endothelium Response and Dysfunction/Epicardial Response/Coronary Endothelial Function/Microvascular Flow
Acetylcholine/Nitroglycerin

Cellular/Molecular Mechanism of Action: Acetylcholine is a neutrotransmitter that binds to and stimulates parasympathetic (cholinergic) receptor complexes.
Physiological Mechanism of Action: Coronary artery spasm can be induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the endothelium is intact by releasing nitric oxide (NO). In conditions of coronary artery spasm, acetylcholine induces a spasmodic response that can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. Such findings imply that some form of abnormality in endothelial integrity exists in conditions of coronary spasm that can be accentuated by acetylcholine and histamine, and to a lesser by ergonovine and serotonin. In general, all ergot alkaloids exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptor complexes. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentrations. Similarly, the contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.
Background Information: Coronary endothelial dysfunction may be an early marker for cardiac allograft vasculopathy (CAV) in orthotopic heart transplant recipients. Using serial studies with intravascular ultrasound and Doppler flow-wire measurements, it has been demonstrated that annual decrements in coronary endothelial function are associated with progressive intimal thickening.
Application: Assessment of epicardial response, microvascular response, and endothelium-dependent responses that can provide valuable information about the integrity and function of vascular graft transplants.
Route of Administration: Adenosine, acetylcholine, and nitroglycerin infused into the left anterior descending coronary artery.
Dosage: Adenosine (32 μg bolus), acetylcholine (54 μg over 2 minutes), and nitroglycerin (200 μg).
Implementation: Evaluation of endothelial dysfunction as a potential parameter capable of predicting subsequent clinical events, including cardiac death and development of CAV conditions.

Clincial cases can be characterized on a yearly basis initially beginning at the time of transplantation procedures until reaching a prespecified end point determined as angiographic evidence of CAV (> 50% stenosis) or cardiac death (graft failure or sudden death). During each study, coronary endothelial function is measured with the aid of intracoronary infusions of adenosine (32 μg bolus), acetylcholine (54 μg over 2 minutes), and nitroglycerin (200 μg) into the left anterior descending coronary artery; intravascular ultrasound images and Doppler velocities are simultaneously recorded during and after diagnostic challenges.

Human Model: Cases of vascular transplant (n = 73).
Interpretation and Analysis: A modest percentage of cases will reach an end point during the evaluation process (e.g., n = 14; n = 6 CAV; n = 4 deaths including n = 4 known CAV; n = 1 graft failure, and n = 3 sudden death). Case group types with an end point will display decreased epicardial acetylcholine responses (constriction of 11.1 ± 2.9% vs dilation of 1.7 ± 2.2%, p = 0.01) and microvascular acetylcholine responses (flow increase of 75 ± 20% vs 149 ± 16%, p = 0.03), as well as endothelium-dependent responses to acetylcholine compared with cases that do not reach an end point. Responses to adenosine and nitroglycerin do not differ significantly. Endothelial dysfunction detected as an abnormal responses to acetylcholine, precedes the development of clinical end points. These data implicate endothelial dysfunction in the development of clinically significant vasculopathy and suggest that serial studies evaluating endothelial function have clinical utility.

Coronary endothelial dysfunction after heart transplantation predicts allograft vasculopathy and cardiac death. Hollenberg SM, Klein LW, Parrillo JE, Scherer M, Burns D, Tamburro P, Oberoi M, Johnson MR, Costanzo MR. Circulation 2001 Dec 18; 104 (25), pp. 3091–6.

Vascular Perfusion: Coronary Microcirculation/Regulation: Vasodilatory and Perfusion Reserve
Adenosine Phosphate/Colored Microspheres

Cellular/Molecular Mechanism of Action: Colored microspheres variably impede/absorb/reflect ultrasound wave energy forms.
Physiological Mechanism of Action: Adenosine phosphate promotes relaxation of vascular smooth muscle.
Application: Evaluation of microvascular perfusion parameters.
Route of Administration: Adenosine phosphate (intravenous/rat), colored microspheres (intravenous).
Dosage: Adenosine phosphage (3 mg/kg/min).
Implementation: Measurement of perfusion parameters based on perfusion-sensitive T1 relaxation after magnetic spin labeling of water protons. Regional blood volume (RBV) is determined from steady-state measurements of T1 before and after administration of an intravascular contrast agent. A colored microsphere technique can be used as a reference method for establishing perfusion measurements. Regional blood volume and perfusion maps are obtained at rest and during administration.

Animal Model: Rat.
Interpretation and Analysis: At MR imaging, perfusion during resting conditions is 3.5 mL/g/min ± 0.1 (SEM), while RBV is 11.6% ± 0.6 (SEM). Adenosine phosphate significantly increases perfusion to 4.5 mL/g/min ± 0.3 (SEM) and decreases mean arterial pressure from 120 mm Hg to 65 mm Hg, which implies a reduction of coronary resistance to 40% of baseline. Regional blood volume increases consistently to 23.8% ± 0.6 (SEM). Quantitative mapping of perfusion and RBV can therefore be performed noninvasively *in vivo* by means of MR imaging. The presented method allows determination of vasodilation and perfusion reserve, which reflects the *in vivo* regulation of coronary microcirculation for a given stimulus.

Myocardial perfusion and intracapillary blood volume in rats at rest and with coronary dilatation: MR imaging *in vivo* with use of a spin-labeling technique. Waller C, Kahler E, Hiller KH, Hu K, Nahrendorf M, Voll S, Haase A, Ertl G, Bauer WR. Radiology 2000 Apr; 215 (1), pp. 189–97.

Vascular Perfusion: Dermal Microvasculature/ Delineation of Perfused to Total Capillary Ratio
Sodium Fluorescein

Application: Delination of the nature of temporal variation in capillary numbers and determination of the proportion of perfused to total nutritional capillaries within normal skin tissues.
Implementation: Using *in vivo* microscopy, the temporal behavior of the number of visible capillaries in the skin of the dorsum of the foot can be observed over periods of time varying from 5 minutes to 55 days. Capillary perfusion is then studied by comparing capillary numbers before and after intravenous injection of sodium fluorescein.

Human Model: Healthy subjects.
Interpretation and Analysis: The mean percent difference in the number of visible capillaries over a mean period of 25.3 days is 5.5%. The percentage ratio of perfused to total capillaries is 54.2%. There appears to be little quantitative change in capillary numbers over periods of up to 50 days, and under physiological conditions, about half of the nutritional capillaries of the skin are not perfused.

Study of temporal and perfusion physiology of skin capillaries in the dorsum of the foot. Lamah M, Mortimer PS, Dormandy JA. J Vasc Res 2001 Jan-Feb; 38 (1), pp. 59–63.

Vascular Perfusion: Dermal Microvascular (Diabetic Foot Ulcers)
Fluorescein

Background Information: Fluorescein dermofluorometry can be used to relate the uptake of fluorescein in the skin to blood flow.
Application: The dye wash-in time constant parameters are inversely proportional to local dermal blood flow.
Implementation: Evaluation of fluorescein dermofluorometry for the detection and characterization of dermal ulcers. The outcomes of foot ulcers have been documented in cases over a 4–14 month period of time. Following intravenous injection of sodium fluorescein, the change in the fluorescein signal with time is continuously measured at the plantar surface of the foot. Both the initial slope of the signal and the wash-in time constant are then calculated.

Human Model: Cases of cutaneous diabetic foot ulcers (n = 4 groups: non-diabetic control patients, diabetic control patients, diabetic patients with chronic foot ulcers, and diabetic patients with acute foot ulcers).
Interpretation and Analysis: Significant differences in the wash-in time constant can be appreciated between diabetic and non-diabetic conditions and between diabetic subjects with and without foot ulcers. In cases with foot ulcers, a relatively large percent may not display an early washout in the dermofluorometer signal and later require amputation (e.g., n = 2/8 or 25%). The fluorescein wash-in time constant demonstrates better correlation with the presence of diabetic microvascular disease than does the initial slope of the signal. Differences in the wash-in time constants of non-diabetic and diabetic subjects support the hemodynamic hypothesis for the development of microvascular disease. The indication of early washout of the fluorescein signal may also be useful in the prediction of ulcer healing.

Fluorescein dermofluorometry for the assessment of diabetic microvascular disease. Oh DK, Jones RN, Marshall W, Magin RL. Skin Res Technol 2001 May; 7 (2), pp. 105–11.

Vascular Perfusion: Dermal Microvasculature (Diabetic Foot Ulcers)/Ulcers: Dermal Vasomotor Responses in Diabetes Associated Foot Lesions
Sodium Fluorescein

Background Information: A loss of sympathetic function could lead to changes in capillary fluid filtration in diabetic conditions.
Application: Delineation of the association between abnormal capillary leakage with decreases in sympathetically mediated dermal vasomotion responses in diabetic conditions.
Implementation: Evaluation of sensory and motor nerve function of the distal extremities assessed utilizing standard neurography and expressed as a sensory-motor nerve function score. Sympathetic vasomotion of the skin microcirculation is assessed by determining the power of blood flow variability in the low-frequency (0.02–0.14 Hz) band by spectral analysis of laser Doppler flowmetry at the median ankle. Skin capillary leakage is evaluated by sodium fluorescein videodensitometry at the same site over the foot.

Human Model: Cases of diabetic cutaneous foot ulcers (n = 3 matched groups; n = 18 diabetic cases with documented peripheral neuropathy [DN], n = 18 diabetic cases without peripheral neuropathy [D], n = 18 healthy control subjects [C]).
Interpretation and Analysis: Sympathetically mediated vasomotion of foot skin microcirculation is lower in diabetic conditions with documented peripheral neuropathy compared with diabetic conditions without peripheral neuropathy and control subjects ($p < 0.001$). Capillary sodium fluorescein leakage is greater in diabetic cases with documented peripheral neuropathy (n = 18) than in diabetic cases without peripheral neuropathy ($p < 0.02$) and C ($p < 0.005$). Multiple regression analysis reveals that reduced sympathetically mediated vasomotion, together with a lower sensory-motor nerve function score, independently contributes to variances in sodium fluorescein leakage [30% ($p < 0.001$); and 17% ($p < 0.01$), respectively]. A loss of sympathetic tone, apart from sensory-motor nerve dysfunction, seems to be a major determinant of increased capillary permeability in diabetic cases with neuropathy.

Sympathetic mediated vasomotion and skin capillary permeability in diabetic patients with peripheral neuropathy. Lefrandt JD, Bosma E, Oomen PH, Hoeven JH, Roon AM, Smit AJ, Hoogenberg K. Diabetologia 2003 Jan; 46 (1), pp. 40–7.

Vascular Permeability: Capillary/Blood Flow and Arterial Input Function
Indocyanine Green (ICG)/Methylene Blue (MB)/[MRI]

Cellular/Molecular Mechanism of Action: Indocyanine green and MB are pigment-based dyes.
Application: Indocyanine green and MB can be utilized as aqueous soluble molecular marker/indicator systems for the evaluation of various physiological processes.
Implementation: Following administration of indocyanine green and methylene blue, measurements are acquired with a combined frequency-domain and steady-state optical technique that facilitates rapid measurement of tissue absorption in the 650–1000 nm spectral range. Tumors can also be imaged by using contrast-enhanced magnetic resonance imaging and coregistered with the location of the optical probe. The absolute concentrations of contrast agent, oxyhemoglobin, deoxyhemoglobin, and water are measured simultaneously each second for a period of approximately 10 minutes.

Animal Model: Rat (adenocarcinoma).
Interpretation and Analysis: The differing tissue uptake kinetics of ICG and MB in late-stage tumors arise from differences in their effective molecular weights. ICG, because of its binding to plasma proteins, behaves as a macromolecular contrast agent with a lower degree of vascular permeability. A compartmental model describing ICG dynamics is used to quantify physiologic parameters related to capillary permeability. In contrast, MB behaves as a small-molecular-weight contrast agent that leaks rapidly from the vasculature into the extravascular, extracellular space and is sensitive to blood flow and the arterial input function.

In vivo quantification of optical contrast agent dynamics in rat tumors by use of diffuse optical spectroscopy with magnetic resonance imaging coregistration. Cuccia DJ, Bevilacqua F, Durkin AJ, Merritt S, Tromberg BJ, Gulsen G, Yu H, Wang J, Nalcioglu O. Appl Opt 2003 Jun 1; 42 (16), pp. 2940–50.

Vascular Permeability (Endothelial Integrity)/ Endothelial Injury and Viability
Evans Blue Dye Staining

Cellular/Molecular Mechanism of Action: Evans blue dye is a pigment-based reagent that becomes differentially associated with viable vs compromised cell populations (e.g., vascular endothelium).
Physiological Mechanism of Action: Tumor necrosis factor-alpha (TNF-α) soluble receptor (TNFsr) has been shown *in vitro* and *in vivo* to neutralize TNF-mediated biological activity.
Background Information: Tumor necrosis factor-α is expressed locally in arteries at sites of balloon injury. *In vitro* studies have shown that TNF inhibits cell cycle progression and induces apoptosis in endothelial cells.
Application: Evans blue dye staining can be applied to evaluate viable *ex vivo* preparations of vascular structures to determine the integrity of endothelial surfaces.
Implementation: Evaluation of the hypothesis that inhibiting TNF could accelerate endothelial recovery after angioplasty. Preparations of TNFsr vs control IgG can be administered by intraperitoneal injection. De-endothelializing balloon injury can then be created, and the resulting induced lesions evaluated 1 week later for evidence of reendothelialization (Evans Blue dye staining); and then after 2 weeks evaluated for reendothelialization and endothelial function.

Animal Model: Rat (Sprague-Dawling strain).
Interpretation and Analysis: At both time points, blockade of TNF biological activity with TNFsr results in increased reendothelialization, as measured as absolute area and percent area reendothelialized. TNFsr also accelerates functional endothelial recovery that manifests as an increase in nitric oxide (NO) production. Neointimal thickening can also be appreciated as being inhibited. Blockade of TNF *in vivo* accelerates functional endothelial recovery after barotraumatic de-endothelializing injury. Locally expressed TNF therefore acts to inhibit functional endothelial recovery after angioplasty and transient blockade of TNF may improve the long-term success of angioplasty.

Evans Blue Staining: The reagent facilitates detection of a demarcation between viable endothelial cell populations and subendothelial collagen.

In vivo blockade of tumor necrosis factor-alpha accelerates functional endothelial recovery after balloon angioplasty. Krasinski K, Spyridopoulos I, Kearney M, Losordo DW. Circulation 2001 Oct 9; 104 (15), pp. 1754–6.

Vascular Permeability/Extravasation Processes
Evans Blue Dye

Cellular/Molecular Mechanism of Action: Evans Blue dye is a pigment-based reagent that can be detected visually within the visible light range.
Background Information: Oxidative stress appears to be relevant to asthma pathogenesis.
Application: Evans blue dye is a pigment-based reagent that can be used as a visual marker or indicator molecule that is compatible with biological systems. In this context, it is often applied as a dilution marker system within the intravascular compartment for measuring total intravascular compartment size and extravasation of plasma proteins and hematologial cell types into the extravascular tissue space.
Implementation: Application of Evans Blue dye to evaluate extravasation into bronchoalveolar lavage fluid following antigen-induced pulmonary responses and the effectiveness of the antioxidant N-acetylcysteine (oral, 1 mmol/kg/day for 7 days before challenge).

Animal Model: Rat (Brown Norway strain that are antigen pre-sensitized).
Interpretation and Analysis: N-acetylcysteine does not reduce the immediate bronchospasm that follows aerosol antigen exposure but does prevent airway hyperreactivity to 5-hydroxytryptamine at 24 hours after antigen challenge. In the absences of N-acetylcysteine, eosinophil numbers are higher (0.178 ± 0.038) than with the administration of N-acetylcysteine ($0.064 \pm 0.020 \times 10^6$ cells/ml) which is significant ($p < 0.05$) and directly corresponds with concentrations of Evans blue dye extravasated into bronchoalveolar lavage fluid. Taurine levels in bronchoalveolar lavage fluid from antigen-challenged *in vivo* models are higher than control values but treatment with N-acetylcysteine fails to further increase these augmented levels. N-acetylcysteine appears to have beneficial effects in *in vivo* models of experimental asthma and serves to validate related findings pertaining to other models of lung injury.

Effectiveness of oral N-acetylcysteine in a rat experimental model of asthma. Blesa S, Cortijo J, Martinez-Losa M, Mata M, Seda E, Santangelo F, Morcillo EJ. Pharmacol Res 2002 Feb; 45 (2), pp. 135–40.

Vascular Permeability: Retinal/Minimally-Invasive Diagnostic Strategy [Diabetes]
Sodium Fluorescein

Background Information: Diabetic retinopathy is the leading cause of blindness in working-age individuals in the United States. Breakdown of the blood-retinal barrier is one of the earliest events in the progression of diabetic retinopathy. Ideally, therapeutic measures should be directed at this early stage of such conditions, but there are few sensitive, quantitative methods to assess the retinal vascular barrier *in vivo*.
Application: Quantitative assessment of the retinal vascular barrier.
Implementation: Utilization of two fluorescent tracers in combination with fluorescence microangiography to quantitatively assess retinal vascular barrier (± infusion of a hyperosmolar solution of 1.6 M mannitol for 5 minutes).

Animal Model: Rat (Long-Evans strain).
Interpretation and Analysis: Permeability × surface area/flow (PS/F) describing the retinal vasculature is 0.086 ± 0.031 ($n = 13$,

avg. ± SD). Based on estimates of flow and surface area, estimated permeability of sodium fluorescein is approximately 1.2 x 10^{-5} cm/sec. Infusion of a hyperosmolar mannitol solution significantly increases PS/F within individual veins and significantly increases flow-weighted PS/F from 0.073 ± 0.028 to 0.16 ± 0.034 (n = 3). Quantitative *in vivo* assessment of retinal vasculature can be delineated applying an adapted indicator dilution technique. Dual-tracer fluorescence angiography appears to be a sensitive indicator for detecting increases in the blood-retinal barrier produced by hyperosmolar mannitol. The methodology may be a promising new minimally-invasive strategy that may be adapted to quantitatively track retinal vascular permeability.

Instrumentation: Fluorescence microangiography.

Retinal vascular permeability determined by dual-tracer fluorescence angiography. Russ PK, Gaylord GM, Haselton FR. Ann Biomed Eng 2001 Aug; 29 (8), pp. 638–47.

Vasodilation Response Capacity: Echocardiography
Nitroglycerin

Cellular/Molecular Mechanism of Action: Activates guanylate cyclase, thereby increasing the synthesis of guanosine 3′,5′ monophosphate (cGMP) within smooth muscle tissues. In part this is mediated by increasing nitric oxide (NO) production that in turn increases cGMP levels. Ultimately light chain myosin is dephosphorylated, which functions in the contractile process. Analogs of cGMP also relax vascular and bronchial smooth muscles.

Physiological Mechanism of Action: Nitroglycerin promotes primarily venodilation properties at lower dosages compared to its effect on arteriole structures.

Application: Echocardiography/pharmaceutical provocative test procedures.

Route of Administration: Nitroglycerin (multiple/variable).

Implementation: Evaluate the effects of non-ECG-triggered imaging. During ultrasound studies of endothelial function, brachial artery diameters can be measured throughout the cardiac cycle at rest, during reactive hyperemia, and after nitroglycerin administration. R-wave-triggered imaging can be performed using a 7.5 MHz ultrasound transducer with data acquisition every 41.7–66.7 ms. Cardiac cycle-related variation is computed as the maximum percent change from the end-diastolic diameter. The range of possible errors in flow-mediated dilation (FMD) and nitroglycerin-mediated vasodilation that may result from ignoring cyclic variations in diameter are determined for each condition.

Human Model: n = 24 subjects.

Interpretation and Analysis: True FMD, true nitroglycerin-mediated vasodilation, and the maximum and minimum values that can be erroneously calculated for FMD if timing is ignored all differ dramatically ($p < 0.05$). The range of apparent FMD values that can be measured is nearly 3 times the true FMD value. Ignoring temporal position within the cardiac cycle artifactually increases calculated FMD into the normal range, despite truly impaired FMD. Peak arterial dilation occurs before end-systole and greater baseline vessel compliance is associated with greater FMD. Brachial arterial diameters vary significantly throughout the cardiac cycle. The magnitude of this variation is similar to the arterial dilation induced by reactive hyperemia and nitroglycerin, making ECG-triggered imaging mandatory for accurate and reproducible measurements of artery diameters and FMD. Measurement of diameters at end-diastole may be preferred to other time points in the cardiac cycle.

Instrumentation: 7.5 MHz ultrasound transducer.

Effect of cardiac cycle on ultrasound assessment of endothelial function. Chuang ML, Douglas PS, Bisinov EA, Stein JH. Vasc Med 2002 May; 7 (2), pp. 103–8.

CARDIOVASCULAR RESPONSE TESTS

Angina
Dipyridamole/[^{201}Thallium]/Stress Test/[Nuclear Scintigraphy]

Cellular/Molecular Mechanism of Action: Influences the metabolism and transport of adenosine and adenosine nucleotides. In this context, dipyridamole, a nucleotide transport blocker, suppresses the intracellular infux of adenosine, which is released from myocardial tissues that have been deprived of adequate oxygen.

Physiological Mechanism of Action: Dipyridamole promotes diminished coronary artery vascular resistance, coronary artery vasodilation, elevates coronary artery blood flow, and increases coronary artery oxygen delivery. One of the most prominent influences of dipyridamole in this regard is its effect on the smaller diameter vascular bed supplied by the coronary artery, but it has minimal effect in regions of ischemia since these same vessels tend to already be maximally dilated.

Application: Characterization of coronary artery function.

Implementation: Evaluation of conditions associated with effort angina using a series of tests including the ECG stress test, stress [^{201}Tl] scintigraphy, coronary arteriography; [^{201}Tl] scintigraphy, coronary arteriography, hemodynamic echo 2-D, and ecographic monitoring performed during a dipyridamole-induced vasodilation test (D).

Human Model: Cases of cardiac-associated angina. Based on coronary arteriography results with the condition categorized in groups classified as Type-A cases (n = 10 patients with significant stenosis ≥ 50%) or Type-B cases (n = 9 subjects with no significant stenosis).

Interpretation and Analysis: Type-A disease states can present with positive stress ECG and scintigraphy images (e.g., n = 9 cases). The dipyridamole test can induce angor and ecographic changes (e.g., n = 5 cases) and left ventricle wall motion disorders (e.g., n = 4 cases). Usually, [^{201}Tl] scans are positive in the vast majority of Type-A cases (e.g., n = 9/9). Type-B disease states are associated with ecographic changes in only a relatively small number of cases (e.g., n = 2/9), but approximately two-thirds will demonstrate positive [^{201}Tl] scan results (e.g., n = 6/9). In Type-B cases, dipyridamole can produce angor (e.g., n = 2/9); ecographic changes (e.g., n = 1/9); left ventricle wall motion disorders (e.g., n = 1); and similar [^{201}Tl] defects as previously detected. In both case group types, identical coronary arteriography findings can be observed immediately before and during dipyridamole infusion. In this context, dipyridamole confirms provocative tests for ischemia. The same ischemic pattern observed at stress and with dipyridamole scintigraphy in cases with no significant coronary stenosis suggests as pathogenetic mechanism involving a regional lack of dilatory reserve.

Instrumentation: Coronary arteriography; [^{201}Tl] scan, coronary arteriography, hemodynamic, echo 2-D, ecographic monitoring.

Angina Pectoris
Dipyridamole/Stress Test/(^{201}Thallium)/[Nuclear Scintigraphy]

Cellular/Molecular Mechanism of Action: Influences the metabolism and transport of adenosine and adenosine nucleotides. In this context, dipyridamole, a nucleotide transport blocker, suppresses the intracellular influx of adenosine released from myocardial tissues that have been deprived of adequate oxygen.
Physiological Mechanism of Action: Dipyridamole promotes diminished coronary artery vascular resistance, coronary artery vasodilation, elevated coronary artery blood flow, and increases coronary artery oxygen delivery. One of the most prominent influences of dipyridamole in this regard is its effect on the smaller diameter vascular beds supplied by the coronary artery; but it has minimal effect in regions of ischemia since these same vessels tend to already be maximally dilated.
Application: Detection and characterization of alterations in vascular blood flow (e.g. coronary artery)
Implementation: Evaluation of effort-induced angina conditions using a spectrum of challenge or stress test procedures including; (i) ECG stress test, (ii) stress [^{201}Tl] scan, (iii) coronary arteriography; (iv) [^{201}Tl] scan; (v) coronary arteriography; (vi) hemodynamic echo 2-D, (vii) ecographic monitoring during the dipyridamole test (D).
Considerations: Based on coronary arteriography results, cases can be divided into groups. In Group A (10 patients with significant stenoses $\geq$ 50%), stress ecg and scintigraphy are positive in a large percentage of cases (e.g. n = 9/10); while the dipyridamole test induces angor and ecographic changes in many cases (e.g. n = 5 cases) and in left ventricle wall motion disorders (e.g. n = 4). In [^{201}Tl] scan results are usually positive in the vast majority of cases affected by these two conditions (e.g. n = 9/9 cases).

In Group B type cases (n = 9 with no significant stenosis), ecographic changes can be observed in a small percentage of cases (e.g n = 2/9) while [^{201}Tl] scans are positive in a larger percentage of cases (e.g. n = 6/9). Dipyridamol can induce angor (e.g. n = 2 cases), ecographic changes (e.g. n = 1), left ventricle wall motion disorders (e.g. n = 1), and the same [^{201}Tl] defects in previously individualized patients (e.g. n = 6).
Interpretation and Analysis: Identical findings have been observed in both case group types administered dipyridamole during coronary arteriography when results are compared to preinjection images. Some clinicians feel confident that dipyridamole has been confirmed as an effective provocative test for ischemia. Markedly similar ischemic patterns observed with stress and dipyridamole scintigraphy in cases with no significant coronary stenosis suggest a lack of regional dilatory reserve as a pathogenic mechanism.
Instrumentation: Coronary arteriography; [^{201}Tl] scan, coronary arteriography, hemodynamic, echo 2-D, ecographic monitoring.

Dipyridamole test and myocardial scintigraphy with Tl-201 in the diagnosis of myocardial ischemic pathology. Caputo V, De Nardo D, Antolini M, Caiazza F, Pitucco G. Radiol Med (Torino) 1987 May; 73 (5), pp. 390–3.

Angina Pectoris/Flow Velocity: Coronary Artery Left Anterior Descending Branch/Coronary Flow Reserve and Vascular Flow Velocity/Transthoracic Echocardiography Assessement
Dipyridamole/[Adenosine]/Echocardiography

Background Information: There is an increasing need for new, functional, and more quantitative parameters to assess coronary arterial function for the purposes of evidence-based medicine. Coronary arterial function has been widely studied using pharmacological stimulation induced by dipyridamole or adenosine. Coronary flow reserve (CFR), defined as the ratio of pharmacologically-induced hyperemic flow divided by basal flow, has been found to be an important functional index in both the clinical and subclinical stages of cardiovascular diseases.
Application: Assessment of coronary blood flow and flow velocity determined through the combined use of dipyridamole and transthoracic echocardiography (TTE). Such methodologies can also be utilized to evaluate the patency of vascular graft sites.
Route of Administration: Dipyridamole (intravascular infusion).
Implementation: Evaluation and comparison of transthoracic Doppler echocardiography and MRI for measuring left anterior descending coronary artery (LAD) flow velocity and CFR. Additional parameters that can be measured include the time-response curve of dipyridamole infusion using transthoracic echocardiography (TTE).

Human Model: Cases without known abnormalities (n = 10 along with n = 5 normal controls for dipyridamole infusion time response curve measurements).
Interpretation and Analysis: Assessment of blood flow velocity, measured as MDV, PDV, and VTI, generates Pearson's correlation coefficients of 0.88, 0.85, and 0.70, respectively, between flow velocity measurements performed using TTE and MRI. The results indicate that, despite minor differences in LAD diastolic velocities measured by TTE and MRI, the correlation of the LAD diastolic velocities measured using both methods is good and both methods are feasible for measuring CFR. Moreover, TTE has the unique capability of acquiring continuous measurements of LAD flow velocity, which allows assessment of the time-response curve for dipyridamole-induced increase in LAD flow velocity. Such findings indicate that the TTE method may be used in sequential or on-line monitoring of LAD blood flow velocity and therefore can be applied to evaluate the time- or dose-response effects of infused drugs in the coronary circulation of humans.

Correlation of transthoracic Doppler echocardiography and magnetic resonance imaging in measuring left anterior descending artery flow velocity and time-course of dipyridamole-induced coronary flow increase. Koskenvuo JW, Saraste M, Niemi P, Knuuti J, Sakuma H, Toikka JO, Komu M, Järvisalo MJ, Hartiala JJ. Scand J Clin Lab Invest 2003; 63 (1), pp. 65–72.

Angina Pectoris: Ischemic Heart Disease/Neurologic Deficits Secondary to Ischemic Heart Disease
Dipyridamole (DPD)/[Acetazolamide]/[Single Photon Emission Computed Tomography (SPECT)]

Background Information: The diagnosis of transient ischemic attack (TIA) is mainly based on the profile of clinical symptoms. Nuclear medicine techniques have been successful in detecting TIA SPECT instrumentation. Improved sensitivity of the examination using a stress test protocol (e.g., acetazolamide) has been well described in the literature.
Application: Dipyridamole has been used to evaluate various aspects of vascular function and response to specific stimuli.
Implementation: Evaluation of the relationship between transient neurological symptoms provoked by the dipyridamole stress test performed in conditions of ischemic heart disease, and changes in regional cerebral blood flow indicated by brain SPECT imaging.

Human Model: Cases of ischemic heart disease evaluated for evidence of secondary alterations in cerebral vascular perfusion (e.g. n = 100).

Interpretation and Analysis: Intravenous dipyridamole injection can create various forms of transient neurological deficits in 23% of ischemic heart disease cases with a considerably higher incidence than previously reported in the literature (1.22/10,000). The so-called "TIA positive" cases can subsequently be examined further using the combination of dipyridamole stress test and brain SPECT imaging.

Dipyridamole test in the early detection of cerebrovascular disorders? Molnár T, Zámbó K, Schmidt E, Tekeres M. Orv Hetil 2000 Dec 10; 141 (50), pp. 2717–22.

Angina Pectoris: Vasodilatory Response Evaluation/ Arterial Graft Vasodilatory Function/Post-Operative Angina

Acetylcholine/Nitroglycerin/[Angiography Adjunct Diagnostic Pharmaceutical]

Cellular/Molecular Mechanism of Action: Acetylcholine binds to and activates its respective postsynaptic membrane receptor complexes. As a neurotransmitter, it is released from postganglionic parasympathetic axons, preganglionic axons, somatic motor axons, and specific CNS synapses.

Acetylcholine: Serves as a neutrotransmitter that influences the function or activation of tissues and organs innervated by the parasympathetic autonomic nervous system in addition to autonomic ganglionic cells (parasympathetic and sympathetic) and cells in the adrenal medulla. Acetylcholine primarily promotes peripheral and pulmonary vasodilation (muscarinic receptors), decreases in heart rate, and suppresses contractile forces (primarily atrial effect). Increased vasodilation causes declines in blood pressure and, therefore, a reflex tachycardia, but acetylcholine is believed to exert a minimal effect at the level of the coronary artery. Bradycardia is observed at the higher range of the Ach dose spectrum.

At the level of the heart, acetylcholine binds to and activates postsynaptic receptor complexes associated with parasympathetic cholinergic synapses found at high densities within the S-A node, A-V node, and atrial tissues. Acetylcholine decreases the heart rate by promoting a hyperpolarization effect, thereby suppressing the frequency of spontaneous S-A node depolarization during diastole (delays action potential reaching threshold potential). Acetylcholine decreases the intensity of atrial contraction and shortens atrial action potential duration in a manner that accentuates atrial flutter and atrial fibrillation induced by increased vagal tone. Alternatively, acetylcholine slows conduction and increases the duration of the refractory period within the A-V node and, to a lesser degree, the Purkinje system. The increase in the duration of the refractory period produced by acetylecholine can decrease the initiation of aberrant atrial impulses transmitted to the ventricles. The negative influence that acetylcholine has on ventricular heart rate is much less that its effect on atrial function, but it does diminish Purkinje system automaticity. In this regard, acetylcholine can diminish or inhibit adrenergic-mediated cardiac stimulation. The latter effect is due to a direct suppression of myocardium adrenergic responsiveness and diminished release of norepinephrine from presynaptic membranes of the autonomic nervous system.

Nitroglycerin: Induces both venous and arterial vasodilation. Relatively selective venous vasodilation can be achieved at the lower dosage ranges of nitroglycerin, thereby promoting declines in end-diastolic left and right ventricular pressures. Such changes are more pronounced than are observed changes in peripheral arterial pressure. Cardiac output is decreased in normal subjects and cases with coronary artery disease when nitroglycerin is given by the sublingual route of administration. High-dose nitroglycerin administered rapidly causes declines in both systolic and diastolic pressures accompanied by lower blood pressure levels, pallor, weakness, dizziness, and initiation of reflex sympathetic-mediated responses (tachycardia, arteriolar vasoconstriction). Transient increases in coronary blood flow (coronary vasodilation) and declines in arterial blood pressure contribute to decreases in cardiac output. Sublingual administration can therefore occasionally produce a significant hypotensive episode.

Background Information: Cigarette smoking is known to promote endothelial dysfunction, thus it can be responsible for an impaired "late" endothelium-dependent vasomotility in arterial grafts after coronary surgery.

Application: Evaluation of endothelial function in the left internal thoracic artery after coronary artery bypass surgery and the identification of predictors or indicators that detect or reflect early dysfunction. Other uses include provocative testing in cases that have undergone routine postoperative coronary angiography.

Route of Administration: Acetylcholine and nitroglycerin (selective intravascular infusion).

Dosage: Acetylcholine (10^{-6} mmol/ml) and nitroglycerin (500 μg).

Considerations: Acetylcholine is rapidly degraded enzymatically by acetylecholineesterase (AchE) and butyrylcholinesterase within the intravascular compartment. Since acetylcholine is a quarternary ammonium compound that is rapidly metabolized within the plasma, very little of this neurotransmitter penetrates the CNS at physiologically significant concentrations. At excessively high doses, acetylcholine can promote a positive effect on cardiac contractility mediated through the release of catecholamine.

Implementation: Evaluation of vascular grafts utilizing vasoactive agents in concert with performing quantitative angiography procedures (long-term follow-up mean time 2.5 years). To assess both endothelium-dependent and independent vasomotility, angiograms can be acquired before and after selective infusions of acetylcholine and nitroglycerin. The predictive value of risk factors, including previous and continued smoking, for impairment of endothelium-dependent vasomotility can also be assessed.

Human Model: Cases previously having undergone coronary bypass surgery utilizing the internal thoracic artery ($n = 20$ with mean age = 64.5 years).

Interpretation and Analysis: The thoracic artery shows a 6.8% ($p < 0.001$) and 9% ($p < 0.001$) increase in mean diameter after acetylcholine and nitroglycerin administration, respectively. Vasodilation responses to acetylcholine and nitroglycerin administration strongly correlate with one another (R: 0.88; $p < 0.001$). Only the common risk factor of age is associated with impairment in the vasodilation response of the arterial graft ($p = 0.001$), and acetylcholine-induced vasodilation is inversely correlated with age (R: 0.69; $p < 0.001$).

Angina following a coronary intervention may be due to vasospasm rather than restenosis. Some cases experience angina following a previously successful recanalization (e.g., $n = 2$ cases). Usually, vasospasm can be documented as the cause of angina (e.g., $n = 2/2$). Validation of this concept can be determined through the use of an ergonovine provocative test or the evaluation of lesion response to nitroglycerin. Rather than another intervention, vasodilator treatment is sometimes instituted, accompanied by effective symptom amelioration.

The digitalis cardiac glycosides promotes increased vagal tone on the function of the heart.

Cholinergic Agonists. Taylor P (Chapter 5: pp. 100–9). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman

LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Continued cigarette smoking after coronary artery bypass surgery reduces endothelium-dependent vasodilation in internal thoracic artery grafts. Amoroso G, Mariani MA, Tio RA, Grandjean JG. Ital Heart J 2001 Feb; 2 (2), pp. 139–41.

Angina Pectoris: Vasospastic/Presynaptic Myocardial Sympathetic Abnormality

Ergonovine/[^{123}I]Iodine-Labelled Metaiodobenzylguanidine (MIBG)/[Nuclear Scintigraphy]

Cellular/Molecular Mechanism of Action: Ergonovine promotes arterial wall smooth muscle contraction. [^{123}I] Iodine-labelled metaiodobenzylguanidine (MIBG) functions as an analog of norepinephrine.

Application: Assess the presence and location of presynaptic myocardial sympathetic abnormalities in conditions of vasospastic angina.

Route of Administration: [^{123}I]-labelled metaiodobenzylguanidine (MIBG) given IV. Ergonovine is administered by intracoronary infusion (ICA).

Dosage: ^{123}Iodine-labelled metaiodobenzylguanidine (MIBG; 111 mBq).

Implementation: Evaluation of conditions of suspected vasospastic angina utilizing single photon emission computed tomography (SPECT) facilitated by the use of MIBG (image agent) and ergonovine provocative challenge. Such diagnostic strategies permit the non-invasive detection and evaluation of suspected vasospastic angina events.

Human Model: Cases of suspected vasospastic angina pectoris (e.g. n = 50) previously evaluated by coronary artery angiography following intracoronary artery ergonovine infusion, with a 99–100% obstructive spasm defined as a positive response.

Interpretation and Analysis: Images aquired 20 minutes and 3 hours following the administration of 111 mBq [^{123}I]-MIBG SPECT can reveal mild reductions in tissue uptake (e.g. n = 5/25). In the majority of the suspect cases, images at 3 hours often demonstrate [^{123}I]-MIBG SPECT abnormalities (e.g. n = 20/25). The location of [^{123}I]-MIBG abnormalities can be completely or partially consistent with the spastic coronary territory (e.g. n = 18/25). Alternatively, reduced [^{123}I]-MIBG uptake may be noted in cases with a negative ergonovine provocative test (e.g. n = 4/25). Accordingly, positive and negative predictive values of MIBG SPECT for the provocative test have been estimated to be 83% (e.g. n = 20/24) and 81% (e.g. n = 21/26), respectively.

Instrumentation: Single photon emission computed tomography.

Value of iodine-123 metaiodobenzylguanidine scintigraphy in patients with vasospastic angina. Taki J, Yasuhara S, Takamatsu T, Nakajima K, Tatami R, Ishise S, Matsunari I, Takayama T, Tonami N. Eur J Nucl Med 1998 Mar; 25 (3), pp. 229–34.

Angina Pectoris: Vasospastic (Coronary Artery Tone)

Acetylcholine/Ergonovine/Nitroglycerin/[Echocardiography]

Cellular/Molecular Mechanism of Action:

Acetylcholine: Binds directly to and positively activates acetylcholine receptor complexes located on the surface of postsynaptic membranes.

Ergonovine: Directly binds and positively but not maximally activates tryptaminergic and a-adrenergic receptor complexes but to a lesser extent than ergotamine. At dopaminergic receptor complexes, ergonovine functions as a weak antagonist. At both tryptaminergic and dopaminergic receptor sites, ergonovine can exert both partial agonist and antagonist properties within certain regions of the central nervous system (CNS). In various smooth muscle tissues, the compound exerts antagonist properties.

Physiological Mechanism of Action:

Nitroglycerin: Induces both venous and arterial vasodilation. Relatively selective venous vasodilation can be achieved at the lower dosage ranges of nitroglycerin, thereby promoting declines in end-diastolic left and right ventricular pressures. Such changes are more pronounced than are the changes in peripheral arterial pressure. Cardiac output is decreased in normal subjects and cases with coronary artery disease when given nitroglycerin by the sublingual route of administration. High-dose nitroglycerin administered rapidly causes declines in both systolic and diastolic pressures accompanied by lower blood pressure levels, pallor, weakness, dizziness, and initiation of reflex sympathetic-mediated responses (tachycardia, arteriolar vasoconstriction). Transient increases in coronary blood flow (coronary vasodilation) and declines in arterial blood pressure contribute to decreases in cardiac output. Sublingual administration can therefore occasionally produce a significant hypotensive episode.

Ergonovine: Vasoconstriction can be induced affecting both venous and arterial vascular structures through direct stimulation of vascular smooth muscle. Systemic administration produces elevations in blood pressure due to peripheral vasoconstriction. Ergonovine can produce coronary artery vascular responses that are detectable as ischemic patterns on EKG tracings and angina pain in cases with coronary artery disease. Bradycardia is often induced by ergot alkaloids even when no elevations in blood pressure are evident, due in part to increased vagal activity and, to a lesser extent, diminished CND sympathetic tone and direct-acting myocardial depression.

Application: Detection and characterization of vasospastic angina. Coronary artery spasm can be induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the endothelium is intact by releasing nitric oxide (NO). In conditions of coronary artery spasm, acetylcholine induces a spasmodic response that can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. Such finding imply that some form of abnormally in endothelial integrity exists in conditions of coronary spasm that can be accentuated by acetylcholine and histamine, and to a lesser extent by ergonovine and serotonin. In general, all ergot alkaloids exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptor complexes. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentrations. Similarly, the contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.

Dosage: Nitroglycerin (0.3 mg sublingual)

Considerations: Basal tone of the left main trunk is elevated in the early morning in vasospastic angina. The definite diagnosis of vasospastic angina is done by a coronary spasm provocative test using ergonovine maleate or acetylcholine during cardiac catheterization. Current non-invasive, non-pharmacologic diagnostic methods are not sensitive enough for the diagnosis of vasospastic angina.

Implementation: Evaluation of the capacity for nitroglycerin to detect and characterize conditions of vasospastic angina pectoris conditions.

One effective approach has been the characterization of the condition at 7 A.M. and at noon on the same day involving measurement of the left main coronary artery trunk diameter by M-mode echocardiography before and after sublingual nitroglycerin administration. Parameters that are calculated include the extent of dilation as a reflection of the degree of coronary artery tone present.

Human Model: Cases with an angiographically normal left main trunk (n = 38). Cases can be classified into 4 groups based on the presence or absence of more than 50% stenosis in the coronary arteries except for the left main trunk and responses the acetylcholine or ergonovine provocative tests.

Interpretation and Analysis: The percent dilation of the left main trunk diameter induced by sublingual nitroglycerin at 7 A.M. and at noon is 22.4 ± 4.7% (mean ± SD) and 18.1 ± 4% in cases with vasospastic angina and without coronary stenosis (e.g. n = 11), 14.9 ± 7.1% and 11.2 ± 6.9% in cases with vasospastic angina and coronary stenosis (e.g. n = 9), 6.1 ± 3.5% and 7.0 ± 5.1% in cases without vasospastic angina but with coronary stenosis (e.g. n = 8), and 8.1 ± 5.6% and 7.8 ± 5.7% in control subjects (e.g. n = 10). The percent dilation at 7 A.M. is significantly greater in case groups with vasospastic angina without coronary stenosis than in the remaining 3 case group types, and in the vasospastic angina groups, the percent dilation at 7 A.M. is significantly greater than that at noon. When percent dilation at 7 A.M. exceeding 15% is defined as positive for the diagnosis of vasospastic angina, the sensitivity is 80% and the specificity 94%.

A new noninvasive method of diagnosing vasospastic angina based on dilation response of the left main coronary artery to nitroglycerin as measured by echocardiography. Morita H, Ohmori K, Matsuyama T, Mizushige K, Matsuo H. J Am Coll Cardiol 1996 May; 27 (6), pp. 1450–7.

Aortic Stenosis: Left Ventricular Contractile Reserve/Cardiac Index/Operative Mortality Rate: Prediction Parameters/Long-Term Survival Predictive Markers
Dobutamine/[Doppler Ultrasound]

Background Information: The prognostic value of dobutamine stress hemodynamic data in the setting of low-gradient aortic stenosis has been addressed in small, single-center studies. Larger studies are needed to define the criteria for selecting the patients who will benefit from valve replacement.

Application: Dobutamine stress Doppler hemodynamics may be factored into the risk-benefit analysis for cases of aortic stenosis.

Implementation: Evaluation of aortic stenosis conditions using dobutamine stress test protocols in combination with Doppler imaging analysis.

Human Model: Aortic stenosis (n = 136 cases; n = 96 males [median age = 72 years; range 65–77 years]; median aortic valve = 0.7 cm^2 [range = 0.6 to 0.8]; mean transaortic gradient = 29 mm Hg [range = 23–34 mm Hg]; cardiac index = 2.11 L × min^{-1} × m^{-2} [range, 1.75 to 2.55 L × min^{-1} × m^{-2}]).

Interpretation and Analysis: Left ventricular contractile reserve on the dobutamine stress Doppler study can be detected in a large number of cases (e.g., n = 92/136; and absent in n = 44/136). Operative mortality is relatively low (e.g., n = 3/64 or 5%) in cases with left ventricular contractile reserve. Alternatively, the operative mortality rate is significantly higher in cases without evidence of left ventricular contractile reserve (e.g., n = 10/31 or 32%; p = 0.0002). Predictors for operative mortality include a lack of contractile reserve (odds ratio, 10.9; 95% confidence interval [CI], 2.6 to 43.4; p = 0.001) and a mean transaortic gradient ≤ 20 mm Hg (odds ratio, 4.7; 95% CI, 1.1 to 21.0; p = 0.04). Predictors for long-term survival are valve replacement (hazard ratio, 0.30; 95% CI, 0.17 to 0.53; p = 0.001) and left ventricular contractile reserve (hazard ratio, 0.40; 95% CI, 0.23 to 0.69; p = 0.001). In the setting of low-gradient aortic stenosis, surgery seems beneficial for most cases with left ventricular contractile reserve.

Low-gradient aortic stenosis: Operative risk stratification and predictors for long-term outcome: A multicenter study using dobutamine stress hemodynamics. Monin JL, Quéré JP, Monchi M, Petit H, Baleynaud S, Chauvel C, Pop C, Ohlmann P, Lelguen C, Dehant P, Tribouilloy C, Guéret P. Circulation 2003 Jul 22; 108 (3), pp. 319–24.

Aortic Stenosis: Aortic Valve Replacement/Coronary Flow Reserve
Dipyridamole/[Angiography]

Physiological Mechanism of Action: Dipyridamole induces vasodilatory responses.

Background Information: In cases with aortic stenosis and a normal coronary angiogram, coronary flow reserve (CFR) is impaired.

Application: Examine the effect of aortic valve replacement (AVR) on CFR after a long-term follow-up

Route of Administration: Dipyridamole (intravascular).

Dosage: Dipyridamole (0.56 mg/kg for 4 minutes).

Implementation: Evaluation of CFR measurements 123 ± 137 days before and 497 ± 167 days after AVR (e.g., n = 21 cases). Coronary flow reserve measurements are performed according to a standardized protocol, with a vasodilator stimulus (e.g. dipyridamole) and peak diastolic velocity measurements recorded at 6 minutes.

Human Model: Cases of aortic stenosis with normal coronary angiogram images (n = 30).

Interpretation and Analysis: The initial average peak gradient of aortic stenosis is 89.5 ± 22.4 mm Hg. After AVR, it decreases to 26.2 ± 9 mm Hg. Left ventricular mass is significantly lower after AVR: 354.9 ± 107.9 g vs 223.8 ± 73.6 g (p < 0.001). The average baseline diastolic velocity measured by pulsed Doppler in the left anterior descending coronary artery amounts to 62.2 ± 25.5 cm/s before and 40.1 ± 13.6 cm/s after AVR. The difference is statistically significant (p < 0.01). The average diastolic velocity at maximum stress equals 117 ± 42.8 cm/s preoperatively, and 91.5 ± 34 cm/s postoperatively (p < 0.005). Calculated CFR before AVR is 1.96 ± 0.5 and increases to 2.37 ± 0.8 postoperatively. The difference is statistically significant (p < 0.05). Prosthetic AVR is of considerable benefit concerning the CFR in cases with a normal coronary angiogram after a long-term follow-up evaluation.

The effect of aortic valve replacement on coronary flow reserve in patients with a normal coronary angiogram. Nemes A, Forster T, Kovács Z, Thury A, Ungi I, Csanády M. Herz 2002 Dec; 27 (8), pp. 780–4.

Atherosclerotic Plaque Deformation: Ultrasound Visualization
Acetylcholine/Isosorbide Dinitrate (Vasodilation)/[Ultrasound]

Cellular/Molecular Mechanism of Action: Acetylcholine functions as a parasympathetic/cholinergic neurotransmitter by physically binding to its corresponding post-synaptic membrane receptor complex.

Physiological Mechanism of Action: Isosorbide dinitrate (vasodilation).

Application: Visualize ultrasonically deformations of atherosclerotic plaques in human coronary arteries during vasoconstriction and vasodilation responses.
Route of Administration: Acetylcholine (selective intravascular); isosorbide dinitrate (intracoronary injection).
Considerations: The cross-sectional area of the atherosclerotic plaque appeared to be constant during vasomotion of human coronary arteries.
Implementation: Evaluation of the "pliability" of atherosclerotic plaque following vasoactive responses induced by acethylcholine and isosorbide dinitrate injection.
Interpretation and Analysis: Intravascular ultrasound can detect occult atherosclerosis in angiographically normal coronary arteries in instances of chest pain at rest (n = 8). During the acetylcholine provocative tests, intravascular ultrasound can be used to monitor deformations of the atherosclerotic plaques. During the last phase of the test, vasodilation can be promoted with intracoronary injection of isosorbide dinitrate. Under control, acetylcholine-treated, and isosorbide dinitrate-treated conditions, the cross-sectional areas of sonolucent circle and vessel lumen can be measured in order to establish the area of atherosclerotic plaques. In the process of vasoconstriction and vasodilation, the plaque area does not change significantly.

Intravascular ultrasonic evidence by constant cross-sectional area of atherosclerotic plaques during coronary vasomotion in humans. Matsu-ura Y, Kijima Y, Hashimura K, Kato Y, Ueda T, Yasuda T, Mori I, Hoshida S, Hori M. Eur Heart J 1997 Jun; 18 (6), pp. 949–53.

Cardiac Failure: Chronic/Inotropic Activity/Cardiac Output/Pressor Effect/Inotropic Agent Selection
Dobutamine/Carvedilol/Metoprolol/ [Echocardiography]

Cellular/Molecular Mechanism of Action: Dobutamine functions as a positive adrenergic (sympathomimetic) agent. Metoprolol is a selective β_1 adrenoreceptor antagonist (blocker), while carvedilol is a non-selective β-adrenoreceptor blocker.
Physiological Mechanism of Action: Dobutamine promotes positive cardiac inotropic responses and can also promote increases in cardiac output and elevations in systemic blood pressure.
Application: Adjunct form of diagnostic intervention during stress echocardiography procedures thereby functioning as a guide when choosing a (therapeutic) inotropic agent.
Route of Administration: Dobutamine (intravenous).
Dosage: Carvedilol and metoprolol CR/XL (target dose 50 mg twice daily and 200 mg once daily, respectively) for 8 weeks. Dobutamine (5 μg/kg/min and high-dose 15 μg/kg/min).
Implementation: Evaluation of carvedilol and metoprolol CR/XL as potential adjunct forms of intervention during dobutamine-stress echocardiography procedures performed at the end of each maintenance period. Parameters evaluated include determining if conditions of congestive heart failure display different hemodynamic responses during dobutamine challenge in the presence of beta adrenoreceptor blocking agents.

Model: Cases with stable chronic congestive heart failure (ejection fraction < 40%) on chronic treatment with metoprolol CR/XL (e.g. n = 10). Single center, single blind, randomized, 2-period crossover study comparing carvedilol with metoprolol CR/XL.
Interpretation and Analysis: No significant hemodynamic differences can be seen at rest after 2 treatments. There is a more pronounced increase in heart rate and cardiac output during dobutamine infusion when cases are given metoprolol in contrast to carvedilol. Mean arterial pressure increases significantly with carvedilol. Cardiac output increases during low-dose dobutamine, without further changes being appreciated with high-dose dobutamine administration. During infusions of dobutamine, there is no significant difference in ejection fraction between carvedilol and metoprolol treatment. Therefore, differences in response to the inotropic drug dobutamine can be appreciated in conditions of congestive heart failure managed with a non-selective β-adenoreceptor blocker compared to β-selective blocker administration. Selective β_1 blockade with metoprolol can be counteracted by dobutamine, whereas with carvedilol a low dose of dobutamine increases cardiac output and a higher dose of dobutamine cause a pressor effect. Such findings may be clinically relevant when choosing an inotropic pharmaceutical agent.

Different responses to dobutamine in the presence of carvedilol or metoprolol in patients with chronic heart failure. Bollano E, Täng MS, Hjalmarson A, Waagstein F, Andersson B. Heart 2003 Jun; 89 (6), pp. 621–4.

Cardiomyopathy: Dilated Cardiomyopathy (DCM) and Coronary Vasospasm/Response Prediction: Antispasmatic Agents/Atrial Fibrillation as an Indicator for DCM
Ergonovine/[Echocardiography]

Cellular/Molecular Mechanism of Action: Coronary artery spasm can be induced by acetylcholine, serotonin, ergonovine, or histamine, all of which cause vasodilation when the endothelium is intact by releasing nitric oxide (NO). In conditions of coronary artery spasm, acetylcholine induces a spasmodic response that can be promptly relieved by nitroglycerin, which promotes vasodilation through a direct action on vascular smooth muscle. Such finding imply that some form of abnormally in endothelial integrity exists in conditions of coronary spasm that can be accentuated by acetylcholine and histamine, and to a lesser by ergonovine and serotonin. In general, all ergot alkaloids exert their biological effect as partial agonists or antagonists at adrenergic, dopaminergic, and tryptaminergic receptor complexes. Effects of full agonists like norepinephrine are usually augmented by low ergot alkaloid concentrations. Similarly, the contractile response to agents like acetylcholine can sometimes be augmented by low ergot alkaloid concentrations.
Background Information: The pathogenesis of dilated cardiomyopathy (DCM) is unknown, but clinical evidence suggests that coronary vasospasm is associated with the development of DCM in some cases.
Application: Detection and characterization of alterations in vascular function.
Route of Administration: Ergonovine (cardiac catheterization).
Implementation: Evaluate and profile the [i] prevalence of coronary vasospasm in DCM conditions; [ii] characteristics of cases with DCM and coronary vasospasm; and [iii] effects of antivasospastic agents on cases with DCM and coronary vasospasm utilizing the ergonovine provocation testing

Human Model: Dilated cardiomyopathy cases (n = 18). Approximately one-third of cases experience coronary vasospasm, thereby requiring secondary administration of antivasospastic agents following cardiac catheterization.
Interpretation and Analysis: A diagnoses of coronary vasospasm can be suspected if ergonovine induces coronary vasoconstriction (≥ 75% diameter narrowing) compared to the diameter after nitroglycerin administration. The prevalence of atrial fibrillation in cases with DCM and coronary vasospasm is greater than that in DCM without coronary vasospasm (67 vs 8% [$p < 0.05$]). The left

ventricular end-diastolic dimension decreases from 61 mm (range 56 to 64) to 55 mm (range 53 to 56) (median, 25th/75th percentile, $p < 0.05$) and the left ventricular ejection fraction increases from 36% (range 32 to 40) to 47% (range 46 to 48) (median, 25th/75th percentile, $p < 0.05$) after the administration of antivasospastic agents ($n = 4/6$ cases improve symptomatically). Ergonovine provocation testing is useful in identifying conditions of DCM and coronary vasospasm, in whom cardiac performance is expected to be improved with antivasospastic agent therapy. DCM patients with atrial fibrillation may be a clue for identifying patients with coronary vasospasm.

Effects of antivasospastic agents in Japanese patients with dilated cardiomyopathy and coronary vasospasm. Nishi I, Ilda K, Kawano S, Masumi T, Fumikura Y, Ohtsuka S, Watanabe S, Yamaguchi I. Jpn Heart J 2002 Jul; 43 (4), pp. 333–42.

Cardiomyopathy: Dilated Cardiomyopathy (DCM)/Left Ventricular Ejection Fraction (LVEF)/Predict Improved Cardiac Sympathetic Nerve Activity/Predict Improved Cardiac Function/Predict Improved Symptoms Posttreatment for Dilated Cardiomyopathy
Dobutamine/[^{123}I]-Meta-Iodobenzylguanidine/[Nuclear Scintigraphy]

Cellular/Molecular Mechanism of Action: [^{123}I]-meta-iodobenzylguanidine functions as an analog of norepinephrine.
Physiological Mechanism of Action: Dobutamine exerts adrenergic/sympathomimetic properties.
Route of Administration: Dobutamine (intravascular).
Dosage: Dobutamine (5, 10, and 15 μg/kg/min).
Implementation: Evaluation of dilated cardiomyopathy conditions by measuring left ventricular ejection fraction (LVEF) at rest and in response to gradient dose increases in dobutamine. Infusions of dopamine can be performed before initiation of therapy in conditions of DCM according to protocols for dobutamine gated blood pool scintigraphy (DOB-GBP). Examinations are performed before and after 1 year of therapeutic intervention. The heart/mediastinum count (H/M) ratio and total defect score (TDS) are determined for [^{123}I]-meta-iodobenzylguanidine images from anterior planar image and single photon emission computed tomography (SPECT) images. Left ventricular ejection fraction and left ventricular end-diastolic dimension (LVDd) are determined by echocardiography.

Human Model: Cases of dilated cardiomyopathy ($n = 22$).
Interpretation and Analysis: After 1 year of treatment, echocardiographic LVEF improves $> 5\%$ (e.g., $n = 11/22$ cases). Before treatment, TDS, H/M, LVEF, and LVDd are similar in both groups. However, there is a greater increase in the LVEF during dobutamine infusion in cases displaying improvement compared to those that do not improve ($21 \pm 8\%$ vs $9 \pm 3\%$, $p < 0.001$). If a critical value of 15% for the DeltaLVEF is used to predict the improvement in LVEF after treatment, sensitivity is 91% and specificity is 82%. The TDS, H/M ratio, LVDd, and New York Heart Association functional class improves in cases demonstrating a post 1–year response that is greater than comparable values for cases that do not demonstrate a response. Dobutamine gated blood pool scintigraphy therefore can be used to predict improved cardiac sympathetic nerve activity, cardiac function, and symptoms after treatment in DCM conditions.

Dobutamine gated blood pool scintigraphy predicts the improvement of cardiac sympathetic nerve activity, cardiac function, and symptoms after treatment in patients with dilated cardiomyopathy. Kasama S, Toyama T, Hoshizaki H, Oshima S, Taniguchi K, Suzuki T, Kurabayashi M. Chest 2002 Aug; 122 (2), pp. 542–8.

Cardiomyopathy: Idiopathic Dilated Cardiomyopathy (IDCM)/Prognostic Parameters for Mortality and Survival
Dobutamine/Low-Dose Dobutamine Echocardiography/[Echocardiography]

Physiological Mechanism of Action: Dobutamine exerts adrenergic agonist properties.
Background Information: Dobutamine echocardiography is widely used to detect and measure myocardial contractile reserve.
Application: Determine the response of the left ventricle to low-dose dobutamine infusion, which adds clinically valuable prognostic information for the evaluation of IDCM.
Dosage: Dobutamine (10 μg/kg/min for 5 minutes).
Implementation: Evaluation of cardiomyopathy conditions as a function of measurements of 2-dimensional and Doppler echocardiographic variables before and after the infusion of dobutamine.

Human Model: Cases suffering from cardiovascular disease (e.g., $n = 77$ IDCM cases; age $= 49 \pm 9$ years; men $= 82\%$) with left ventricular (LV) ejection fractions of $< 40\%$. Study population: $n = 30/77$ died; $n = 5/77$ successful heart transplant over a 63 ± 7-month follow-up period (range, 49 to 75 months).
Interpretation and Analysis: Using multivariate regression analysis, the only significant factors related to fatal outcome or the need for cardiac transplantation were the following: (i) LV end-systolic volume of > 150 mL after low-dose dobutamine infusion (odds ratio [OR], 2.2; confidence interval [CI], 1.2 to 4.1; $p = 0.011$); (ii) no decrease of LV end-diastolic volume after dobutamine infusion (OR, 1.9; CI, 1.1 to 3.4; $p = 0.031$); (iii) atrial fibrillation (OR, 2.7; CI, 1.4 to 5.3; $p = 0.003$); and (iv) male gender (OR, 2.6; CI, 1.2 to 5.5; $p = 0.017$). A scoring system has been proposed with 1 point assigned for each of the above-mentioned factors. The mortality rates for total scores of 0, 1, 2, 3, and 4 were 0%, 19%, 48%, 83%, and 100%, respectively. The response of the LV to low-dose dobutamine infusion adds clinically valuable prognostic information to the evaluation of the patient with IDCM.

Prognostic value of low-dose dobutamine echocardiography in patients with idiopathic dilated cardiomyopathy. Drozdz J, Krzemińska-Pakula M, Plewka M, Ciesielczyk M, Kasprzak JD. Chest 2002 Apr; 121 (4), pp. 1216–22.

Cardiomyopathy: Idopathic Dilated Cardiomyopathy (IDCM)/Contractile Reserve (CR)/ Cyclic Variation/Prognostic Indicator
Dobutamine/[Echocardiography]

Cellular/Molecular Mechanism of Action: Dobutamine possesses adrenergic agonist properties.
Background Information: Conventional resting echocardiography is not able to predict the CR of cases with IDCM.
Application: Dobutamine has been utilized in combination with ultrasonic myocardial texture analysis in IDCM for the purpose of discriminating cases with and without preserved CR and cases with an unfavorable prognosis.
Route of Administration: Dobutamine infusion (intravenous).
Dosage: Dobutamine infusion (10 μg/kg/min).
Implementation: Myocardial texture analysis can be performed on echocardiographic digitized images in conditions of IDCM through a calibrated 256 gray level digitization system. Cyclic variation (CV) index of myocardial mean gray level (MGL) is calculated

according to the formula ($MGL_{diast} - MGL_{syst}$)/ $MGL_{diast} \times 100$. Contractile reserve is defined as the %-change of ejection fraction by 10 μg/kg/min dobutamine infusion. A clinical follow-up is also performed for all cases for an average of 8 ± 3 months.

Human Model: Idiopathic dilated cardiomyopathy (IDCM n = 27 cases).

Interpretation and Analysis: Contractile reserve ranges between −1.8% and 50.3%. Cyclic variation index of both septum and posterior wall (PW) is the parameters that most significantly correlates with CR ($r = 0.69$ and $r = 0.77$, respectively, $p < 0.0001$ for both). The mean resting CV index of septum and PW are significantly lower in cases with CR < 17% median value of all subjects (5.7 ± 5.6 vs 16.9 ± 7.9 and 5.4 ± 5.9 vs 16.1 ± 6.4, respectively, $p < 0.0001$ for both). A CV index of 10% for both septum and PW yields a sensitivity of 77% and 84%, and a specificity of 84% and 84% for predicting diminished CR, respectively. The event-free survival rate is significantly lower in patients with CR less than 17% (61.5 vs 92.8%, $p < 0.05$). The CV index of both septum and PW are also significantly lower in cases with cardiac events (13.6 ± 8 vs 4.3 ± 6, $p < 0.05$ and 14.7 ± 7 vs 4.1 ± 7, $p < 0.01$, respectively). Ultrasonic myocardial texture analysis in IDCM has a good ability to discriminate between cases with and without preserved CR, and cases with unfavorable outcome as well.

Instrumentation: Videodensitometric myocardial texture analysis.

Myocardial texture analysis in idiopathic dilated cardiomyopathy: Prediction of contractile reserve on dobutamine echocardiography. Dagdeviren B, Akdemir O, Bolca O, Eren M, Gürlertop Y, Tezel T. J Am Soc Echocardiogr 2002 Jan; 15 (1), pp. 36–42.

Cardiomyopathy: Ischemic/Low-Dose Wall Motion Scores: Risk Stratification/Prediction of Benefit from Revascularization
Dobutamine/[Echocardiography]

Physiological Mechanism of Action: Dobutamine exerts adrenergic/sympathetic agonist properties.

Application: Evaluation of dobutamine low-dose wall motion scores for estimating risk stratification of cases with ischemic cardiomyopathy and identifying those who do and do not benefit from revascularization.

Route of Administration: Dobutamine (intravascular).

Dosage: Dobutamine (low-dose = 10 μg/kg/min and high-dose/maximal dose 50 μg/kg/min) infusion.

Implementation: Delineation of the value of wall motion scores at rest and with low- and high-dose dobutamine infusion for predicting the outcome and benefit from revascularization in cases with ischemic cardiomyopathy. Complementary follow-up evaluation of ischemic cardiomyopathy cases can entailechocardiography image acquision at rest and during low-dose and high-dose/maximum dobutamine infusion.

Human Model: Cases of ischemic cardiomyopathy (n = 139).

Interpretation and Analysis: Both rest and low-dose wall motion scores are multivariate predictors of cardiac death, but ischemia and peak dose scores are not predictors. Cases can be classified into resting case scores that have been risk stratified into 3 groups: (i) score (1.00 to 1.99) with 11% cardiac death; (ii) score (2.00 to 2.49) with 30% death; and (iii) score ≥ 2.50 with 47% death. One-third of cases with rest scores ≥ 2.50 have improvement in scores to < 2.50 with low-dose dobutamine. Their frequency of cardiac death is reduced to 23% compared with 60% ($p = 0.04$) in those that remain with low-dose scores ≥ 2.50. Low-dose scores also can identify those cases that will benefit from revascularization. In cases with low-dose scores (1.00 to 1.99), the frequency of cardiac death is marginally lower in revascularized than non-revascularized cases (10 vs 21%, $p = 0.28$). In cases with scores (2.00 to 2.49), revascularized subjects have a significantly lower frequency of cardiac death than non-revascularized cases (15 vs 41%, $p < 0.05$). The frequency of death in those with low-dose scores ≥ 2.50 is very high in both revascularized (75%) and non-revascularized (56%, $p = 0.42$) cases.

Usefulness of rest and low-dose dobutamine wall motion scores in predicting survival and benefit from revascularization in patients with ischemic cardiomyopathy. Sawada SG, Lewis SJ, Foltz J, Ando A, Khouri S, Kaser S, Gradus-Pizlo I, Gill W, Fineberg N, Segar D, Feigenbaum H. Am J Cardiol 2002 Apr 1; 89 (7), pp. 811–6.

Functional Reserve: Cardiovascular/Global Ventricular Function/Tei Index
Dobutamine Stress Echocardiography

Cellular/Molecular Mechanism of Action: Dobutamine exerts adrenergic/sympathomimetic properties.

Background Information: Dobutamine stress echocardiography has become accepted as an evaluation modality for assessing cardiac functional reserve. Although the Doppler-derived index of combined systolic/diastolic myocardial performance (Tei index) has been reported to be easily obtainable and useful for predicting left ventricular performance, the effect of dobutamine on the Tei index has not been thoroughly established in children.

Application: Dobutamine can be applied in combination with diagnostic ultrasound to characterize various aspects of cardiac physiology (e.g., ventricular function).

Route of Administration: Dobutamine (infusion).

Dosage: Dobutamine (5 μg/kg/min).

Implementation: Assess the effect of dobutamine on the Tei index in cases of postoperative ventricular septal defect and Kawasaki disease. Echocardiographic recordings are obtained before and after dobutamine infusion. Variables measured include transmitral flow velocities (E, A, E/A), rate-corrected mean velocity of circumferential fiber shortening (rate-corrected Vcf), and IMP. Isovolumic contraction time (ICT), isovolumic relaxation time (IRT), and ejection time (ET) are measured and then applied to calculate the Tei index using the following formula: Tei index = (ICT + IRT)/ET.

Human Model: Postoperative ventricular septal defect (n = 8 cases); and cases of Kawasaki disease (n = 7).

Interpretation and Analysis: Dobutamine infusion increases rate-corrected Vcf (29%, $p < 0.01$), peak E (7%, $p < 0.05$), and peak A (13%, $p < 0.05$). E/A ratios do not change during dobutamine infusion. Ejection time, ICT, and IRT decrease during dobutamine infusion. The magnitude of the change in ICT (−21%, $p < 0.01$) is greater than those in IRT (−12%, $p < 0.01$) and ET (−8%, $p < 0.05$). The decrease in Tei index (−16%, $p < 0.01$) results in a decrease in ICT/ET (−21%, $p < 0.01$) and a slight decrease in IRT/ET (−12%, $p < 0.05$). The Tei index has the potential of functioning as a sensitive indicator of the effects of inotropic stimulation on global left ventricular function.

Effect of dobutamine on a Doppler echocardiographic index of combined systolic and diastolic performance. Harada K, Tamura M, Toyono M, Yasuoka K. Pediatr Cardiol 2002 Nov-Dec; 23 (6), pp. 613–7.

Hemodynamic Responses: Heart Rate and Systolic Blood Pressure/Cardiac Death: Independent Predictive Parameters
Adenosine/[Single Photon Emission Computed Tomography (SPECT)]

Physiological Mechanism of Action: Adenosine phosphate promotes relaxation of vascular smooth muscle and a negative chronotropic effect on cardiac function.
Application: Detection of hemodynamic responses to adenosine that add incremental prognostic value to myocardial perfusion single photon emission computed tomography imaging procedures.
Implementation: Evaluation of outcome in cases subjected to adenosine stress myocardial perfusion SPECT (without additional exercise). Multivariable Cox proportional hazards analysis can be used to assess the prognostic value of hemodynamic variables in predicting cardiac death (CD).

Human Model: Postevaluation cases of adenosine stress myocardial perfusion studies (MPS; n = 3444 cases; women = 53.5%; mean age 74 ± 8.4 years) during a 2 ± 0.8 year follow-up (monitoring) period.
Interpretation and Analysis: During an extended monitoring period of 2 ± 0.8 years, a relatively small percentage of cardiac deaths may be appreciated (e.g., n = 224/3444 or 6.5%). Review of multivariable analysis of various parameters reveals a higher resting heart rate (HR) and to a lesser extent lower peak HR as viable markers of impending CD. In addition, peak/rest HR ratio is an independent predictor of CD when added to the multivariable model in place of peak and rest heart rate. Peak/rest HR ratios can also be used to risk stratify cases within each MPS category. A significant interaction can be appreciated between gender and peak systolic blood pressure (SBP), in which there is an increased risk associated with a low peak systolic blood pressure (SBP < 90 mm Hg at end of adenosine infusion) in men but not in women. Cases undergoing adenosine stress MPS with high rest HR and low peak/rest HR ratio have an increased risk of CD, as do male cases with a low peak SBP measurement. Detection of the hemodynamic response to adenosine adds incremental prognostic value to MPS results and enhances identification of cases at risk for CD.

Prognostic impact of hemodynamic response to adenosine in patients older than age 55 years undergoing vasodilator stress myocardial perfusion study. Abidov A, Hachamovitch R, Hayes SW, Ng CK, Cohen I, Friedman JD, Germano G, Berman DS. Circulation 2003 Jun 17; 107 (23), pp. 2894–9.

Myocardial Dysfunction: Left Ventricular (Chronic)/ Ventricular Wall Motion Score Index (WMSI)
Glucose-Insulin-Potassium/Dobutamine/ [Echocardiography]

Background Information: Glucose-insulin-potassium (GIK) infusion improves cardiac function and outcome during acute ischemia.
Application: To determine whether GIK infusion benefits conditions of chronic ischemic left ventricular dysfunction, and if so whether this is related to qualitative and quantitative parameters pertaining to viable myocardium.
Route of Administration: Dobutamine (intravenous) and G-I-K (4-hour intravenous infusion).
Implementation: Dobutamine echocardiography combined with a 4-hour infusion of GIK. Segmental responses are quantified by improvement in WMSI and peak systolic velocity using tissue Doppler. Global responses are assessed by left ventricular volume and ejection fraction, measured using a 3-dimensional reconstruction. Myocardial perfusion is also determined using contrast echocardiography.

Human Model: Chronic ischemic left ventricular dysfunction (n = 30 cases).
Interpretation and Analysis: Ventricular WMSI (mean ± SD) improves with dobutamine (from 1.8 ± 0.4 to 1.6 ± 0.4, $p < 0.001$) and with GIK (from 1.8 ± 0.4 to 1.7 ± 0.4, $p < 0.001$); there is a similar increment for both. Improvement in wall motion score with GIK is observed in 55% of the 62 segments classed as viable by dobutamine echocardiography, and in 5% of 162 segments classed as non-viable. There is an increment in peak systolic velocity after both dobutamine echocardiography (from 2.5 ± 1.8 to 3.2 ± 2.2 cm/s, $p < 0.01$) and GIK (from 3.0 ± 1.6 to 3.5 ± 1.7 cm/s, $p < 0.001$). The GIK effects are not mediated by changes in pulse, mean arterial pressure, lactate, or catecholamines, nor do they correlate with myocardial perfusion. End systolic volume improves after GIK ($p = 0.03$), but only in cases that have a viable myocardium appreciated by dobutamine echocardiography. Therefore, for cases with viable myocardium and chronic left ventricular dysfunction, GIK improves wall motion score, myocardial velocity, and end systolic volume, independent of effects on hemodynamics or catecholamines. The response to GIK is observed in areas of normal and abnormal perfusion assessed by contrast echocardiography.

Effects of glucose-insulin-potassium infusion on chronic ischemic left ventricular dysfunction. Khoury VK, Haluska B, Prins J, Marwick TH. Heart 2003 Jan; 89 (1), pp. 61–5.

Myocardial Dysfunction: Left Ventricular (Doxorubicin)/Screening Test
Dobutamine (Low Dose)/[Echocardiography]

Cellular/Molecular Mechanism of Action: Dobutamine possesses adrenergic agonist/sympathomimetic properties.
Background Information: Late cardiotoxicity after anthracycline chemotherapy administered for childhood cancer is a well recognized sequelae. Many long-term survivors may have subclinical cardiac dysfunction undetectable at a baseline evaluation. Various tests have been utilized for the diagnosis of left ventricular impairment. Recently, low-dose dobutamine stress echocardiography has been proposed as a more sensitive screening test.
Application: Detections of alterations in myocardial dysfunction precipitated by doxorubicin-mediated injury.
Route of Administration: Dobutamine (intravenous).
Dosage: Dobutamine (5–10 μg/kg/min).
Implementation: Evaluation of low-dose dobutamine stress echocardiography in asymptomatic survivors.

Human Model: Cases treated with doxorubicin (e.g., n = 36; n = 20 male; n = 16 female aged 14.6 ± 4.7 years) administered a cumulative dose of 226 ± 106 mg/m^2 of doxorubicin. Note: Median follow-up 5 years. Reference control group (20 sex- and age-matched volunteers (n = 12 male; n = 8 female age = 12.6 ± 4.9 years).
Interpretation and Analysis: Significant differences can be appreciated in mean velocity of circumferential fiber shortening, myocardial performance index (Tei index), left ventricular posterior wall thickening, and end-systolic wall stress at a baseline. The stress response is significantly blunted only with regard to the following parameters: end-systolic wall stress, isovolumic relaxation time, and

myocardial performance index. The threshold response is abnormal (0–5% improvement of a variable only) in 45% of reference controls subjects for only 1 or 2 parameters. In contrast, 63% of subjects from case groups respond in a pathological manner (variable worsening) pertaining to 1 or more parameters. A correlation between risk factors of late cardiotoxicity and stress changes of left ventricular function parameters have not been appreciated. Low-dose dobutamine stress echocardiography therefore appears to be a safe and feasible diagnostic tool in children and adolescents. Dobutamine significantly increases the differences in cardiac variables between healthy populations and asymptomatic survivors for childhood cancer. Relative to control subjects, most asymptomatic patients reveal subclinical myocardial damage. The predictive value for the development of clinical symptoms and cardiac complications needs to be assessed in a large prospective study.

The evaluation of left ventricular function in childhood cancer survivors by pharmacological stress echocardiography. Elbl L, Hrstkova H, Chaloupka V, Novotny J, Michalek J. Neoplasma 2003; 50 (3), pp. 191–7.

Myocardial Infarction (Uncomplicated)/Prediction of Favorable Outcome and Risk Stratification/High Negative Predictive Value Assessment
Dobutamine/Electropharmacology/ [Echocardiography]

Cellular/Molecular Mechanism of Action: Dobutamine is an adrenergic agonist/sympathomimetic agent.
Background Information: Risk stratification after uncomplicated myocardial infarction is a major clinical problem. In particular, the prognostic value of residual inducible ischemia is still controversial.
Application: Exercise ECG and dobutamine stress echocardiography procedures can be utilized to prognostically evaluate uncomplicated myocardial infarction during the early postinfarction period.
Route of Administration: Dobutamine (intravenous).
Implementation: Evaluation of maximal exercise ECG tracings and dobutamine stress echocardiography within 10 days of an uncomplicated myocardial infarction in the absence of therapeutic intervention in concert with prospective monitoring over an 8.8 month period. The variables of age, sex, diabetes, smoking habit, hypertension, dyslipidaemia, infarct location, thrombolysis, and resting wall motion score index are taken into account as relevant clinical variables. Prognostic correlations are made versus spontaneous events (cardiac death, non-fatal reinfarction, and unstable angina requiring hospitalization) while cases undergo revascularization procedures (e.g. percutaneous transluminal coronary angioplasty or coronary artery bypass surgery).

Human Model: Cases of an uncomplicated myocardial infarction (off therapy) and prospectively monitored for 8.8 months ($n = 406$ with $n = 53$ females; aged 57 ± 9 years).
Interpretation and Analysis: A relatively high number of events usually occur during the monitoring period (e.g., $n = 127$; $n = 41$ or 10% spontaneous [5 deaths; 12 reinfarctions; 24 unstable angina]; $n = 86$ procedural related [27 angioplasty; 59 bypass surgery]). Spontaneous events cannot be predicted by any clinical, exercise ECG, or dobutamine stress echocardiography variable. However, the negative predictive value of both tests is excellent (91% and 90%, respectively). With multivariate Cox analysis, male gender, positive low-workload (< 100 W) exercise ECG ($p < 0.0001$), positive low-dose dobutamine stress echocardiography ($p < 0.0001$), and rest-stress wall motion score index variation ($p < 0.001$) are capable of predicting cumulative cardiac events with an independent and additive value.

Model: Dobutamine stress echocardiography is significantly more sensitive (p , 0.05) and less specific (p , 0.01) in predicting the outcome of cases with anterior infarction, while exercise ECG is significantly more sensitive (p , 0.05) in cases with non-Q wave infarction. The most valuable concepts related to these observations include (i) spontaneous events are poorly predicted by provocative tests in low-risk cases after uncomplicated myocardial infarction; (ii) however, both exercise ECG and dobutamine stress echocardiography can predict a favorable outcome with a very high negative predictive value; (iii) dobutamine stress echocardiography should be considered a secondary option in cases where the exercise ECG is equivocal or when the location of ischemia is a relevant issue; and (iv) the possibility that the 2 tests have a differential utility depending on the infarct location and type (Q wave vs non-Q wave) may be clinically relevant and deserves further evaluation.

Prognostic value of residual ischemia assessed by exercise electrocardiography and dobutamine stress echocardiography in low-risk patients following acute myocardial infarction. Bigi R, Galati A, Curti G, Coletta C, Barlera S, Partesana N, Bordi L, Ceci V, Occhi G, Fiorentini C. Eur Heart J 1997 Dec; 18 (12), pp. 1873–81.

Myocardial Ishchemia: Functional Markers/ Hypokinesia, Akinesia, or Dyskinesia
Dobutamine/[Magnetic Resonance Imaging (MRI)]

Cellular/Molecular Mechanism of Action: Dobutamine is an adrenergic agonist/sympathomimetic pharmaceutical agent.
Application: Detection or exclusion of myocardial ischemia and viability in cases with suspected coronary artery disease.
Dosage: Dobutamine (increasing doses of 10, 20, 30, and 40 μg/kg/min).
Implementation: Assessment of the clinical applicability of DS-MRI for the detection of myocardial ischemia and myocardial viability. Three left ventricular short-axis planes are examined for occurrence of disorders in wall movement during infusion of increasing larger doses of dobutamine (10, 20, 30, and 40 μg/kg/min). Temporary recovery of wall thickening in a previously diminished or non-contracting segment under 5 μg/kg/min of dobutamine is considered proof of viability. Development of hypokinesia, akinesia, or dyskinesia at higher doses of dobutamine serves as an indicator of ischemia. If the DS-MRI test is positive for ischemia, then coronary angiography is performed. Such evaluations, if indicated, are subsequently subjected to revascularization. If DS-MRI does not reveal ischemia, then cases may be monitored on an outpatient basis.

Human Model: Cases of suspected coronary artery disease subjected to breath-hold DS-MRI (1 Tesla) 4 days after cessation of anti-ischemic medication that can not be studied by means of conventional bicycle ergometry.

Human Model: Cases of suspected coronary artery disease ($n = 100$; $n = 62$ men; $n = 38$ female; mean age $= 62$ years $\pm$ 12). All cases with non-ischemic DS-MRI scans (e.g., $n = 53$) are followed up for 11 to 23 months (mean 17 months). One case expired suddenly 2 weeks after the MRI test. Other cases may not experience any coronary events or sudden cardiac death (e.g., $n = 52/100$).
Interpretation and Analysis: A high percentage of cases subjected to DS-MRI will yield diagnostic information ($n = 95/100$ or 95%). In cases with DS-MRI scans considered positive for ischemia and in which coronary angiography is subsequently performed, essentially all affected individuals have coronary abnormalities that

suggest revascularization is necessary (n = 41/42; n = 1 false positive). The predictive value of a positive DS-MRI scan for ischemia is 98% and the predictive value of a negative DS-MRI scan is also 98%. DS-MRI is a safe diagnostic method for the detection or exclusion of myocardial ischemia and viability in cases with suspected coronary artery disease.

Dobutamine stress magnetic resonance imaging (DS-MRI), a valuable tool for the diagnosis of ischemic heart disease. van Dijkman PR, Kuijpers TJ, Blom BM, van Herpen G. Ned Tijdschr Geneeskd 2002 Jul 13; 146 (28), pp. 1327–32.

Myocardial Ischemia: Cardiomyopathy (Ischemic)/ Diabetes Mellitus
Acipimox/[^{18}F]-FDG Single Photon Emission Computed Tomography (SPECT)

Cellular/Molecular Mechanism of Action: The agent [^{18}F]-FDG functions as an analog of glucose. Acipimox is a nicotinic acid derivative.
Application: Evaluation of differences in glucose metabolism using [^{18}F]-FDG. Acipimox utilized in combination with [^{18}F]-FDG SPECT metabolic imaging can be used to assess conditions of ischemic cardiomyopathy even in cases suffering from diabetes mellitus.
Implementation: Evaluation of ischemic cardiomyopathy cases applying [^{18}F]-FDG SPECT imaging analysis in combination with acipimox to assess myocardial viability. Complementary image analysis involves performing resting 2-dimensional echocardiography in an effort to identify dysfunctional myocardial tissues. Image quality is then scored visually and quantitatively, while the myocardium-to-background ratio is determined by region-of-interest analysis. The plasma concentrations of glucose and free fatty acids are determined to evaluate metabolic status before and during [^{18}F]-FDG imaging.

Human Model: Ischemic cardiomyopathy (n = 70; n = 34/70 diabetes mellitus; n = 12/34 insulin-dependent diabetes mellitus; n = 22/34 non-insulin-dependent diabetes mellitus; n = 36/70 non-diabetic).
Interpretation and Analysis: Acipimox significantly lowers plasma levels of free fatty acids in both categories of case type. Approximately 50% of diabetic cases (e.g., n = 15/34) have plasma glucose levels of > 9 mmol/L, which is lowered successfully in essentially all cases when administered additional insulin. Visual evaluation of [^{18}F]-FDG images reveals variable quality in cases with either diabetes (good n = 27/34; moderate n = 5/34; poor n = 2/34; p = ns) or individuals without diabetes (non-diabetic: good n 32/34; moderate n = 4/34; poor n = 0/34; p = ns). The myocardium-to-background ratio of [^{18}F]-FDG SPECT images is comparable in cases with and without diabetes mellitus (3.1 ± 1 vs 3.5 ± 0.9; p = ns). The type of diabetes has no influence on [^{18}F]-FDG image quality. Note: Severe side effects usually are not appreciated with [^{18}F]-FDG SPECT procedures. [^{18}F]-FDG SPECT metabolic imaging after acipimox appears to be safe and practical for routine assessment of viability in cases with ischemic cardiomyopathy. Image quality is good, even in patients with diabetes, although additional insulin is sometimes needed.

Effect of diabetes mellitus on myocardial 18F-FDG SPECT using acipimox for the assessment of myocardial viability. Schinkel AF, Bax JJ, Valkema R, Elhendy A, van Domburg RT, Vourvouri EC, Bountioukos MA, Krenning EP, Roelandt JR, Poldermans D. J Nucl Med 2003 Jun; 44 (6), pp. 877–83.

Myocardial Ischemia
Dipyridamole/[^{99m}Tc]-SestaMIBI/[Nuclear Scintigraphy]

Cellular/Molecular Mechanism of Action: Dipyridamole influences the metabolism and transport of adenosine and adenosine nucleotides. In this context, dipyridamole, a nucleotide transport blocker, suppresses the intracellular infux of adenosine released from myocardial tissues that have been deprived of adequate oxygen. Dipyridamole also induces the release of hydrogen peroxide and chemotactic factors from peripheral blood leukocyte populations.
Physiological Mechanism of Action: Dipyridamole promotes diminished coronary artery vascular resistance, increased coronary artery vasodilation, elevated coronary artery blood flow, and increased coronary artery oxygen delivery. One of the most prominent influences of dipyridamole in this regard is its effect on small-diameter vascular beds supplied by the coronary artery. However, it has a minimal effect in regions of ischemia since these same vessels tend to already be maximally dilated.
Application: Dipyridamole can be applied to characterize conditions of angina pectoris either in stress tests or during the course of performing advanced forms of imaging analysis.
Considerations: Radionuclide myocardial perfusion imaging has been shown to have favorable diagnostic and prognostic value in this setting. Early sensitivity is excellent for the detection of acute myocardial infarction not achieved by other diagnostic/testing modalities.
Route of Administration: Intravenous.
Interpretation and Analysis: Elevation in plasma leukocyte chemotactic factors correspond with positive imaging results obtained with the application of [^{99m}Tc]-sestaMIBI. Sensitivity for detecting early acute myocardial infarction (MI) has been superior to findings achieved with other testing modalities based on the validation of findings applying [^{99m}Tc]-sestaMIBI scintigraphy.
Instrumentation: Boyden leukocyte chemotactic chamber and nuclear medicine scintigraphy imaging instrumentation.
Toxicity: Relatively low but includes peripheral vascular hypotension, nausea, vomiting, diarrhea.

Myocardial Perfusion Assessment
Dipyridamole/[^{201}Thallium] Single Photon Emission Computed Tomography (SPECT)/[Nuclear Scintigraphy]

Cellular/Molecular Mechanism of Action: Dipyridamole is classified as a adenosine nucleotide trasport blocker whereby it prevents adenosine influx into myocardial cells.
Application: Detection and characterization of myocardial ischemia.
Route of Administration: Dipyridamole (slow IV).
Dosage: Dipyridamole.
Considerations: A dipyridamole stress test might be considered appropriate as a provocative test when physical exercise cannot be carried out.
Implementation: Evaluate the diagnostic capability of SPECT to detect diseased coronary vessels utilizing dipyridamole in concert with [^{201}thallium]/SPECT imaging.

Assessment of myocardial infusion (+ maximal ergometric exercise) can be facilitated by infusion of dipyridamole in concert with myocardial imaging techniques employing [^{201}thallium]/SPECT. To evaluate the diagnostic capability of SPECT in the detection of

diseased coronary vessels, the left ventricular myocardium can be subdivided into 6 segments related to the 3 major coronary arteries. Images using SPECT are acquired after dipyridamole infusion (Methodology Type 1 with n = 41 cases) or after tracer injections at maximal ergometric exercise (Methodology Type 2 with n = 162 cases). Based on findings from [^{201}Tl] and dipyridamole evaluations, cases are then subdivided into 2 groups for diagnostic coronary angiography.

Human Model: Cases suffering from cardiovascular disease involving the coronary artery(n = 203).

Interpretation and Analysis: Sensitivity for characterizing the left anterior descending artery is 77% with Methodology Type 1 and 79% for Methodology Type II; while specificity is 80% and 96%, respectively. Sensitivity for the right coronary artery is 91% with Method 1 and 85% with Method 2; while specificity is 81% and 73%. For the left circumflex artery sensitivity is 65% with Method 1 and 67% for Method 2, while specificity is 89% and 88%, respectively. Myocardial SPECT results after ergometric exercise and dipyridamole infusion turned out to be almost superimposable (identical).

Diagnostic accuracy of single photon emission computed tomography with thallium-201 for the identification of diseased coronary arteries. Comparison between dipyridamole infusion and exercise stress test. Dondi M, Monetti N, Levorato M, Corbelli C, Zagni P, Tartagni F, Maiello L, Fallani F, Ortolani P. J Nucl Med Allied Sci 1990 Jan-Mar; 34 (1), pp. 19–23.

Myocardial Perfusion/Microvascular Permeability/ Renal Hypertension/Hypercholesterolemia Model *Dobutamine/Adenosine/[Computed Tomography]*

Cellular/Molecular Mechanism of Action: Explanations have been provided for review in a previous citation entry for the molecular and physiological mechanisms of actions for adenosine and dobutamine (adrenoreceptor agonist/sympathomimetic agent).

Physiological Mechanism of Action: Adenosine phosphate promotes relaxation of vascular smooth muscle and exerts a negative chronotropic effect on the heart.

Background Information: Hypercholesterolemia (HC) and hypertension (HT) are both major risk factors for the development and progression of atherosclerotic heart disease. Simultaneous occurrence of both conditions has been associated with an increased incidence of cardiac events in clinical studies. Hypercholesterolemia and HT are individually associated with abnormal myocardial vascular function, but whether HT exacerbates HC-induced myocardial vascular dysfunction remains uncertain.

Application: Evaluation of myocardial perfusion and microvascular permeability.

Route of Administration: Adenosine (intravenous) and dobutamine (intravenous).

Implementation: Evaluation of myocardial perfusion and microvascular permeability *in vivo* applying electron-beam computed tomography in response to cardiac challenge with adenosine and dobutamine administration. The involvement of systemic and myocardial tissue oxidative stress *in vitro* is assessed by oxidizability of LDL, levels of endogenous antioxidants, and tissue activity levels of radical-scavenger systems.

Animal Model: Swine (dietary hypercholesterolemia and renovascular hypertension).

Interpretation and Analysis: While in normal control *in vivo* models myocardial perfusion increases in response to adenosine (+36 ± 13%, $p < 0.05$), in HC and HT alone the increase is blunted. In HC + HT myocardial perfusion response is further attenuated and significantly lower than normal, while myocardial vascular resistance fails to decrease (+7.6 ± 8.8 vs −21 ± 5.8%, p = 0.02 vs normal). HC + HT also show a blunted response to dobutamine, and augmented *in vivo* increases in microvascular permeability. These functional abnormalities are associated with increases in systemic and myocardial tissue oxidative stress compared to HC or HT alone, and synergistic decreases in endogenous antioxidant defenses in myocardial tissue. Furthermore, chronic antioxidant vitamin supplementation in combined HC and HT improves myocardial vascular responses. Hypertension amplifies hypercholesterolemia-induced myocardial microvascular dysfunction hypercholesterolemia *in vivo* and increases oxidative stress *in vitro*. These alterations may potentially play a role in the increased incidence of cardiac events observed when HC and HT co-exist.

Hypertension exacerbates the effect of hypercholesterolemia on the myocardial microvasculature. Rodriguez-Porcel M, Lerman A, Herrmann J, Schwartz RS, Sawamura T, Condorelli M, Napoli C, Lerman LO. Cardiovasc Res 2003 Apr 1; 58 (1), pp. 213–21.

Myocardial Perfusion: Kawasaki Disease (KD)/ Regional Hypoperfusion *Dipyridamole/[^{99m}Tc]-Tetrofosmin/[Nuclear Scintigraphy]*

Cellular/Molecular Mechanism of Action: Dipyridamole is classified as a adenosine nucleotide transport blocker whereby it prevents adenosine influx into myocardial cells.

Physiological Mechanism of Action: Dipyridamole promotes vasodilator activity.

Background Information: The coronary artery abnormalities in KD often cause myocardial ischemia. The radionuclide [^{201}thallium] has been applied for the purpose of imaging myocardial perfusion in order to determine the extent of ischemia in KD cases. The technetium perfusion agents offer better resolution and may offer additional information about ventricular function in KD conditions.

Application: Tetrofosmin myocardial perfusion imaging can detect regional areas of myocardial hypoperfusion (e.g., Kawasaki disease of children).

Dosage: Dipyridamole (high-dose = 0.70 mg/kg); [^{99m}technetium]-tetrofosmin (first dose = 74 to 370 MBq; second dose = 148 to 740 MBq).

Implementation: Evaluation of myocardial perfusion in cases of KD applying [^{99m}technetium]-tetrofosmin (TF) in conjunction with performing a dipyridamole vasodilator stress test. Significant coronary artery stenosis is classified as being ≥ a 75% reduction in lumen diameter. After the administration of high-dose dipyridamole by infusion, [^{99m}Tc]-tetrofosmin is injected followed by as second [^{99m}Tc]-tetrofosmin injection at rest 4 hours later. Single photon emission computed tomography (SPECT) images are then obtained more than 30 minutes after TF injection.

Human Model: Kawasaki disease (n = 86 cases 11.5 ± 6.4 years; n = 20 age-matched controls without heart disease).

Interpretation and Analysis: Significant coronary artery stenosis, coronary aneurysm without stenosis, and an absence of coronary lesions can be appreciated in a moderate number of cases (e.g., n = 20/86, n = 37/86, and n = 29/86, respectively). Regional myocardial hypoperfusion can be observed in a number of cases that have coronary artery stenosis of 75% or greater (n = 18/86; sensitivity, 90%, and specificity, 100%). A small percentage of cases do not demonstrate ischemic changes in the presence of coronary artery stenosis (n = 2) and some of these cases have good collateral flow (n = 1 of 2).

Myocardial ischemia in Kawasaki disease: Evaluation with dipyridamole stress technetium 99m tetrofosmin scintigraphy. Fukuda T, Ishibashi M, Yokoyama T, Otaki M, Shinohara T, Nakamura Y, Miyake T, Kudoh T, Oku H. J Nucl Cardiol 2002 Nov-Dec; 9 (6), pp. 632–7.

Ventricular (Myocardial) Function *Dobutamine/Nitroglycerin/[Echocardiography]*

Cellular/Molecular Mechanism of Action: Dobutamine is an adrenergic agonist/sympathomimetic agent.
Physiological Mechanism of Action: Nitroglycerin exerts vasodilatory properties.
Background Information: Dobutamine-inducible left ventricular obstruction lacks sound clinical meaning. The phenomenon may be related to an abnormal response of the heart to sympathetic stimulation. Head-up tilt can elicit intraventricular obstruction in cases known to develop such responses during dobutamine administration, through the synergistic effects of reduced preload, hypercontractility, and reflex increases in cardiac sympathetic tone.
Application: Dobutamine echocardiography analyses for the evaluation of ventricular function.
Route of Administration: Dobutamine (infusion), and nitroglycerin (infusion).
Dosage: Dobutamine and nitroglycerin (0.3 mcg/kg/min).
Implementation: Evaluation of dobutamine stress echocardiography and 60-degrees head-up tilt on echocardiographic and electrocardiographic monitoring in drug-free and during nitroglycerin infusion (0.3 mcg/kg/min) protocols. Doppler echocardiographic measurements of left ventricular diameters, function, and outflow tract flow velocity are acquired in addition to assessment of power heart rate variability analyzed on short electrocardiographic recordings (low-/high-frequency ratio). In addition, plasma catecholamines can be determined at baseline and during tilt maneuvers.

Human Model: Normal coronaries (n = 21; of which n = 12 cases with left ventricular obstruction during dobutamine stress echocardiography [n = 7/12 males, aged 61 ± 7 years]; n = 9 failed to develop complications [n = 3 males, aged 60 ± 9 years]).
Interpretation and Analysis: Intraventricular obstruction does not develop in group 1 type cases. Baseline differences can be appreciated in ventricular dimensions and mitral early-to-late velocity ratios between case group types 1 and 2 in contrast to hemodynamic patterns during either tilt maneuver which appear to be very similar Low/high frequency ratios, that are similar at baseline and similarly increased with the head-up tilt test, conversely increase significantly less in nitroglycerin-tilt tests for group 1 type cases. Differences in plasma catecholamine concentrations can usually not be appreciated. In summary, reflex cardiovascular adaptive responses such as those elicited by passive tilt are not involved in dynamic intraventricular obstruction in cases with dobutamine-inducible obstruction, and data indicates that left ventricular geometry and hypercontractility are not sufficient pathophysiological determinants.

Dobutamine-inducible left ventricular obstruction: Head-up tilt and autonomic balance. Barletta G. Int J Cardiol 2003 Feb; 87 (2–3), pp. 245–52.

Ventricular Wall Motion: Post–Heart Transplant/ Myocardial Contractile Response *Dipyridamole/[Echocardiography]*

Background Information: Coronary allograft vasculopathy (CAV) remains a major factor limiting long-term survival after heart transplantation. The diagnosis of CAV is still based on serial coronary angiography imaging.
Application: Evaluation of CAV applied in combination with echocardiography image analysis. Dipyridamole echocardiography is a simple, non-invasive test that after heart transplantation may identify cases with altered wall motion that require stricter surveillance.
Dosage: Dipyridamole (high-dose).
Implementation: Evaluation of the prognostic value of high-dose dipyridamole echocardiography in heart transplant cases. Coronary allograft vasculopathy is defined as CAV 1 (focal or diffuse stenosis $< 50\%$) or CAV 2 (focal or diffuse stenosis $\geq 50\%$). Wall motion score index (WMSI) is evaluated at rest and after dipyridamole challenge.

Human Model: Cases 48 hours prior to scheduled annual coronary angiography (n = 68 cases).
Interpretation and Analysis: Results of coronary angiography imaging are normal in a large number of cases (n = 43/68 or 63%), approximately 16% show CAV 1 (n = 11/68), and CAV 2 can be appreciated in about 21% of cases (e.g., n = 14/68). Similary, resting wall motion is normal in a large percentage of cases (n = 39/68 normal; n = 29/68 abnormal). Following dipyridamole administration, wall motion remains unchanged in normal cases (e.g., n = 39/39; Group 1, no CAV in n = 34, and CAV 1 in n = 5). In cases with resting wall motion abnormalities (e.g., n = 29), dipyridamole creates no changes or worsening in most instances (e.g., n = 21/29; Group 3, CAV 2 in n = 14, and no CAV or CAV 1 in n = 7), but can reverse conditions to normal in a modest number of cases (n = 8/29; Group 2, no CAV in n = 7 and CAV 1 in n = 1). During a 6-year (± 3 years) monitoring period, a large number of cases will experience a major cardiac event (n = 15; n = 11 Group 3; n = 4 Groups 1 and 2). Wall motion at rest and after dipyridamole administration and CAV are independent predictors for cardiac events; only dipyridamole WMSI > 1 remains significant ($p < 0.0001$) when evaluated using multivariate analysis.

Prognostic value of contractile response during high-dose dipyridamole echocardiography test in heart transplant recipients. Ciliberto GR, Parodi O, Cataldo G, Mangiavacchi M, Alberti A, Parolini M, Frigerio M. J Heart Lung Transplant 2003 May; 22 (5), pp. 526–32.

Vasodilation Response: Pharmaceutical-Mediated *Nitroglycerin/[Ultrasound]*

Physiological Mechanism of Action: Nitroglycerin promotes vasodilatory/venodilation properties with venodilation most predominant at lower dosages.
Application: Provocative pharmaceutical challenge for enhancing the diagnostic accuracy of echocardiography procedures.
Route of Administration: Nitroglycerin (multiple).
Dosage: Nitroglycerin.
Implementation: Investigate the effects of non-ECG-triggered imaging during ultrasound studies assessing endothelial function. Brachial artery diameters are measured throughout the cardiac cycle at rest, during reactive hyperemia, and after nitroglycerin administration. R wave-triggered imaging using a 7.5 MHz ultrasound transducer can be utilize with image acquisition every 41.7–66.7 ms. Cardiac cycle-related variation is computed as the maximum percent change from the end-diastolic diameter. The range of possible errors in flow-mediated dilation (FMD) and nitroglycerin-mediated vasodilation that may result from ignoring cyclic variations in diameter are then determined for each condition.

Human Model: n = 24 subjects.
Interpretation and Analysis: True FMD, true nitroglycerin-mediated vasodilation, and the maximum and minimum values that

can be erroneously calculated for FMD if timing is ignored all differ dramatically ($p < 0.05$). The range of apparent FMD values that can be measured is nearly 3 times the true FMD value. Ignoring temporal position within the cardiac cycle artifactually increases calculated FMD into the normal range, despite the presence of truly impaired FMD. Peak arterial dilation occurs before end-systole and greater baseline vessel compliance is associated with greater FMD. Brachial artery diameters vary significantly throughout the cardiac cycle. The magnitude of this variation is similar to the arterial dilation induced by reactive hyperemia and nitroglycerin, making ECG-triggered imaging mandatory for acquiring accurate and reproducible clinical and research measurements of artery diameters and FMD. Measurement of diameters at end-diastole may be preferred to other time points in the cardiac cycle.

Instrumentation: 7.5 MHz ultrasound transducer.

Effect of cardiac cycle on ultrasound assessment of endothelial function. Chuang ML, Douglas PS, Bisinov EA, Stein JH. Vasc Med 2002 May; 7 (2), pp. 103–8.

2 Central Nervous System

AUTONOMIC NERVOUS SYSTEM

Dynamic Time-Frequency Analysis of the Autonomic Nervous System
Sodium Amobarbitol/Wada Test

Background Information: Recently, time-frequency analysis has become very popular for examining non-stationary time series and for researching fast-changing phenomena.
Application: Utilized in performing the Wada test which involves the (selective) injection of sodium amobarbitol into the internal carotid artery resulting in the inactivation of cerebral structures supplied by the ipsilateral anterior and middle cerebral arteries. The intra-carotid instillation of amobarbital can be combined with electrocardiographic analyses (electrocardiopharmacology test).
Route of Administration: Amobarbitol (catheter instillation into the internal carotid artery).
Implementation: Utilization of a smoothed-pseudo Wigner-Ville distribution to model the underlying dynamic autonomic nervous system changes that occur during the intracarotid sodium amobarbital (ISA Wada test). Electrocardiogram (EKG) data are recorded during the entire procedure and 10 minutes prior to each injection which are used as baseline values for that hemisphere. Interbeat-interval time series are created from such data for further analyses (smoothed-pseudo Wigner-Ville distribution to model).
Human Model: 13-year-old patient.
Interpretation and Analysis: Evaluations of data generated in the fashion described reveals that sodium amobarbitol injection to either side of the brain produces decreased power in the vagally mediated high frequency band (.14-.40 Hz). Importantly, the decrease is greater when the right hemisphere is inactivated compared to the left side. Such results are consistent with the known lateralized innervation of the heart such that right-sided autonomic inputs have greater influence on cardiac chronotropy. Very rapid changes in autonomic control occur that characterize the inactivation and subsequent recovery of the cerebral hemispheres and serve to confirm the utility of time-frequency analysis in the investigation of cardiac time series.

Time frequency analysis of the cardiovascular response during the intracarotid amobarbital test. Sollers JJ 3rd, Ahern GL, Merritt MM, Thayer JF. Biomed Sci Instrum 2002; 38, pp. 267–71.

Ileus: Postoperative/Myasthenia Gravis
Neostigmine

Cellular/Molecular Mechanism of Action: Inhibits the biochemical activity of acetylecholine esterase, resulting in accumulation of acetylcholine neutrotransmitter. As a result, there is an elevated level of parasympathetic activity within the autonomic nervous system.
Diagnostic Application: (i) verification of the presence of normal gastrointestinal function in conditions of postoperative and pain-induced ileus. (ii) diagnostic detection of myasthenia gravis conditions.
Route of Administration and Dosage: Subcutaneous injection usually preferred.
Considerations: Rapid intravenous injection can promote spastic (painful) contractions of gastrointestinal smooth muscle. Administration in cases suffering from myasthenia gravis can exacerbate the severity of the condition through depletion of axonal acetylcholine stores.
Interpretation and Analysis: Alleviation of gastrointestinal ileus based upon detection of auscultatable borborygmi, palpable contractions, and visualization of fecal production. Myasthenia gravis cases will temporarily demonstrate an improvement in generalized motor function and a reduction in the severity of apparent paresis.

Ileus: Reversible (Postoperative):
Adenosine Receptor Antagonist Ligands
FK352/DPCPX

Cellular/Molecular Mechanism of Action: (R)-1-[(E)-3-(2-phenylpyrazolo[1,5-a]pyridin-3-yl) acryloyl]-piperidin-2-yl acetic acid (FK352) and 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX) function as adenosine A_1 receptor antagonists.
Physiological Mechanism of Action: Reversal of declines in large intestine motility.
Application: Diagnostic interpretation of postoperative ileus or hypomotility related to pain.
Implemenation: Evaluation of colonic propulsive motility as a function of dye migration following injection into the lumen of the colon. Relevant experimental stimuli include pentobarbital anesthesia or surgical trauma (partial gastrectomy, cecectomy, or gentle touching of the colon with fingers).
Animal Model: Rat (unrestrained).
Interpretation and Analysis: Propulsive motility significantly decreases after anesthesia or partial gastrectomy. Intravenous administration of either (R)-1-[(E)-3-(2-phenylpyrazolo[1,5-a]pyridin-3-yl) acryloyl]-piperidin-2-yl acetic acid (FK352) or 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX) function as adenosine A_1 receptor antagonists and reverse the slowing of colonic propulsion activity in experimental ileus models. Blockade of adenosine A_1 receptors therefore has both diagnostic benefits for assessing the integrity of parasympathetic regulation of gastrointestinal motility, and intestinal smooth muscle function relevant to conditions including post-operative ileus. Adenosine A_1 receptor blockade also has several potential therapeutic applications.

Adenosine A1 receptor blockade reverses experimental postoperative ileus in rat colon. Kadowaki M, Nagakura Y, Tokita K, Hanaoka K, Tomoi M. Eur J Pharmacol 2003 Jan 1; 458 (1–2), pp. 197–200.

Sympathetic Nervous System/Reflex Sympathetic Dystrophy: Cocaine-Induced Reflex
Phentolamine

Cellular/Molecular Mechanism of Action: Phentolamine (α-adrenergic blocker).
Physiological Mechanism of Action: Cocaine (vasoconstrictor activity); phentolamine (vasodilator activity).
Background Information: Reflex sympathetic dystrophy (RSD) usually follows traumatic injuries or neurologic disorders.

Human Model: Rare cases of RSD following intra-arterial administration of cocaine in the presence of a history of intravenous drug abuse. An example of a representative case includes cocaine self-administered inadvertently into the femoral artery rather than into the femoral vein.
Interpretation and Analysis: Despite the intense pain, swelling, and dermatologic changes that can accompany intra-arterial carotid cocaine injection, a diagnosis of RSD should be validated by scintigraphic imaging. A combination of normal radiographic finding, a normal leukocyte study, and abnormal bone scan lesions in the region of tenderness and swelling serve to exclude other possibilities and suggest a diagnosis of RSD. In some conditions of RSD, the disorder is likely caused by an ischemic autonomic injury induced by the vasoconstrictor activity of cocaine. Clinical follow-up and relief using phentolamine, an α-adrenergic blocker and vasodilator, make the diagnosis of RSD most likely.

Cocaine-induced reflex sympathetic dystrophy. Gay D, Singh A. Clin Nucl Med 2000 Nov; 25 (11), pp. 863–5.

PERIPHERAL NERVOUS SYSTEM AND NEUROMUSCULAR FUNCTION

Neurologic vs Musculoskeletal Differentiation
Lidocaine/Mepivicaine/Bupivicaine

Cellular/Molecular Mechanism of Action: Inhibits axonal membrane-associated Na^+/K^+ pump activity, thereby diminishing the rate or completely preventing transmission of neurologic impulses.
Diagnostic Application: Localization of sites of musculoskeletal pain associated with bone and joint structures. In instances where the origin of pain is uncertain (e.g. veterinary medicine), then injections of local anesthetic are initially injected at the most distal regions of a limb with progressively more proximal injections made until the painful foci is desensitized. Local peripheral nerve blocks can also be applied to differentiate musculoskeletal pain from neurologic deficit/disorders.

Localization of pain to specific anatomical foci or regions improves the accuracy of detecting pathological lesions and facilitates more efficient if not accurate radiographic examinations. The procedure is of particular applicability in lameness examinations performed in veterinary medicine (e.g., equine gait abnormalities).
Route of Administration and Dosage: Local injection either intra-articular or in a juxta-position along peripheral nerves.
Considerations: Lidocaine has an onset of action of approximately 5 minutes with a duration of action that approaches 30 to 45 minutes. Mepivicaine (Carbocaine) has a slightly longer onset of action and a duration of activity lasting approximately 45 to 60 minutes. Bupivicaine (Marcaine) has the longest onset of action and the longest duration of activity; on average it can last between 3 and 4 hours.

Onset of action and duration of activity are inversely related and are dependent upon the individual diffusion characteristics of each local anesthetic agent across axonal membrane structures. Duration of activity can be enhanced with preparations containing epinephrine that promote vasoconstriction which inhibits diffusion away from the site of local injection. Dextrose has also been included in preparations of local anesthetic agents to increase their osmotic characteristics and promote accumulation of tissue fluid at the site of injection, thereby inhibiting diffusion of local anesthetic agent away from the site of injection.
Implementation: Injection of a local anesthetic agent in joint structures or in a juxta-position along peripheral nerves that directly correspond to anatomical regions suspected of being a source of pain.

Clinical Example: Evaluation of confirmed cases of osteoarthrosis (osteoarthritis, OA) of the hip and spine with concomitant lower extremity pain below the knee that is coxalgic or neuropathic. One approach to determining if leg symptoms are coxalgic or neuropathic can involve the administration of Bupivicaine by intra-articular injection into the hip as a provocative test.

Human Model: Cases with roentgenographically proven osteoarthrosis (osteoarthritis, OA) of the hip and spine (e.g. n = 18 consecutive patients).

Interpretation: Alleviation of pain or signs of abnormal locomotion or gait which directly corresponds to the anatomical site of anesthetic agent injection (e.g., intra-articular or peripheral nerve branch). In the clinical example provided pertaining to osteoarthritis lesions affecting the hip and spine, such tests allow correct identification of the source of the pain with a sensitivity of 87%, a specificity of 100%, and an efficiency of 88%. A provocative test of this nature provides significant savings in terms of diagnostic tests and patient discomfort.

The value of bupivicaine hip injection in the differentiation of coxarthrosis from lower extremity neuropathy. Kleiner JB; Thorne RP; Curd JG. J Rheumatol] 1991 Mar; Vol. 18 (3), pp. 422-7.

Neuromuscular Disorder: Botulinum Neurotoxins: Type A/[Botulinum Neurotoxins B and E]/[Tetanus Toxin]/[Myasthenia Gravis]
3,4-Diaminopyridine/4-Aminopyridine

Cellular/Molecular Mechanism of Action: Both 3,4-diaminopyridine (3,4-DAP) and 4-aminopyridine function as A-type K^+ channel blockers.
Diagnostic Application: Partial or substantial response appreciated in cases of botulism intoxication caused by *Clostridium botulinum* exotoxin.
Application: Detection, characterization, and differentiation of neuromuscular disorders due to botulinum neurotoxin types A, B, and E, in addition to tetanus toxin.
Implementation: Evaluation of the potential for 4-aminopyridine and 3,4-diaminopyridine to delay the onset of paralysis due to botulinum neurotoxin types A, B, and E. Complementary investigations can involve evaluation of the potential for 3,4-diaminopyridine to reverse paralysis due to botulinum toxin using phrenic nerve-hemidiaphragm *ex vivo* preparations previously poisoned *in vivo*. Analogous types of experiments can be done using tetanus toxin.

Animal Model: Murine (mouse excised phrenic nerve-hemidiaphragm preparations).
Interpretation and Analysis: At a concentration that produces enhancement of muscle twitch amplitude, 4-aminopyridine and 3,4-diaminopyridine delay the onset of paralysis due to botulinum toxin type A. Under the same conditions, the drugs do little to protect tissues against botulinum toxin types B and E. The agent 3,4-diaminopyridine produces transient increases in neuromuscular transmission, with the

"reversal" effect being greater for botulinum neurotoxin type A than for botulinum neurotoxin types B and E. Results with 3,4-diaminopyridine show that tetanus toxin resembles botulinum toxins type B and E. Such findings clarify the role of aminopyridines as potential diagnostic/therapeutic agents in the treatment of botulism. They also provide insights into the mechanism of action of clostridial neurotoxins.

A preclinical evaluation of aminopyridines as putative therapeutic agents in the treatment of botulism. Simpson LL. Infect Immun 1986 Jun; 52 (3), pp. 858–62.

Neuromuscular Disorders/Lambert-Eaton Myasthenic Syndrome/Botulism
4-Aminopyridine/Neostigmine/[d-Tubocurarine]

Cellular/Molecular Mechanism of Action: Neostigmine inhibits the biochemical activity of acetylecholine esterase. D-tubocurarine competitively inhibits binding of acetylcholine to its corresponding receptor complex.
Application: Classification and characterization of presynaptic disorders, including Lambert-Eaton myasthenic syndrome and botulism.
Implementation: Evaluation of pre- and postsynaptic events at the neuromuscular junction in muscle tissue exposed to clinically significant concentrations of 4-aminopyridine (4-AP), neostigmine, or combinations of the 2 drugs.
Animal Model: Rat (skeletal muscle tissue).
Interpretation and Analysis: Simultaneous application of 4-AP and neostigmine produces increases in the amplitudes of nerve-evoked end plate potentials that are significantly greater than the summed effects of the drugs applied individually. Such synergism is present at junctions where transmission is blocked either postsynaptically by d-tubocurarine or presynaptically by low $[Ca^{2+}]0$ and high $[Mg^{2+}]0$. Quantal content analysis of the latter preparation indicates that the evoked release of acetylcholine is potentiated significantly more than the amplitude of spontaneous miniature end plate potentials, suggesting that the site of synergism is predominantly presynaptic. For symptomatic relief and long-term management of the neuromuscular junction disorders, it has been proposed that when applied in combination, aminopyridine and anticholinesterase agents should be applied at reduced dosages, thereby minimizing adverse effects. Such combinations should be particularly effective in presynaptic disorders, including Lambert-Eaton myasthenic syndrome and botulism.

Synergistic interaction of 4-aminopyridine with neostigmine at the neuromuscular junction. Tierney PC, Kim YI, Johns TR. Eur J Pharmacol 1985 Sep 24; 115 (2–3), pp. 241–7.

The value of bupivicaine hip injection in the differentiation of coxarthrosis from lower extremity neuropathy. Kleiner JB; Thorne RP; Curd JG. J Rheumatol] 1991 Mar; Vol. 18 (3), pp. 422-7.

Peripheral Nerve Function Post–Crush Injury/ Sensorimotor Recovery/Temporary -vs- Permanent Nerve Injury
$ACTH_{1\text{-}24}$/$ACTH_{1\text{-}16}NH_2$ / $ACTH_{4\text{-}10}$/[Met(O_2) 4,D-Lys_8,Phe_9]$ACTH_{4\text{-}9}$ (Org. 2766)

Cellular/Molecular Mechanism of Action: The most well known mechanism of adrenalcorticotropin hormone (ACTH) pertains to its ability to promote the release of cortisol from the adrenal cortex. Other physiological properties of ACTH extend beyond this most prominent function (e.g., "crossover" melanocyte maturation).
Application: Discovery of the potential for peripheral nerve crush injury to regain sensorimotor function.
Implementation: Evaluation of sensorimotor function recovery using the "foot-flick" response test after crushing of the sciatic nerve. Every other day, $ACTH_{1\text{-}24}$ is administered by subcutaneous injection immediately after the foot-flick test.
Animal Model: Rat (foot-flick response test following mechanical crushing of the sciatic nerve).
Interpretation and Analysis: Administration of $ACTH_{1\text{-}24}$ promotes a faster recovery of sensorimotor function compared to reference controls. Such beneficial effects are dose-dependent. In contrast, no effect on toe-spreading can be detected. To investigate what part of the peptide molecule contains the active site and is devoid of steroid properties, smaller sequences of $ACTH_{1\text{-}24}$ without corticotrophic activity are then tested. Treatment with $ACTH_{1\text{-}16}NH_2$, $ACTH_{4\text{-}10}$, or [Met(O_2)4_4,D-Lys_8,Phe_9] $ACTH_{4\text{-}9}$ (Org. 2766) mimic the effects of $ACTH_{1\text{-}24}$, whereas treatment with $ACTH_{1\text{-}24}$ does not affect the return of sensorimotor function. The stimulatory effects of corticotrophin$_{1\text{-}24}$ and congeners on recovery of sensorimotor function have been described in terms of the direct effect of these peptides on nervous tissue, probably through enhanced brain and spinal cord protein synthesis.

Effects of corticotrophin (ACTH) on recovery of sensorimotor function in the rat: Structure-activity study. Bijlsma WA, Jennekens FG, Schotman P, Gispen WH. Eur J Pharmacol 1981 Nov 19; 76 (1), pp. 73–9.

CEREBRAL CORTEX AND CEREBRAL HEMISPHERES

Acetylcholine Receptor Complexes: Nicotinic Ligands
Bis-Nicotinium, Bis-Pyridinium/Bis-Picolinium, Bis-Quinolinium

Cellular/Molecular Mechanism of Action: Recognize and bind to nicotinic acetylcholine receptor complexes (nAChRs).
Physiological Mechanism of Action: Nicotinic acetylcholine receptor antagonist (parasympatholytic) and could potentially be applied as an indicator/marker molecule in non-imaging diagnostic procedures, or as a carrier imaging agent in nuclear medicine oriented investigations.
Application: Nictotinic acetylcholine receptor antagonist activity. Variation of N-n-alkyl chain length together with structural modification of the azaaromatic quaternary ammonium moiety affords selective antagonist activity for the $\alpha_4\beta_2$* nAChR subtype, as well as the formation of ligands with selectivity at α_7* nAChRs. The experimental findings provide a foundation for investigations devoted to the molecular design of nicotinic acetylcholine receptor ligands.
Implementation: Evaluation of a series of bis-nicotinium, bis-pyridinium, bis-picolinium, bis-quinolinium and bis-isoquinolinium compounds for their binding affinity at nAChRs expressed in neurologic tissues.
Ex-Vivo Model: Preparations of rat brain membranes.
Interpretation and Analysis: N,N′-Decane-1,12-diyl-bis-nicotinium diiodide (bNDI) exhibits the highest affinity for [^{3}H]-nicotine binding sites (K_i = 330 nM), but does not inhibit [^{3}H]-methyllycaconitine binding (K_i > 100 μM), indicative of an interaction with $\alpha_4\beta_2$*, but not α_7* receptor subtypes, respectively. In addition, bNDI inhibits (IC_{50} = 3.76 microM) nicotine-evoked [$^{86}Rb^+$] efflux from thalamic synaptosomes, indicating antagonist activity at $\alpha_4\beta_2$* nAChRs. N,N′-Dodecane-1,12-diyl-bis-quinolinium dibromide (bQDDB) exhibits the highest affinity for [^{3}H]-methyllycaconitine binding sites (K_i = 1.61 μM), but does not inhibit [^{3}H]-nicotine binding (K_i > 100 μM), demonstrating an interaction with α_7* but not $\alpha_4\beta_2$* nAChRs.

Bis-Azaaromatic quaternary ammonium analogues: Ligands for $\alpha4\beta_2$* and α_7* subtypes of neuronal nicotinic receptors. Ayers JT, Dwoskin LP, Deaciuc AG, Grinevich VP, Zhu J, Crooks PA. Bioorg Med Chem Lett 2002 Nov 4; 12 (21), pp. 3067–71.

Acetylcholine Receptors/Neurodegenerative Disease States: Acethylcholine Nictotinic $\alpha_4\beta_2$ Receptors/Tobacco Dependency
(S)-5-[^{123}I]Iodo-3-(2-Azetidinylmethoxy)Pyridine

Cellular/Molecular Mechanism of Action: (S)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine selectively binds to $\alpha_4\beta_2$ subtype nicotinic acetylcholine receptor complexes.
Application: The iodinated analog (S)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine of A-85380 can be used for the specific detection and characterization of $\alpha_4\beta_2$ subtype nicotinic acetylcholine receptor complexes in neurodegenerative disease states including conditions of tobacco dependence.
Route of Administration: (S)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine (intravenous).
Implementation: Accurate measurement of (S)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine plasma concentrations in order to determine its capacity to function as an "tracer" molecule for *in vivo* quantification of $\alpha_4\beta_2$ subtype nicotinic acetylcholine receptor complexes.
Human Model: Non-human and human primates.
Interpretation and Analysis: In human/non-human primate whole blood as well as in plasma, the parent compound is only stable for approximately 5 minutes, after which it decomposes. In contrast, the radioligand is stable in the injection solution and in protein-free (> 30-kDa M.W.) plasma ultrafiltrate for at least 18 hours. To preserve the parent compound in plasma the radioactive plasma must be mixed with equal volumes of acetonitrile within 5 minutes of collection or, alternatively, radioactive blood should be collected and mixed with sodium azide (3 mg/ml blood). The *in vivo* metabolism of [^{123}I]5-IA generates 2 distinct components including (i) a radiometabolite that is less lipophilic than the parent compound; and (ii) a polar radiometabolite that is not free radioiodide because of the absence of accumulated radioactivity within the thyroid.

Measurement of plasma metabolites of (S)-5-[123I]iodo-3-(2-azetidinylmethoxy)pyridine (5-IA-85380), a nicotinic acetylcholine receptor imaging agent, in nonhuman primates. Zoghbi SS, Tamagnan G, Baldwin MF, Al-Tikriti MS, Amici L, Seibyl JP, Innis RB. Nucl Med Biol 2001 Jan; 28 (1), pp. 91–6.

Acetylcholine Receptors: Nicotinic Ligands
5-(3-Pyridinyl)-4-Azahomoadamantane 2a

Cellular/Molecular Mechanism of Action: Recognizes and has binding avidity for α_7 nicotinic acetylcholine receptor complexes.
Physiological Mechanism of Action: Competitive antagonist of acetylecholine at nicotinic receptor complexes (parasympatholytic).
Application: Potential carrier molecule for imaging the distribution and expression density of nicotinic-class acetylcholine receptor complexes.
Semi-Synthetic Methods: Applying a general synthetic route, nicotinic ligands can be produced that bear a variety of bulky azaring groups and yield 5-(3-pyridinyl)- and 5-(3-quinolinyl)-4 azahomoadamantanes 2a, 3a and 2b, 3b.
Interpretation and Analysis: The parent compound, 5-(3-pyridinyl)-4-azahomoadamantane 2a (K_i = 5.0 μM) binds with about a 100 times lower affinity than (+)-epibatidine 1 (K_i = 0.045 μM) to α_7 nicotinic acetylcholine receptors (nAChRs). N-methyl substitution of 2a gives rise to compound 3a, which has about 9 times lower binding affinity. The replacement of pyridinyl with a quinolinyl ring (compounds 2b, 3b) results in a dramatic reduction in potency (Ki > 1000 μM).

Synthesis and binding affinities of 5-(3-pyridinyl)- and 5-(3-quinolinyl)-4-azahomoadamantanes to α_7 nicotinic acetylcholine receptors. Tataridis D, Kolocouris A, Fytas G, Kolocouris N, Foscolos GB, Poulas K, Tzartos SJ. Farmaco. 2002 Dec; 57 (12), pp. 979–84.

Alcoholism: Genetic-Associated Sensitivity/Central Nervous System EEG Patterns
Ethanol/Electroencephalogram Analysis/ (Electropharmacogenetics)

Cellular/Molecular Mechanism of Action: Ethanol functions as a central nervous system depressant.
Background Information: Electroencephalogram (EEG) analysis offers several advantages for performing pharmacogentic-related studies since it simultaneously evaluates both genetic traits and challenge characteristics.
Application: Delineation of alterations in electroencephalogram tracings induced by ethanol that are associated with specific genetic characteristics that may be related to a predisposition of alcoholism.
Route of Administration: Ethanol is administered orally to effect.
Implementation: Pharmacogenetic studies employing electroencephalogram analysis have been applied to characterize the influence of acute ethanol dosing on brain function. Performance of such investigations in twin individuals facilitates the discovery of certain ethanol-induced alterations in brain wave profiles that are directly associated with genetic-related characteristics.
Interpretation and Analysis: Pharmacogenetic studies employing electroencephalogram analysis combined with acute ethanol dosing in twin individuals reveals that certain alterations in EEG tracings profiles induced by this depressant are genetic regulated in nature. Among the various genetically determined variants of EEG tracings, the poorly synchronized alpha EEG exhibits the most pronounced response. Examination of a series of alcoholics of both sexes as well as their 1st-degree relatives reveals that in females the poorly synchronized alpha EEG may be a predictive indicator reflecting a predisposition to alcoholism. Such observations support the perception that the disposition to alcoholism is under genetic influence not only at the metabolic but also at the pharmacodynamic level.

Pharmacogenetics of alcohol's CNS effect: Implications for the etiology of alcoholism. Propping P. Pharmacol Biochem Behav 1983;18 Suppl 1, pp. 549–53.

Alcoholism: Genetic-Associated Sensitivity/ Cholinergic Function
Ethanol/[^{3}H]-Quinuclidinyl Benzilate

Cellular/Molecular Mechanism of Action: Ethanol functions as a central nervous system depressant.
Application: Characterization of pharmacogentic properties associated with vulnerability or resistance to the development of alcoholism pertaining to different aspects of cholinergic function within the autonomic nervous system.
Route of Administration: Ethanol is traditionally administered orally (per os).
Dosage: Ethanol (4.6 g/kg).
Implementation: Evaluation of alterations in choline acetyltransferase (ChAT), acetylcholinesterase (AChE), aand [^{3}H]-quinu-

clidinyl benzilate (QNB) binding within different regions of the brain following ethanol administration.

Animal Model: Murine (C57BL/6 and BABLB/c inbred strains of mice)

Interpretation and Analysis: Choline acetyltransferase in the striatum and septum of C57BL/6 *in vivo* models exhibit greater sensitivity to ethanol compared to BALB/c *in vivo* models. The limbic system and related structures of BALB/c *in vivo* models show greater sensitivity to ethanol compared to C57BL/6 *in vivo* models. Acute ethanol administration in C57BL/6 *in vivo* models increases striatal ChAT activity (up to 22% with 60-minute latency). Induction of ethanol-induced augmentation of ChAT activity in this manner is associated with non-synchronous decreases in kinetic characteristics of stratum QNB binding. In contrast, no such changes are seen in BALB/c *in vivo* models, except the observation of an increase in K_d up to 90 min after acute ethanol treatment. Similar significant increases in ChAT activity can also be observed in the septum of C57BL/6 *in vivo* models 165 minutes after ethanol administration. However, the septum in BALB/c *in vivo* models does not exhibit comparable changes. Ethanol increases ChAT activity in several brain areas of both C57BL/6 and BALB/c *in vivo* models. Regions of the CNS that are affected include the hippocampus, temporal limbic cortex, and piriform cortex or paleocortex. Interestingly, the latencies to increased ChAT activity in these areas are much shorter in BALB/c than in C57 *in vivo* models. The kinetic characteristics of QNB binding sites (B_{max} and K_d) and AChE activity are unchanged in all regions of the brain and do not differ between strains (except where previously indicated). Genetic differences in ethanol preference and sensitivity in these strains therefore are accompanied by differential sensitivity of ChAT to acute ethanol. Genotypic variations in dopaminergic-cholinergic interactions in striatum and hippocampus, septum, and temporal limbic and piriform cortex could contribute to genetic differences in cholinergic sensitivity to ethanol. In addition, different blood-brain barrier and membrane properties may also contribute to genetic differences in the sensitivity of cholinergic function to ethanol. The differential effects on ChAT activity could possibly be responsible for genetic differences in memory disorders (limbic system and related structures) and motor incoordination (basal ganglia) following high-dose alcohol administration.

Genetically-determined responses of central cholinergic markers: The effects of ethanol on inbred strains of mice. Hashemzadeh-Gargari H, Mandel P. Neurotoxicology. 1989 Fall; 10 (3), pp. 555–68.

Alcoholism: Neuroendocrinology
Ethanol Challenge/[Angiotensin II]

Application: Detection of variations in responses to ethanol intoxication for the purpose of detecting and characterizing conditions of alcoholism.

Route of Administration: Ethanol (oral).

Implementation: Simultaneous evaluation of multiple circulating neurohormones, osmolality, thirst, and fluid balance following oral ethanol challenge for the purpose of identifying [i] aberrant hormonal responses related to Ang II and AVP.; and [ii] diagnostic parameters that can potentially be utilized to detect and characterize conditions of chronic alcoholism. Parameters and blood samples are collected prior to and 12 hours after ethanol challenge.

Human Model: Cases of alcoholism (e.g. n = 8 actively drinking, alcoholic males) and n = 7 controls.

Interpretation and Analysis: Basal levels of serum osmolality and thirst are significantly higher in alcoholics compared to controls, yet actively drinking alcoholics have normal vasopressin levels, plasma angiotensin II (Ang II), plasma renin activity, plasma aldosterone, and plasma catecholamines. In response to ethanol, serum osmolalities increases are significantly higher, while plasma vasopressin levels are significantly suppressed in alcoholics. After ethanol stimulus, plasma angiotensin II levels of alcoholics are significantly higher than those of controls at 11 A.M. (12.15 ± 4.49 vs 1.83 ± 0.6 pg/ml; $p < 0.02$) and noon (14.93 ± 6.81 vs 1.37 ± 0.17 pg/ml, $p < 0.04$). Neither plasma renin activity nor aldosterone change in accordance with the elevated plasma angiotensin II in alcoholics. Diuresis in alcoholics (assessed by the sum of urine output following the challenge dose) is significantly less than that of controls. Thirst scores and fluid intakes after the ethanol challenge do not differ between alcoholics and controls. The lack of an angiotensin II mediated increase in plasma aldosterone or thirst response suggests that ethanol may have a specific blunting effect on angiotensin II receptors.

Neuroendocrine, fluid balance, and thirst responses to alcohol in alcoholics. Collins GB, Brosnihan KB, Zuti RA, Messina M, Gupta MK. Alcohol Clin Exp Res 1992 Apr; 16 (2), pp. 228–33.

Alcohoism/Seizures/Recurrent Alcoholic Detoxification Episodes
Chlordiazepoxide/Pharmaco-Electroencephalography

Application: Delineation of recurrent alcoholic detoxification seizures.

Route of Administration: Per os (oral) during detoxification events (associated with the elimination of withdrawal seizures).

Implementation: Evaluation of the potential increased risk of seizure activity during conditions of recurrent alcohol detoxifications. Structural brain damage, focal neurologic deficits, epileptogenic findings on electroencephalograms, and provocative drug use are considered pertinent variables for seizure risk.

Human Model: Review of detoxification and seizure histories (n = 256 men; n = 45 women voluntarily admitted to a 5-day inpatient detoxification program).

Interpretation and Analysis: Administration of chlordiazepoxide is usually associated with the elimination of withdrawal seizures. Some cases have a history of seizures (e.g., n = 64/301) while others have significantly abnormal electroencephalograms during detoxification (e.g., n = 42/301), or have focal slowing on the electroencephalogram (e.g., n = 30/301). No correlation exists between the duration of alcohol abuse or lifetime consumption of alcohol and seizure activity. There is, however, a significant correlation between the number of inpatient alcohol detoxifications and the prevalence of seizure disorders. This correlation holds true even when provocative drug use is taken into consideration.

Seizure risk with recurrent alcohol detoxification. Lechtenberg R, Worner TM. Arch Neurol 1990 May; 47 (5), pp. 535–8.

Alcoholism/Sensitivity to Ethanol
Ethanol/Calcium Chloride/A23187 (Calcium Ionophor)

Cellular/Molecular Mechanism of Action: Ethanol functions as a central nervous system depressant.

Application: Characterization of differences in central nervous system ethanol metabolism.

Route of Administration: Ethanol is administered orally (per os).

Implementation: Sensitivity to ethanol is measured as a function of the blood ethanol concentration at the point where the righting reflex is lost.

Animal Model: Murine (SS and LS mice)

Interpretation and Analysis: Sensitivity to ethanol is increased significantly in SS but not LS mice following intracerebroventricular (ICV) administration of calcium chloride or a calcium ionophore (e.g. A23187). Magnesium chloride or lanthanum chloride, ICV, does not alter sensitivity to ethanol in either SS or LS mice, further indicating a specificity for calcium cation. Calcium does not affect sensitivity to halothane narcosis in LS or SS mice. Endogenous brain calcium content is similar in these mouse lines, and ethanol administration either *in vivo* or *in vitro* does not alter brain calcium concentration. Differences in brain sensitivity to ethanol are therefore mediated, in part, by genetic differences in calcium-related processes and support the hypothesis that ethanol-induced narcosis may be due to alterations in calcium metabolism in the CNS.

Calcium influence on neuronal sensitivity to ethanol in selectively bred mouse lines. Morrow EL, Erwin VG. Pharmacol Biochem Behav 1986 Apr; 24 (4), pp. 949–54.

Alcoholism/Salsolinol: Striatal Production/Alcohol Dehydrogenase/Aldehyde Dehydrogenase
Cyanamide/4-Methylpyrazole/Ethanol

Cellular/Molecular Mechanism of Action: Cyanamide (CY) is a potent inhibitor of aldehyde dehydrogenase. 4-methylpyrazole (4-MP) is a strong inhibitor of alcohol dehydrogenase.
Application: The *in-vivo* formation of salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquionoline), an endogeneous condensation product of dopamine (DA) with acetaldehyde (AcH), can be characterized following the administration of cyanamide (CY) plus ethanol (EtOH) using microdialysis-high-performance liquid chromatography with electrochemical detection.
Route of Administration: Cyanamide (50 mg/kg); 4-MP (82 mg/kg); EtOH (1 g/kg).
Implementation: Evaluation of the capacity for [i] CY to inhibit aldehyde dehydrogenase; [ii] 4-MP to inhibit alcohol dehydrogenase; and [iii] the combined effect of CY + 4-MP administration. One hour latter, EtOH, CY and 4-HP is administered (only by intraperitoneal administration).
Animal Model: Rat (microdialysis probe inserted into the striatum).
Interpretation and Analysis: Administration of CY + EtOH promotes elevation in the amount of salsolinol detected in striatal (CNS) dialysates, and high AcH concentrations are found in the blood. The time course of changes in salsolinol concentrations correlates with blood AcH concentrations. Alternatively, salsolinol in the dialysates and high AcH concentrations in the blood are not detected with other treatment challenges. Such metabolite profiles indicate that (i) high AcH concentrations induce the formation of salsolinol in the striatum; and (ii) there is no effect of EtOH or AcH on striatal dialysate concentrations of DA and 5-hydroxytryptamine.

In vivo formation of salsolinol induced by high acetaldehyde concentration in rat striatum employing microdialysis. Jamal M, Ameno K, Kubota T, Ameno S, Zhang X, Kumihashi M, Ijiri I. Alcohol 2003 May–Jun; 38 (3), pp. 197–201.

Alzheimer's Disease (AD): Acethylcholine Receptors (Muscarinic)/Muscarinic-Cholinergic Investigative Probe
RS 86/2-Ethyl-8-Methyl-2,8-Diazaspiro-[4,5]-Decan-1,3-Dion Hydrobromide/Oxotremorine/Arecoline/Pilocarpine /Aceclidine

Cellular/Molecular Mechanism of Action: The agent 2-ethyl-8-methyl-2,8-diazaspiro-[4,5]-decan-1,3-dion hydrobromide (RS 86) recognizes and physically binds to cholinergic muscarinic receptor complexes as a component of the parasympathetic autonomic nervous system. In this context, it functions as a potent, centrally acting, selective muscarinic receptor agonist.
Application: The agent 2-ethyl-8-methyl-2,8-diazaspiro-[4,5]-decan-1,3-dion hydrobromide (RS 86) appears to be an adequate probe for the clinical examination of the cholinergic hypothesis for the molecular pathogenesis of AD.
Implementation: Evaluate and compare the effects of the compound 2-ethyl-8-methyl-2,8-diazaspiro-[4,5]-decan-1,3-dion hydrobromide (RS 86) to those of classical muscarinic receptor agonists utilizing *in vitro* and *in vivo* test systems for muscarinic cholinergic activity.
Animal Model: Guinea pig (*ex vitro* ileum preparations).
Animal Model: Rat (superior cervical ganglion).
Animal Model: Murine.
Interpretation and Analysis: In radioligand binding assays RS 86 displays high nanomolar apparent affinity only for sites labeled by [^{3}H]-muscarinic receptor agonists while its apparent affinity for sites labeled by [^{3}H]-muscarinic receptor antagonists including [^{3}H]QNB, [^{3}H]NMS, and [^{3}H]pirenzepine is in the micromolar range. RS 86 has no or only low affinity (IC_{50} greater than 10 μM) for other neurotransmitter or drug receptor sites. The compound induces scopolamine-sensitive contractions of isolated guinea pig ileum showing a pD2 of 6 in this model. In the isolated rat superior cervical ganglion RS 86 also functions as an agonist with a pD2 of 6.7. When given to mice or rats by different routes RS 86 induces central and peripheral effects typical of a muscarinic receptor agonist, such as hypothermia, tremor, mydriasis, salivation, lacrimation, diarrhea, and modification of behavior as observed in an "open-field" environment. In several of these tests RS 86 is about 10 times less potent than oxotremorine but more potent than arecoline, pilocarpine, aceclidine, or the compound (cis) AF-30. The ED_{50} values for some central effects, including the induction of hypothermia and alert non-mobile behavior, are lower than those for tremor and peripheral effects. Some of the effects last for up to 6 hours depending on dose. Finally, RS 86 administration results in modifications of brain acetylcholine turnover and high affinity choline uptake typical of a central muscarinic receptor agonist. Taken together these results demonstrate clearly that RS 86 is a potent, centrally acting, selective muscarinic receptor agonist. RS 86 appears to be an adequate tool for the clinical examination of the cholinergic hypothesis of Alzheimer's disease.

The pharmacological assessment of RS 86 (2-ethyl-8-methyl-2,8-diazaspiro-[4,5]-decan-1,3-dion hydrobromide). A potent, specific muscarinic acetylcholine receptor agonist. Palacios JM, Bolliger G, Closse A, Enz A, Gmelin G, Malanowski J. Eur J Pharmacol 1986 Jun 5; 125 (1), pp. 45–62.

Alzheimer's Disease (AD): Amyloid (A-beta) Fibrils/Senile Plaques and Neurofibrillary Tangles
Methoxy-X04/Derivative of Congo Red and Chrysamine-G

Cellular/Molecular Mechanism of Action: Similar to Congo red, methoxy-X04 is a fluorescent dye that possesses binding avidity for molecular amyloid beta fibrils.
Background Information: The identification of amyloid deposits in living AD cases is important for both early diagnosis of the condition and for monitoring the efficacy of newly developed antiamyloid therapies.
Application: Visual fluorescent detection and characterization of amyloid beta fibrils deposits contained within plaques, tangles, and cerebrovascular amyloid deposits.

Route of Administration: Methoxy-X04 (IV = 5 to 10 mg/kg; IP = 10 mg/kg in mice).
Dosage: Methoxy-X04 (intravenous, intraperitoneal in mice).
Semi-Synthetic Methods: Methoxy-X04 is a derivative of Congo red and chrysamine-G that contains no acid groups and is therefore smaller and much more lipophilic than Congo red or chrysamine-G.
Implementation: Evaluation of the *in vivo* ability of methoxy-X04 to selectively "associate" with individual plaques in a manner that can facilitate non-invasive detection applying multiphoton microscopy.
Animal Model: Mouse (PSI/APP).
Interpretation and Analysis: Methoxy-X04 retains *in vitro* binding affinity for amyloid beta (a-beta) fibrils (K_i = 26.8 nM) very similar to that of chrysamine-G (K_i = 25.3 nM). The fluorescent properties of methoxy-X04 stained plaques, tangles, and cerebrovascular amyloid in postmortem sections of AD brain reveals good levels of specificity. In an *in vivo* environment (mice), multiphoton microscopy can be applied to obtain high-resolution (1 μm) fluorescent images of the brain. Under such conditions, it is possible to detect individual plaques that can be recognized within 30 to 60 minutes after a single intravenous injection (e.g. PSI/APP mouse brain). A single intraperitoneal methoxy-X04 injection at 10 mg/kg also produces high contrast images of plaques and cerebrovascular amyloid (e.g. PSI/APP mouse brain). Quantitative studies using tracer doses of [^{11}C]labeled methoxy-X04 show that it enters rat brain in amounts that suggest it is a viable candidate as a positron emission tomography (PET) amyloid-imaging agent for *in vivo* human diagnostic studies.
Instrumentation: Multiphoton microscopy.

Imaging a-beta plaques in living transgenic mice with multiphoton microscopy and methoxy-X04, a systemically administered Congo red derivative. Klunk WE, Bacskai BJ, Mathis CA, Kajdasz ST, McLellan ME, Frosch MP, Debnath ML, Holt DP, Wang Y, Hyman BT. J Neuropathol Exp Neurol 2002 Sep; 61 (9), pp. 797–805.

Alzheimer's Disease (AD): Amyloid Beta/Dense Plaque Oxidative Properties
Oxidation-Activated Fluorogenic Probes/N-Tert-Butyl-Alpha-Phenylnitrone

Cellular/Molecular Mechanism of Action: N-tert-butyl-alpha-phenylnitrone functions as a free radical spin trap.
Background Information: Amyloid beta, the primary constituent of senile plaques in AD, is hypothesized to cause neuronal damage and cognitive failure, but the mechanisms that mediate such alterations remain largely unknown.
Application: Fluorogenic compounds can be used *in vivo* to determine the degree of association between amyloid beta deposits and free radical production. The compound N-tert-butyl-alpha-phenylnitrone can function as a means of validating the effectiveness and accuracy of tracers and probes designed to detect anatomical regions of elevated oxidation potential.
Implementation: Utilization of compounds that actively fluoresce following exposure to tissues/anatomical regions that have elevated oxidative potential thereby facilitating their detection utilizing infrared laser imaging techniques. As a validation procedure, N-tert-butyl-alpha-phenylnitrone (a free radical spin trap) can be systemically administered to determine the accuracy of oxidation-activated fluorogenic probes. Collectively, these agents can be applied to delineate if a direct association exists between amyloid-beta deposits and free radical production.
Animal Model: Mouse (*in vivo* transgenic AD).
Human Model: Brain tissue from cases of AD (*ex vivo*).
Interpretation and Analysis: Fluorescence can be appreciated in dense core plaques, but not diffuse plaques, as determined by subsequent addition of thioflavine S and immunohistochemistry analysis for amyloid beta. Systemic administration of N-tert-butyl-alpha-phenylnitrone greatly reduces the detection of probes activated by tissue oxidative processes. It is therefore believed that a subset of amyloid plaques produce free radical species in living Alzheimer's models and in human Alzheimer tissue sections. Antioxidant therapy neutralizes these highly reactive molecules and may therefore be of both diagnostic and therapeutic value in AD.
Instrumentation: Multiphoton imaging.

In vivo imaging of reactive oxygen species specifically associated with thioflavine S-positive amyloid plaques by multiphoton microscopy. McLellan ME, Kajdasz ST, Hyman BT, Bacskai BJ. J Neurosci 2003 Mar 15; 23 (6), pp. 2212–7.

Alzheimer's Disease (AD): Amyloid/A-beta$_{1\text{-}40}$ Fibrils/Senile Plaques and Neurofibrillary Tangles
[^{18}F]FDDNP

Cellular/Molecular Mechanism of Action: The agent 2-(1-(6-[(2-[^{18}F]fluoroethyl)-(methyl)amino]-2-naphthyl)ethylidene) malononitrile ([^{18}F]FDDNP) is a hydrophobic dual-function semi-synthetic compound that emits fluorescent light and possess binding avidity for certain types of molecular amyloid.
Background Information: Senile plaques (SPs) and neurofibrillary tangles (NFTs) are hallmark pathologies that accompany the neurodegeneration associated with AD. Beta-amyloid (a-beta) peptide is a major constituent of SPs.
Application: Hydrophobic, fluorescent molecular-imaging probe [^{18}F]FDDNP, crosses the blood-brain barrier and determines the localization and load of SPs and NFTs *in vivo* in cases of AD.
Route of Administration: Intravascular.
Implementation: Determination of the binding avidity of FDDNP and its analog, 1-(6-[(2-[^{18}F]fluoroethyl)(methyl) amino] naphthalen-2-yl)ethanone ([^{18}F]FENE), to synthetic fibrils of a-beta$_{1\text{-}40}$ applying fluorimetric and radioactive binding assays.
Interpretation and Analysis: Both FDDNP and FENE bind to kinetically distinguishable binding sites (n = 2 sites) on a-beta$_{1\text{-}40}$ fibrils. Fluorescence titrations yield apparent K_d values of 0.12 and 0.16 nm associated with high-affinity binding sites for FDDNP and FENE, respectively, and apparent K_d values of 1.86 and 71.2 nm for low-affinity binding sites. The traditional radioactive binding assays also produce apparent K_d values in the low nanomolar range. The presence of 2 kinetically distinguishable binding sites for FDDNP and FENE suggests multiple binding sites for SPs and identifies the parameters that allow for the structural optimization of this family of probes for *in vivo* use. The high-affinity binding of the probes to multiple binding sites on fibrils is consistent with results obtained with digital autoradiography, immunohistochemistry, and confocal fluorescence microscopy using human brain specimens of AD cases.

Binding characteristics of radiofluorinated 6-dialkylamino-2-naphthylethylidene derivatives as positron emission tomography imaging probes for beta-amyloid plaques in Alzheimer's disease. Agdeppa ED, Kepe V, Liu J, Flores-Torres S, Satyamurthy N, Petric A, Cole GM, Small GW, Huang SC, Barrio JR. J Neurosci 2001 Dec 15; 21 (24), pp. RC189.

Alzheimer's Disease (AD): Amyloid Beta-Sheet Proteins/Amyloid Plaques: A-beta Peptide/ Neurofibrillary Tangles: Tau Protein
Thioflavin Derivatives

Cellular/Molecular Mechanism of Action: Thioflavin-T (ThT) derivatives possess binding avidity for beta-sheet proteins deposited in amyloid plaques (a-beta peptide) and neurofibrillary tangles (tau protein).

Background Information: Assessment of the beta-sheet proteins deposited *in vivo* in amyloid plaques (a-beta peptide) or neurofibrillary tangles (tau protein) represents a target for the development of biological markers for AD.

Application: Detection and characterization of beta-sheet proteins deposited in amyloid plaques (a-beta peptide) and neurofibrillary tangles (tau protein) similar to those associated with the pathogenesis of AD.

Route of Administration: [Intravenous] if eventually applied in a clinical environment.

Semi-Synthetic Methods: Production of thioflavin-T (ThT) derivatives and the carbon-11-labeled compound [N-methyl-^{11}C]6-Me-BTA-1.

Implementation: Evaluate and characterize the ability for [N-methyl-^{11}C]6-Me-BTA-1 to enter into and be cleared from the brain.

Animal Model: Mouse (Swiss-Webster).

Interpretation and Analysis: These compounds lack the positively charged quaternary heterocyclic nitrogen of ThT and are therefore uncharged at physiological pH. They are 600-fold more lipophilic than ThT. Derivatives of ThT bind to a-beta$_{1\text{-}40}$ fibrils with higher affinity (K_i = 20.2 nM) than ThT (K_i = 890 nM). The uncharged ThT derivatives stain both plaques and neurofibrillary tangles in postmortem AD brain, showing some preference for plaque staining.

The compound [N-methyl-^{11}C]6-Me-BTA-1 enters the brain at levels comparable to commonly used neuroreceptor imaging agents (0.223 %ID-kg/g or 7.61 %ID/g at 2 minutes postinjection) and shows good clearance of free and non-specifically bound radioactivity in normal rodent brain tissue (brain clearance $T_{1/2}$ = 20 minutes). The combination of relatively high affinity for amyloid, specificity for staining plaques and neurofibrillary tangles in postmortem AD brain, and good brain entry and clearance makes [N-methyl-^{11}C]6-Me-BTA-1 a promising candidate as an *in vivo* positron emission tomography beta-sheet imaging agent.

Uncharged thioflavin-T derivatives bind to amyloid-beta protein with high affinity and readily enter the brain. Klunk WE, Wang Y, Huang GF, Debnath ML, Holt DP, Mathis CA. Life Sci 2001 Aug 17; 69 (13), pp. 1471–84.

Alzheimer's Disease (AD): Amyloid/Extracellular Amyloid-Beta Protein/Abnormal Tau Protein/ Abnormal Synuclein Proteins/[Alzheimer's Disease]
[Trans/Trans]-1-Bromo-2,5-bis-(3-Hydroxycarbonyl-4-Hydroxy)Styrylbenzene/Thioflavin S

Cellular/Molecular Mechanism of Action: Congo red-derived fluorescent probe [trans/trans]-1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (BSB) binds to molecular amyloid.

Background Information: The novel Congo red-derived fluorescent probe BSB binds to amyloid plaques of postmortem Alzheimer's disease brains and *in vivo* in the brains of transgenic mice.

Application: Prototype imaging agent for detecting and characterizing amyloid deposits/plaques (e.g. AD).

Implementation: Evaluation of postmortem tissues from cases with various neurodegenerative diseases possessing diagnostic lesions using BSB as a probe. Additionally, BSB can be assessed for its ability to characterize fibrillar intra- or extracellular lesions and compare its properties with standard histochemical dyes such as thioflavin S and immunohistochemical stains specific for the same lesion type.

Interpretation and Analysis: Laboratory results reveals that BSB binds not only to extracellular amyloid beta protein but also to many intracellular lesions composed of abnormal tau and synuclein proteins which suggests that radioiodinated BSB derivatives or related ligands may be useful *in vivo* imaging agents for detecting and monitoring diverse types of amyloid.

The fluorescent Congo red derivative, (trans, trans)-1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (BSB), labels diverse beta-pleated sheet structures in postmortem human neurodegenerative disease brains. Schmidt ML, Schuck T, Sheridan S, Kung MP, Kung H, Zhuang ZP, Bergeron C, Lamarche JS, Skovronsky D, Giasson BI, Lee VM, Trojanowski JQ. Am J Pathol 2001 Sep; 159 (3), pp. 937–43.

Alzheimer's Disease (AD): Amyloid Plaques (A-beta)
Stilbene Derivatives/N-[^{11}C]Methylamino-4′-Hydroxystilbene

Cellular/Molecular Mechanism of Action: Stilbene derivatives recognize and physically bind to amyloid plaques analogous to those associated with AD.

Application: N-[^{11}C]methylamino-4′-hydroxystilbene, or [^{11}C]4 may be useful as a positron emission tomography (PET) imaging agent for mapping a-beta plaques in the brain of cases with AD.

Route of Administration: Intravenous.

Semi-Synthetic Methods: Synthesis of a series of stilbene derivative can be successfully achieved by performing a simple Wadsworth-Emmons reaction between diethyl (4-nitrobenzyl)phosphonate and 4-methoxybenzaldehyde. The [^{11}C] labeled 4-N-methylamino-4′-hydroxystilbene, [^{11}C] 4, is prepared by [^{11}C] methylation of 4-amino-4′-hydroxystilbene.

Analysis and Interpretation: 4-N,N-dimethylamino-4′-methyoxy and the corresponding 4-N-monomethylamino-, 4′-hydroxy stilbenes show good binding affinities toward a-beta aggregates *in vitro* (K_i < 10 nM). The [^{11}C]4 displays a moderate lipophilicity (log p = 2.36) and shows very good brain penetration and washout from normal rat brain following intravenous injection. *In vitro* autoradiography of transgenic AD mouse brain sections shows a high specific labeling of beta-amyloid plaques, whereas control sections show no binding.

11C-labeled stilbene derivatives as A-beta-aggregate-specific PET imaging agents for Alzheimer's disease. Ono M, Wilson A, Nobrega J, Westaway D, Verhoeff P, Zhuang ZP, Kung MP, Kung HF. Nucl Med Biol 2003 Aug; 30 (6), pp. 565–71.

Alzheimer's Disease (AD): Amyloid Deposits (Related Conditions)/Differential Stain Recognition: Non-Fibrillar vs Fibrillar Amyloid/Transthyretin: Amyloidic Protein
X-34/Congo Red/Thioflavin S

Cellular/Molecular Mechanism of Action: The agents Congo red, thioflavin S, and X-34 all possess binding avidity for molecular amyloid.

Background Information: Transgenic *Caenorhabditis elegans* animals can be engineered to express high levels of human beta amyloid peptide (a-beta). Histochemistry of fixed tissue reveals deposits reactive with the amyloid-specific dyes Congo red and thioflavin S.
Implementation: Discovery of the potential for X-34 to bind to amyloid deposits in *Caenorhabditis elegans* applying immunoelectron microscopy as a validation methodology.
Interpretation and Analysis: Immunoelectron microscopy can detect intracellular immunoreactive deposits with classic amyloid fibrillar ultrastructure. These deposits can be visualized in living animals using the newly developed, intensively fluorescent, amyloid-specific dye X-34. *In vivo* staining allows monitoring of amyloid deposition over time. The specificity of X-34 staining is demonstrated by examining transgenic models expressing high levels of a non-fibrillar beta peptide variant, the beta single-chain dimer. These animals have deposits immunoreactive with antibeta antibodies but do not have X-34 positive-staining deposits or deposits with a fibrillar ultrastructure. Visualization of putative amyloid deposits resulting from accumulation of human transthyretin, another amyloidic protein, can also be achieved *in vivo* using X-34 dye reagent. *In vivo* amyloid staining with X-34 may be a useful tool for monitoring anti-amyloidic treatments in real time or screening for genetic alterations that affect amyloid formation.

Visualization of fibrillar amyloid deposits in living, transgenic Caenorhabditis elegans animals using the sensitive amyloid dye, X-34. Link CD, Johnson CJ, Fonte V, Paupard M, Hall DH, Styren S, Mathis CA, Klunk WE. Neurobiol Aging 2001 Mar–Apr; 22 (2), pp. 217–26.

Alzheimer's (Dementia Type): Cerebral Cortical Function/Mini-Mental State Examination/ Prediction Classification: Stable/Improvement vs Deterioration
Pharmaco-EEG/Tetrahydroaminoacridine

Cellular/Molecular Mechanism of Action: Tetrahydroaminoacridine (THA) is a reversible cholinesterase inhibitor.
Physiological Mechanism of Action: Tetrahydroaminoacridine (THA) can function as an antidementia agent that is capable of reversing the decreases in acetylcholine release, and improve the spatial memory deficits associated with the administration of Delta9-tetrahydrocannabinol, a major psychoactive component of marijuana.
Background Information: Previous investigations have indicated that a single-dose pharmaco-EEG may predict the outcome of 4 to 7 weeks of THA treatment in Alzheimer's type of dementia (DAT).
Application: Characterization of alterations in cerebral cortical function in DAT conditions. The pharmaceutical has been used in the treatment of Alzheimer's disease.
Route of Administration: Tetrahydroaminoacridine (oral).
Dosage: Tetrahydroaminoacridine (30 mg).
Implementation: Evaluate the influence of THA on cerebral cortex function in conditions of DAT applying quantitative EEG analyses. Tracings are recorded 2 hours after an intial oral dose and then again after 12 weeks of THA.

Human Model: Probable cases of DAT (n = 24). Investigations can be designed in the format of an open trial study.
Interpretation and Analysis: The EEG in conditions of DAT that exhibit stable or improved scores on Mini-Mental State Examinations (MMSE) at 12 weeks display a significantly faster baseline mean alpha frequency as well as significant reductions in relative theta power following a single THA dose compared to conditions displaying evidence of deterioration. Discriminant analysis using test dose response EEG variables can correctly classify 75–79% of these 2 patient group types, suggesting that the procedure may be a useful approach for optimizing the individual selection for anti-dementia treatments.

Pharmaco-EEG test dose response predicts cholinesterase inhibitor treatment outcome in Alzheimer's disease. Knott V, Mohr E, Mahoney C, Ilivitsky V. Methods Find Exp Clin Pharmacol 2000 Mar; 22 (2), pp. 115–22.

Alzheimer's Disease (AD): Histamine H_3 Receptors
A-304121/A-317920/Thioperamide, Ciproxifan, and GT-2331

Cellular/Molecular Mechanism of Action: Physically bind to and functionally antagonize H_3 receptor sites.
Physiological Mechanism of Action: Pharmacological blockade of central histamine H_3 receptors (H3Rs) enhances cognition in rodents.
Application: Potentially beneficial for the clinical diagnosis (imaging) and treatment of certain neurological disorders (Alzheimer's-like conditions).
Route of Administration: [(4-(3-(4-((2R)-2-aminopropanoyl)-1-piperazinyl)-propoxy)-phenyl)-cyclopropylmethanone] and A-317920 [N-((1R)-2-(4-(3-(4-(cyclopropylcarbonyl)-phenoxy)-propyl)-1-piperazinyl)-1-methyl-2-oxo-ethyl)-2-furamide].
Dosage: Agent #1—[(4-(3-(4-((2R)-2-aminopropanoyl)-1-piperazinyl)-propoxy)-phenyl)-cyclopropylmethanone] (A-304121: dipsogenia (1 mg/kg); rat-pup avoidance (10 mg/kg)]. Agent #2—[N-((1R)-2-(4-(3-(4-(cyclopropylcarbonyl)-phenoxy)-propyl)-1-piperazinyl)-1-methyl-2-oxo-ethyl)-2-furamide] [A-317920: dipsogenia (0.45 mg/kg); rat-pup avoidance 3 mg/kg)].
Considerations: Several previously characterized H_3R antagonists are either not selective for H_3Rs or have potentially significant tolerability issues.
Interpretation and Analysis: Both A-304121 [(4-(3-(4-((2R)-2-aminopropanoyl)-1-piperazinyl)propoxy)-phenyl)-cyclopropylmethanone] and A-317920 [N-((1R)-2-(4-(3-(4-(cyclopropylcarbonyl)-phenoxy)-propyl)-1-piperazinyl)-1-methyl-2-oxo-ethyl)-2-furamide] evoke functional blockade of central H_3 receptors based on their ability to significantly attenuate acute dipsogenia responses mediated by the selective H_3R agonist (R)-alpha-methylhistamine [(R)-alpha-MeHA].

Behavioral and neurophysiological research (*in vivo*) has demonstrated that cognitive performance is improved in 5-trial rat-pup avoidance tests following the administration of A-304121 (10 mg/kg) or A-317920 (3 mg/kg), with efficacy levels comparable with previously published observations for the reference H_3R antagonists thioperamide (10 mg/kg), ciproxifan (3 mg/kg), and GT-2331 [(1R,2R)-4-(2-(5,5-dimethylhex-1-ynyl)cyclopropyl)imidazole] (1 mg/kg). Social memory is also significantly enhanced in the adult rat with A-304121 (3, 10 mg/kg) and A-317920 (1, 3 mg/kg) at doses that produce no significant change in electroencephalogram slow-wave amplitude activity. Relative therapeutic indices (TIs) of 30 and 42 have been estimated for A-304121 and A-317920, respectively, by comparing doses producing adverse effects in general observation studies with potency in inhibitory avoidance, which are superior to TIs of 8, 10, and 18 observed for the reference antagonists thioperamide, ciproxifan, and GT-2331, respectively. The compounds A-304121 and A-317920 represent a series of novel, H_3R-selective piperazine amides that enhance cognition *in vivo*, which could offer advantages over existing H_3R antagonists or cognition-enhancing agents.

Two novel and selective nonimidazole H3 receptor antagonists A-304121 and A-317920: II. *In vivo* behavioral and neurophysiological characterization. Fox GB, Pan JB, Radek RJ, Lewis AM, Bitner RS, Esbenshade TA, Faghih R, Bennani YL, Williams M, Yao BB, Decker MW, Hancock AA. J Pharmacol Exp Ther 2003 Jun; 305 (3), pp. 897–908. Epub 2003 Feb 20.

Antidepressant Profiling Influences on Cortex Function
Pharmaco-Electroencephalography Psychometric Tests/S-Adenosyl-L-Methionine (SAMe)/L-Methionine (SAMe)/Ademetionine

Physiological Mechanism of Action: S-adenosyl-L-methionine promotes EEG patterns similar to the thymoleptic-type properties associated with imipramine and amitriptyline.
Application: S-adenosyl-L-methionine, L-methionine (SAMe), or ademetionine.
Route of Administration: S-adenosyl-L-methionine (infusion over 30 minutes for 7 days with a washout period of 3 weeks enforced between serial EEG recordings).
Dosage: S-adenosyl-L-methionine (800 mg).
Implementation: Evaluation of the influence of SAMe, L-methionine (SAMe), and ademetionine on brain function and behavior utilizing EEG mapping and psychometry analyses. S-adenosyl-L-methionine is administered with an enforced washout period of 3 weeks in between serial analyses. EEG recordings and psychometric analyses are performed 0, 1, 3, and 6 hours after drug administration on days 1 and 7.

Human Model: Cases of normal healthy elderly volunteers (n = 10; n = 5 males; n = 5 females; age = 56–71 years, mean: = 59.3 years). Investigation design: Double blind, placebo-controlled crossover study.
Interpretation and Analysis: Multivariate analysis based on MANOVA/Hotelling T_2 tests demonstrate significant central effects of SAMe compared to placebo after acute, subacute, and superimposed drug administration. Acute SAMe induces changes that are characterized by a decrease in total power, an increase in absolute delta, and a decrease in absolute alpha power; further by an increase in relative delta and a decrease in relative alpha power; and a slowing of the delta/theta centroid as well as a slowing of the centroid of the total power spectrum. These changes are typical of classical antidepressants of the thymoleptic type (e.g. imipramine and amitriptyline). After 1 week of daily infusions there are marked increases in total power, reminiscent of nootropic drug effects. One additional superimposed dosage mitigates these effects in the direction of an antidepressant profile, with the inter-drug differences waning in the 6^{th} hour. Pharmaco-EEG findings therefore reveal that both inhibitory and excitatory drug effects underlying the antidepressant properties of SAMe are well documented in clinical trials. Psychometric tests concerning noopsychic and thymopsychic measures as well as critical flicker frequency generally demonstrate a lack of differences between SAMe and placebo in a manner that reflects the good tolerability of the agent in elderly subjects.

Pharmacodynamic studies on the central mode of action of S-adenosyl-L-methionine (SAMe) infusions in elderly subjects, utilizing EEG mapping and psychometry. Saletu B, Anderer P, Linzmayer L, Semlitsch HV, Lindeck-Pozza E, Assandri A, di Padova C, Saletu-Zyhlarz GM. Ther Drug Monit 2002 Aug; 24 (4), pp. 532–6.

Arteriovenous Malformations: Central Nervous System (CNS)
Methohexital

Physiological Mechanism of Action: Methohexital is a barbiturate that acts as a central nervous system depressant.
Application: Existing reports suggest that methohexital is a safe provocative agent for use prior to embolotherapy for brain arteriovenous malformations. However, the full effects of methohexital within the cerebral circulation remain to be elucidated.
Route of Administration: Methohexital (intra-arterial).
Dosage: Methohexital (1% concentration with individual doses of 1–6 mg/injection).
Interpretation and Analysis: Arterovenous malformation can initially be characterized with the aid of digital vascular imaging methods performed just prior to and just after intra-arterial methohexital injection.

Human Model: Cases without angiographic or clinically detectable evidence of vasospasms (e.g. n = 66 vascular pedicle injections in n = 30 cases).
Interpretation and Analysis: Perfusion of functional tissue with methohexital can often immediately precipitate evidence of altered neurologic status that fortunately almost invariably resolves within 2 minutes of the initial onset. No signs of either prolongation of the induced clinical symptoms or seizures are evident to suggest any adverse effects related to either methohexital crystallization or the effects of injecting an alkaline solution into the cerebral vascular circulation.

Though the full effects of methohexital in the cerebral circulation remain to be elucidated, existing reports suggest it is a safe provocative agent for use prior to embolotherapy for brain arteriovenous malformations.

Intra-arterial use of sodium methohexital for provocative testing during brain embolotherapy. Peters KR, Quisling RG, Gilmore R, Mickle P, Kuperus JH. AJNR Am J Neuroradiol 1993 Jan–Feb; 14 (1), pp. 171–4.

Autism (Infantile): Hypothalamic Dysfunction/ Parkinson's Disease
L-dopa

Cellular/Molecular Mechanism of Action: Systemically, L-dopa is converted to dopamine, which is responsible for the primary physiological properties of L-dopa preparations.
Application: Delineation of hypothalamic dysfunction and possible relationship to dopaminergic abnormalities in cases of infantile autism fulfilling the DSM-III criteria for this disorder.
Considerations: Following oral administration, approximately 95% of the L-dopa ingested is decarboxylated to dopamine (poorly penetrates the blood-brain barrier). In order to achieve changes in neurological function through the accumulation of L-dopa in the CNS, relatively large doses have to be administered. Over time, a tolerance to L-dopa begins to develop. The conversion of L-dopa to dopamine, however, can be inhibited by the co-administration of inhibitors of hepatic dopa decarboxylase (L-amino acid decarboxylase inhibitors: carbidopa [USA] benserazide [Europe]).

One side effect of L-dopa is an inhibition of prolactin secretion presumably through a direct effect on the adenohypophysis and possibly the release of prolactin inhibitor factor. Other side effects are

rather common when L-dopa is applied as a therapeutic modality (vomiting, nausea).

Drug interactions may occur with pyridoxine since L-dopa is decarboxylated by the pyridoxine-dependent enzyme L-amino acid decarboxylase. Excess amounts of pyridoxine enhance extracerebral metabolism of L-dopa, thereby neutralizing its biological/physiological effect (e.g., pyridoxine in vitamin supplements). Reserpine depletes intracellular dopamine stores.

Implementation: Evaluation of suspected cases of autism and Parkinson's disease.

Interpretation and Analysis: Results indicate a high incidence (at least 30%) of blunted plasma growth hormone responses following oral L-doap administration. These data suggest an alteration of hypothalamic dopamine receptor sensitivity in cases with blunted responses. Thus, a subgroup of autistic cases within a descriptively homogeneous diagnostic category shows evidence of hypothalamic dysregulation and dopaminergic abnormalities.

In conditions of Parkinson's disease, approximately 75% of the cases respond favoriably to L-dopa administration and may appear essentially symptom free. To some degree, all of the clinical signs of Parkinson's disease respond to L-dopa administration except episodes of dementia.

Growth hormone response to L-dopa and clonidine in autistic children. Realmuto GM, Jensen JB, Reeve E, Garfinkel BD. J Autism Dev Disord 1990 Dec; 20 (4), pp. 455–65.

Plasma growth hormone response to oral L-dopa in infantile autism. Deutsch SI, Campbell M, Sachar EJ, Green WH, David R. J Autism Dev Disord 1985 Jun; 15 (2), pp. 205–12.

Autonomic Nervous System Dynamic Time-Frequency Analysis
Sodium Amobarbitol/Wada Test

Background Information: Recently, time-frequency analysis has become very popular for examining non-stationary time series and for researching fast-changing phenomena.

Application: Utilized in performing the Wada test which involves the (selective) injection of sodium amobarbitol into the internal carotid artery resulting in the inactivation of cerebral structures supplied by the ipsilateral anterior and middle cerebral arteries. The intra-carotid instillation of amobarbital can be combined with electrocardiographic analyses (electrocardiopharmacology test).

Route of Administration: Amobarbitol (catheter instillation into the internal carotid artery).

Implementation: Utilization of a smoothed-pseudo Wigner-Ville distribution to model the underlying dynamic autonomic nervous system changes that occur during the intracarotid sodium amobarbital (ISA Wada test). Electrocardiogram (EKG) data are recorded during the entire procedure and 10 minutes prior to each injection which are used as baseline values for that hemisphere. Interbeat-interval time series are created from such data for further analyses (smoothed-pseudo Wigner-Ville distribution to model).

Human Model: 13-year-old patient.

Interpretation and Analysis: Evaluations of data generated in the fashion described reveals that sodium amobarbitol injection to either side of the brain produces decreased power in the vagally mediated high frequency band (.14-.40 Hz). Importantly, the decrease is greater when the right hemisphere is inactivated compared to the left side. Such results are consistent with the known lateralized innervation of the heart such that right-sided autonomic inputs have greater influence on cardiac chronotropy. Very rapid changes in autonomic control occur that characterize the inactivation and subsequent recovery of the cerebral hemispheres and serve to confirm the utility of time-frequency analysis in the investigation of cardiac time series.

Time frequency analysis of the cardiovascular response during the intracarotid amobarbital test. Sollers JJ 3rd, Ahern GL, Merritt MM, Thayer JF. Biomed Sci Instrum 2002; 38, pp. 267–71.

Behavior: Depression/REM Latency Disturbances
Pharmaco-Electroencephalography/Growth Hormone/DST

Application: Provocative stimulation tests that promote increased growth hormone secretion can be applied to detect and characterize certain types of behavioral depression.

Implementation: Evaluate the influence of provocative neuroendocrine stimulation tests with DST and growth hormone (GH) on cerebral cortical function during sleep analyzed by EEG recordings.

Human Model: Cases of prepubueral monozygotic twins (n = 2 males) with Major Depressive Disorder. Results are compared with results from a normal puberal control boy (e.g., n = 1), and an adolescent female (e.g., n = 1) suffering from major depression.

Interpretation and Analysis: The REM latency is reduced in the adolescent depressed girl but not in the puberal depressed twins when compared to normal controls (e.g., n = 1). Sleep continuity and sleep architecture are, however, disturbed in examples of puberal and adolescent depression and a function of or directly related to the severity of the original depressive state. The results of the DST reveal abnormal cortisol values in severely depressed individuals (e.g., most severely depressed twin and in the depressed adolescent). Growth hormone secretion after DMI shows a clear GH response in the less depressed twin and in normal subjects (e.g., n = 1) while in the depressed adolescent (e.g., n = 1), the GH response is blunted. Therefore, REM latency disturbances in at least some cases of depression do not appear before adolescence, while neuroendocrine dysfunction can already be present in puberal depression.

Electroencephalogram and neuroendocrine parameters in pubertal and adolescent depressed children. A case report study. Mendlewicz J, Hoffmann G, Kerkhofs M, Linkowski P. J Affect Disord 1984 Jun; 6 (3–4), pp. 265–72.

Behavior: Depression
L-5-Hydroxytryptophan (L-5HTP)

Background Information: Phenylalanine, tyrosine, L-dopa, L-tryptophan, and L-5-hydroxytryptophan (L-5HTP) have been found to induce differential behavioral and biochemical effects in both healthy subjects and endogenous depressed individuals. Indoleamine precursors predominantly cause mood changes. However, the efficacy of these amino acids as antidepressants has been neither clearly established nor refuted, probably due to insufficient consideration of the following criteria: lack of adequately high plasma levels to facilitate passive transport of enough agent into the brain; lack of effective serotoninergic system stimulation; and selective increase of serotonin turnover with minimal interaction with other neurotransmitters.

Application: Characterization of depressive behavior patterns.
Route of Administration: L-5HTP (intravenous).
Implementation: Evaluation of strategies for systemic loading with precursor amino acids of the monoamines that are postulated to be involved in the molecular pathogenesis of mental disorders. One approach towards achieving this objective involves delineating the concentration profile for intravenous L-5HTP utilized as a provocative test for cases of depression, administered in concert with the concomitant measurement of neuroendocrinological and psychometric parameters.

Theoretical and therapeutic potential of indolamine precursors in affective disorders. Wirz-Justice A. Neuropsychobiology 1977; 3 (4), pp. 199–233.

Behavior: Depression
Sulpiride/(Prolactin Response)

Cellular/Molecular Mechanism of Action: Sulpiride is a dopamine D2 receptor antagonist.
Background Information: Multiple lines of investigation have implicated the role of the dopaminergic system in disorders of depression.
Application: Detection and characterization of the molecular basis for depression behavior (e.g. cerebral hemisphere disfunction).
Implementation: Characterization of dopamine D_2 receptor sensitivity status in depressed vs negative control cases by means of a neuroendocrine challenge test involving the measurement of prolactin response to sulpiride challenge. Cases are diagnosed as positive according to DSM-IV criteria, in concert with the application of the Montgomery Asberg and Zung scales.

Human Model: Cases of behavioral central nervous system depression (n = 10 with n = 10 age-matched male volunteer controls). No significant difference in baseline levels of prolactin can be appreciated between the depressed and control case groups.
Interpretation and Analysis: Significantly higher prolactin levels after sulpiride challenge can be appreciated in cases suffering from depression in contrast to unaffected controls at all time points following sulpiride administration. Such a neuroendocrine challenge paradigm suggests that the prolactin response to sulpiride is enhanced in depression, and suggests that this receptor might be supersensitive in depression disorders compared to controls. Observations of this nature add to the data implicating the dopaminergic system in the pathophysiology of depression and suggest that dopaminergic mechanisms might be a target of diagnostic and therapeutic interest.

The prolactin response to sulpiride in major depression: The role of the D2 receptor in depression. Verbeeck WJ, Berk M, Paiker J, Jersky B. Eur Neuropsychopharmacol 2001 Jun; 11 (3), pp. 215–20.

Behavior: Depression
Thyrotropin-Releasing Hormone (TRH)

Cellular/Molecular Mechanism of Action: One of the biological effects of hypothalamic thyrotropin-releasing hormone (TRH, rifathyroin) is induced production/release of pituitary thyroid stimulating hormone.
Physiological Mechanism of Action: Possesses a peculiar spectrum of stimulating action bringing it nearer to such psychotropic agents as psychostimulants and antidepressants.
Application: Thyrotropin-releasing hormone (TRH, rifathyroin) can be applied to characterize conditions of mental depression.
Considerations: In animals, TRH increases locomotivity without induction of aggression, stereotypia, or epileptic disorders; while it enhances the effects of amphetamine, apomorphine, and L-dopa; and reduces the action of reserpine, haloperidol, and clonidine.
Experimental Interpretation: Thyrotropin-releasing hormone facilitates the teaching of rats in a T-form labyrinth causing rotating behavior of rats during selective administration to the head of the caudate nucleus. In this context, experimental data has revealed that TRH possess adreno- and dopamine-stimulating properties in TRH.
Clinical Interpretation: Euphoristic and stimulating effects have been noted with the clinical application of TRH as a diagnostic agent in cases with depressive disturbances (e.g. n = 42).

Behavior: Depression: Cerebral Hemisphere Function/Pharmaco-EEG Profile/Delta and Alpha Power/Delta/Theta Centroid/Centroid of the Total Power Spectrum/Noopsychic and Thymopsychic Measures/Critical Flicker Frequency
S-adenosyl-L-Methionine/Ademetionine/[Imipramine]/[Amitriptyline]

Physiological Mechanism of Action: S-adenosyl-L-methionine promotes EEG patterns similar to the thymoleptic-type properties associated with imipramine and amitriptyline.
Application: S-adenosyl-L-methionine, L-methionine (SAMe), or ademetionine.
Route of Administration: S-adenosyl-L-methionine (infusion over 30 minutes for 7 days with a washout period of 3 weeks enforced between serial EEG recordings).
Dosage: S-adenosyl-L-methionine (800 mg).
Implementation: Evaluation of the influence of SAMe, L-methionine (SAMe), and ademetionine on brain function and behavior utilizing EEG mapping and psychometry analyses. S-adenosyl-L-methionine is administered with an enforced washout period of 3 weeks in between serial analyses. EEG recordings and psychometric analyses are performed 0, 1, 3, and 6 hours after drug administration on days 1 and 7.

Human Model: Cases of normal healthy elderly volunteers (n = 10; n = 5 males; n = 5 females; age = 56–71 years, mean: = 59.3 years). Investigation design: Double blind, placebo-controlled crossover study.
Interpretation and Analysis: Multivariate analysis based on MANOVA/Hotelling T_2 tests demonstrate significant central effects of SAMe compared to placebo after acute, subacute, and superimposed drug administration. Acute SAMe induces changes that are characterized by a decrease in total power, an increase in absolute delta, and a decrease in absolute alpha power; an increase in relative delta and a decrease in relative alpha power; and a slowing of the delta/theta centroid as well as a slowing of the centroid of the total power spectrum. These changes are typical of classical antidepressants of the thymoleptic type (e.g. imipramine and amitriptyline). After 1 week of daily infusions there are marked increases in total power, reminiscent of nootropic drug effects. One additional superimposed dosage mitigates these effects in the direction of an antidepressant profile, with the inter-drug differences waning in the 6^{th} hour. Pharmaco-EEG findings therefore reveal that both inhibitory and excitatory drug effects underlying the antidepressant properties of SAMe are well documented in clinical trials. Psychometric tests concerning noopsychic and thymopsychic measures as well as critical flicker frequency generally demonstrate a lack of differences between SAMe and placebo in a manner that reflects the good tolerability of the agent in elderly subjects.

Pharmacodynamic studies on the central mode of action of S-adenosyl-L-methionine (SAMe) infusions in elderly subjects, utilizing EEG mapping and psychometry. Saletu B, Anderer P, Linzmayer L, Semlitsch HV, Lindeck-

Pozza E, Assandri A, di Padova C, Saletu-Zyhlarz GM. Ther Drug Monit 2002 Aug; 24 (4), pp. 532–6.

Behavior: Depression
L-dopa/TRH/[Propanolol]

Cellular/Molecular Mechanism of Action: Induces growth hormone (GH) release/secretion
Background Information: Suboptimal concentrations of GH can be found in serum following the administration of L-dopa/propanolol.
Application: Detection of deficiencies in systemic GH production.
Implementation: Evaluation of endocrine factors in conditions of mental depression using the dexamethasone suppression test (DST), thyroid-stimulating hormone test (TSH), prolactin (PRL) responses to thyrotropin-releasing hormone (TRH), in addition to GH responses to L-dopa challenge. Provocative tests are performed before commencement of antidepressant treatment and after recognition of a clinical response to determine if; (i) a functional relationship exists between the hypothalamic-pituitary-adrenal (HPA) axis, the hypothalamic-pituitary-thyroid (HPT) axis, and the dopaminergic system in disorders of depression, in addition to determining if (ii) alterations in these hormonal functions occur during treatment with antidepressants.

Human Model: Cases of mental depression (n = 19).
Interpretation and Analysis: Thyroid-stimulating hormone responses to TRH show a tendency to increase from pre- to post-treatment periods, while TRH-induced PRL and L-dopa-induced GH responses do not change during treatment for depression in cases that noticeably respond favorably to intervention. Females show significantly higher TSH and PRL responses to TRH compared to males. No interconnections can be appreciated between responses in DST, TRH stimulation test, and L-dopa-induced GH test. Therefore, there appears to be no support that an interrelationship exists between the abnormalities in the HPT and HPA axes and central dopaminergic activity in depression.

Effects of antidepressant treatment on thyrotropin-releasing hormone stimulation, growth hormone response to L-dopa, and dexamethasone suppression tests in major depressive cases. Esel E, Kartalci S, Tutus A, Turan T, Sofuoglu S. Prog Neuropsychopharmacol Biol Psychiatry 2004 Mar; 28 (2), pp. 303–9.

Behavior Modification
Naloxone

Cellular/Molecular Mechanism of Action: Competitively blocks binding of endogenous endorphins to membrane-associated opiod receptor sites.
Diagnostic Application: Management of endorphin-reinforced "self-reward" behavior. Examples include horses displaying "cribbing," "weaving," and self-mutilation behaviors (e.g. equine species); and excessive licking, tail-chasing, and biting/chewing in dogs and cats.
Route of Administration and Dosage: Parenteral/systemic ideally administered using a slow "timed-release" type of delivery modality. An example of such a preparation is an impregnated resin pellet.
Interpretation and Analysis: A positive response is detected by a reversal or prevention of behavior abnormalities (pharmacological behavior modification). Naloxone has a relatively short duration of action which complicates the practicality of administering the agent for the purpose of behavior modification. Slow timed-release forms appear to be one alternative approach to avoiding the disadvantages of short duration of action.

Behavior: Neuropsychological Testing/Cholinergic Receptor Complexes: Muscarinic
Scopolamine/Physostigmine/Methylphenidate

Cellular/Molecular Mechanism of Action: Scopolamine binds to muscarinic cholinergic receptor complexes in a manner that competitively inhibits the biological activity of acetylcholine as a neurotransmitter, thereby functioning as anticholinergic/parasympatholytic agent. Physostigmine binds to and inactivates the biochemical activity of acetylcholine esterase in a manner that promotes accumulation of acetylchline neurotransmitter (anticholinesterase properties). Methylphenidate, like other amphetamines, promotes the release of biogenic amines from storage granules located within presynaptic membranes.
Physiological Mechanism of Action: Scopolamine (anticholinergic), scopolamine plus physostigmine (anticholinesterase), scopolamine plus methylphenidate (adrenergic: mild CNS stimulant and produces convulsions at higher dosage levels). Methylphenidate promotes locomotor stimulation that is generally associated with norepinephrine release in contrast to the release of dopamine from dopaminergic nerve terminals within the CNS (e.g., neostiatum). Higher doses of methylphenidate are believed to cause the release of 5-hydroxytryptamine (5-HT or serotonin) from tryptaminergic presynaptic membranes (mesolimbic system) in a manner that promotes psychotic behavioral changes.
Application: Neurophysiological testing utilized in combination with evoked potential analyses to evaluate various types of dysfunction, including age-related cognitive impairment and Alzheimer's like dementia conditions (DAL) that are linked to alterations in the cholinergic system.
Examples of Clinical Implementation: Scopolamine prolongs the P3 latency and reduces the spectral power of long latency evoked potentials without significantly altering earlier components. Physostigmine partially reverses these effects, but methylphenidate does not. Motor speed is slowed by scopolamine but not by scopolamine/methylphenidate combinations. The cholinergic systems appear to be involved in the generation or modulation of the P3 potential, possibly secondary to the cholinergic role in selective attention. Similar techniques and pharmaceutical agents may be useful as *in vivo* probes of central cholinergic function and thus as research and diagnostic aids in aging and disease.

In vivo probe of central cholinergic systems. Meador KJ, Loring DW, Lee GP, Taylor HS, Hughes DR, Feldman DS. J Gerontol 1988 Nov; 43 (6), pp. M158–62.

Norepinephrine, Epinephrine, and the Sympathomimetic Amines. Weiner N (Chapter 8: pp. 145–80). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Behavior/Opioid Abstinence (Acute Morphine Withdrawl)
Naloxone/Nalorphine

Cellular/Molecular Mechanism of Action: Morphine, naloxone, and nalorphine bind to opioid receptor complexes residing within the central nervous system.
Implementation: Evaluation of the effects of morphine, naloxone, and nalorphine on "responses" when *in vivo* models are maintained under variable-interval schedules for food presentation.

Observations are assessed before and after successive periods of daily morphine maintenance (15 mg/kg/day SC). Withdrawal from morphine dependence is performed gradually following 2 initial maintenance periods and abruptly following a third period. Schedule-controlled "response" is disrupted when morphine maintenance is abruptly discontinued but not when the maintenance dosage is gradually reduced to zero.

Animal Model: Primate (rhesus monkeys).

Interpretation and Analysis: Tolerance to the acute effects of morphine (IV) on "responses" develop during morphine maintenance and dissipate when daily injections are discontinued. Effects of naloxone and nalorphine following each period of morphine maintenance are generally similar to their effects in initial periods of evaluation. Such observations indicate that tolerance-producing regimens of repeated daily injections with morphine do not necessarily produce enduring changes in the effects of opiate antagonists on schedule-controlled behavior. Additionally, gradual withdrawal from morphine maintenance can minimize the behavioral disruptions that attend abrupt abstinence.

Behavioral effects of naloxone and nalorphine preceding and following morphine maintenance in the rhesus monkey. Bergman J, Schuster CR. Psychopharmacology (Berl)] 1985; 86 (3), pp. 324–7.

Behavior/Panic Disorder/Cerebral Cortex Function
Sodium Lactate/Carbon Dioxide/Caffeine/Yohimbine/Isoprenaline/Cholecystokinin

Background Information: The pharmacological challenge strategy involves giving a provoking agent according to controlled protocols to clarify some aspect of behavioral or biological function.

Implementation: Various agents such as sodium lactate, carbon dioxide, caffeine, yohimbine, isoprenaline, and cholecystokinin have been used as agents to provoke panic disorder in healthy volunteers as well as in panic cases.

Interpretation and Analysis: Results obtained in this field of study have been updated, with emphasis on the potential mechanisms of action. It has been concluded that there may be a final pathway between carbon dioxide, sodium lactate, and cholecystokinin inducing panic attacks.

Provocative agents in panic disorder. Bourin M, Malinge M, Guitton B. Therapie 1995 Jul–Aug; 50 (4), pp. 301–6.

Behavior/Pharmaco-Electroencephalogram Mapping
Diazepam

Cellular/Molecular Mechanism of Action: Diazepam inhibits central nervous system gamma aminobutyrate (GABA), which functions as a neurotransmitter. Suppression is associated with the known ability of diazepam to bind to membrane-associated GABA receptor complexes.

Application: Diazepam (central nervous system depressant).

Dosage: Diazepam (10 mg).

Implementation: Evaluate the influence of diazepam on brain (cerebral cortex) function applying EET topographical mapping techniques (recordings applied from 4 different reference sites). Four referential derivations including ipsilateral earlobe (A1A2), average reference (AV), source derivation (SD), and balanced noncephalic electrode (BNE) can be used. One-minute vigilance-controlled EEGs before drug administration and 2 hours after drug administration are analyzed using Fourier transformation followed by the calculation of absolute power and relative power for 4 frequency bands.

Human Model: Cases of normal healthy volunteers (n = 10 right-handed males; 21–25 years of age). Double blind crossover trials with a single oral dose of 10 mg diazepam or placebo control administered in random sequence at intervals of 1 week.

Interpretation and Analysis: Diazepam causes a widespread increase of beta-frequency relative power, and a widespread decrease of theta-frequency absolute power when A1A2 and SD are taken as references. From the comparison of different reference electrodes, the selection of the reference and of spectral parameters (absolute power or relative power) play an important role in pharmaco-EEG studies.

Pharmaco-EEG mapping of diazepam effects using different references and absolute and relative power. Yamadera H, Kato M, Ueno T, Tsukahara Y, Okuma T. Pharmacopsychiatry 1993 Nov; 26 (6), pp. 254–8.

Behavior/Psychotropic (Central) Side Effects
Pharmaco-Electroencephalogram/Loderix (Setastine)/Terfenadine (Teldane)/Chloropyramine HCl (Suprastin)

Physiological Mechanism of Action: The pharmaceutical agents, terfenadine, Teldane, chloropyramine HCl, and Suprastin possess sedative effects of very different degrees.

Application: Manipulation of cerebral cortex function and evaluation of pharmaceutical intervention in neurological disorders.

Implementation: Evaluate the influence of Loderix (setastine), in addition to a "vehicle" and with 2 reference pharmaceuticals (terfenadine, Teldane; and chloropyramine HCl, Suprastin) on cerebral cortex function applying EEG analysis as a means of monitoring efficacy.

Human Model: Cases of normal healthy male volunteers (n = 10) in a double blind, placebo-controlled, crossover arrangement.

Interpretation and Analysis: The different effects of the drugs on the central nervous system (CNS) are precisely indicated by the posterior alpha/theta ratio in the EEG. This marker parameter is affected by Loderix in the same direction as by Teldane and in the opposite direction relative to Suprastin. In addition, Loderix increases the beta frequency range in the median areas of both hemispheres; moreover, it increases the total EEG power. The latter changes raise the question about the possibility that Loderix has an "own" action on EEG analyses. (The observed "own" effect could not even be brought into connection with a decrease of the vigilance level). Observations made to date strengthen the view that the action of Loderix on the EEG is similar to that induced by Teldane, a drug very favorable with respect to sedative side effects. Moreover, the drug does not affect the EEG power spectra in a direction that corresponded to a sedative action. The spectral parameters in the "pharmaco" EEG recordings seem to be useful in the objective definition of the central (psychotropic) side effects of drugs.

Assessing the sedative (adverse) effects of antiallergic drugs by quantitative electroencephalography: Effects of setastine, a non-sedating antihistaminic drug. Rajna P, Veres J. Ther Hung 1994; 42 (1), pp. 14–20.

Behavior: Schizophrenia/Pellegra
Niacin Challenge

Application: Clinical and biochemical evidence for alterations in phospholipid function in schizophrenia conditions. Used in cases with frank psychoses that may also simultaneously be suffering from pellagra-associated neurological abnormalities.

Dosage: Relatively large doses of niacin are required to induce neurologic signs, particularly when the psychosis is associated with conditions of encephalopathy.

Interpretation and Analysis: In human cases of pellagra with neurological signs of confusion, inappetence, nausea, and vomiting, the administration of nicotinic acid results in an abalation of signs within a period of 12 hours (approximately). Pellegra may be accompanied by conditions of peripheral neuritis precipitated by a thiamine deficiency.

The niacin challenge test: Clinical manifestation of altered transmembrane signal transduction in schizophrenia? Hudson CJ, Lin A, Cogan S, Cashman F, Warsh JJ. Biol Psychiatry 1997 Mar 1; 41 (5), pp. 507–13.

Behavior: Schizophrenia
Niacin (Skin Flush Response)/Niacin Challenge

Cellular/Molecular Mechanism of Action: Niacin selectively mediates the release of vasodilatory prostaglandins in the skin, creating a "flush" type response or sensation.

Application: Detection and characterization of schizophrenia conditions.

Implementation: Detection and characterization of schizophrenia cases.

Interpretation and Analysis: A normal skin flush response to niacin is attenuated in many individuals with schizophrenia (SCZ). Such findings suggest abnormal prostaglandin signaling in SCZ. Since prostaglandins are derived from arachidonic acid (AA), the finding of an abnormal skin flush response is consistent with biochemical data suggesting relative depletion of AA, and other essential fatty acids (EFAs), in a substantial portion of people with SCZ. Reports have described the mechanism of the skin flush response to niacin and why it is abnormal in SCZ conditions. The disease state is not related to psychotropic medications, and it may be a marker of EFA deficiency, which has been documented to be present in many SCZ cases.

Relationship between the niacin skin flush response and essential fatty acids in schizophrenia. Messamore E. Prostaglandins Leukot Essent Fatty Acids 2003 Dec; 69 (6), pp. 413–9.

Behavior: Schizophrenia (Familial Clusters of Disease)
Lithium

Background Information: Selecting a drug according to the treatment response in a blood relative has been widely accepted advice in the management of mood disorders. However, this recommendation has not been adequately substantiated in the literature. Minimal information is known about to what extent the response to long-term lithium treatment is a familial trait.

Implementation: Evalation and comparison of the response to long-term lithium treatment in bipolar blood relatives of bipolar lithium responders and bipolar controls. Validation of a diagnosis for bipolar disorder can be determined using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) and Research Diagnostic Criteria (RDC). Lithium response is evaluated on a rating scale reflecting the quality and quantity of available data.

Human Model: Relatives of cases with bipolar disorders (n = 24) identified in families of 106 cases with lithium-response disorder; and lithium-treated bipolar cases serving as a comparison control group (e.g. n = 40).

Interpretation and Analysis: Relatives with bipolar disorder identified in families with lithium-responsive bipolar disorder, compared to lithium-treated bipolar cases (comparison controls) reveals a prevalence of unequivocal response among the relatives of 67%, compared to a response rate of 35% in the comparison group (chi^2 = 6.04, df = 1, p = 0.014). The highly significant difference in observed response between relatives and reference control groups supports the perspective that lithium prophylaxis clusters exist within families and the general population.

Is response to prophylactic lithium a familial trait? Grof P, Duffy A, Cavazzoni P, Grof E, Garnham J, MacDougall M, O'Donovan C, Alda M. J Clin Psychiatry 2002 Oct; 63 (10), pp. 942–7.

Behavior: Tourette's Syndrome (Tic Disorders)
Amphetamine/Dextroamphetamine/Haloperidol/ Levamfetamine Succinate/Apomorphine/Piribedil/ [^{11}C]-Raclopride

Cellular/Molecular Mechanism of Action: Apomorphine is postulated to interact with presynaptic dopamine receptors. Dopamine functions as a inhibitory neurotransmitter.

Background Information: Dopaminergic abnormalities in frontal-subcortical circuits have been hypothesized as the underlying pathophysiologic mechanism in Tourette's syndrome.

Application: Administration is based on the hypothesis that presynaptic dopamine/catecholamine release from anatomical structures like the striatum is abnormal in adults with Tourette's syndrome.

Implementation: Evaluation of Tourette's syndrome conditions utilizing 2 positron emission tomography (PET) scans with high specific activity [^{11}C]-raclopride. The 1st scan is performed after an intravenous injection of saline, while the 2nd scan is made after intravenous amphetamine injection. Relative dopamine release is estimated as the percentage difference in binding potential between the post-saline and post-amphetamine scans.

Interpretation and Analysis:

Nuclear Medicine Studies: Binding potential determined after initial [^{11}C]-raclopride scans do not significantly differ between Tourette's syndrome cases and control subjects. After amphetamine challenge, the mean value of intrasynaptic dopamine in the putamen (as determined by true equilibrium bolus estimation) increases by 21% in subjects with Tourette's syndrome but does not change in control subjects; the mean values increase by 16.9% and decrease by 1.8%, respectively (constrained method of measurement). Dopamine release in the caudate region is not significantly different with Tourette's syndrome and comparison subjects. Greater putamen dopamine release is seen in adults with Tourette's syndrome than in comparison subjects after a pharmacologic challenge with amphetamine. These results suggest that the underlying pathobiology in Tourette's syndrome is a phasic dysfunction of dopamine transmission.

Behavioral Studies: Both dextroamphetamine and levamfetamine increase the severity of the symptoms; dextroamphetamine is more potent. Haloperidol controls the symptoms and also antagonizes the effect of dextroamphetamine. Apomorphine injections reduce the severity of symptoms, even in the presence of dextroamphetamine. Therefore, dopamine rather than norepinephrine is the principal catecholamine responsible for observed symptoms. The effect of apomorphine may be understood through its action on postulated presynaptic inhibitory dopamine receptors or other presynaptic mechanisms of action.

Effects of dopamine agonists and antagonists in Tourette's disease. Feinberg M, Carroll BJ. Arch Gen Psychiatry 1979 Aug; 36 (9), pp. 979–85.

Benzodiazepine/Receptor Ligands
2, 9-Disubstituted Quinolino[2′, 3′-5, 4](3-Pyrazolino)Pyrimidin-2-Ones/2, 9-Disubstituted Quinolino[2′, 3′-5, 4](3-Pyrazolino)Purin-4-Ones

Cellular/Molecular Mechanism of Action: The compounds 2, 9-disubstituted quinolino[2′, 3′-5, 4](3-pyrazolino)pyrimidin-2-ones and purin-4-ones recognize and bind to benzodiazepine receptor complexes (GABA).
Physiological Mechanism of Action: The compounds 2, 9-disubstituted quinolino[2′, 3′-5, 4](3-pyrazolino)pyrimidin-2-ones and purin-4-ones displace [^{3}H]R015-1788 from its specific binding site in bovine brain membranes.
Interpretation and Analysis: Compound 5c causes 83% (± 8%) inhibition of [^{3}H]R015-1788 specific benzodiazepine receptor binding followed by compounds 5f, 5h, and 5i, in decreasing order of binding avidity. Other analogs are inactive at 10 μM.

Synthesis and benzodiazepine receptor binding activity of 2, 9-disubstituted quinolino[2′, 3′-5, 4](3-pyrazolino)[3, 2-b]purin-4-ones. El-Sayed OA, Habib NS, Aboul-Enein HY, Costa B, Lucacchini A, Martini C. Arch Pharm (Weinheim) 2002 May; 335 (5), pp. 207–12.

Bladder Function/Over-Active Bladder Conditions/ Serotonin (5-HT) Production in the Central Nervous System/Physiological Processes Mediated by Alterations in Serotonin Production
Clomipramine/Fluoxetine

Cellular/Molecular Mechanism of Action: Clomipramine modifies (reduces) concentrations of serotonin (5-HT) within the central nervous system (CNS). Fluoxetine is a selective CNS serotonin uptake inhibitor.
Physiological Mechanism of Action: Clomipramine promotes endogenous CNS depression while fluoxetine can reverse these CNS properties induced by clomipramine.
Background Information: Serotonin (5-HT) in the CNS may inhibit bladder activity. Based on this consideration, it has been postulated that CNS depression associated with altered 5-HT production may be associated with or be the cause of conditions such as overactive bladder (OAB) activity.
Application: Detection and evaluation of physiological functions or conditions (e.g., OAB) that may be associated with alterations in serotonin (5-HT) production within the CNS.
Dosage: Clomipramine hydrochloride (22.5 mg/kg body weight) compared to an equal volume of sterile physiological saline administered by injection to negative reference controls. Fluoxetine (20 mg/kg daily).
Implementation: Evaluation of the influence of clomipramine hydrochloride on urinary voiding frequency at 10 and 15 weeks. Behavioral correlates of depression are assessed using a forced-swim challenge. At age 15 weeks cystometry (CMG) analysis is performed. Fluoxetine is then co-administered with clomipramine or saline followed by the measurement of void frequency (VF) and reevaluation of CMG analyses.

Animal Model: Rat (Wistar pups, postnatal days 8 to 21) endogenous model for depression promoted by lowering serotonin (5-HT) concentrations.
Interpretation and Analysis: Clomipramine increases immobility when combined with forced-swim challenge testing (indication of depression). Clomipramine promotes more frequent voiding compared to negative saline controls at 10 and 15 weeks. Interestingly, the difference is significant only in females. Cystometry of clomipramine/females reveals decreased bladder capacity, micturition volume, and inter-micturition contractions compared with negative saline reference controls. Fluoxetine reverses these changes (comprable to saline negative reference controls). Such observations support the hypothesis that OAB may be associated with altered 5-HT function and may explain previous descriptions of an association between depression and OAB.

Alterations in voiding frequency and cystometry in the clomipramine induced model of endogenous depression and reversal with fluoxetine. Lee KS, Na YG, Dean-McKinney T, Klausner AP, Tuttle JB, Steers WD. J Urol 2003 Nov; 170 (5), pp. 2067–71.

Blood-Brain Cerebrospinal Fluid (CSF) Barrier/ Carrier Mediated Efflux Transport System
[^{3}H]-Oestrone-3-Sulfate

Cellular/Molecular Mechanism of Action: Oestrone-3-sulfate is a steroid hormone.
Application: Evaluation and characterization of blood-brain barrier transport mechanisms.
Implementation: Evaluation of the efflux transport of oestrone-3-sulfate, a steroid hormone sulfate, across the blood-cerebrospinal fluid barrier has been examined following its intracerebroventricular administration. To characterize the oestrone-3-sulfate transport process, an *in vitro* uptake experiment can be performed using isolated rat choroid plexus.

Animal Model: Rat (*in vivo* and applying isolated rat *ex vivo* choroid plexus preparation).
Interpretation and Analysis: [^{3}H]oestrone-3-sulfate is eliminated from CSF with an apparent efflux clearance of 205 μL min^{-1}. Approximately 25% of unmetabolized [^{3}H]oestrone-3-sulfate is in the plasma 5 minutes after intracerebroventricular administration, indicating that at least a fraction of [^{3}H]oestrone-3-sulfate is transported from the CSF, across the CSF-blood barrier and into the circulating blood (intravascular compartment). This efflux transport is inhibited by the co-administration of excess oestrone-3-sulfate (25 mM 10 μL = 0.25 μmol) into the cerebral ventricle.

Oestrone-3-sulfate uptake by isolated choroid plexus (*ex vivo* preparation) is a saturable process with a Michaelis-Menten constant (K_m) of 18.1 ± 6.3 μM, and a maximum uptake rate (V_{max}) of 48.0 ± 15.1 pmol/min/μL of tissue. The oestrone-3-sulfate transport process is temperature dependent and inhibited by metabolic inhibitors such as 2,4-dinitrophenol and rotenone, suggesting that the process is energy dependent. Such uptake events are also inhibited by steroid hormone sulfates (1 mM dehydroepiandrosterone sulfate at 1 mM; and oestrone sulfate at 1 mM), bile acids (taurocholic acid at 1 mM; and cholic acid at 1 mM), and organic anions (sulphobromophthalein at 1 mM; and phenolsulphonphthalein at 1 mM), whereas *p*-aminohippuric acid at 1 mM, *p*-nitrophenol sulfate at 1 mM, methotrexate at 0.1 mM, and the cardiac glycoside digoxin at 2.5 μM have little effect. Therefore, oestrone-3-sulfate appears to be transported from the CSF into the circulating blood across the blood-CSF barrier via a carrier-mediated efflux transport system.

In vivo and *in vitro* evidence of a carrier-mediated efflux transport system for oestrone-3-sulfate across the blood-cerebrospinal fluid barrier. Kitazawa T, Hosoya K, Takahashi T, Sugiyama Y, Terasaki T. J Pharm Pharmacol 2000 Mar; 52 (3), pp. 281–8.

Caffeine/Cerebral Cortex Function/Environmental Psychostimulant Interference
Caffeine/Topographic Quantitative EEG

Cellular/Molecular Mechanism of Action: Caffeine is classified as a xanthine analog that promotes stimulation of the central nervous system.
Physiological Mechanism of Action: Caffeine (psychostimulants and stimulate the medullary respiratory centers).
Background Information: Despite the widespread use of caffeine as a central nervous stimulant, the central pharmacodynamic properties of the drug have not yet been conclusively evaluated in humans.
Application: Evaluation of the interference of environmental psychostimulant on topographic quantitative electroencephalography analyses.
Route of Administration: Caffeine (oral powder in water).
Dosage: Caffeine (200 mg equivalent to 2 cups of coffee).
Implementation: Evaluate the influence of caffeine on cerebral cortex function by topographically measuring quantitative electroencephalogram (EEG) profiles. Oral caffeine is administered under randomized, double blind crossover conditions on 2 different occasions. Before administration and 30 minutes afterward, a 17-channel quantitative EEG is recorded during relaxation with eyes open and closed (15 minutes each).
Human Subjects: Cases of normal cerebral cortex function (n = 10 healthy subjects; mean age = 25 and SD ± 4 years).
Interpretation and Analysis: Caffeine causes a significant reduction of total EEG power at fronto-parieto-occipital and central electrode positions of both hemispheres when the eyes are kept open. Absolute power of the slow and fast alpha and slow beta activities are diminished in various regions of the brain ($p < 0.05$). The effect is more pronounced when the eyes are kept open than when the eyes are kept closed. Quantitative EEG appears to be a sensitive method for assessing the effects of psychostimulants on the human brain. Therefore, in pharmaco-EEG studies, environmental factors such as caffeine have to be excluded.

Effects of caffeine on topographic quantitative EEG. Siepmann M, Kirch W. Neuropsychobiology 2002; 45 (3), pp. 161–6.

Cerebrospinal Fluid (CSF)Rhinorrhea: Cranial Trauma
Fluorescein Dye

Background Information: Cerebrospinal fluid rhinorrhea typically results from trauma to the base of the skull, producing leaks through either the cribiform plate region or the sphenoid bone. Traditional approaches to the repair of such leaks include a frontal craniotomy or external ethmoidectomy. An endoscopic approach through the nose has also proven to be successful.
Implementation: Evaluation of traumatic CSF rhinorrhea and development of a meningocele in the region of the cribiform plate using fluorescein dye to demonstrate the site of CSF leakage.
Interpretation and Analysis: Visualization of the defect with fluorescein dye can fasciliate repair of the defect via a transnasal endoscopic approach. Given such a scenario, a variety of complementary diagnostic procedures (radiographic assessment) and approaches for repair and postoperative care can subsequently be employed.

Endoscopic repair of traumatic CSF rhinorrhea in a pediatric patient. Wetmore RF, Duhaime AC, Klausner RD. Int J Pediatr Otorhinolaryngol 1996 Jul; 36 (2), pp. 109–15.

Reconstruction of the lacrimal drainage system. Holt JE, Holt GR. Arch Otolaryngol 1984 Apr; 110 (4), pp. 211–20.

Chorea-Acanthocytosis (Familial)/Hypothalamic Disorder/Dopaminergic System
Levodopa/Thyroid-Stimulating Hormone (TSH)/ Thyrotropin-Releasing Hormone (TRH)

Cellular/Molecular Mechanism of Action: Levodopa promotes the release of growth hormone releasing hormone.
Application: Assessment of hypothalamic/pituitary function (acromegaly, gigantism, anterior secretion, somatropin secretion). L-dopa can be used to assess growth hormone releasing hormone synthesis/release. Thyroid-stimulating hormone can be used to evaluate thyroid function, while thyrotropin releasing hormone (TRH) can be utilized to assess plasma prolactin responses (also promotes TSH release).
Dosage: Glucose (oral tolerance test 75 grams).
Implemenation: Hypothalamic/pituitary interactions and functionality can be assessed by the collective administration of L-dopa, TSH, TRH, and oral glucose tolerance test. Additionally, it is possible to evaluate endocrinology aspects of dopaminergic regulation of the hypothalamic-pituitary axis in conditions of familial chorea-acanthocytosis.
Human Model: Cases of familial chorea-acanthocytosis (n = 3 females, 38–47 years of age).
Interpretation and Analysis: Many cases of familial chorea-acanthocytosis (e.g., n = 3/3) will exhibit low basal levels of triiodothyronine (T3), in addition to slightly elevated baseline plasma prolactin levels (e.g., n = 2/3). Cases will have delayed plasma TSH responses and plasma PRL excessive responses to TRH, and a low plasma growth hormone releasing hormone (GRF) response to L-dopa. Such TSH, PRL, and GRF responses represent a secretion pattern that is associated with a hypothalamic disorder that suggests the presence of impaired regulation of hormone secretion by the dopaminergic system, primarily in the level of the hypothalamus. In addition, an increased growth hormone (GH) secretory response following TRH (paradoxical response) can also be observed in some cases (e.g., n = 2/3), suggesting pituitary involvement. The vast majority of affected cases may display a diabetic profile when evaluated using an oral glucose tolerance test.

Disturbance of hypothalamic-pituitary hormone secretion in familial chorea-acanthocytosis. Terao S, Sobue G, Takahashi M, Miura N, Mitsuma T, Takeda A, Sakakibara T. No To Shinkei (Brain and Nerve) 1995 Jan; 47 (1), pp. 57–61.

Cough Reflex Induction: Brainstem/Internal Branches of the Superior Laryngeal Nerve/Forced Vital Capacity/Identification of Postanesthesia Complications/Resumption of Oral Feeding: Decision Criteria
Tartaric Acid (Nebulized)/Lidocaine

Physiological Mechanism of Action: Tartaric acid as applied for the activation of the cough reflex is perceived as a chemical irritant by receptors within the upper respiratory system.
Background Information: The internal branch of the superior laryngeal nerve (ibSLN) conveys impulses for the laryngeal cough reflex, which protects the laryngeal aditus and prevents the development of aspiration pneumonia.

Application: Determine the effect of bilateral anesthesia of the ibSLN on the cough reflex after inhalation of a nebulized chemoirritant solution of tartaric acid.
Route of Administration: Tartaric acid (inhaled/nebulization).
Implementation: Lidocaine (2% without epinephrine) is injected bilaterally into the paraglottic space containing internal branches of the superior laryngeal nerve (ibSLN). Tidal volume after inhalation of nebulized tartaric acid solution (20%) followed by the measurement of forced vital capacity (FVC) before and after injection. Data is analyzed using the Wilcoxon signed ranks, Mann-Whitney, and sign tests.

Human Model: Cases are normal healthy volunteers (n = 9).
Interpretation and Analysis: Complete anesthesia of the ibSLN abolishes the laryngeal cough reflex. Postinjection tidal volumes are significantly lower than preinjection volumes ($p < .01$). The decrease in tidal volume with complete bilateral anesthesia is significantly larger than decreases in tidal volume with partial anesthesia (n = 6/9 vs n = 3/9 @ $p < .05$). Force vital capacity measurements for both complete bilateral anesthesia and partial anesthesia do not significantly change from pre-injection to post-injection. Complications or adverse respiratory sequelae rarely develop. Tartaric acid–induced coughing may be useful in assessing the integrity of the laryngeal cough reflex after anesthesia or in cases with neurologic injury that are at risk of developing aspiration pneumonia. It may also be useful in making the decision whether to resume oral feeding schedules.

Anesthesia for the superior laryngeal nerves and tartaric acid-induced cough. Addington WR, Stephens RE, Goulding RE. Arch Phys Med Rehabil 1999 Dec; 80 (12), pp. 1584–6.

Dystonia (Childhood)/Encephalopathic and Neurologic Acute Crisis/Cerebral Cortex Function/ Glutaryl-CoA Dehydrogenase Deficiency/Glutaric Acidemia, Type I (GA-I)/Acute Encephalopathic/ Neurologic Crisis
Promethazine/[Diphenhydramine]

Background Information: The characteristic clinical feature of glutaric academia Type I (GA-I) is an acute encephalopathic and neurologic crisis typically occurring during a catabolic state. Despite slow improvement, many cases do not fully recover from a neurologic crisis, and residual neurologic morbidity can be significant.
Application: Promethazine may possibly be applied to characterize conditions of childhood dystonia associated with glutaric acidemia Type I (glutaryl-CoA dehydrogenase deficiency) in situations when the disease state can be detected by newborn screening with tandem mass spectrometry.

Human Model: Case of childhood dystonia (n = 1; promethazine-induced).
Interpretation and Analysis: Promethazine can induce dystonia in childhood cases while displaying a lack of a response to diphenhydramine administration. Such response profiles suggests a diagnosis of glutaric acidemia, type I (GA-I), classified as an autosomal recessive inborn error of metabolism caused by a deficiency of glutaryl-CoA dehydrogenase. Although newborn screening using tandem mass spectrometry is expected to enable presymptomatic diagnosis of GA-I, the case in the present report could not be detected by newborn screening with tandem mass spectrometry. Therefore, a high suspicion of GA-I must be maintained in the evaluation of childhood dystonia, even when newborn screening results are reportedly normal.

Glutaric acidemia, type I, missed by newborn screening in an infant with dystonia following promethazine administration. Smith WE, Millington DS, Koeberl DD, Lesser PS. Pediatrics 2001 May; 107 (5), pp. 1184–7.

Electroencephalography: Pharmacology Assisted Mapping/Behavior
Diazepam

Cellular/Molecular Mechanism of Action: Diazepam inhibits central nervous system gamma aminobutyrate (GABA), which functions as a neurotransmitter. Suppression is associated with the known ability of diazepam to bind to membrane-associated GABA receptor complexes.
Application: Diazepam (central nervous system depressant).
Dosage: Diazepam (10 mg).
Implementation: Evaluate the influence of diazepam on brain (cerebral cortex) function applying EET topographical mapping techniques (recordings applied from 4 different reference sites). Four referential derivations including ipsilateral earlobe (A1A2), average reference (AV), source derivation (SD), and balanced noncephalic electrode (BNE) can be used. One-minute vigilance-controlled EEGs before drug administration and 2 hours after drug administration are analyzed using Fourier transformation; followed by the calculation of absolute power and relative power for 4 frequency bands.

Human Model: Cases of normal healthy volunteers (n = 10 right-handed males; 21–25 years of age). Double blind crossover trials with a single oral dose of 10 mg diazepam or placebo control administered in random sequence at intervals of 1 week.
Interpretation and Analysis: Diazepam causes a widespread increase of beta-frequency relative power, and a widespread decrease of theta-frequency absolute power when A1A2 and SD are taken as references. From the comparison of different reference electrodes, the selection of the reference and of spectral parameters (absolute power or relative power) play an important role in pharmaco-EEG studies.

Pharmaco-EEG mapping of diazepam effects using different references and absolute and relative power. Yamadera H, Kato M, Ueno T, Tsukahara Y, Okuma T. Pharmacopsychiatry 1993 Nov; 26 (6), pp. 254–8.

Electroencephalography: Pharmacology Assisted/ Behavior/Depression: Antidepressants/ $GABA_A$ Receptor Complexes/ Serotonin Uptake: Cerebral Cortex Function/Hypocampal Electroencephalogram Profiles
Pharmaco-Electroencephalogram Analyses/ Serotoninertic Agents/Pentylenetetrazol/ Imipramine/Fluoxetine/Trazodone/ Desipramine/Nortriptyline

Cellular/Molecular Mechanism of Action: The antidepressants imipramine, fluoxetine, or trazodone inhibit serotonin uptake. Conversely, the antidepressants desipramine and nortriptyline inhibit norepinephrine uptake.
Application: Evaluate the influence of antidepressants with serotonergic and adrenergic effects on the function of $GABA_A$ receptor complexes.
Route of Administration: Pentylenetetrazol, imipramine, fluoxetine, or trazodone (intraperitoneal in rat *in vivo* model).
Dosage: Pentylenetetrazol (PTZ; 27.5 mg/kg) and imipramine, fluoxetine, or trazodone (7 days at 10 mg/kg given twice a day).

Implementation: Evaluate power spectrum changes induced by PTZ (chloride ionophore antagonist) in hippocampal electroencephalograms in order to better delineate the antidepressant drug effects on $GABA_A$ receptor complexes (e.g. $GABA_A$ receptor, chloride ionophore, and benzodiazepine receptor).

Animal Model: Rat (hippocampal EEG).

Interpretation and Analysis: In control recording, PTZ increases EEG power at frequencies under 12 Hz up to 5 times. After *in vivo* models are pretreated with imipramine, fluoxetine, or trazodone for 7 days (10 mg/kg i.p. twice a day), PTZ does not increase EEG power to more than 3 times the power before injection; this effect is not observed after 3 days of pretreatment. These 3 antidepressants inhibit serotonin uptake, while antidepressants that inhibit norepinephrine uptake (desipramine, nortriptyline) fail to counter the effect of PTZ. Antidepressants with serotonergic effects enhance the function of the $GABA_A$ receptor complex.

Electrophysiologic analysis of antidepressant drug effects on the $GABA_A$ receptor complex based upon antagonist-induced encephalographic power spectrum changes. Matsubara M, Suzuki S, Miura K, Terashima M, Sugita S, Kimura H, Hatsuda S, Mori T, Murakami H, Hayashi T, Ohta T, Ohara M. Neuropsychobiology 2000; 42 (3), pp. 149–57.

Electroencephalography: Pharmacology Assisted/ Behavior/Psychometry Tests
CATEEM/IBIS Psychometry

Background Information: Both psychometric tests and quantitative pharmaco-EEG are accepted as necessary tools in different fields of pharmacology. However, using these methods separately, neither is able to generate information about the behavioral performance and the central nervous demand simultaneously. The solution to this problem is a complex psychophysiological investigative design. Such complex measurement setups are a rarity. The main reason is the impossibility of EEG recordings during psychometric tests without the occurance of different artefacts.

Implementation: Utilization of a CATEEM-System in connection with the IBIS-Psychometry-system to determine the possibility of acquiring artifact-free EEG recordings during psychometric test conditions. The different mental loads at different tests produce a graduated increase in the delta and theta frequency range of the EEG of the fronto-temporal region and the occipital region of the cortex.

Interpretation and Analysis: During test evaluations a decrease of alpha activity is generally appreciated. These effects can be described quantitatively as the topographical distribution of frequency changes. The present data are in line with the knowledge about the functional and anatomical structure of the human brain.

Relation between psychometric tests and quantitative topographic EEG in pharmacology. Schober F, Dimpfel W. Int J Clin Pharmacol Ther Toxicol 1992 Nov; 30 (11), pp. 428–30.

Electroencephalography: Pharmacology Assisted/ Behavior/Psychometric Tests/ Antidepressant Profiling Influences on Cortex Function
S-Adenosyl-L-Methionine (SAMe)/L-Methionine (SAMe)/Ademetionine

Physiological Mechanism of Action: S-adenosyl-L-methionine promotes EEG patterns similar to the thymoleptic-type properties associated with imipramine and amitriptyline.

Application: S-adenosyl-L-methionine, L-methionine (SAMe), or ademetionine.

Route of Administration: S-adenosyl-L-methionine (infusion over 30 minutes for 7 days with a washout period of 3 weeks enforced between serial EEG recordings).

Dosage: S-adenosyl-L-methionine (800 mg).

Implementation: Evaluation of the influence of SAMe, L-methionine (SAMe), and ademetionine on brain function and behavior utilizing EEG mapping and psychometry analyses. S-adenosyl-L-methionine is administered with an enforced washout period of 3 weeks in between serial analyses. EEG recordings and psychometric analyses are performed 0, 1, 3, and 6 hours after drug administration on days 1 and 7.

Human Model: Cases of normal healthy elderly volunteers (n = 10; n = 5 males; n = 5 females; age = 56–71 years, mean: = 59.3 years). Investigation design: Double blind, placebo-controlled crossover study.

Interpretation and Analysis: Multivariate analysis based on MANOVA/Hotelling T_2 tests demonstrate significant central effects of SAMe compared to placebo after acute, subacute, and superimposed drug administration. Acute SAMe induces changes that are characterized by a decrease in total power, an increase in absolute delta, and a decrease in absolute alpha power; an increase in relative delta and a decrease in relative alpha power; and a slowing of the delta/theta centroid as well as a slowing of the centroid of the total power spectrum. These changes are typical of classical antidepressants of the thymoleptic type (e.g., imipramine and amitriptyline). After 1 week of daily infusions there are marked increases in total power, reminiscent of nootropic drug effects. One additional superimposed dosage mitigates these effects in the direction of an antidepressant profile, with the inter-drug differences waning in the 6^{th} hour. Pharmaco-EEG findings therefore reveal that both inhibitory and excitatory drug effects underlying the antidepressant properties of SAMe are well documented in clinical trials. Psychometric tests concerning noopsychic and thymopsychic measures as well as critical flicker frequency generally demonstrate a lack of differences between SAMe and placebo in a manner that reflects the good tolerability of the agent in elderly subjects.

Pharmacodynamic studies on the central mode of action of S-adenosyl-L-methionine (SAMe) infusions in elderly subjects, utilizing EEG mapping and psychometry. Saletu B, Anderer P, Linzmayer L, Semlitsch HV, Lindeck-Pozza E, Assandri A, di Padova C, Saletu-Zyhlarz GM. Ther Drug Monit 2002 Aug; 24 (4), pp. 532–6.

Electroencephalography: Pharmacology Assisted/ Behavior/Psychotropic (Central) Side Effects
Pharmaco-Electroencephalogram/Loderix (Setastine)/Terfenadine (Teldane)/ Chloropyramine HCl (Suprastin)

Physiological Mechanism of Action: The pharmaceutical agents, terfenadine, Teldane, chloropyramine HCl, and Suprastin possess sedative effects of very different degrees.

Application: Manipulation of cerebral cortex function and evaluation of pharmaceutical intervention in neurological disorders.

Implementation: Evaluate the influence of Loderix (setastine), in addition to a "vehicle" and with 2 reference pharmaceuticals (terfenadine, Teldane; and chloropyramine HCl, Suprastin) on cerebral cortex function applying EEG analysis as a means of monitoring efficacy.

Human Model: Cases of normal healthy male volunteers (n = 10) in a double blind, placebo-controlled, crossover arrangement.

Interpretation and Analysis: The different effects of the reference drugs on the central nervous system (CNS) are precisely indicated

by the posterior alpha/theta ratio in the EEG. This marker parameter is affected by Loderix in the same direction as by Teldane and in the opposite direction relative to Suprastin. In addition, Loderix increases the beta frequency range in the median areas of both hemispheres; moreover, it increases the total EEG power. The latter changes raise the question about the possibility that Loderix has an "own" action on EEG analyses. (The observed "own" effect could not even be brought into connection with a decrease of the vigilance level). Observations made to date strengthen the view that the action of Loderix on the EEG is similar to that induced by Teldane, a drug very favorable with respect to sedative side effects. Moreover, the drug does not affect the EEG power spectra in a direction that corresponded to a sedative action. The spectral parameters in the "pharmaco" EEG recordings seem to be useful in the objective definition of the central (psychotropic) side effects of drugs.

Assessing the sedative (adverse) effects of antiallergic drugs by quantitative electroencephalography: Effects of setastine, a non-sedating antihistaminic drug. Rajna P, Veres J. Ther Hung 1994; 42 (1), pp. 14–20.

Electroencephalography: Pharmacology Assisted/ Caffeine/Environmental Psychostimulant Interference
Caffeine/Topographic Quantitative EEG

Cellular/Molecular Mechanism of Action: Caffeine is classified as a xanthine analog that promotes stimulation of the central nervous system.
Physiological Mechanism of Action: Caffeine (psychostimulants and stimulate the medullary respiratory centers).
Background Information: Despite the widespread use of caffeine as a central nervous stimulant, the central pharmacodynamic properties of the drug have not yet been conclusively evaluated in humans.
Application: Evaluation of the interference of environmental psychostimulant on topographic quantitative electroencephalography analyses.
Route of Administration: Caffeine (oral powder in water).
Dosage: Caffeine (200 mg equivalent to 2 cups of coffee).
Implementation: Evaluate the influence of caffeine on cerebral cortex function by topographically measuring quantitative electroencephalogram (EEG) profiles. Oral caffeine is administered under randomized, double blind crossover conditions on 2 different occasions. Before administration and 30 minutes afterward, a 17-channel quantitative EEG is recorded during relaxation with eyes open and closed (15 minutes each).

Human Subjects: Cases of normal cerebral cortex function (n = 10 healthy subjects; mean age = 25 and SD ± 4 years).
Interpretation and Analysis: Caffeine causes a significant reduction of total EEG power at fronto-parieto-occipital and central electrode positions of both hemispheres when the eyes are kept open. Absolute power of the slow and fast alpha and slow beta activities are diminished in various regions of the brain ($p < 0.05$). The effect is more pronounced when the eyes are kept open than when the eyes are kept closed. Quantitative EEG appears to be a sensitive method for assessing the effects of psychostimulants on the human brain. Therefore, in pharmaco-EEG studies, environmental factors such as caffeine have to be excluded.

Effects of caffeine on topographic quantitative EEG. Siepmann M, Kirch W. Neuropsychobiology 2002; 45 (3), pp. 161–6.

Electroencephalography: Pharmacology Assisted/ Epilepsy: Uncontrollable/Pharmacokinetic and Pharmacodynamic Analyses of Antiepileptic Agents
Quantitative Pharmaco-Electroencephalography/ Clonazepam/Valproate

Cellular/Molecular Mechanism of Action: Clonazepam inhibits central nervous system gamma aminobutyrate (GABA), which functions as a neurotransmitter. In this context, diazepam is known to bind to membrane-associated GABA receptor complexes.
Route of Administration: Clonazepam (orally/per os).
Dosage: Clonazepam (CZP 0.05 mg/kg single dose).
Implementation: Evaluation of the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of valproate (VPA) and clonazepam (CZP) in epileptic children in addition to its effect on cerebral cortex function applying quantitative pharmaco-EEG (QPEEG) techniques. Following a single clonazepam dose, blood samples are collected at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 8.0, 12.0, and 24.0 hours. At each blood collection time point, EEG signals (60 s) are recorded for brain electrical activity mapping, and the power percentage average (PPA) of each frequency band calculated. The relationship between drug concentrations and their corresponding PPA of each frequency band are then analyzed.

Human Model: Cases of childhood epilepsy (n = 4) suffering from uncontrolled seizures despite long-term VPA therapy.
Interpretation and Analysis: Valproate steady-state drug concentrations (Css) are within the therapeutic range and not affected by CZP. The peak concentration (C_{max}) of CZP and the time intervals from dosing to C_{max} (T_{max}) are 20.9 ng/mL to 113.8 ng/mL and 1 hour to 1.5 hours, respectively. There is no significant correlation between VPA concentrations and the PPA of any of the EEG frequency bands. Clonazepam blood concentrations show significant correlation with PPA in a high percentage of cases (e.g., n = 3/4 cases). Clonazepam may therefore affect fast wave activities in proportion to CZP blood concentrations. Quantitative pharmaco-electroencephalography appears to be a promising technique for studying the pharmacokinetics and pharmacodynamics of selected antiepileptic drugs.
Instrumentation: High-pressure liquid chromatography (clonazepam) and gas-chromatography (valproate).

Pharmacokinetic and pharmacodynamic effects of clonazepam in children with epilepsy treated with valproate: A preliminary study. Wang L, Wang XD. Ther Drug Monit 2002 Aug; 24 (4), pp. 532–6.

Electroencephalography: Pharmacology Assisted/ Epilepsy
Pharmaco-Electroencephalogram/ Carbamazepine (CBZ)

Application: Delineating the influence of CBZ on cerebral cortex function in epileptic disease states.
Dosage: Carbamazepine (300 mg, single dose).
Implementation: Evaluate the influence of CBZ on cerebral cortex function in epileptic conditions utilizing quantitative EEG (qEEG) techniques and instrumentation. Sample EEG tracings are recorded before and at regular intervals within 24 hours following pharmaceutical administration. The EEG activity is processed by power spectral analysis and the drug serum concentration assessed concomitantly.

Human Model: Cases of epilepsy (n = 10 epileptics; n = 10 healthy adult volunteers).
Interpretation and Analysis: Increases in serum CBZ concentrations correspond with the percentage of power progressively increasing in the theta band while it decreases in the alpha2 band. The percentage of theta and alpha2 power is found to reach maximum and minimum levels, respectively, when the peak values for serum concentration of CBZ are attained. The effects on qEEG in anterior scalp areas are more evident than in posterior scalp areas.

Quantitative pharmaco-EEG of carbamazepine in volunteers and epileptics. Wu X, Xiao CH. Clin Electroencephalogr 1996 Jan; 27 (1), pp. 40–5.

Electroencephalography: Pharmacology Assisted/ Epilepsy
Pharmaco-Electroencephalogram/Valproate

Application: Characterization of cerebral cortical function in epilepsy conditions.
Dosage: Valproate (single-dose administration).
Implementation: Evaluation of the influence of sustained-release valproate (SR-VPA) on cerebral cortex function in epilepsy conditions applying quantitative background electroencephalography (EEG). Complementary investigations include establishing SR-VPA serum concentrations.

Human Model: Cases of CNS epilepsy (n = 10 untreated with generalized tonic-clonic seizures).
Interpretation and Analysis: Pharmaco-electroencephalography reveals increased power of alpha2 band over occipital areas, a decrease of power of beta band (diffuse and preponderant on posterior scalp areas) with increasing serum concentrations. Both effects reach their maxima and minima, respectively, when the peak values of serum concentration are reached. A positive correlation exists between alpha2 power on the occipital areas and serum SR-VPA concentrations, and a negative correlation of serum concentration and beta power are detected over the posterior scalp areas.

Quantitative pharmaco-EEG of sustained release valproate in epileptics. Wu X, Xiao CH. Clin Electroencephalogr 1997 Apr; 28 (2), pp. 117–20.

Electroencephalography: Pharmacology Assisted/ Epilepsy/Cononical Correlation (Semilinear): Anesthesiology/Responders vs Non-Responders: Psychiatry/1st-Order Markov Model of Sleep Stage Transitions: Neurophysiology
Pharmaco-Electroencephalograpy (Pharmaco-EEG)

Background Information: In the clinical application and interpretation of pharmaco-EEG, it is important to appreciate its value in the context of other diagnostic parameters such as biomarkers, surrogate end points, clinical end points, and clinical outcomes.
Application: Detection and characterization of neurologic disorders through the combined application of specific pharmaceutical agents and measurement of their influence on central nervous function detected with EEG instrumentation.
Considerations: Much work still needs to be conducted to fully develop the potential capabilities that pharmaco-EEG together with pharmacokinetic-pharmacodynamic modeling can bring to therapeutics in neuroscience.
Interpretation and Analysis: State-of-the-art applications of pharmaco-EEG, together with pharmacokinetic-pharmacodynamic modeling in everyday clinical practice, is becoming a reality pertaining to the fields of anesthesiology (semilinear canonical correlation), psychiatry (discrimination between responders and non-responders to pharmacological treatment using the test dose), neurology (antiepileptic field), and neurophysiology (1st-order Markov model of sleep stage transitions). The combination of both procedures, although successfully used during some drug development programs (opioids or benzodiazepines), it is not widely applied in clinical conditions affecting the central nervous system.

Uses of pharmaco-EEG and pharmacokinetic-pharmacodynamic modeling in the clinical scenario. Barbanoj MJ, Valle M, Kulisevsky J, Perez V, Gambus P. Methods Find Exp Clin Pharmacol 2002; 24 Suppl D, pp. 139–44.

Electroencephalography: Pharmacology Assisted/ Epilepsy: Anticonvulsive Therapy Response/Relative Efficacy Evaluation/ Anticonvulsants for Petit mal/ Generalized Non-Convulsive Epilepsy
Pharmaco-Electroencephalography/Milacemide (CP 1552 S)/Sodium Valproate/Piracetam

Cellular/Molecular Mechanism of Action: Milacemide (CP 1552 S) is a derivative of glycine that exerts an anticonvulsant action by increasing GABA concentrations and endogenous glycine pools in the brain.
Physiological Mechanism of Action: Milacemide exerts a brain anticonvulsant action by increasing GABA concentrations.
Application: Compare and contrast the electroencephalography profiles and behavioral anticonvulsant properties of various therapeutic agents.
Route of Administration: Milacemide (oral).
Dosage: Milacemide (400 mg, 800 mg, 1600 mg). Reference compounds (placebo; sodium valproate at 600 mg; and piracetam at 1600 mg).
Implementation: Evaluation of the encephalotropic and psychotropic properties of milacemide by means of quantitative EEG and psychometric analyses. Electroencephalogram recordings and monitoring of blood pressure, heart rate, and side effects are performed at 0, 1, 2, 4, 6, and 8 hours. Psychometric tests are performed at 0, 2, 4, 6, and 8 hours.

Human Model: Cases functioning as healthy normal reference controls (n = 12). Study Design: Double blind placebo-controlled study.
Interpretation and Analysis: Computer-assisted spectral analysis of EEG profiles shows significant effects of milacemide on the central nervous system (CNS) in contrast to placebo, characterized by an attenuation of delta activity accompanied by an acceleration of the centroid of the slow activity but also of the total activity in response to all 3 dosage levels. Other alterations noted include increases in beta activity after 400 mg, an increase of alpha activity after 800 mg, as well as an increase of alpha activity but a decrease in beta activity after a 1600 mg dose. Such changes, also seen after 600 mg sodium valproate, are reminiscent of quantitative EEG changes described after antiepileptic drugs used in the treatment of petit mal or generalized non-convulsive epilepsy. Moreover, they are also indicative of improvement in "vigilance" in the sense of Head, which is also seen at the behavioral level, specifically after the lowest doses of milacemide, as psychometric tests demonstrate an improvement in attention, concentration, psychomotor activity, and after-effect (indicating CNS activation) as measured by means of the Archimedean spiral. Such a beneficial influence on performance declines with increasing dose. Evaluation of pulse, blood pressure,

and side effects demonstrate good tolerance after all administered substances.

Early clinical pharmacological trials with a new antiepileptic, milacemide, using pharmaco-EEG and psychometry. Saletu B, Grünberger J. Methods Find Exp Clin Pharmacol 1984 Jun; 6 (6), pp. 317–30.

Electroencephalography: Pharmacology Assisted/ Ethanol and Thiopental Cerebral Cortex Depression/Pharmacological Interactions *Pharmaco-Electroencephalography/ Captopril/Enalaprilat*

Cellular/Molecular Mechanism of Action: Captopril and enalaprilat function as angiotensin converting enzyme inhibitors.
Background Information: The influence of captopril and enalaprilat on the central nervous system has been investigated in laboratory animals.
Route of Administration: Captopril (intraperitoneal); enalaprilat (intraperitoneal); ethanol (intraperitoneal); and thiopental (intraperitoneal).
Dosage: Captopril (5 and 20 mg/kg); enalaprilat (5 and 20 mg/kg); ethanol (4 g/kg); and thiopental (70 mg/kg) single administration or repeated SID for 10 days.
Implementation: Evaluation of the effect captopril and enalaprilat have on the duration of ethanol- and thiopental-induced changes in sleep, body temperature, spontaneous locomotor activity, and analgesic properties (hot plate and tail-flick test, and pharmaco-EEG profiling).
Animal Model: Murine (hot plate and tail-flick test).
Animal Model: Rabbit (pharmaco-EEG profiles).
Interpretation and Analysis: Single administration of captopril (both doses) and single or prolonged administration of enalaprilat decrease the duration of ethanol- and thiopental-induced sleep. Captopril (5 mg/kg) and enalaprilat (5 and 20 mg/kg) increase pain thresholds. Both captopril and enalaprilat after single or repeated administration do not influence spontaneous locomotor activity (mice). Captopril and enalaprilat decrease body temperature in mice. Examined ACEIs produce changes in EEG recording, more profoundly exhibited after administration of enalaprilat.

An attempt to assess the central action of captopril and enalaprilat. Czarnecka E, Strzelec J, Pietrzak B. Med Sci Monit 2000 May–Jun; 6 (3), pp. 491–7.

Electroencephalography: Pharmacology Assisted/ Seizures: Alcoholic Detoxification/ Recurrent Episodes *Chlordiazepoxide/Pharmaco-Electroencephalography*

Application: Delineation of recurrent alcoholic detoxification seizures.
Route of Administration: Per os (oral) during detoxification events (associated with the elimination of withdrawal seizures).
Implementation: Evaluation of the potential increased risk of seizure activity during conditions of recurrent alcohol detoxifications. Structural brain damage, focal neurologic deficits, epileptogenic findings on electroencephalograms, and provocative drug use are considered pertinent variables for seizure risk.
Human Model: Review of detoxification and seizure histories (n = 256 men; n = 45 women voluntarily admitted to a 5-day inpatient detoxification program).
Interpretation and Analysis: Administration of chlordiazepoxide is usually associated with the elimination of withdrawal seizures. Some cases have a history of seizures (e.g., n = 64/301) while others have significantly abnormal electroencephalograms during detoxification (e.g., n = 42/301), or have focal slowing on the electroencephalogram (e.g., n = 30/301). No correlation exists between the duration of alcohol abuse or lifetime consumption of alcohol and seizure activity. There is, however, a significant correlation between the number of inpatient alcohol detoxifications and the prevalence of seizure disorders. This correlation holds true even when provocative drug use is taken into consideration.

Seizure risk with recurrent alcohol detoxification. Lechtenberg R, Worner TM. Arch Neurol 1990 May; 47 (5), pp. 535–8.

Electroencephalography: Pharmacology Assisted/ Seizures/Epilepsy Induction *Pentylenetetrazol/Pharmaco-Electroencephalography*

Mechanisms of Action: Pentylenetetrazol diminishes the influence of GABA on chloride ion conductance at concentrations that do not alter resting neuronal membrane conductance in the absence of GABA. Additional attributes of pentylenetetrazol include inhibition of picrotoxin association with binding sites, and it has the ability to block the enhancement of GABA and benzodiazepine binding promoted by hypnotic barbiturates. No direct stimulatory effect has been associated with pentylenetetrazol.
Diagnostic Application: Primarily of value in facilitating the diagnostic induction of epileptic episodes for the purpose of acquiring EEG recordings.
Route of Administration: Pentylenetetrazol (subcutaneous).
Dosage: Pentylenetetrazol (PTZ seizure dose = 65 mg/kg).
Considerations: Pentylenetetrazol induces seizure activity through CNS stimulation. Because of the potential hazards that seizure episodes might precipitate, appropriate antiseizure medications should always be readily (immediately) available for administration as a safety precaution.
Implementation: Truly simultaneous electroencephalogram (EEG) and functional magnetic resonance imaging (fMRI) are recorded following the induction of convulsions with pentylenetetrazol. Rigorous control of physiological parameters like body temperature and ventilation with control of blood gasses help avoid potential interference between systemic parameters and central PTZ-induced blood oxygenation level-dependent (BOLD) changes.
Animal Model: Rat (curarized rats).
Interpretation and Analysis: In experimental *in vivo* models, simultaneous EEG/fMRI recordings demonstrate progressive epileptiform EEG discharges with concomitant BOLD changes, with the latter gradually affecting most of the fore- and midbrain. Approximately 15 minutes after PTZ injection, the 1st BOLD contrast changes mainly occur in the neocortex, and coincide with the 1st minor EEG alterations. Most regions that display BOLD changes are areas with reportedly high $GABA_A$ receptor densities. Full-blown epileptiform discharges occur on the EEG tracing, approximately 30 minutes after PTZ injection, and coincide with bilateral positive and/or negative BOLD contrast changes in cortical and subcortical regions. Behavioral observations demonstrate that the 1st of several generalized clonic or clonic-tonic seizure episodes occur also around this same time period. Approximately 90 minutes after injection, the electrographic paroxysms gradually decrease in amplitude and duration, whereas BOLD signal changes still extended with alternating positive and negative traces and spread to subcortical regions like caudate-putamen and globus pallidus.

In humans, the induction of an epileptic seizure or analogous episodes following administration constitutes a positive diagnosis that can be more fully characterized through analysis of EEG recordings.

Laboratory Instrumentation: Electroencephalogram recording.

Simultaneous electroencephalographic recording and functional magnetic resonance imaging during pentylenetetrazol-induced seizures in rat. Van Camp N, D'Hooge R, Verhoye M, Peeters RR, De Deyn PP, Van der Linden A. NeuroImage 2003 Jul; 19 (3), pp. 627–36.

Electroelectroencephalography: Pharmacology Assisted/Sleep Quality
Pharmaco-Electroencephalogram (EEG) Mapping/ d-Fenfluramine (d-FEN)/l-Fenfluramine (l-FEN)/ Chlorpromazine (CPZ)/d-Amphetamine (AMPH)/Prolactin

Route of Administration: Single oral dose of each *d*-fenfluramine, l-fenfluramine, chlorpromazine, and *d*-amphetamine (AMPH @ 50 mg).
Dosage: Single dose of *d*-fenfluramine (d-FEN at 15 and 30 mg); l-fenfluramine (l-FEN at 30 mg); chlorpromazine (CPZ at 50 mg); and *d*-amphetamine (AMPH at 50 mg).
Implementation: Pharmaco-EEG mapping of the pharmacodynamic properties of *d*-FEN, l-FEN compared to CPZ, and AMPH as well as prolactin and safety assessments. Blood samples are collected to determine concentrations of AMPH, FEN and its main metabolite norFEN, as well as plasma prolactic levels; EEG recordings and evaluation of hemodynamics and spontaneous side effects are performed at 0, 2, 4, 6, and 8 hours after drug administration. Before and 24 hours after drug intake, morning subjective sleep quality is then also assessed.

Human Model: Cases of young healthy volunteers (n = 18) receiving pharmaceutical agents at weekly intervals.
Interpretation and Analysis: Blood concentration levels measured after d-FEN demonstrate a dose-dependent blood concentrations peaking after 2–4 hours and slowly declining thereafter. NorFEN, the main metabolite, exhibits a steady increase in plasma concentrations up to the 8th hour. After l-FEN a similar time course is observed, but the concentrations of the parent compound are higher and those of the metabolite lower in comparison with the *d*-isomer, suggesting stereoselective kinetics. *d*-FEN induces significant and dose-dependent pharmaco-EEG changes characterized by a decrease in total power and combined delta/theta activity; an increase in beta activity; and an acceleration of the centroid of the total activity with no changes in alpha power that are very different from those of the reference drugs. l-FEN produces only minimal changes (of the same type as d-FEN). CPZ (50 mg) and *d*-AMPH (20 mg) produce significant changes compared to placebo but with quite opposite effect profiles; the former exhibits a typical sedative-neuroleptic pattern while the latter is typical of psychostimulants. Dose/treatment and time-efficacy calculations based on all variables show in the V-EEG that only *d*-FEN (30 mg) and CPZ (50 mg) are different from placebo (with peak effects in the 8th and 4th hour, respectively), while in the resting condition (R-EEG) all drugs/dosages but l-FEN (30 mg) differ from placebo (with the peak effect occurring in the 4th–6th hour after *d*-FEN and l-FEN; in the 2nd hour after CPZ; and in the 4th–6th hour after AMPH). Plasma prolactin levels are only modified by CPZ (maximal increase in the 2nd hour).

Clinical-pharmacological study with the two isomers (d-, l-) of fenfluramine and its comparison with chlorpromazine and d-amphetamine: Blood levels, EEG mapping and safety evaluation. Saletu B, Barbanoj MJ, Anderer P, Sieghart W, Grünberger J. Methods Find Exp Clin Pharmacol 1993 Jun; 15 (5), pp. 291–312.

Endomorphins-2/Mu(1)-Opioid and Mu(2)-Opioid Receptors (Agonists/Antagonists)/Nociceptive Properties
D-Pro(2)-Endomorphin-2

Cellular/Molecular Mechanism of Action: Analogs of endomorphin-2 function as competitive antagonists at their corresponding membrane-associated receptor complex.
Physiological Mechanism of Action: Antinociception activity achieved through antagonism of endomorphin-2.
Application: Endomorphin analogues containing D-Pro(2) are considered able to discriminate between the antinociceptive actions of mu_1- and mu_2-opioid receptor agonists at the level of the spinal cord.
Implementation: Characterization of opioid/endorphin receptor complexes.

Animal Model: Use of the paw withdrawal response in mice to detect/evaluate endomorphin antagonism.
Interpretation and Analysis: D-Pro_2-endomorphin-1 and D-Pro_2-endomorphin-2, injected intrathecal (i.t.), have no significant effect on the nociceptive thermal threshold when administered individually. Alternatively, when D-Pro_2-endomorphin-1 (0.05–0.1 pmol) is injected simultaneously with i.t. endomorphin-1 (5.0 nmol) or endomorphin-2 (5.0 nmol), antinociception induced by endomorphin-1 is reduced significantly, whereas endomorphin-2-induced antinociception is not affected by D-Pro_2-endomorphin-1. Antinociception induced by i.t. endomorphin-2 (5.0 nmol) is reduced significantly by its analogue, D-Pro_2-endomorphin-2 (100 pmol), but not by D-Pro_2-endomorphin-1. D-Pro_2-endomorphin-1 also antagonizes the antinociceptive effect of i.t. DAMGO, a mu-opioid receptor agonist, whereas D-Pro_2-endomorphin-2 does not reduce the effect of DAMGO.

Endomorphin analogues containing D-Pro2 discriminate different mu-opioid receptor mediated antinociception in mice. Sakurada S, Watanabe H, Hayashi T, Yuhki M, Fujimura T, Murayama K, Sakurada C, Sakurada T. Br J Pharmacol 2002 Dec; 137 (8), pp. 1143–6.

Epilepsy: Cerebral Cortex Function/Early-Onset Epilepsy with Pleomorphic Seizures/ Guanidinoacetate N-Methyltransferase Deficiency/ Arginine/Glycine Amidinotransferase Deficiency/ Creatine Transporter 1 Dysfunction/Strategy for Case Selection for Proton Magnetic Resonance Spectroscopy Evaluation
Creatine Monohydrate

Cellular/Molecular Mechanism of Action: Creatine monohydrate is a biochemical pathway end product.
Background Information: Creatine metabolism disorders have so far been described at the level of 2 synthetic steps, (i) guanidinoacetate N-methyltransferase and arginine/glycine amidinotransferase, and at the (ii) creatine transporter 1 complex. Arginine/glycine amidinotransferase and creatine transporter 1 deficiencies are mainly present with mental retardation and severe language disorders. Guanidinoacetate N-methyltransferase deficiency results in the development of a severe neurologic condition (age–of–onset 3 months to 2 years) characterized by developmental arrest, neurologic deterioration, movement disorders, mental retardation, autistic-like behavior, and epilepsy. Severe early-onset epilepsy with pleomorphic seizures is a key symptom of such disorders.
Application: Creatine monohydrate can identify enzyme deficiencies in biochemical pathways for guanidinoacetate N-methyltransferase or arginine/glycine amidinotransferase, in addition to

disorders in creatine transporter 1 level dysfunction. Such biochemical pathway–based disorders precipitate early-onset epilepsy conditions characterized by pleomorphic seizure disorders. Creatine monohydrate can be utilized in strategies for selecting appropriate cases suitable to evaluation by proton magnetic resonance spectroscopy.

Implementation: Complementary diagnostic strategy for evaluating epilepsy conditions caused by dysfunction in 2 synthetic steps involved in creatinine metabolism including (i) guanidinoacetate N-methyltransferase and arginine/glycine amidinotransferase deficiencies; and (ii) creatine transporter 1 function.

Interpretation and Analysis: Guanidinoacetate N-methyltransferase and arginine/glycine amidinotransferase deficiencies respond positively to substitutive treatment with creatine monohydrate. Data suggest that in cases with guanidinoacetate N-methyltransferase deficiency, epilepsy and associated electroencephalographic abnormalities are more responsive to creatine supplementation than to conventional antiepilepsy drugs. All cases of creatine disorders reported to date have been detected by brain proton magnetic resonance spectroscopy, an expensive technique not routinely used in pediatric neurology. A potential diagnostic strategy to select cases for evaluation using proton magnetic resonance spectroscopy have been described.

Inborn errors of creatine metabolism and epilepsy: Clinical features, diagnosis, and treatment. Leuzzi V. J Child Neurol 2002 Dec; 17 Suppl 3, pp. 3S89–97; discussion 3S97.

Epilepsy: Cerebral Cortex Function (Uncontrollable)/ Pharmacokinetic and Pharmacodynamic Analyses of Antiepileptic Agents
Quantitative Pharmaco-Electroencephalography/ Clonazepam/Valproate

Cellular/Molecular Mechanism of Action: Clonazepam inhibits central nervous system gamma aminobutyrate (GABA), which functions as a neurotransmitter. In this context, diazepam is known to bind to membrane-associated GABA receptor complexes.

Route of Administration: Clonazepam (orally/per os).

Dosage: Clonazepam (CZP 0.05 mg/kg single dose).

Implementation: Evaluation of the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of valproate (VPA) and clonazepam (CZP) in epileptic children in addition to its effect on cerebral cortex function applying quantitative pharmaco-EEG (QPEEG) techniques. Following a single clonazepam dose, blood samples are collected at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 8.0, 12.0, and 24.0 hours. At each blood collection time point, EEG signals (60 s) are recorded for brain electrical activity mapping, and the power percentage average (PPA) of each frequency band calculated. The relationship between drug concentrations and their corresponding PPA of each frequency band are then analyzed.

Human Model: Cases of childhood epilepsy (n = 4) suffering from uncontrolled seizures despite long-term VPA therapy.

Interpretation and Analysis: Valproate steady-state drug concentrations (Css) are within the therapeutic range and not affected by CZP. The peak concentration (C_{max}) of CZP and the time intervals from dosing to C_{max} (T_{max}) are 20.9 ng/mL to 113.8 ng/mL and 1 hour to 1.5 hours, respectively. There is no significant correlation between VPA concentrations and the PPA of any of the EEG frequency bands. Clonazepam blood concentrations show significant correlation with PPA in a high percentage of cases (e.g. n = 3/4 cases). Clonazepam may therefore affect fast wave activities in proportion to CZP blood concentrations. Quantitative pharmaco-electroencephalography appears to be a promising technique for studying the pharmacokinetics and pharmacodynamics of selected antiepileptic drugs.

Instrumentation: High-pressure liquid chromatography (clonazepam) and gas-chromatography (valproate).

Pharmacokinetic and pharmacodynamic effects of clonazepam in children with epilepsy treated with valproate: A preliminary study. Wang L, Wang XD. Ther Drug Monit 2002 Aug; 24 (4), pp. 532–6.

Epilepsy: Cerebral Cortex Function/Medial Temporal Lobe/Left-Sided Speech Dominance/ Atypical Speech Dominance/Psychic Auras: Limbic Seizure Spread/Epileptic Activity: Speech Reorganization
Amobarbital (Wada Test)

Physiological Mechanism of Action: Amobarbital is a barbiturate that generally exerts a depressant effects on the central nervous system.

Background Information: Factors influencing atypical speech lateralization have theoretical importance in understanding the organization and reorganization of higher cognitive functions, as well as having practical implications, especially in brain surgery and neurorehabilitation. Atypical (right-sided or bilateral) language representation is more frequent in focal epilepsy than in healthy people. This difference is thought to be related to early childhood brain injuries localized in regions near speech centers. The effect of epileptic activity on speech lateralization has not been investigated, although several investigations have suggested that epileptic activity may interfere with higher brain functions. Ideally, it can only be evaluated in a homogeneous human population with epilepsy having the same lesion type in the same anatomical localization.

Application: Detection and characterization of neurological function/dysfunction in conditions of medial temporal lobe epilepsy. The Wada test can also be applied to accurately localize foci within the CNS responsible for epileptic activity and for predicting the potential and severity of residual postoperative deficits.

Implementation: Evaluation of medial temporal lobe epilepsy (MTLE) due to unilateral hippocampal sclerosis (HS), but without other epileptogenic lesions using superselective delivery of amobarbital (Wada test). Comprehensive presurgical evaluations are performed as a baseline reference to guide decision making processes related to surgical intervention. In MTLE, the influence of age at the time of brain damage (e.g., initial precipitating injury or IPI) can be evaluated separately from other timing factors.

Human Model: Cases of MTLE due to unilateral HS, but without other epileptogenic lesions (n = 184; n = 100 Wada test procedures; n = 83 left-sided MTLE cases).

Interpretation and Analysis: The Wada test can reveal left-sided speech patterns in 76% of the left-sided MTLE and in 100% of the right-sided MTLE cases (n = 100 Wada test cases; $p < 0.05$).The mean age at seizure onset is approximately 10.1 ± 7.8 years (range 1–37 years); the mean age at evaluation is approximately 35.7 ± 9.8 years. Applying the Wada test, it is possible to detect cases with left-sided speech (e.g., n = 63) in addition to identifying cases of atypical speech dominance (e.g., n = 20 or 24%). Atypical speech representation in left MTLE is associated with a higher spiking frequency ($p < 0.05$) and with sensory auras representing an ictal involvement of the lateral temporal structures ($p < 0.01$). Psychic auras suggesting limbic seizure spread show a significant association with left-sided speech dominance in left MTLE ($p < 0.05$). Neither age at epilepsy onset nor age at IPI is associated with atypical speech in left MTLE. Therefore, cases with focal epilepsy, not only the known factors

(i.e., the age at which the brain injury occurred and its localization) but also the epileptic activity itself (i.e., interictal discharges and seizure spread), may influence speech reorganization. Such findings also suggest that not only structural elements but also functional factors have an effect on the language organization within the brain.

Epileptic activity influences the speech organization in medial temporal lobe epilepsy. Janszky J, Jokeit H, Heinemann D, Schulz R, Woermann FG, Ebner A. Brain 2003 Sep; 126 (Pt 9), pp. 2043–51.

Epilepsy: Cerebral Cortex Function/Presurgical Evaluation of Refractory Conditions/Arterial-Venous Malformations
Methohexital/[Wada Test]

Cellular/Molecular Mechanism of Action: Methohexital is a barbiturate and functions as a central nervous system depressant.
Application: Sodium methohexital (Brevital) can be applied as a local anesthetic agent in the Wada test for assessing language and memory in presurgical epilepsy cases.
Implementation: Evaluation of the diagnostic effectiveness of methohexital compared to the more commonly used anesthetic agent, sodium amobarbital (Amytal).
Human Model: Cases of presurgical epilepsy (n = 86; and n = 173 procedures).
Interpretation and Analysis: Despite differences between the methohexital and amobarbital test protocols, the behavioral and neurologic effects of the 2 anesthetics are similar. Because of the brief duration of methohexital, 2 successive injections are made on each side rather than 1, to lengthen the time available for testing both language and memory. Behavioral and EEG indices return to baseline more quickly and more completely with methohexital than with amobarbital, allowing several repetitions of the procedure without incremental drowsiness, and the total time taken for the procedure is less with methohexital than with amobarbital. Therefore, language and memory testing in the Wada test are equivalent for amobarbital and methohexital, except that methohexital has a briefer duration of action and is associated with less sedation.

Sodium methohexital (brevital) as an anesthetic in the Wada test. Buchtel HA, Passaro EA, Selwa LM, Deveikis J, Gomez-Hassan D. Epilepsia 2002 Sep; 43 (9), pp. 1056–61.

Epilepsy: Cerebral Cortex Function (Seizures)/ Refractory Status Epilepticus/Neurologic Outcomes and Survival Prediction
Pentobarbital (Coma)

Physiological Mechanism of Action: Pentobarbital is a barbiturate that promotes depression of the central nervous system.
Background Information: Status epilepticus is a life-threatening condition requiring emergency medical attention. Although initial therapies with antiepileptic drugs generally terminate seizures within 30 to 60 minutes, cases with refractory status epilepticus require additional intervention. High-dose pentobarbital has been the most commonly prescribed agent for the management of refractory status epilepticus in children.
Application: Management and assessment of projected neurologic outcomes and survival in cases of refractory status epilepticus.
Route of Administration: Pentobarbital (intravenous).
Implementation: Evaluate the association between the response of pentobarbital coma and neurologic outcomes in refractory status epilepticus. A pentobarbital-induced coma is induced for a period of at least 48 hours. Relevant information entered into medical records is reviewed in an effort to identify an association between different variables and to comparatively assess responses.
Human Model: Cases of refractory status epilepticus (n = 23).
Interpretation and Analysis: In cases classified as refractory status epilepticus, approximately (i) 50% can be controlled with pentobarbital (e.g., n = 12/23); (ii) 30% will not respond to pentobarbital (e.g., n = 6/23); and (iii) slightly greater than 20% relapsed after discontinuation or during tapering of pentobarbital administration (e.g., n = 5/23). The mortality rate among relapse cases and nonresponder cases combined is 90.9%, but the survival rate among the responder cases is very high (e.g., 100% at $p < 0.001$). The survival rate is greater among toddlers compared with neonates or older children. Failure of seizure control after pentobarbital coma is associated with a poor prognosis. The potential for serious complications of pentobarbital therapy among neonates highlights the need for careful dosing in this age group.

Neurologic outcomes of pediatric epileptic cases with pentobarbital coma. Kim SJ, Lee DY, Kim JS. Pediatr Neurol 2001 Sep; 25 (3), pp. 217–20.

Epilepsy: Cerebral Cortex Function/Early-Onset Epilepsy with Pleomorphic Seizures/ Guanidinoacetate N-Methyltransferase Deficiency/ Arginine-Glycine Amidinotransferase Deficiency/ Creatine Transporter 1 Dysfunction/Strategy for Case Selection for Proton Magnetic Resonance Spectroscopy Evaluation
Creatine Monohydrate

Cellular/Molecular Mechanism of Action: Creatine monohydrate is a biochemical pathway end product.
Background Information: Creatine metabolism disorders have so far been described at the level of 2 synthetic steps, (i) guanidinoacetate N-methyltransferase and arginine/glycine amidinotransferase, and at the (ii) creatine transporter 1 complex. Arginine/glycine amidinotransferase and creatine transporter 1 mainly present with mental retardation and severe language disorder. Guanidinoacetate N-methyltransferase deficiency results in the development of a severe neurologic condition (age of onset 3 months to 2 years) characterized by developmental arrest, neurologic deterioration, movement disorders, mental retardation, autistic-like behavior, and epilepsy. Severe early-onset epilepsy with pleomorphic seizures is a key symptom of such disorders.
Application: Creatine monohydrate can identify enzyme deficiencies in biochemical pathways for guanidinoacetate N-methyltransferase or arginine/glycine amidinotransferase, in addition to disorders in creatine transporter 1 level dysfunction. Such biochemical pathway–based disorders precipitate early—onset epilepsy conditions characterized by pleomorphic seizure disorders. Creatine monohydrate can be utilized in strategies for selecting appropriate cases suitable to evaluation by proton magnetic resonance spectroscopy.
Implementation: Complementary diagnostic strategy for evaluating epilepsy conditions caused by dysfunction in 2 synthetic steps involved in creatinine metabolism including (i) guanidinoacetate N-methyltransferase and arginine/glycine amidinotransferase deficiencies; and (ii) creatine transporter 1 function.
Interpretation and Analysis: Guanidinoacetate N-methyltransferase and arginine/glycine amidinotransferase deficiencies respond positively to substitutive treatment with creatine monohydrate. Data suggest that in cases with guanidinoacetate N-methyltransferase deficiency, epilepsy and associated electroencephalographic abnormalities are more responsive to creatine supplementation than to

conventional antiepilepsy drugs. All cases of creatine disorders reported to date have been detected by brain proton magnetic resonance spectroscopy, an expensive technique not routinely used in pediatric neurology. A potential diagnostic strategy to select cases for evaluation using proton magnetic resonance spectroscopy have been described.

Inborn errors of creatine metabolism and epilepsy: Clinical features, diagnosis, and treatment. Leuzzi V. J Child Neurol 2002 Dec; 17 Suppl 3, pp. 3S89–97; discussion 3S97.

Epilepsy: Cerebral Cortex Function/Intractable Childhood Epilepsy/Side-of-Seizure Onset Prediction/Memory Asymmetries/Temporal and Extra-Temporal Lobe Epilepsy Lateral Seizure Prediction

Amobarbital/Wada Test

Cellular/Molecular Mechanism of Action: Amobarbital is a barbiturate.

Physiological Mechanism of Action: Amobarbital functions as a depressant of central nervous system function.

Application: Selective localization of deficits and predicting the extent of undesirable side effects if surgical intervention is attemped to correct intractable neurologic disorders (e.g. uncontrollable epilepsy).

Background Information: Because of the capacity of intracarotid amobarbital (Wada) memory assessment to predict seizure-onset laterality in children has not been thoroughly investigated, 3 comprehensive epilepsy surgery centers have pooled their data and examined Wada memory asymmetries to predict side-of-seizure onset in children being considered for epilepsy surgery.

Route of Administration: Amobarbital (Wada test) involves the selective delivery of the barbiturate into specific arterial branches within the central nervous system facilitated by catheterization of the carotid artery.

Implementation: Evaluation of intractable epilepsy conditions utilizing Wada testing protocols. After return to neurologic baseline, recognition memory for the stimuli is assessed. Seizure onset is determined by simultaneous video-EEG recordings of multiple seizures.

Human Model: Cases of intractable epilepsy (n = 152 children) underwent Wada testing.

Interpretation and Analysis: Despite variations in the type of memory stimuli, number of memory stimuli, and methods applied, all children presented with 6 to 10 items soon after amobarbital injection. In children with unilateral temporal lobe seizures (n = 87), Wada memory asymmetries accurately predicts seizure laterality to a statistically significant degree. Wada memory asymmetries also correctly predict side-of-seizure onset in children with extra-temporal lobe seizures (n = 65). Although individual case prediction accuracy is statistically significant in temporal lobe cases, onset laterality is incorrectly predicted in approximately $\leq$ 52% of children with left temporal lobe seizure onset, depending on the methods and asymmetry criterion used. There also are significant differences between Wada prediction accuracy across the 3 epilepsy centers. Results suggest that Wada memory assessment is useful in predicting side-of-seizure onset in many children. However, Wada memory asymmetries should be interpreted more cautiously in children than in adults.

Prediction of seizure-onset laterality by using Wada memory asymmetries in pediatric epilepsy surgery candidates. Lee GP, Park YD, Hempel A, Westerveld M, Loring DW. Epilepsia 2002 Sep; 43 (9), pp. 1049–55.

Epilepsy: Cerebral Hemisphere Function/Intractable Epilepsy/Preoperative Foci Localization/Prediction of Residual Postoperative Deficits

Amobarbital (Wada Test)/[^{99m}Tc]-Hexamethylpropylene Aminoxime ([^{99m}Tc]-HMPAO)

Physiological Mechanism of Action: Amobarbital is a barbiturate that has a general depressant effect on central nervous system function.

Background Information: The IAP is used presurgically in cases with temporal lobe epilepsy to predict the effects of planned temporal lobectomy on LTM and language. This prognosis presumes that a similar pattern of perfusion will result in anesthesia of the same cerebral regions in most cases. Co-injection of [^{99m}Tc]-HMPAO with the barbiturate during the IAP has been used to ascertain whether this actually is true, with variable results. Moreover, most studies document only unilateral IAPs and do not report on behavioral performance.

Application: Amobarbital can be applied to assist in accurately localizing central nervous system lesions and predicting the probability and severity of postoperative deficits.

Route of Administration: Amobarbital (superselective intracarotid delivery to specific regions of the central nervous system).

Implementation: Evaluation of central nervous system disorders utilizing [^{99m}Tc]-HMPAO co-injected with amobarbital (e.g., n = 33 intracarotid amobarbital procedures; n = 15/18 bilateral, n = 3/18 unilateral). Important aspects of the procedure pertain to delineating the relationship between perfusion pattern and behavioral performance. Such objectives can be address applying SPECT analysis and comparison of results with angiographic evaluations obtained at the time of catheter placement. SPECT perfusion data is rated for presence/absence and intensity of perfusion to the ACA, MCA, and PCA territories and to H, i, or c to the injection site. V, STM, and LTM are graded according to a standardized protocol.

Human Model: Cases of epilepsy (n = 18).

Interpretation and Analysis: MCAi is perfused in 100% of cases, ACAi in 91%, PCAi in 21%, and Hi in only 6%. Crossover flow can be detected in a small percent of cases (e.g., n = 9) while 50% of cases in whom both sides are injected (on different days) have crossover, involving the ACAc territory in 80% of cases. As expected, injection on the non-ES is associated with a significantly worse LTM performance than on the ES (p = 0.006). There is no relationship between the perfusion pattern and the V level of cases (a potential confounding variable in memory/language evaluation) during IAP, nor between perfusion pattern and LTM. STM is significantly adversely affected by the presence of crossover perfusion. Angiography in general overestimates the extent of cerebral perfusion demonstrated by SPECT, most probably because of the markedly different injection conditions. Despite the best efforts to standardize injections, the perfusion pattern has been mostly unpredictable in cases. Moreover, it has little bearing on their behavioral performance, except for the prediction of poor STM performance (the clinical implications of this remain dubious). Marked LTM alterations after non-ES injections confirm remote hippocampal effects in the presence of only rare direct perfusion of that region. [^{99m}Tc]-HMPAO/amobarbital co-injection is unhelpful from a clinical perspective, most probably because a large part of the effects of amobarbital arise from deafferentation of regions not directly perfused by the anesthetic agent.

Absence of correlation between amobarbital distribution as assessed with SPECT brain perfusion imaging and behavioral manifestations during the intracarotid amobarbital procedure (Wada test). Soucy JP, Rouleau I, Roy D, Robidoux J, Laflamme K, Laflamme L. Prog Neuropsychopharmacol Biol Psychiatry 1999 Feb; 23 (2), pp. 259–74.

Epilepsy: Cerebral Cortex Function/Pharmaco-Electroencephalograpy
Pharmaco-Electroencephalogram/ Carbamazepine (CBZ)

Application: Delineating the influence of CBZ on cerebral cortex function in epileptic disease states.
Dosage: Carbamazepine (300 mg, single dose).
Implementation: Evaluate the influence of CBZ on cerebral cortex function in epileptic conditions utilizing quantitative EEG (qEEG) techniques and instrumentation. Sample EEG tracings are recorded before and at regular intervals within 24 hours following pharmaceutical administration. The EEG activity is processed by power spectral analysis and the drug serum concentration assessed concomitantly.

Human Model: Cases of epilepsy (n = 10 epileptics; n = 10 healthy adult volunteers).
Interpretation and Analysis: Increases in serum CBZ concentrations correspond with the percentage of power progressively increasing in the theta band while it decreases in the alpha2 band. The percentage of theta and alpha2 power is found to reach maximum and minimum levels, respectively, when the peak values of serum concentration of CBZ are attained. The effects on qEEG in anterior scalp areas are more evident than in posterior scalp areas.

Quantitative pharmaco-EEG of carbamazepine in volunteers and epileptics. Wu X, Xiao CH. Clin Electroencephalogr 1996 Jan; 27 (1), pp. 40–5.

Epilepsy: Cerebral Cortex Function/Pharmaco-Electroencephalograpy
Valproate/Pharmaco-Electroencephalography

Application: Characterization of cerebral cortical function in epilepsy conditions.
Dosage: Valproate (single-dose administration).
Implementation: Evaluation of the influence of sustained-release valproate (SR-VPA) on cerebral cortex function in epilepsy conditions applying quantitative background electroencephalography (EEG). Complementary investigations include establishing SR-VPA serum concentrations.

Human Model: Cases of CNS epilepsy (n = 10 untreated with generalized tonic-clonic seizures).
Interpretation and Analysis: Pharmaco-electroencephalography reveals increased power of alpha2 band over occipital areas, a decrease of power of beta band (diffuse and preponderant on posterior scalp areas) with increasing serum concentrations. Both effects reach their maxima and minima, respectively, when the peak values of serum concentration are reached. A positive correlation exists between alpha2 power on the occipital areas and serum SR-VPA concentrations, and a negative correlation of serum concentration and beta power are detected over the posterior scalp areas.

Quantitative pharmaco-EEG of sustained release valproate in epileptics. Wu X, Xiao CH. Clin Electroencephalogr 1997 Apr; 28 (2), pp. 117–20.

Epilepsy: Cerebral Cortex Function/Pharmaco-Electroencephalograpy/Cononical Correlation (Semilinear): Anesthesiology/Responders vs Non-Responders: Psychiatry/1st-Order Markov Model of Sleep Stage Transitions: Neurophysiology
Pharmaco-Electroencephalograpy (Pharmaco-EEG)

Background Information: In the clinical application and interpretation of pharmaco-EEG, it is important to appreciate its value in the context of other diagnostic parameters such as biomarkers, surrogate end points, clinical end points, and clinical outcomes.
Application: Detection and characterization of neurologic disorders through the combined application of specific pharmaceutical agents and measurement of their influence on central nervous function detected with EEG instrumentation.
Considerations: Much work still needs to be conducted to fully develop the potential capabilities that pharmaco-EEG together with pharmacokinetic-pharmacodynamic modeling can bring to therapeutics in neuroscience.
Interpretation and Analysis: State-of-the-art applications of pharmaco-EEG, together with pharmacokinetic-pharmacodynamic modeling in everyday clinical practice, is becoming a reality pertaining to the fields of anesthesiology (semilinear canonical correlation), psychiatry (discrimination between responders and non-responders to pharmacological treatment using the test dose), neurology (antiepileptic field), and neurophysiology (1st-order Markov model of sleep stage transitions). The combination of both procedures, although successfully used during some drug development programs (opioids or benzodiazepines), is not widely applied in clinical conditions affecting the central nervous system.

Uses of pharmaco-EEG and pharmacokinetic-pharmacodynamic modeling in the clinical scenario. Barbanoj MJ, Valle M, Kulisevsky J, Perez V, Gambus P. Methods Find Exp Clin Pharmacol 2002; 24 Suppl D, pp. 139–44.

Epilepsy (Intractable): Cerebral Cortex-Hemisphere Function/Preoperative Foci Localization/Prediction and Probability of Residual Postoperative Deficits/Hemiplegia Duration and Relationship to SPECT Hypoperfusion/Drug-Induced Delta EEG Activity/HMPAO-SPECT Assessment of Methohexital Distribution
Methohexital (Wada Test)/[^{99m}Tc]-Hexamethylpropylene Aminoxime

Physiological Mechanism of Action: Methohexital is a barbiturate that functions as a central nervous system depressant.
Application: Methohexital can be applied to accurately localize foci within regions of the CNS responsible for intractable epilepsy conditions. Methohexital can also simultaneously function as a means for predicting the probability and potential severity of postoperative deficits. [^{99m}Tc]hexamethylpropylene aminoxime (HMPAO) can be administered intravenously during the procedure to determine the intracerebral distribution of methohexital while performing the Wada test.
Route of Administration: Methohexital (superselective intracarotic delivery into specific predetermined regions of the central nervous system).
Implementation: Evaluation of epileptic conditions being considered for surgery for intractable epilepsy utilizing a preoperative

evaluation protocol employing intracarotid sodium methohexital delivery. The lipophilic brain SPECT agent, HMPAO is administered intravenously during the procedure to determine the intracerebral distribution of methohexital.

The validity of the ISM depends on consistent delivery of the anesthetizing agent to 1 hemisphere, including the mesial structures of the temporal lobe. To prevent postoperative language and memory deficits, correct interpretation of the test results supposes a reliable knowledge of which regions of the brain have been anesthetized. Results of HMPAO-SPECT are compared with clinical and EEG video-monitoring data and with results of digital subtraction angiography (DSA) performed during the test in an effort to establish criteria for determining the level and topographical extent of anesthesia.

Human Model: Case being considered for surgery for intractable epilepsy (n = 25).

Interpretation and Analysis: In the vast majority of cases, the effect of sodium methohexital (SM) is ipsilateral cerebral hypoperfusion on SPECT and crossed cerebellar diaschisis (e.g., n = 25/25). The distribution of HMPAO varies from patient to patient. The delivery of SM to mesial temporal lobe structures is not constant. Using non-parametric tests, it has been demonstrated that a statistically significant relationship exists between hypoperfusion on SPECT and duration of hemiplegia but not with the duration of aphasia. Hypoperfusion on SPECT is also related to the onset and duration of drug-induced delta activity on EEG. SPECT reveals a statistically different distribution of SM in the brain from that predicted with digital subtraction angiography (DSA). HMPAO-SPECT can be used for mapping the distribution of methohexital's effects during intracarotid sodium methohexital (ISM) Wada tests. Several investigations have confirmed that SPECT assessment may be an excellent way of determining the distribution of barbiturate during examinations. It increases confidence in interpreting results of speech and memory testing by detecting either contralateral diffusion of the drug due to crossflow between hemispheres or insufficient quantitative delivery to the homolateral hemisphere.

Monitoring sodium methohexital distribution with [99mTc]HMPAO with single photon emission computed tomography during Wada test. Coubes P, Baldy-Moulinier M, Zanca M, Boire JY, Child R, Bourbotte G, Frerebeau P. Epilepsia 1995 Oct; 36 (10), pp. 1041–9.

Epilepsy: Cerebral Cortex Function/Resistant Focal Epilepy/Accuracy of Amobarbital Target Delivery/ Temporary Neurologic Deficits/Risk Assessment of Postsurgical Motor Deficits Before Functional Hemispherectomy/Language Area Identification

Amobarbital (Sodium) Wada Test/[^{99m}Tc]-HMPAO/ Electrical Stimulation Mapping

Cellular/Molecular Mechanism of Action: Amobarbital is classified as a barbiturate agent.

Physiological Mechanism of Action: Amobarbital has a generalized suppressant effect on neurons within the central nervous system.

Application: Analyze the role of selective middle cerebral artery (MCA) Wada tests in the presurgical workup of cases with drug-resistant focal epilepsies.

Route of Administration: Amobarbital is administered selectively into arterial branches supplying specific regions of the cerebral cortex using a catheter introduced into the carotid artery.

Implementation: Evaluation of cerebral cortex function in conditions of (i) motor cortex cerebral hemisphere lesions (connatal MCA infarct; unilateral cortical dysplasia; Rasmussen encephalitis); (ii) circumscribed neoplastic or non-neoplastic lesions adjacent to the motor cortex or classical language regions; and (iii) sleep electrical status epilepticus. Sodium amobarbital and [^{99m}Tc]-HMPAO are simultaneously injected through a microcatheter system into the distal M1 segment, the inferior MCA trunk, or the middle cerebral artery branches in order to identify eloquent cortex.

Human Model: Cases of cerebral cortex dysfunction including (i) hemispheric lesions involving the motor cortex (n = 9 cases, of which n = 5/9 connatal MCA infarct; n = 3/9 unilateral cortical dysplasia; n = 1/9 Rasmussen encephalitis); (ii) circumscribed neoplastic or non-neoplastic lesions adjacent to the motor cortex or classic language areas (n = 5 cases); and (iii) purely electrophysiologic analyses for conditions of electrical status epilepticus in sleep (n = 2 cases). Total Wada test performed, n = 20.

Interpretation and Analysis: Co-registered single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI; n = 18) reveals that sodium amobarbital does not reach the target area during some procedures (e.g., n = 3/20). Temporary neurologic deficits can occur in slightly more than half of total Wada procedures performed (e.g., n = 12/20). Cases subjected to surgical intervention requires the use of several different surgical approaches, including functional hemispherectomy (n = 3); partial or extended lesionectomy (n = 4); anterior temporal lobectomy (n = 1); and multiple subpial transsections (n = 3). Seizure freedom (Engel class I) can be achieved in a modest percentage of cases (e.g., n = 5/16 cases). The MCA Wada tests can therefore contribute to risk assessment concerning postsurgical motor deficits before functional hemispherectomy. Other indications are less clear: The identification of language areas is targeted primarily by electrical-stimulation mapping after subdural grid implantations, and selective MCA Wada tests in ESES cases but they still ideally should be validated in a larger patient groups.

Selective middle cerebral artery Wada tests as a part of presurgical evaluation in cases with drug-resistant epilepsies. Urbach H, Von Oertzen J, Klemm E, Koenig R, Linke DB, Kurthen M, Schramm J, Elger CE. Epilepsia 2002 Oct; 43 (10), pp. 1217–23.

Epilepsy: Cerebral Cortex Function/Response to Anticonvulsive Therapy/Relative Efficacy Evaluation/ Anticonvulsants for Petit mal/Generalized Non-Convulsive Epilepsy

Pharmaco-Electroencephalography/Milacemide (CP 1552 S)/Sodium Valproate/Piracetam

Cellular/Molecular Mechanism of Action: Milacemide (CP 1552 S) is a derivative of glycine that exerts an anticonvulsant action by increasing GABA concentrations and endogenous glycine pools in the brain.

Physiological Mechanism of Action: Milacemide exerts a brain anticonvulsant action by increasing GABA concentrations.

Application: Compare and contrast the electroencephalography profiles and behavioral anticonvulsant properties of various therapeutic agents.

Route of Administration: Milacemide (oral).

Dosage: Milacemide (400 mg, 800 mg, 1600 mg). Reference compounds (placebo; sodium valproate at 600 mg; and piracetam at 1600 mg).

Implementation: Evaluation of the encephalotropic and psychotropic properties of milacemide by means of quantitative EEG and psychometric analyses. Electroencephalogram recordings and monitoring of blood pressure, heart rate, and side effects are per-

formed at 0, 1, 2, 4, 6, and 8 hours. Psychometric tests are performed at 0, 2, 4, 6, and 8 hours.

Human Model: Cases functioning as healthy normal reference controls (n = 12). Study Design: Double blind placebo-controlled study.

Interpretation and Analysis: Computer-assisted spectral analysis of EEG profiles shows significant effects of milacemide on the central nervous system (CNS) in contrast to placebo, characterized by an attenuation of delta activity accompanied by an acceleration of the centroid of the slow activity but also of the total activity in response to all 3 dosage levels. Other alterations noted include increases in beta activity after 400 mg, an increase of alpha activity after 800 mg, as well as an increase of alpha activity but a decrease in beta activity after a 1600 mg dose. Such changes, also seen after 600 mg sodium valproate, are reminiscent of quantitative EEG changes described after antiepileptic drugs used in the treatment of petit mal or generalized non-convulsive epilepsy. Moreover, they are also indicative of improvement in "vigilance" in the sense of Head, which is also seen at the behavioral level, specifically after the lowest doses of milacemide, as psychometric tests demonstrate an improvement in attention, concentration, psychomotor activity, and after-effect (indicating CNS activation) as measured by means of the Archimedean spiral. Such a beneficial influence on performance declines with increasing dose. Evaluation of pulse, blood pressure, and side effects demonstrate good tolerance after all administered substances.

Early clinical pharmacological trials with a new antiepileptic, milacemide, using pharmaco-EEG and psychometry. Saletu B, Grünberger J. Methods Find Exp Clin Pharmacol 1984 Jun; 6 (6), pp. 317–30.

Epilepsy: Cerebral Cortex Function/Spatiotemporal Patterns of Spontaneous Epileptiform Activity/ Cerebral Cortext Seizures
RH414 (Fluorescent Voltage-Sensitive Dye)/Bicuculline ($GABA_A$ Receptor Antagonist)

Application: RH414 functions as a fluorescent voltage-sensitive dye and the $GABA_A$ receptor antagonist bicuculline can be applied to induce acute spontaneous epileptoform discharges.

Implementation: Characterization of spatiotemporal patterns of *in vivo* spontaneous epileptiform activity in the cerebral cortex. The surface of the cerebral cortex is exposed under anesthesia and stained with a fluorescent voltage-sensitive dye, RH414, and optical recording techniques applied for the detection of transmembrane voltage changes. Acute spontaneous epileptiform discharges can be induced by the application of bicuculline ($GABA_A$ receptor antagonist). Changes in the intensity of fluorescence are recorded from the cerebral cortex using a photodiode system.

Animal Model: Rat cerebral cortex.

Interpretation and Analysis: Recordings can be acquired that detect spontaneous epileptiform discharges in a manner that can faciliate the construction of initiation-site maps. Initiation sites are neither unique nor randomly located but instead exhibit a multimodal distribution pattern. The incidence of epileptiform discharges is different between the initiation sites, and some sites show dominance in the induction of spontaneous epileptiform discharges.

Instrumentation: Optic fiber photodiode system (464-channel).

Optical analysis of acute spontaneous epileptiform discharges in the *in vivo* rat cerebral cortex. Iyakawa N, Yazawa I, Sasaki S, Momose-Sato Y, Sato K. Neuroimage. 2003 Mar; 18 (3), pp. 622–32.

Ethanol and Thiopental Cerebral Cortex Depression/ Pharmacological Interactions
Pharmaco-Electroencephalography/ Captopril/Enalaprilat

Cellular/Molecular Mechanism of Action: Captopril and enalaprilat function as angiotensin converting enzyme inhibitors.

Background Information: The influence of captopril and enalaprilat on the central nervous system has been investigated in laboratory animals.

Route of Administration: Captopril (intraperitoneal); enalaprilat (intraperitoneal); ethanol (intraperitoneal); and thiopental (intraperitoneal)

Dosage: Captopril (5 and 20 mg/kg); enalaprilat (5 and 20 mg/kg); ethanol (4 g/kg); and thiopental (70 mg/kg) single administration or repeated SID for 10 days.

Implementation: Evaluation of the effect captopril and enalaprilat have on the duration of ethanol- and thiopental-induced changes in sleep, body temperature, spontaneous locomotor activity, and analgesic properties (hot plate and tail-flick test, and pharmaco-EEG profiling).

Animal Model: Murine (hot plate and tail-flick test).

Animal Model: Rabbit (pharmaco-EEG profiles).

Interpretation and Analysis: Single administration of captopril (both doses) and single or prolonged administration of enalaprilat decrease the duration of ethanol- and thiopental-induced sleep. Captopril (5 mg/kg) and enalaprilat (5 and 20 mg/kg) increase pain threshold. Both captopril and enalaprilat after single or repeated administration do not influence spontaneous locomotor activity (mice). Captopril and enalaprilat decrease body temperature in mice. Examined ACEIs produce changes in EEG recording, more profoundly exhibited after administration of enalaprilat.

An attempt to assess the central action of captopril and enalaprilat. Czarnecka E, Strzelec J, Pietrzak B. Med Sci Monit 2000 May–Jun; 6 (3), pp. 491–7.

Headache Pain: Cluster Episodes/Cerebral Cortex
Sumatriptan/Prolactin/Adrenocorticotropic Hormone (ACTH)

Cellular/Molecular Mechanism of Action: Sumatriptan is a selective 5-HT(1B/1D) agonist that stimulates the secretion of growth hormone and inhibits the release of prolactin, ACTH, and cortisol.

Application: Assess the sensitivity of 5-HT(1D) receptors in cases with episodic cluster headache using sumatriptan as a pharmacological probe. These effects may be used to explore the function of serotonergic systems *in vivo*.

Route of Administration: Sumatriptan (subcutaneous).

Implementation: Evaluation of the influence of sumatriptan on cluster headaches.

Animal Model: Cases of headache (n = 20; n = 10 active phase; n = 10 remission period; n = 12 negative reference controls).

Interpretation and Analysis: Sumatriptan-induced increases in growth hormone concentrations are significantly ($p < 0.05$) blunted in cases with active cluster headache. Prolactin and ACTH responses to the drug are significantly ($p < 0.05$) reduced in cluster headache conditions in both the active and in the remission period. Therefore, cerebral serotonergic functions mediated by 5-HT(1D) receptors become altered in episodic cluster headache conditions.

Abnormal 5-HT1D receptor function in cluster headache: A neuroendocrine study with sumatriptan. Pinessi L, Rainero I, Valfrè W, Lo Giudice R, Ferrero M, Rivoiro C, Arvat E, Gianotti L, Del Rizzo P, Limone P. Cephalalgia 2003 Jun; 23 (5), pp. 354–60.

Hemorrhage: Intracranial/Cerebral Cortex
Hirudin

Cellular/Molecular Mechanism of Action: Hirudin has a relatively strong binding avidity for thrombin and functions as an inhibitor of most biological properties associated with this proteolytic enzyme fraction.
Background Information: In humans, intracerebral hemorrhage (ICH) causes marked perihematomal edema formation and neurological deficits. A rat ICH model, involving infusion of autologous blood into the caudate, has been used extensively to study mechanisms of edema formation, but an examination of behavioral outcome would improve its preclinical utility and provide a more rigorous assessment of the pathological cascade of events over time.
Application: Detection and characterization of hemorrhagic conditions affecting the central nervous system.
Route of Administration: Intravenous.
Implementation: Evaluate a battery of sensorimotor function tests to determine the neurological effects of ICH and to examine which components of the hematoma are involved in generating those effects. One experimental approach to delineating these features involves assessment from day 1 to day 28 postinjection the influence of (i) autologous whole blood injection; (ii) injection of blood plus hirudin (thrombin inhibitor); (iii) packed red blood cells; (iv) thrombin; (v) saline; and (vi) needle placement only.

Animal Model: Rat (behavioral tests employing forelimb placing, preference for forelimb use for weight shifts during vertical exploration of a cylindrical enclosure, and a corner turn test).
Interpretation and Analysis: Radioisotope-labeled hirudin accumulates at sites of intravascular thrombosis. Behavioral-oriented tests can detect the presence of marked neurological deficits by day 1 after ICH, with progressive recovery of function over 4 weeks. The forelimb placing scores parallel changes in edema. Injection of thrombin causes (promotes), while injected hirudin reduces, ICH-induced neurological deficits. Injection of packed red blood cells (promotes delayed edema formation) induces delayed neurological deficits. Such tests allow continuous monitoring of neurological deficits after rat ICH and assessment of therapeutic interventions. The time course of the neurological deficit closely matches the time course of cerebral edema for both ICH and injection of blood components. There is marked recovery of function after ICH, which may be amenable to therapeutic manipulation.

Behavioral tests after intracerebral hemorrhage in the rat. Hua Y, Schallert T, Keep RF, Wu J, Hoff JT, Xi G. Stroke 2002 Oct; 33 (10), pp. 2478–84.

Hepatoencephalopathy Coma
Bromocriptine

Diagnostic Application: Detection and characterization of coma associated with conditions of hepatoencephalopathy.
Dosage: Bromocryptine is rapidly absorbed from gastrointestinal tract and is extensively removed by 1st-pass hepatic perfusion. Peak plasma concentration (human) occurs approximately 3 hours following oral administration and the plasma $T_{1/2}$ is about 3 hours.
Considerations: Induces less dyskinesia than levodopa. A 1st-dose rapid-onset cardiovascular collapse event has been reported with bromocriptine, but this is not a feature associated with levodopa administration. Adverse effects from bromocriptine are usually related to dopaminergic agonist activity. Complications are usually recognized during the initial phases of administration of bromocriptine given for the first time in cases that are eventually managed with chronic therapy.
Interpretation and Analysis: Creates non-specific arousal of the CNS (similar to levodopa) that may be effective in treating comatose cases whose coma is caused by hepatoencephalopathy.

Hepatoencephalopathy: Coma/Non-Hepatoencephalopathy-Induced Neurologic Deficits
Flumazenil

Background Information: The pathogenesis of hepatic encephalopathy is unknown. It has been suggested that liver failure leads to the accumulation of substances that bind to receptor complexes within the brain, resulting in neural inhibition that may progress to coma. Several trials have assessed the effectiveness of benzodiazepine receptor antagonists for conditions of hepatic encephalopathy, but the results are conflicting.
Application: Flumazenil can function as a valuable diagnostic agent for characterizing conditions of coma but only with clear indications.
Considerations: Eligible trials have been/can be identified through the Cochrane Hepato-Biliary Group Controlled Trials Register, the Cochrane Controlled Trials Register, MEDLINE, EMBASE, reference lists of relevant articles, authors of trials, and the pharmaceutical company known to produce benzodiazepine receptor antagonists. Randomized trials comparing any benzodiazepine receptor antagonist vs placebo or no intervention for hepatic encephalopathy can be included, regardless of language or publication status. Trial inclusion and data extraction are made independently by multiple contributors (e.g. n = 2). Depending on the presence or absence of significant heterogeneity ($p < 0.1$), a random or fixed effect model can be used. Potential causes for heterogeneity should be explored by sensitivity analyses. Extraction of data on all outcomes from all trials may not be possible.
Implementation: Retrospective evaluation of the response, efficacy and safety of benzodiazepine receptor antagonists in conditions of acute or chronic hepatic encephalopathy. A secondary objective is to determine if any prognostic variables or factors can be identified.

Human Model: Cases with hepatoencephalopathy (n = 765).

Study Design: Twelve randomized trials with eight trials having a crossover design. All trials are double blind and assess flumazenil vs placebo.
Interpretation and Analysis: The vast majority of cases classified as having a favorable prognosis survive (flumazenil = 92% or n = 341/379 vs placebo = 91% or n = 325/356). Flumazenil has no significant effect on full recovery (2 trials), survival (9 trials), or on the occurrence of adverse events (5 trials). However, flumazenil is associated with a significant effect on improvement of hepatic encephalopathy compared to placebo at the end of treatment (30% or n = 103/346 versus 7% or 23/332), risk difference 0.23, 95% confidence interval 0.18 to 0.28 (n = 9 trials).

Flumazenil has no significant effect on recovery or survival from hepatic encephalopathy. However, flumazenil had a significant effect on short-term improvement of hepatic encephalopathy in some cases with chronic liver disease and a highly favorable prognosis. Considering the fluctuating nature of hepatic encephalopathy, future trials should use a parallel design and assess if flumazenil can promote sustained improvement or increased recovery and survival. Until this

has been demonstrated, flumazenil may be considered for cases with chronic liver disease and hepatic encephalopathy but cannot be recommended for routine clinical use.

Benzodiazepine receptor antagonists for acute and chronic hepatic encephalopathy. Als-Nielsen B, Kjaergard LL, Gluud C. Cochrane Database Syst Rev 2001 (4), p. CD002798.

Hepatoencephalopathy: Cerebral Cortical Function/Acute Hepatic Encephalopathy/ Encephalopathy Associated with Hemorrhage/ Potential Prognostic Indicators
Flumazenil/Pharmaco-Electroencephalography

Background Information: Randomized controlled trials testing flumazenil in hepatic encephalopathy have shown conflicting results. The aim of the tests is to compare flumazenil and placebo in hepatic encephalopathy in cases with cirrhosis.
Application: Evaluate the effects of flumazenil on hepatic encephalopathy in cases with liver cirrhosis and the potential of therapeutic response to correlate with prognosis.
Route of Administration: Flumazenil (intravenous).
Dosage: Flumazenil (2 mg).
Implementation: Evaluation of the response to flumazenil in conditions of encephalopathy associated with hepatic cirrhosis. Administration of flumazenil or placebo can be accompanied by the co-administration of conventional treatments (e.g., branched-chain amino acid, saline, glucose, and lactulose). Alterations in clinical signs are assessed over a 24-hour observation period. Clinical improvement is defined as a 3-point decrease in the Glasgow coma score at any time within 24 hours.

Human Model: Cases of encephalopathy associated with hepatic cirrhosis ($n = 54$ cases with hepatic encephalopathy grades III and IV). For each end point, heterogeneity and treatment efficacy are assessed.
Interpretation and Analysis: In double blind randomized controlled trials ($n = 6$ trials; with $n = 641$ cases; $n = 326/641$ treated with flumazenil; $n = 315/641$ placebo) the treatment duration can range between 5 minutes to 3 days. (Note: heterogeneity tests between control groups not significant.) In trials (e.g., $n = 5$) the mean percentage of clinical improved is approximately 27% with pharmaceutical challenge and about 3% with placebo reference controls (Peto methodology: odds ratio = 6.15; 95% confidence interval, 4.0–9.5; $p < 0.001$; Der Simonian methodology: mean rate difference, 29%; 95% confidence interval, 17–41; $p < 0.001$). The mean percentage of cases with electroencephalographic improvement is approximately 19% with flumazenil but 2% with placebo. The difference is significant only with the Peto method of analysis (odds ratio = 5.8; 95% confidence interval, 3.4–9.7; $p < 0.001$). Sensitivity analysis reveals similar findings. Meta-analysis of this type reveals that flumazenil induces clinical and electroencephalographic improvement of hepatic encephalopathy in cases with cirrhosis.

In related investigations, clinical improvement can be appreciated in a large percentage of affected cases (e.g., $n = 22/28$ with flumazenil; $n = 14/26$ placebo @ $p < 0.05$). Improvement is observed within the 1st 6 hours in the vast majority of cases given flumazenil (e.g., $n = 21/22$ cases vs $n = 3/14$ placebo). Unfortunately, the mortality rate is not different between case groups receiving either flumazenil challenge of placebo. However, a large percentage of non-responders subjected to flumazenil challenge died within 24 hours (e.g., $n = 6/6$ or 100%) in contrast to only slightly less than half of placebo-treated cases (e.g., $n = 5/12$ non-responders).

In conditions of postbleeding encephalopathy, over half of affected cases challenged with flumazenil improve ($n = 11/17$) compared to a very low number of placebo case (e.g., $n = 2/14$ @ $p < 0.001$). Flumazenil therefore exerts a beneficial effect in a subset of cases with acute hepatic encephalopathy; encephalopathy associated with bleeding is more likely to respond to flumazenil; and responders to the treatment usually improve within the 1st 6 hours while lack of response usually represents a bad prognostic sign.

Flumazenil in the treatment of acute hepatic encephalopathy in cirrhotic cases: A double blind randomized placebo controlled study. Laccetti M, Manes G, Uomo G, Lioniello M, Rabitti PG, Balzano A. Dig Liver Dis 2000 May; 32 (4), pp. 335–8.

Flumazenil vs placebo in hepatic encephalopathy in cases with cirrhosis: A meta-analysis. Goulenok C, Bernard B, Cadranel JF, Thabut D, Di Martino V, Opolon P, Poynard T. Aliment Pharmacol Ther 2002 Mar; 16 (3), pp. 361–72.

Hippocampus Neurotransmitter Function/ Dopamine, Norepinephrine, and Serotonin Induction
Bupropion

Cellular/Molecular Mechanism of Action: Bupropion is a dual dopamine/norepinephrine reuptake blocker.
Dosage: Bupropion (17 mg/kg).
Implementation: Evaluation of the influence of an acute dose of bupropion on extracellular neurotransmitter concentrations within the hippocampus collected *in vivo* using a microdialysis system. Peripheral hormonal concentrations are assessed using an indwelling catheter. A microdialysis probe is inserted in the hippocampus, and samples for serotonin, dopamine, and norepinephrine are collected every 20 minutes before and after injection of bupropion, for a total sampling time of 180 minutes. In a separate group of subjects, a catheter is placed in the vena femoralis, and blood samples collected before and after bupropion injection for quantification of growth hormone, prolactin, corticosterone, adrenocorticotropic hormone, and beta-endorphins.

Animal Model: Rat (free moving).
Interpretation and Analysis: All neurotransmitter levels (dopamine, norepinephrine, and serotonin) significantly increase after bupropion injection. This is accompanied by a significant decrease in prolactin concentrations, whereas the other hormones show no statistically significant variation. It can, therefore, be concluded that, although bupropion has dual reuptake properties, the observed effects both at the central and at the peripheral level seem to be ruled by the dopaminergic system.

Effect of bupropion on hippocampal neurotransmitters and on peripheral hormonal concentrations in the rat. Piacentini MF, Clinckers R, Meeusen R, Sarre S, Ebinger G, Michotte Y. J Appl Physiol 2003 Aug; 95 (2), pp. 652–6. Epub 2003 Apr 11.

Hippocampal Neurotransmitter Function/GABA Receptor Complex Expression Density
Muscimol-[Alexa Fluor 532]

Cellular/Molecular Mechanism of Action: Muscimol-[Alexa Fluor 532] functions as a fluorescent-labeled GABA receptor ligand.
Application: Detection and characterization of $GABA_A$ binding sites within hippocampal neurons (rat) using fluorescence correlation spectroscopy.
Semi-Synthetic Methods: Labeling of Muscimol with the fluorophore Alexa Fluor 532.

Animal Model: Rat.

Interpretation and Analysis: Based on binding constant parameters, the binding affinity of dye-labeled Muscimol-[Alexa Fluor 532] to $GABA_A$ receptors is highly specific with a K_D value of 3.4 ± 0.5 nM and a ligand-receptor dissociation rate constant (k_{diss}) of $(5.37 \pm 0.95) \times 10^{-2}$/sec. The ligand-receptor association rate constant (K_{ass})has been calculated to be $(1.57 \pm 0.28) \times 10^7$ L mol^{-1} /sec while observed diffusion coefficients are estimated to be $D_{Free} = 233 \pm 20$ μm^2/sec (n = 66) for free diffusing μ-Alexa; $D_{bound1} = 2.8 \pm 0.9$ μm^2/sec (n = 64) for the lateral mobility; and $D_{bound2} = 0.14 \pm 0.05$ microm^2/sec (n = 56) for hindered mobility of the $GABA_A$ receptor-ligand complex in the cell membrane. Saturation of Muscimol-Alexa binding is observed at a concentration of 50 nM while the maximum number of binding sites has been projected to be [$B_{max} = 18.4 \pm -0.4$ nM (n = 5)]. Similar K_i values of 4.5 ± 1.0 nM for non-labeled muscimol and 8.8 ± 1.8 nM for Muscimol-Alexa have been found by RRAs using [^{3}H]muscimol as a radiolabeled ligand. Concentration-dependent increases in the level of specific Muscimol-Alexa binding have been observed as demonstrated by the positive cooperative activity of co-incubated midazolam, which is selectively found in $GABA_A$ receptor-ligand complexes with hindered mobility.

Instrumentation: Fluorescence correlation spectroscopy.

Lateral mobility and specific binding to $GABA_A$ receptors on hippocampal neurons monitored by fluorescence correlation spectroscopy. Meissner O, Haberlein H. Biochemistry 2003 Feb 18; 42 (6), pp. 1667–72.

Histamine Receptor Agonist: H3 Selective Avidity
Histamine H_3 Ligand

Cellular/Molecular Mechanism of Action: Highly selective H_3 receptor agonist with virtually no H_4 subtype receptor affinity.

Application: Evaluation of histamine function and image analysis of histamine receptor sites.

Semi-Synthetic Methods: Production of a series of cyclopropane-based conformationally restricted analogs of histamine including the "folded" cis-analogs (1S,2R)-2-(aminomethyl)-1-(1H-imidazol-4-yl)cyclopropane (11), (1S,2S)-2-(2-aminoethyl)-1-(1H-imidazol-4-yl)cyclopropane (13), and their enantiomers ent-11 and ent-13, and the "extended" trans-analogsues (1R,2R)-2-(aminomethyl)-1-(1H-imidazol-4-yl)cyclopropane (12) and its enantiomer ent-12, have been designed as histamine H_3 receptor agonists. These target compounds were synthesized from the versatile chiral cyclopropane units, (1S,2R)- and (1R,2R)-2-(tert-butyldiphenylsilyloxy)methyl-1-formylcyclopropane (14 and 15, respectively) or their enantiomers ent-14 and ent-15.

Interpretation and Analysis: Among the conformationally restricted analogues, the "folded" analogue 13 (AEIC) having the *cis*-cyclopropane structure has been identified as a potent H_3 receptor agonist, which shows a significant binding affinity ($K_i = 1.31 \pm 0.16$ nM) and has an agonist effect (EC_{50}) value of 10 ± 3 nM) on receptor complexes. The compound owes its importance to being the 1st highly selective H_3 receptor agonist to have virtually no effect on the H_4 subtype receptor. Preliminary studies have therefore demonstrated that the cis-cyclopropane structure is very effective in the conformational restriction of histamine to improve the specific binding to the histamine H_3 receptor.

Cyclopropane-based conformational restriction of histamine. (1S,2S)-2-(2-aminoethyl)-1-(1H-imidazol-4-yl)cyclopropane, a highly selective agonist for the histamine H3 receptor, having a cis-cyclopropane structure. Kazuta Y, Hirano K, Natsume K, Yamada S, Kimura R, Matsumoto S, Furuichi K, Matsuda A, Shuto S. J Med Chem 2003 May 8; 46 (10), pp. 1980–8.

Huntington's Disease (HD)
Physostigmine/L-dopa

Cellular/Molecular Mechanism of Action: Physostigmine inhibits the biochemical activity of acetylcholine esterase, thereby increasing basal levels of acetylcholine neurotransmitter (substrate for acetylcholine esterase). The agent levodopa (L-dopa) is the immediate synthetic precursor to dopamine and melanine. Dopamine is a precursor in the production of norepinephrine.

Background Information: Huntington's disease is a dominantly inherited disorder that affects mental and motor systems and includes a rigid form as well as the better known choreic form. Many articles have been devoted to predicting the future onset of the disease in cases who are at risk, but none of the suggested predictors is currently considered completely reliable. Members from individual families do tend to show a similar age of onset, and similar intellectual and motor abnormalities do develop within a single family; but the presence or absence of this dominant gene of high penetrance is not usually certain until the obvious physical signs appear.

Psychological testing abnormalities are often seen abnormally early in the course of the disease in some cases of HD, particularly when motor dexterity or apraxia is tested. Family members often insist that various psychological traits enable them to predict which members are affected by the gene. Neurophysiologic tests have been described, including new data on the increased liklihood of H-reflexes in Huntington's disease. Electroencephalography at one time was advocated as a possible predictive test but, although there is frequently an association of a low voltage EEG activity with HD, such changes are too variable for certainty in prediction. Pneumoencephalography with specific measurements of caudate atrophy is of clinical interest, but a pneumoencephalogram is rarely needed for diagnosis and caudate atrophy may not actually be an early sign. Metabolic changes in HD include the biochemical effects of hypothalamic dysfunction, changes in growth hormone production/regulation, and reported change in GABA levels within the CSF or brain.

Application: Characterization of motor dexterity and apraxia parameters in suspected cases of Huntington's disease and their potential value as predictive indicators.

Considerations: Predictive tests are of importance not only to decide which person may develop the disorder, but they may also offer a clue to associated or causal features of the disease.

Implementation: Provocative tests have utilized numerous drugs in an attempt to predict the onset of the disease. Most commonly, this includes the diagnostic application of physostigmine and L-dopa.

Interpretation and Analysis: All of the tests elucidate peculiarities of the disease, and all are of ethical as well as neurological interest. Many of the provocative tests utilize quantification of known neurologic features of the disease, such as reduction in saccadic movements of the eye, increased reflexes, or patterns of movement. The ethical problems in predictive tests, especially tests intended to provoke features of the disease, have been a matter of quiet controversy.

Predictive tests in Huntington's disease. Paulson GW Res Publ Assoc Res Nerv Ment Dis] 1976; Vol. 55, pp. 317-29.

Huntington's Disease Chorea/Parkinsons Disease Dyskinesis/Striatal Medium Spiny Neurons/ Glutamate N-Methyl-D-Aspartate Receptors (NMDA)
Amantadine

Cellular/Molecular Mechanism of Action: Amantadine functions as an antagonist at glutamate N-methyl-D-aspartate (NMDA) receptor complexes. Amantadine also promotes release of dopamine from central neurons, particular from the nigrostriatal neurons.

Physiological Mechanism of Action: Compared to levodopa or anticholinergic agents, amantadine is relatively free of side effects.

Background Information: Chorea in Huntington's disease (HD) and in the levodopa-induced dyskinesias of Parkinson's disease (PD) may be clinically indistinguishable. In PD, hyperphosphorylation of NMDA receptors expressed on striatal medium spiny neurons contributes to peak-dose dyskinesias, and drugs that block these receptors can diminish the severity of chorea. Since striatal medium spiny neurons are the primary target of the neurodegenerative process in HD, sensitization of NMDA receptors on residual striatal neurons might also participate in the generation of motor dysfunction in HD.

Since amantadine is believed to promote the release of dopamine from intact dopaminergic terminals that remain in the nigrostriatium in conditions of Parkinson's disease, the therapeutic efficacy of this pharmaceutical can be enhanced by the concurrent administration of levodopa.

Application: Characterization of motor and cognitive function in HD.

Dosage: Amantadine (usually 400 mg/d).

Considerations: Some clinicians have entertained concerns regarding the ethical aspects of provocative testing for HD.

Implementation: Determination of the influence of acute NMDA receptor inhibition on motor and cognitive function in HD.

Interpretation and Analysis: Chorea scores are lower with amantadine treatment (usually 400 mg/d) than observed with placebo, with a median reduction in extremity chorea at rest of 36% ($p = 0.04$) and 56% in cases with the highest plasma drug levels. Improvement correlates with plasma amantadine concentrations ($p = 0.01$) but not CAG repeat length. Parkinsonian rating scores do not worsen and there is no consistent change in cognitive measures. The adverse event profile of amantadine is benign. Observations noted in preliminary investigations imply that NMDA receptor supersensitivity may contribute to the clinical expression of choreiform dyskinesias in HD and that selective antagonists at that site can safely confer palliative benefit.

A randomized trial of amantadine in Huntington's disease. O'Suilleabhain P, Dewey RB Jr. Arch Neurol 2003 Jul; 60 (7), pp. 996–8.

Ischemia Lesions/Parkinson's Disease Research
Stem Cells/Gadolinium Rhodamine Dextran (GRID)

Cellular/Molecular Mechanism of Action: Rhodamine emits light energy while gadolinium functions as an element that can be selectively detected by magnetic resonance imaging. Stem cells have an innate ability to migrate within the central nervous system to regions of neuronal injury or degradation (e.g. ischemica, Parkinson's disease).

Background Information: The ability to track stem cell transplants in the brain by *in vivo* neuroimaging will improve current understanding about how these cells mediate functional recovery after neural transplantation. One major challenge for the development and refinement of stem cell transplantation is to map the spatial distribution and rate of *in situ* migration.

Application: Gadolinium rhodamine dextran is a bifunctional contrast agent that can emit light energy and be detected in a magnetic field. These attributes allow it to be applied as a tracer or marker molecule for profiling the migration of transplanted stem cell populations within the central nervous system. Stem cells can be applied to target various types of lesions within the central nervous system including ischemic injury and neuronal degeneration associated with Parkinson's disease.

Route of Administration: Intravenous.

Semi-Synthetic Methods: Conjugation of rhodamine to dextran applying cross-linking strategies. Addition of gadolinium to dextran can potentially be achieved by covalent bond formation or through the application of chelation groups.

Implementation: Assessment of stem cell migration applying *in vitro* labeling of populations with the bifunctional contrast agent GRID prior to transplantation. Detection is achieved applying both MRI and fluorescence microscopy, or with PKH26 for visualization exclusively under fluorescence microscopy. Brains can be evaluated by both histology and *ex vivo* MR imaging at different time points following engraftment.

Interpretation and Analysis: Transplanted stem cells can be identified by MRI only if prelabeled with GRID, whereas fluorescence microscopy can detect transplanted cells using either labeling reagent. Distribution of GRID-labeled stem cells established applying MRI corresponds to results using fluorescence microscopy. Stem cells and their migration can therefore be reliably identified applying GRID-enhanced MRI methodologies.

Tracking transplanted stem cell migration using bifunctional, contrast agent-enhanced, magnetic resonance imaging. Modo M, Cash D, Mellodew K, Williams SC, Fraser SE, Meade TJ, Price J, Hodges H. Neuroimage 2002 Oct; 17 (2), pp. 803–11.

Motion Sickness
Scopolamine (Transdermal)

Application: Characterization of motion sickness disorders.

Dosage: Scopolamine 1.0 mg over a period of 3 days.

Implementation: Evaluation of the response of motion sickness disorders to scopolamine. Subjects are exposed to a standardized slow-rotation-room test after 6 apparently identical treatments comprised of 4 placebos and 2 medications. Efficacy can be categorized as beneficial, inconsequential, or detrimental. None of the responses are detrimental. The difference in efficacy after 12 and 72 hours has practical and theoretical significance.

Interpretation and Analysis: In one clinical trial, the first administration of a scopolamine protocol, resulted in 4 beneficial responses after 12 hours, but no beneficial effect appreciated after 72 hours. Following the 2nd exposure regimen, there were 4 beneficial responses after 12 hours and 3 beneficial responses after 72 hours.

Great individual differences can be demonstrated in most test subject groups (e.g. n = 2 cases accounting for 6 beneficial responses; n = 2 cases accounting for only 1 beneficial response).

Multiple Sclerosis (MS)
Thyrotropin-Releasing Hormone (TRH) Stimulation/Bromocriptine/(Prolactin)

Cellular/Molecular Mechanism of Action: Thyrotropin-releasing hormone (TRH) promotes prolactin secretion in addition to the release of thyroid stimulating hormone (TSH).
Application: Multiple sclerosis cases reportedly display elevations in plasma prolactin concentrations.
Considerations: Before the onset and during experimental allergic encephalomyelitis (EAE; the animal counterpart of MS), the peripheral concentrations of prolactin can be found to be elevated and bromocriptine has been found to attenuate such attacks. Prolactin may play a role in the immunology of MS.
Implementation: Evaluation of prolactin levels in cases of multiple sclerosis as a diagnostic parameter.

Animal Model: Experimental allergic encephalomyelitis can be applied as a model for human MS.
Interpretation and Analysis: Multiple sclerosis cases have slightly but significantly higher prolactin levels at baseline (10.2 ± 1.6 vs 6.44 ± 0.57 ng/ml, p = 0.042) than do matched reference controls; however, values are within the normal range. Prolactin levels post-TRH are significantly higher in cases with MS with peak prolactin levels being higher than negative reference controls (57.08 ± 6.144 vs 32.94 ± 4.92 ng/ml, p = 0.006). The area under the curve of prolactin is also higher in cases than in controls (3421.87 ± 394.53 vs 2317.62 ± 257.22 ng/ml, p = 0.030).

Prolactin secretion is increased in cases with multiple sclerosis. Azar ST, Yamout B. Endocr Res 1999 May; 25 (2), pp. 207–14.

Multiple Sclerosis (MS)
Metoclopramide/Bromocriptine

Cellular/Molecular Mechanism of Action: Bromocriptine blunts elevations in prolactin while thyrotropin-releasing hormone (TRH) promotes the release of prolactin. Prolactin (PRL) belongs to the growth and lactogenic hormone family and has potent immunomodulating properties.
Background Information: Mild hyperprolactinemia has been found to enhance several autoimmune diseases, and increased PRL plasma levels have been described in natural and experimental models of MS models. The PRL antagonist bromocriptine suppresses the disease.
Application: Detection and characterization of cases suspected of suffering from MS.
Considerations: Prolactin concentrations are elevated in multiple sclerosis. Before the onset and during experimental allergic encephalomyelitis (EAE), the animal counterpart of MS, prolactin levels are elevated and bromocriptine attenuates the severity of attacks.
Implementation: Evaluation of the prolactin baseline values as a function of disease course and activity in a large population of MS cases. Furthermore, inhibitory (bromocriptine) and stimulatory (metoclopramide) tests are performed in a population subgroups to gain functional information.

Human Model: Cases suffering from MS (n = 132; n = 39/132 receiving alternating bromocriptine and metoclopramide).
Interpretation and Analysis: No correlation exists between baseline values and disease course or activity. Nevertheless, in the regression analysis of stimulatory test results, 14% of the variance is attributable to disease activity. In conclusion, PRL does not seem to be relevant as an activity marker in the whole MS population but may be relevant to certain MS subpopulations.

Prolactin stimulation in multiple sclerosis—An indicator of disease subtypes and activity? Heesen C, Gold SM, Bruhn M, Möxnch A, Schulz KH. Endocr Res 2002 Feb–May; 28 (1–2), pp. 9–18.

Multiple Sclerosis (MS)
Arginine Vasopressin (AVP)

Background Information: In the LEW/N rat model, a decreased hypothalamic-pituitary-adrenal (HPA) axis response to inflammatory and immune mediators confers susceptibility to the development of a variety of inflammatory and immune diseases, including experimental allergic encephalomyelitis. In humans with optic neuritis, early intervention with steroids is associated with a decrease in the number of cases who go on to develop MS.
Application: Multiple sclerosis cases demonstrate a blunted release of ACTH in response to AVP stimulation test.
Implementation: Evaluate the potential for MS conditions to show evidence of hypoactive HPA axis function before (baseline) and following provocative testing with the following: (i) ovine corticotrophin releasing hormone (CRH); (ii)] AVP; and (iii) ACTH stimulation. Results are then compared to negative reference controls.

Human Model: Cases of MS (n = 13).
Interpretation and Analysis: Compared to matched controls, MS cases have significantly higher plasma cortisol levels at baseline. Despite this hypercortisolism and in contrast to cases with depression that have similar elevations in plasma cortisol levels, cases with MS show normal, rather than blunted, plasma ACTH responses to ovine CRH, suggesting that the pathophysiology of hypercortisolism in MS is different than for depression. Cases with MS also show a blunted ACTH response to AVP stimulation and normal cortisol responses to high and low dose ACTH stimulation. Taken together, these findings are compatible with data from studies of experimental *in vivo* models exposed to chronic inflammatory stress, that show mild increased activation of the HPA axis with increased relative activity of AVP in the regulation of the pituitary-adrenal axis. These data do not support a role for hypocortisolism in MS once the disease is established.

Multiple sclerosis is associated with alterations in hypothalamic-pituitary-adrenal axis function. Michelson D, Stone L, Galliven E, Magiakou MA, Chrousos GP, Sternberg EM, Gold PW. J Clin Endocrinol Metab 1994 Sep; 79 (3), pp. 848–53.

Multiple Sclerosis (MS)/Inflammatory Medicator Profile (TNF-α; IL-2; IL-4; IL-6; IL-10; IgG)/Clinical Course and Severity
Azathioprine/Cyclosporin/Cyclophosphamide/ IFN-b 1a/Corticosteroids (CST)

Physiological Mechanism of Action: Azathioprine (AZA), cyclosporin, cyclophosphamide, interferon-beta 1a (IFN-β 1a) and/or corticosteroids (CST) each exert immunosuppressive properties through different individual mechanisms.
Implementation: Discover the relationship between various therapies and the clinical activity and course of MS on the *in vitro* production of (i) tumor necrosis factor-alpha (TNF-α); (ii) interleukin-2 (IL-2); (iii) IL-4; (iv) IL-6; (v) IL-10; and (vi) oligoclonal IgG (IgG OB). Experimental samples analyzed include whole blood stimulated with lipopolysaccharide or concanavalin A.

Human Model: Cases of relapsing-remitting, relapsing-progressive, or chronic progressive MS (n = 61) receiving no therapy, AZA, cyclosporin, cyclophosphamide, subcutaneous IFN-β 1a, and/or CST.

Interpretation and Analysis: The cinetics of cytokine y production show that (i) in the absence of treatment, TNF-α and IL-6 production drops after and during the periods surrounding relapse, while IL-4 is increasing before and IL-10 after relapse; (ii) with AZA, levels of TNF-α and IL-6 decrease before exacerbation, IL-4 is produced at high levels for prolonged periods before, while IL-10 is produced at high levels after relapse episodes; and (iii) along with IFN-β 1a, IL-10 is already increasing before relapse, and TNF-α is higher after relapse. When cytokine levels are analyzed independently from MS clinical activity, the use of AZA inhibits IgG OB and TNF-α synthesis ($p = 0.002$) but increases IL-4 ($p = 0.0024$), whereas IFN-β 1a stimulates TNF-α and inhibits IgG OB and inhibits IL-4 production. CST inhibits TNF-α, IL-6, IL-4, and IgG OB synthesis. Such findings illustrate the potential relevance of cytokine profiling in MS conditions.

In vitro cytokine profiles as indicators of relapse activity and clinical course in multiple sclerosis. Schluep M, van Melle G, Henry H, Städler C, Roth-Wicky B, Magistretti PJ. Mult Scler 1998 Jun; 4 (3), pp. 198–202.

Narcolepsy
Imipramine/Methylphenidate/[Food Intake]

Cellular/Molecular Mechanism of Action: Imipramine is classified as a tertiary amine.

Background Information: Narcolepsy afflicts more than 200,000 Americans. In most cases the 1st symptom of the disease, excessive daytime sleepiness, develops during childhood or adolescence. This initial presentation is followed by cataplexy or other auxiliary symptoms several years later. Not infrequently, many years pass before the proper diagnosis of narcolepsy is made. Narcolepsy is a chronic lifelong disease without periods of remission. Excessive daytime sleepiness, inappropriate sleep attacks, and the pathognomonic symptom of cataplexy are diagnostic of narcolepsy.

Diagnostic Application: Applied in the diagnosis and therapy of narcolepsy conditions. Response to imipramine and methylphenidate could potentially be used to differentiate narcolepsy from other neurologic/behavioral abnormalities.

Considerations (Equine Pharmacokinetics): In horses (e.g., n = 5 narcoleptics) pharmacokinetic profile studies can be performed by collecting blood samples before (time 0) and 3, 5, 10, 15, 20, 30, and 45 minutes and 1, 2, 3, 4, 6, 8, 12, and 24 hours after IV administration of imipramine hydrochloride (2 or 4 mg/kg of body weight). Imipramine concentrations are analyzed by HPLC for the estimation of serum concentration as a function of time.

Implementation: Evaluation of narcoleptic conditions based on diminished clinical signs in response to imipramine.

Interpretation and Analysis: Confirmation of the disease is made by performing a multiple sleep latency test. Although still not being used for diagnostic purposes, the association between narcolepsy and the human leukocyte group A (HLA) antigen DR2 is the strongest so far described for any disease. With the help of psychosocial support, therapeutic naps, and medications, cases with narcolepsy may be able to lead a normal life. Methylphenidate and imipramine are the 2 most widely used drugs for the treatment of daytime somnolence and cataplexy, respectively.

Administration of imipramine to normal individuals results in unsteady gait, clumsiness, tiredness or sleepiness, lightheadedness, quiet behavior, and drop in blood pressure. Such sequela are often accompanied by signs of blurry vision due to a decrease in the size of pupils, and a dry mouth (anticholinergic-like activity). In humans, individuals feel unhappy and experience an increased level of anxiety.

In horses, potentially serious adverse effects may be seen with doses of imipramine exceeding 2 mg/kg. Total body clearance of imipramine in horses is slower than that in humans; thus, the interval between subsequent doses should be longer in horses. Adverse effects (muscle fasciculations, tachycardia, hyperresponsiveness to sound, and hemolysis) are detected in most horses when serum imipramine concentrations are high, and these effects are most severe in horses receiving 4 mg of imipramine/kg. Residual adverse effects are usually not apparent. Values (mean ± SD) for area under the curve estimates are 3.9 ± 0.7 hours × μg/ml, while the volume of distribution is 584 ± 161.7 ml/kg, total body clearance is 522 ± 102 ml/kg/hour, and mean residence time is 1.8 ± 0.6 hours. Some horses may have signs of narcolepsy 6 and 12 hours after imipramine administration; and corrresponding serum imipramine concentrations are in such instances below the accepted therapeutic range.

Narcolepsy. Chaudhary BA, Husain I. J Fam Pract 1993 Feb; 36 (2), pp. 207–13.

Pharmacokinetics of imipramine in narcoleptic horses. Peck KE, Hines MT, Mealey KL, Mealey RH. Am J Vet Res 2001 May; 62 (5), pp. 783–6.

Neoplasia (Glioma)
EtNBA Fluorescent Dye

Refer to Section II, Neoplastic Disease.

Fluorescent imaging in a glioma model *in vivo*. Nikas DC, Foley JW, Black PM. Lasers Surg Med 2001; 29 (1), pp. 11–7.

Neoplasia (Gliosarcoma)
Hoechst 33258 Dye/Rhodaminated LCDIO/Green Fluorescent Protein

Refer to Section II, Neoplastic Disease.

Tumoral distribution of long-circulating dextran-coated iron oxide nanoparticles in a rodent model. Moore A, Marecos E, Bogdanov A Jr, Weissleder R. Radiology 2000 Feb; 214 (2), pp. 568–74.

Neoplasia/Ophthalmic Artery Dependent Vision/Preoperative Risk Evaluation Prior to Selective Ophthalmic Artery Embolization/[Arteriovenous Malformations of the Orbit/Dural Arteriovenous Fistulae]/[Orbital Meningiomas/Planum Sphenoidale Meningioma/Juvenile Nasal Angiofibroma]
Lidocaine/Amytal

Background Information: A number of anteriorly located cranial base and extracranial lesions receive their vascular supply wholly or in part from the ophthalmic artery, and embolization of the ophthalmic artery can be helpful in the management of these lesions, either as the primary treatment or as an adjunct to surgery.

Application: Determination of the dependency of visual function on patency of the ophthalmic artery.

Route of Administration: Lidocaine/amytal (intra-arterial catheterization).

Implementation: Evaluation of the degree visual function is impared by local delivery of anesthetic agents into branches of the ophthalmic artery that supply intra-occular and extra-occular lesions. Such preliminary examinations can be used to predict to what extent

visual sight will be affected if selective embolization therapy is implemented for partial or total resolution of lesions. In this context, lidocaine and amytal can be used as a provocative test prior to embolization procedures to assess the role of the ophthalmic artery in vision. In each case, a Tracker No. 18 microcatheter (Target Therapeutics, Inc. Fremont, CA) is navigated into the ophthalmic artery using a steerable guidewire and digital road mapping. Embolic agents include polyvinyl alcohol particles ranging from 350 to 1500 μm in diameter, 2 mm platinum microcoils, and n-butyl-cyanoacrylate. Examples of clinical conditions that can benefit from lidocaine/amytal diagnostic procedures include (i) arteriovenous malformations of the orbit; (ii) dural arteriovenous fistulae; (iii) orbital meningiomas; (iv) planum sphenoidale meningioma; and (v) juvenile nasal angiofibroma.

Interpretation and Analysis: In conditions where provocative testing with lidocaine and amytal indicate that the ophthalmic artery has a substantial effect on vision, then embolization procedures are not performed. Applying this protocol, proper case selection, judicious use of embolic agents, and use of provocative testing agents can result in safe embolization of lesions supplied by the ophthalmic artery.

Embolization of neurosurgical lesions involving the ophthalmic artery. Lefkowitz M, Giannotta SL, Hieshima G, Higashida R, Halbach V, Dowd C, Teitelbaum GP. Neurosurgery 1998 Dec; 43 (6), pp. 1298–303.

Neurotransmitter Dynamics/Relative Cerebral Blood Flow/Epilepsy/Drug Abuse/Schizophrenia/ Neurodegeneration

Superparamagnetic Iron Oxide/Amphetamine/Cocaine Analog 2β-Carbomethoxy-3-β-(4-Fluorophenyl) Tropane

Background Information: The use of functional magnetic resonance imaging (fMRI) techniques for evaluation of pharmacologic stimuli has great potential for understanding neurotransmitter dynamics for a number of brain disorders, such as drug abuse, schizophrenia, epilepsy, or neurodegeneration. Unfortunately, blood oxygenation level-dependent (BOLD) imaging at common fields strengths, such as 1.5 or 3 T, has very low sensitivity and contrast-to-noise ratios (CNRs).

Application: Detection and characterization of alterations in neurotransmitter dynamics and relative cerebral blood flow related to various conditions affecting the central nervous system including drug abuse, schizophrenia, epilepsy, and neurodegeneration.

Route of Administration: Intravascular.

Implementation: Evaluation of the ability for intravascular superparamagnetic iron oxide contrast agent with a long plasma half-life to detect hemodynamic changes related to dopaminergic stimuli using amphetamine or the cocaine analog 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (CFT) as provocative diagnostic agents. The technique is sometimes referred to as increased relaxation with iron oxide nanoparticles (IRON).

Interpretation and Analysis: Results indicate that even at field strengths as high as 4.7 T, it is possible to obtain increases in CNR by factors of 2 to 3 over BOLD imaging. Benefits include greater than an order of magnitude increase in statistical power with greatly increased sensitivity to hemodynamic changes in brain regions difficult to observe using BOLD imaging. Furthermore, use of the intravascular contrast agent allows for a meaningful physiologic parameter to be measured (relative cerebral blood volume [rCBV]), compared to conventional BOLD imaging.

Improved mapping of pharmacologically induced neuronal activation using the IRON technique with superparamagnetic blood pool agents. Chen YC, Mandeville JB, Nguyen TV, Talele A, Cavagna F, Jenkins BG. J Magn Reson Imaging 2001 Nov; 14 (5), pp. 517–24.

Opioid Receptors/Delta-OR Receptors/Mu-Opioid Receptors

Fluo-Deltorphin/d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH_2/Fentanyl Citrate

Cellular/Molecular Mechanism of Action: Fluo-deltorphin is a fluorescent compound that physically binds to delta opioid receptors in a highly selective manner. Similarly, d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH_2 selectively binds to mu-opioid receptor complexes. Fentanyl citrate also binds to mu receptors.

Physiological Mechanism of Action: Fluo-deltorphin is a highly selective delta opioid receptor agonist. d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH_2 is a selective mu-opioid receptor antagonist, while fentanyl citrate alternatively functions as a mu receptor agonist.

Background Information: Opioid receptors are known to undergo complex regulatory changes in response to ligand exposure.

Application: Examine the effect of morphine on the *in vitro* and *in vivo* density and trafficking of delta opioid receptors (deltaORs).

Route of Administration: Intravascular (traditionally).

Implementation: Prolonged exposure of cortical neurons in culture to morphine (10 μm) for a period of 48-hours with and without the presence of d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH_2 or fentanyl citrate. Alterations in the expression density of membrane associated deltaORs can be detected applying immunogold electron microscopy.

Interpretation and Analysis: Prolonged exposure of cortical neurons to morphine results in a profound increase in the internalization of fluo-deltorphin. Such an effect is mu-mediated because it is entirely blocked by d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH_2 and is reproduced using the selective mu agonist fentanyl citrate. Based on results from immunogold electron microscopy analysis, marked increases in the expression density of surface membrane-associated deltaORs occur in neurons exposed to morphine and indicates that increases in fluo-deltorphin internalization are caused by elevations in receptor availability. Prolonged morphine exposure has no effect on delta opioid receptor complex levels, as assessed by immunocytochemistry and Western blot analysis, suggesting that the increase in bioavailable delta opioid receptors is caused by recruitment of reserve receptors from intracellular stores and not from receptor neosynthesis. Chronic treatment of adult rats with morphine (5–15 mg/kg, s.c. every 12 hours) similarly augments targeting of delta opioid receptors to neuronal plasma membranes in the dorsal horn of the spinal cord. Such exposure markedly potentiates intrathecal d-[Ala_2]deltorphin II-induced antinociception. Therefore, prolonged *in vitro* and *in vivo* stimulation of neurons with morphine markedly increases recruitment of intracellular delta opioid receptors to the cell membrane surface. Receptor subtype cross-mobilization of this type may widen the transduction repertoire of G-protein-coupled receptors and offer insight into the development of new diagnostic and therapeutic strategies.

Prolonged morphine treatment targets delta opioid receptors to neuronal plasma membranes and enhances delta-mediated antinociception. Cahill CM, Morinville A, Lee MC, Vincent JP, Collier B, Beaudet A. J Neurosci 2001 Oct 1; 21 (19), pp. 7598–607.

Opioid Tolerance/Mu-Opioid Tolerance and Receptor Down Regulation
$G_i\alpha 2$ Protein Antisense Oligodeoxynucleotide/ Etorphine/Morphine

Application: Characterization of morphine tolerance phenomenon.
Route of Administration: Etorphine or morphine administered by continuous subcutaneous (SC) infusion. Antisense oligonucleotides (intracerebroventricularly and spinal intrathecal space).
Dosage: Etorphine (200 μg/kg/day) or morphine (40 mg/kg/day + 25 mg pellet). Antisense oligonucleotides (30 μg/site/day) for 4 to 5 consecutive days.
Considerations: Although opioid receptors are G-protein coupled, the role that specific G-protein subunits play in the development of opioid tolerance and the regulation of opioid receptor number is not well understood.
Implementation: Evaluation of the influence of antisense ODN or a mismatch ODN directed at mRNA encoding for the $G_i\alpha 2$ subunit of G-proteins (controls = dH_2O). On day 2 of ODN treatment, etorphine (200 μg/kg/day) or morphine (40 mg/kg/day + 25 mg pellet) is administered by continuous subcutaneous (SC) infusion. On day 5 (72 hours later), pumps and pellets are removed and mouse whole brains are tested for morphine analgesia or mu-opioid receptor density. Reference controls initially receive injections of dH_2O and are implanted with inert placebo pellets.
Animal Model: Murine.
Interpretation and Analysis: Etorphine produces significant tolerance (ED_{50} shift = approximately 11-fold) and downregulates mu-opioid receptors (approximately 25%). Morphine produces significant tolerance (ED_{50} shift approximately 9-fold), but no mu-opioid receptor downregulation. Antisense treatment reduces $G_i\alpha 2$ protein levels in striatum and spinal cord by approximately 25%. $G_i\alpha 2$ antisense reduces the acute potency of morphine. $G_i\alpha 2$ antisense blocks the development of tolerance to morphine treatment and reduces the development of tolerance to etorphine treatment. Antisense does not have any effect on etorphine-induced mu-opioid receptor downregulation. In other investigations, 7-day treatment with morphine or etorphine similarly increases $G_i\alpha 2$ mRNA and protein abundance within spinal cord tissues. Such findings support the concept that $G_i\alpha 2$-protein has an important role in the acute effects of opioids and opioid tolerance. However, $G_i\alpha 2$ is not required for agonist-induced mu-opioid receptor density regulation *in vivo*.

Role of G(i)α2-protein in opioid tolerance and mu-opioid receptor downregulation *in vivo*. Yoburn BC, Gomes BA, Rajashekara V, Patel C, Patel M. Synapse 2003 Feb; 47 (2), pp. 109–16.

Organophosphate Intoxication
Atropine

Cellular/Molecular Mechanism of Action: Parasympatholytic activity mediated through blockade of muscarinic receptor sites.
Diagnostic Application: (i) Systemic and topical occular application as a means of non-specifically detecting cases suffering from organophosphate (OP) intoxication. (ii) Reverses or prevents narcoleptic episodes.
Considerations: Atropine can promote gastrointestinal ileus and possible luminal obstruction. Due to its molecular form, it is capable of traversing the blood-brain barrier.
Interpretation and Analysis: Alleviation of abnormal signs associated with organophosphate intoxication (specific and non-specific).

CNS involvement in acute organophosphate poisoning: Specific pattern of toxicity, clinical correlates and antidotal treatment. Finkelstein Y, Taitelman U, Biegon A. Ital J Neurol Sci 1988 Oct; 9 (5), pp. 437–46.

Parkinson's Disease (PD): Dopamine Transporter (DAT)/Attention Deficit Hyperactivity Disorder (ADHD)
Fluoratec/[^{99m}Tc]-Based Tropane Ligand

Cellular/Molecular Mechanism of Action: The DAT, is located presynaptically on dopaminergic neurons and is involved in the trans-membrane transport of dopamine.
Background Information: In ADHD, DAT density levels are elevated, while in PD these levels are depleted. The depletion of DAT levels also corresponds with the loss of dopamine. An additional feature of PD is a depletion of dopaminergic neurons within the substantia nigra accompanied by fibrillar Lewy bodies comprised primarily of alpha-synuclein.
Application: Characterization of the density of dopamine transporter complex expression within the central nervous system. Dopamine transporters are located presynaptically on dopamine neurons and can conceptually function as a biological marker for PD and ADHD.
Semi-Synthetic Methods: Design and synthesize a 2nd-generation [^{99m}technetium]-based tropane ligand [e.g. N-[(2-((3′-N′-propyl-(1′′R)-3′′alpha-(4-fluorophenyl)tropane-2′′beta-1-propanoyl)(2-mercaptoethyl)amino)acetyl)-2-aminoethanethiolato]technetium(V) oxide].
Implementation: Evaluation of the biology and SPECT imaging potential of [^{99m}technetium]-based tropane ligands based on their potent and selective binding to DATs.
Animal Model: Non-human primates.
Interpretation and Analysis: [^{99m}Technetium]-based tropane ligands possess improved selectivity and biological stability representing qualities that facilitate sufficient entry of the agent into the brain and label dopamine transporter complexes in a manner that can provide a quantitative measure of DAT densities (non-human primates). The agent N-[(2-((3′-N′-propyl-(1′′R)-3′′alpha-(4-fluorophenyl)tropane-2′′beta-1-propanoyl)(2-mercaptoethyl)amino) acetyl)-2-aminoethanethiolato]technetium(V) oxide, also known as FLUORATEC, functions as a DAT imaging agent that is a representative [^{99m}technetium]-based tropane ligand.

A second-generation 99m technetium single photon emission computed tomography agent that provides *in vivo* images of the dopamine transporter in primate brain. Meltzer PC, Blundell P, Zona T, Yang L, Huang H, Bonab AA, Livni E, Fischman A, Madras BK. J Med Chem 2003 Jul 31; 46 (16), pp. 3483–96.

Parkinson's Disease (PD): Dopamine Transporter (DAT)
3,6-Disubstituted Piperidine Derivatives

Application: The 3,6-disubstituted piperidine derivatives can function as novel templates for high-affinity inhibitors of the dopamine transporter molecule. Ultimately, the compounds could feasibly be utilized to characterize the distribution and expression density of dopamine transporter complexes.

Synthetic Methodologies: Applying the concepts of structure-activity relationships (SAR), a novel conformationally constrained lead *cis*-3,6-disubstituted piperidine derivative derived from (2,2-diphenylethyl)-[1-(4-fluorobenzyl)piperidine-4-ylmethyl]amine (I) can facilitate the design of a series of compounds synthesized by derivatizing the exocyclic N-atom at the 3-position of the lead compound. Related substituted phenyl and heterocyclic derivatives have been produced and evaluated for their affinity for DAT, serotonin transporter (SERT), and norepinephrine transporter (NET) in the brain by measuring their potency by assessing their ability to compete for the binding of [^{3}H]-WIN 35 428, [^{3}H]-citalopram, and [^{3}H]-nisoxetine, respectively. Analogous compounds have also been evaluated for their activity in inhibiting the uptake of [^{3}H]DA. The SAR results demonstrate that the nature of substitutions on the phenyl ring is important for evoking activity at the DAT with the presence of an electron-withdrawing group having the maximum effect on potency. Replacement of the phenyl ring in the benzyl group by heterocyclic moieties results in the development of compounds with moderate activity for the DAT. The 2 most potent racemic compounds can be separated by a diastereoisomeric separation procedure, and differential affinities can be observed for the enantiomers. Absolute configuration of the enantiomers can be obtained unambiguously by x-ray crystal structural study. One of the enantiomers, compound S,S-(-)-19a, exhibits the highest potency for the DAT (IC_{50} = 11.3 nM) among all the compounds tested and is as potent as GBR 12909 (1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine). However, the compound (-)-19a is more selective than GBR 12909 in binding to the DAT compared with binding to the SERT and NET.

Interpretation and Analysis: The newly developed 3,6-disubstituted piperidine derivatives are novel templates for high-affinity inhibitors of DAT. Structurally, these molecules are more constrained compared to earlier flexible piperidine molecules and, thus, should provide greater insight into their bioactive conformations.

Interaction of cis-(6-benzhydrylpiperidin-3-yl)benzylamine analogues with monoamine transporters: structure-activity relationship study of structurally constrained 3,6-disubstituted piperidine analogues of (2,2-diphenylethyl)-[1-(4-fluorobenzyl)piperidin-4-ylmethyl]amine. Kolhatkar RB, Ghorai SK, George C, Reith ME, Dutta AK. J Med Chem 2003 May 22; 46 (11), pp. 2205–15.

Parkinson's Disease (PD): Early vs Late Onset
Bromocriptine/Thyrotropin-Releasing Hormone (TRH)

Route of Administration: Thyrotropin-releasing hormone (intravascular) following bromocriptine pretreatment.

Implementation: Evalution of early-onset and late-onset conditions of Parkinson's disease as a function of basal plasma prolactin (PRL) concentrations in response to and their changes after TRH administration following bromocriptine (BCT) pretreatment (BCT/TRH test).

Human Model: Cases with early- and late-onset Parkinson's disease (n = 12 cases with young-onset < 40 years of age; and n = 10 cases with late-onset PD at > 40 years of age; compared to n = 10 healthy age-matched reference controls.

Interpretation and Analysis: Basal PRL levels are normal in both groups of cases. However, following BCT/TRH, significantly lower prolactin increases are induced in older-onset parkinsonians than in controls. Such a response becomes even more blunted in young-onset cases, being significantly more attenuated than in older-onset PD cases.

Function of dopamine receptors in young-onset Parkinson's disease: Prolactin response. Kostic VS, Marinkovic Z, Filipovic S, Momcilovic D. Mov Disord 1993 Apr; 8 (2), pp. 227–9.

Parkinson's Disease: Idiopathic/Type Differentiation of Parkinson's Disease/Parkinsonian Multiple System Atrophy/Parkinsonian Multiple Systemic Atrophy: Cerebellar
Clonidine/[GHRH-Arg]/L-dopa

Cellular/Molecular Mechanism of Action: Clonidine is a centrally active alpha 2-adrenoceptor agonist.

Physiological Mechanism of Action: Clonidine is a centrally active alpha 2-adrenoceptor agonist that raises concentrations of GH in serum.

Background Information: Idiopathic PD is a common neurodegenerative disease that is difficult to distinguish from other parkinsonian syndromes such as multiple-system atrophy (MSA). In MSA, autonomic dysfunction is common and is associated with either parkinsonian or cerebellar features, or both. Differentiation of idiopathic PD from MSA is important because prognosis, complications, and response to therapy vary according to disorder.

Diagnostic Application: Clonidine can be used to diagnostically differentiate between MSA and idiopathic PD.

Implementation: Evaluate the various conditions of Parkinson's disease as a function of growth hormone secretion in response to clonidine challenge.

Human Model: Cases of PD with clonidine administered to n = 14 idiopathic Parkinson's disease without autonomic deficits; in addition to n = 31 cases with MSA comprised of 3 different clinical forms [e.g. parkinsonian, cerebellar, and mixed]; n = 19 cases with pure autonomic failure; and n = 27 healthy participants. In a subgroup, n = 9 cases of parkinsonian MSA (MSA-P) are assessed for GH response to levodopa challenge.

Interpretation and Analysis: Clonidine raises serum GH concentrations in cases with idiopathic PD (median increase 8.98 [IQR 6.6–6.6] mU/L), normal participants (13.2 [7.0–18.6] mU/L), and cases with pure autonomic failure (12.5 [5.6–18.2] mU/L). In those with MSA that have central autonomic failure, GH concentrations remain unchanged (MSA-P; 0.41 [−0.30–2.09] mU/L and cerebellar MSA [MSA-C] 1.67 [0–4.49] mU/L). The GH response to clonidine in idiopathic Parkinson's disease is significantly different from that in MSA-P ($p < 0.0002$). In MSA-P, the dopamine precursor levodopa raises GH concentrations (from mean 2.7 [SE 1.0] mU/L to a mean of 18.2 [6.0] mU/L, $p < 0.05$) and GH-releasing hormone (GHRH) concentrations (from mean 20.6 [3.25] ng/L to a mean 68.0 [10.6] ng/L, $p < 0.05$), excluding dysfunction of pituitary somatotrophs or GHRH neurons as a cause for the absent GH response to clonidine in MSA.

Clonidine raises concentrations of GH in serum in healthy people and those with pure autonomic failure (with peripheral lesions), but not in Parkinson's cases with MSA (MSA with a central autonomic deficit). The GH responses to clonidine can clearly differentiate idiopathic PD from MSA-C and MSA-P. Together with the levodopa studies they indicated a specific alpha 2-adrenoceptor-hypothalamic deficit in MSA. The clonidine-GH test may provide further insight into central neurotransmitter and alpha 2-adrenoceptor-hypothalamic abnormalities in MSA.

Distinction of idiopathic Parkinson's disease from multiple-system atrophy by stimulation of growth-hormone release with clonidine. Kimber JR, Watson L, Mathias CJ. Lancet 1997 Jun 28; 349 (9069), pp. 1877–81.

Parkinson's Disease (PD)/Prediction of Sustained Long-Term L-dopa Responsiveness/Major Diagnostic Criteria
Levodopa (L-dopa)

Background Information: Establishing a diagnosis of clinical idiopathic PD requires following strict criteria. Final definitive diagnosis can only be made after pathological confirmation and, despite following clinical criteria, several cases can be misdiagnosed.
Application: Evaluation of the sensitivity and specificity of acute levodopa (L-dopa) challenge to predict sustained long-term L-dopa responsiveness as a major criterion for clinical diagnosis of PD.
Dosage: L-dopa-carbidopa (250/50 mg).
Implementation: Evaluation of the sensitivity and specificity of acute challenge with L-dopa to predict sustained long-term L-dopa responsiveness as a major criterion for clinical diagnosis of PD. Provocative testing involves acute challenge with L-dopa-carbidopa and results rated as positive or negative according to whether values reached a minimal 30% of improvement on UPDRS scores. Positive tests are considered supportive of a presumptive clinical diagnosis of Parkinson's disease. Blind to test results and according to clinical presumption, an examinter/evaluator initiates treatment with the necessary L-dopa dose or, alternatively, until reaching 1 g for 1 month in those who failed to display a positive test response. At 24-month follow-up, they are retested with 1 g for 1 month when required. At this point, clinical criteria of the U.K. Parkinson's Disease Society Brain Bank are applied and definitive clinical diagnosis of PD determined. Sensitivity, specificity, and positive predictive ratio for acute challenge are calculated.

Human Model: Cases of PD (n = 82) first evaluated at a movement disorders clinic. A second examiner, blind to the presumptive diagnosis, performs the prescribed provocative tests.
Interpretation and Analysis: Overall sensitivity and specificity of acute L-dopa challenge to predict a clinical diagnosis of PD is approximately 70.9% and 81.4%, respectively; positive predictive ratio is close to 88.6%. When cases are divided into 3 groups according to their UPDRS motor section score at initial examination, sensitivity and specificity vary: Group I ($\leq$ 10), 71.4% and 100%; Group II (11–20), 75% and 75%; and Group III ($\geq$ 21), 36.4% and 87%, respectively. Positive predictive ratios increase to 100% in Group I and to 87.5% in Group III. The positive result of initial acute L-dopa challenge predicts chronic L-dopa responsiveness as a major criterion of PD in all cases with UPDRS motor scores lower than 10.

Accuracy of acute levodopa challenge for clinical prediction of sustained long-term levodopa response as a major criterion for idiopathic Parkinson's disease diagnosis. Merello M, Nouzeilles MI, Arce GP, Leiguarda R. Mov Disord 2002 Jul; 17 (4), pp. 795–8.

Pharmaceutical Elimination (Brain): Suppression/(Capillary Endothelial rOat3 Organic Anion Transporter)
para-Aminohippuric Acid/Cimetadine/Pravastatin/Benzylpenicillin

Cellular/Molecular Mechanism of Action: Para-aminohippuric acid (PAH) and benzylpenicillin (PCG) are substrate competitive inhibitors of organic anion transporter 3 (rOAT3) complexes expressed by brain capillary endothelium. Cimetidine (H_2 histamine blocker) and pravastatin are also substrate competitive inhibitors of organic anion transporter 3 (rat rOat3).

Physiological Mechanism of Action: Para-aminohippuric acid and PCG can serve as tracer molecules to evaluate central nervous system elimination mechanisms (efflux transport) involving rOat3 organic anion transporter molecules expressed by brain capillary endothelium. In contrast, cimetidine and pravastatin delay the elimination of diagnostic pharmaceutical agents across the blood-brain barrier.
Background Information: Based on RT-PCR analysis, the organic anion transporter complexes rOat1 and rOat2 do not appear to be expressed in the brain capillary endothelial cells (BCEC) in contrast to rOat3 (moderate expression levels). The expression of rOat3 in the BCEC can be further confirmed by Western blot analysis and immunohistochemical staining reveals that rOat3 is located on the abluminal and, possibly, luminal membrane of the BCEC.
Application: Potential uses of pharmaceutical agents like cimetadine, *para*-aminohippuric acid (PAH), pravastatin, benzylpenicillin (PCG) include prolongation of the exposure of diagnostic pharmaceuticals to specific function targets or lesions within the central nervous sytem.
Interpretation and Analysis: As a reference model, the rOat3 substrates PAH and PCG can be used to evalate the functionality of this organic anion transporter protein. Applying these pharmaceutical marker systems, the Brain Efflux Index method can be used to determine the extent and time course of PAH and PCG elimination from the cerebrum and into the peripheral intravascular compartment. In this context, PAH and PCG are eliminated from the cerebrum with rate constants of 0.039 and 0.043/min, respectively, and the elimination processes become saturated at high substrate concentrations. Taking account of the dilution effect within the brain, the K_m values for the elimination of PAH and PCG is estimated to be 168 and 29 μM, respectively. Efflux of PAH and PCG across the BBB is inhibited in a dose-dependent manner by unlabeled PCG and PAH, respectively. The K_i value of PAH for the efflux of PCG is 106 μM and that of PCG for the efflux of PAH is 58 μM. These values are comparable with their K_m values, suggesting that they share the same efflux mechanism at the blood-brain barrier. Furthermore, cimetidine and pravastatin, which are also substrate competitive inhibitors of rOat3, significantly inhibited the efflux of PAH and PCG from the cerebrum. These results suggest that rOat3 is responsible for the elimination of PAH and PCG from the brain across the BBB.

Contribution of organic anion transporter 3 (Slc22a8) to the elimination of p-aminohippuric acid and benzylpenicillin across the blood-brain barrier. Kikuchi R, Kusuhara H, Sugiyama D, Sugiyama Y. J Pharmacol Exp Ther 2003 Jul; 306 (1), pp. 51–8. Epub 2003 Apr 8.

Polioencephalomalacia: Cerebral Cortical Dysfunction
Thiamine

Physiological Mechanism of Action: Certain strains of rumenal bacterial can produce thiaminase, thereby diminishing systemic and central nervous system concentrations of thiamine.
Background Information: Polioencephalomalacia (PEM) induced in sheep has been compared with the disease found in naturally occurring cases. Blood biochemical indicators include pyruvate, lactate, glucose, erythrocyte transketolase (TK), and stimulation of TK by addition of thiamine pyrophosphate (TPP effect). Feces and rumen contents can be assayed for thiaminase activity. The effect of treating affected sheep with thiamine is also noted. Amprolium treatment can induce thrombocytopenia, but once sheep become accustomed to amprolium in the diet they seem to be resistant to this

effect. In sheep receiving amprolium significant weight loss can serve as a variable that consistently precedes the onset of clinical signs. Further weight loss continues throughout the recovery period despite removal of amprolium from the diet and treatment with thiamine. Blood glucose levels are variable, and elevated only when marked clinical signs are present. Pyruvate and lactate levels show marked variation throughout trials. TK values are depressed and TPP effects increase well before the onset of clinical signs, although some naturally occurring cases have normal levels. Fecal thiaminase activity is negligible in all sheep on amprolium but most field cases have a high level. High fecal thiaminase is observed in about 5% of clinically normal animals from affected flocks. Depression of erythrocyte TK activity coupled with the presence of fecal thiaminase appears to be the most reliable diagnostic biochemical parameter for PEM.

Implementation: Detection of neurologic cases of PEM based on response to thiamine administration.

Interpretation and Analysis: Administration of thiamine to PEM affected sheep rapidly brings the biochemical status of animals back to normal. However, in conditions of advanced brain lesions and when the damage is permanent, such sheep treated with thiamine remain partially decorticate.

Schematic Description: Decreased RBC TK → thiamine → rapid correction of decreased RBC TK in PEM.

Biochemistry of natural and amprolium-induced polioencephalomalacia in sheep. Spicer EM, Horton BJ. Aust Vet J 1981 May; 57 (5), pp. 230–5.

Seizures: Alcoholic Detoxification/Recurrent Episodes
Chlordiazepoxide/Pharmaco-Electroencephalography

Application: Delineation of recurrent alcoholic detoxification seizures.

Route of Administration: Per os (oral) during detoxification events (associated with the elimination of withdrawal seizures).

Implementation: Evaluation of the potential increased risk of seizure activity during conditions of recurrent alcohol detoxifications. Structural brain damage, focal neurologic deficits, epileptogenic findings on electroencephalograms, and provocative drug use are considered pertinent variables for seizure risk.

Human Model: Review of detoxification and seizure histories (n = 256 men; n = 45 women voluntarily admitted to a 5-day inpatient detoxification program).

Interpretation and Analysis: Administration of chlordiazepoxide is usually associated with the elimination of withdrawal seizures. Some cases have a history of seizures (e.g., n = 64/301) while others have significantly abnormal electroencephalograms during detoxification (e.g., n = 42/301), or have focal slowing on the electroencephalogram (e.g., n = 30/301). No correlation exists between the duration of alcohol abuse or lifetime consumption of alcohol and seizure activity. There is, however, a significant correlation between the number of inpatient alcohol detoxifications and the prevalence of seizure disorders. This correlation holds true even when provocative drug use is taken into consideration.

Seizure risk with recurrent alcohol detoxification. Lechtenberg R, Worner TM. Arch Neurol 1990 May; 47 (5), pp. 535–8.

Seizures: Central Nervous System Activity
Pentylenetetrazol/Pharmaco-Electroencephalography

Mechanisms of Action: Pentylenetetrazol diminishes the influence of GABA on chloride ion conductance at concentrations that do not alter resting neuronal membrane conductance in the absence of GABA. Additional attributes of pentylenetetrazol include inhibition of picrotoxin association with binding sites, and it has the ability to block the enhancement of GABA and benzodiazepine binding promoted by hypnotic barbiturates. No direct stimulatory effect has been associated with pentylenetetrazol.

Diagnostic Application: Primarily of value in facilitating the diagnostic induction of epileptic episodes for the purpose of acquiring EEG recordings.

Route of Administration: Pentylenetetrazol (subcutaneous).

Dosage: Pentylenetetrazol (PTZ seizure dose = 65 mg/kg).

Considerations: Pentylenetetrazol induces seizure activity through CNS stimulation. Because of the potential hazards that seizure episodes might precipitate, appropriate antiseizure medications should always be readily (immediately) available for administration as a safety precaution.

Implementation: Truly simultaneous electroencephalogram (EEG) and functional magnetic resonance imaging (fMRI) are recorded following the induction of convulsions with pentylenetetrazol. Rigorous control of physiological parameters like body temperature and ventilation with control of blood gasses help avoid potential interference between systemic parameters and central PTZ-induced blood oxygenation level-dependent (BOLD) changes.

Animal Model: Rat (curarized rats).

Interpretation and Analysis: In experimental *in vivo* models, simultaneous EEG/fMRI recordings demonstrate progressive epileptiform EEG discharges with concomitant BOLD changes, with the latter gradually affecting most of the fore- and midbrain. Approximately 15 minutes after PTZ injection, the 1st BOLD contrast changes mainly occur in the neocortex, and coincide with the 1st minor EEG alterations. Most regions that display BOLD changes are areas with reportedly high $GABA_A$ receptor densities. Full-blown epileptiform discharges occur on the EEG tracing, approximately 30 minutes after PTZ injection, and coincide with bilateral positive and/or negative BOLD contrast changes in cortical and subcortical regions. Behavioral observations demonstrate that the 1st of several generalized clonic or clonic-tonic seizure episodes occur also around this same time period. Approximately 90 minutes after injection, the electrographic paroxysms gradually decrease in amplitude and duration, whereas BOLD signal changes still extended with alternating positive and negative traces and spread to subcortical regions like caudate-putamen and globus pallidus.

In humans, the induction of an epileptic seizure or analogous episodes following administration constitutes a positive diagnosis that can be more fully characterized through analysis of EEG recordings.

Laboratory Instrumentation: Electroencephalogram recording.

Simultaneous electroencephalographic recording and functional magnetic resonance imaging during pentylenetetrazol-induced seizures in rat. Van Camp N, D'Hooge R, Verhoye M, Peeters RR, De Deyn PP, Van der Linden A. NeuroImage 2003 Jul; 19 (3), pp. 627–36.

Seizures: Hypoglycemic-Induced
Glucose/Glucagon

Cellular/Molecular Mechanism of Action: Carbohydrates (starches, monosaccharides, disaccharides, polysaccharides) are broken down to monosaccharides by pancreatic saccahridases secreted into the gastrointestinal track. Monosaccharides are then absorbed from the small intestine into the blood stream. Once in the blood stream, monosaccharides are "pumped" across cellular membranes

and either (i) enter the tricarboxylic acid (TCA) cycle, where they generate the production of ATP, GTP, and lactic acid; or (ii) are transformed into hepatic or muscle glycogen, which is a storage form (pool) of glucose available for cellular metabolism at a later date.

Diagnostic Applications: (i) Glucose Tolerance Test can be utilized to detect conditions of diabetes mellitus or prediabetic conditions. (ii) Glucose Absorption Test has been applied to identify conditions of gastrointestinal malabsorption. (iii) Glucose Suppression Test: can serve as an approach for depressing growth hormone (GH) in cases with (feline) acromegaly.

Route of Administration: (i) Glucose Tolerance Test: Oral administration at a specific dose; (ii) Glucose Absorption Test: oral administration of a specific dose; (iii) Glucose Suppression Test: Oral or intravenous administration.

Dosage: (i) Glucose Tolerance Test: 75 g or approximately 1 g/kg body weight; (ii) Glucose Absorption Test: 1 g/kg body weight; (iii) Glucose Suppression Test: 1 g/kg body weight.

Considerations: Glucose given orally can be degraded by normal bacterial flora residing within the gastrointestinal tract. In addition, glucose can inhibit gastrointestinal emptying at concentrations above 10%, and stimulate pancreatic insulin secretion. Each of these confounding variables can contribute to the development of false negative results.

Semi-Synthetic Methods: A glucagon radioimmunoassay can be employed using antiserum specific for pancreatic glucagon. In this context, glucagon can be radioiodinated utilizing the chloramine T technique and purified on QAE-Sephadex A 25 to a specific activity of approximately 225 μCi/μg. The standard curve generated allows measurements to be acquired from 12 to 500 pg/ml with a sensitivity of 17.5 pg/ml, and a precision level of 6.3 to 14.9% (CV, within-assay) and 5.6 to 10.7% (CV, between-assay). Recovery is between 82 and 112%.

Interpretation and Analysis: (i) Glucose Tolerance Test: Excessively high blood glucose concentrations may imply the presence of diabetes mellitus. An excessive glucose rebound phenomenon may also be appreciated, which is interpreted by some physicians as a prediabetic condition. (ii) Glucose Absorption Test: Serum (blood) glucose levels should at least double within 2 hours of oral administration. Fasting plasma glucagon levels in diabetics, obese subjects, acromegalics, and cases with Cushing's syndrome are greater than in normals (22.0 ± 91 pg/ml; mean ± SD). Very low glucagon levels can be appreciated after oral glucose suppression (15.2 ± 3.1 pg/ml) in normal cases, while greatly increased values are detected after arginine in insulin-dependent diabetics (271.0 ± 132.3 pg/ml).

Laboratory Instrumentation: Standard clinical pathology methods for analysis and measurement of glucose concentrations within serum and urine samples.

Seizures/Insecticides and Nerve Agents/(Tabun, Sarin, Soman, Cyclosarin, VR, VX)/Relative Vulnerability to Seisure Termination: Sarin and VX Compared to Soman, Cyclsarin and VR

Anticholinergics: Atropine, Birperiden, Pyridostigmine, 2-PAM, Trihexyphenidyl/Benzodiazepines: Diazepam, Midazolam

Application: Detection of seizure episodes induced by nerve agents including tabun, sarin, soman, cyclosarin, VR, and VX. Results can possibly function as adjunct information to high-performance liquid chromatography (HPLC) analysis in efforts to differentiate between exposures to different types of nerve agents.

Route of Administration: Pyridostigmine bromide (intramuscular); soman (subcutaneously); atropine SO_4 (intramuscular).

Dosage: Pyridostigmine bromide (0.026 mg/kg); atropine SO_4 is implemented (2 mg/kg); 2-PAM Cl (25 mg/kg).

Implementation: Evaluation of pyridostigmine bromide pretreatment 30 minutes prior to challenge with 2 × LD_{50} dose of a given nerve agent (SQ). Complementary evaluations include challenges with 5 × LD_{50} of soman. One minute after agent challenge, exposure to atropine SO_4 is implemented admixed with 2-PAM Cl. Five minutes after the start of EEG seizures, different doses of anticholinergics (IM) or benzodiazepines (IM) are administered and observations made to detect termination of seizure episodes. The time to seizure onset, the time to seizure termination, and 24-hour lethality are recorded. The anticonvulsant ED_{50} of each drug for termination of seizures induced by each agent is calculated and compared. Brain tissue from *in vivo* models that survive 24 hours is examined for pathology.

Animal Model: Guinea pigs (implanted with cortical electrodes for EEG recording).

Interpretation and Analysis: All drugs are capable of terminating seizure activity, with midazolam and trihexyphenidyl being significantly more potent than the other pharmaceutical agents, and midazolam being more rapid in controlling seizure than atropine, trihexyphenidyl, or diazepam against each agent. Seizures induced by sarin or VX require lower doses of all the test anticonvulsants. The dose of a given drug that is an effective anticonvulsant against a 2 × LD_{50} challenge of soman is equally effective against seizures induced by a 5 × LD_{50} challenge. All of the nerve agents (tabun, sarin, soman, cyclosarin, VR, VX) are capable of producing neuropathology. Seizure control is strongly associated with protection against acute lethality and brain pathology.

Control of nerve agent-induced seizures is critical for neuroprotection and survival. Shih TM, Duniho SM, McDonough JH. Toxicol Appl Pharmacol 2003 Apr 15; 188 (2), pp. 69–80.

Seizures/Intractable (Medical): Surgical Intervention/Essential Tremors/Cerebral Resuscitation/Seisures: Partial, Complex Partial, and Secondary General

Amobarbital/Methohexital/Phenobarbital

Cellular/Molecular Mechanism of Action: The primary mechanism of action of barbiturates is to increase inhibition mediated through the gamma-aminobutyric acid (GABA) system. Anesthetic barbiturates also decrease excitation via a decrease in calcium conductance.

Application: Barbiturates have been used as adjunct injectable general anesthetics and as anticonvulsant agents. In this context, barbiturates have been used in the evaluation of medically intractable seizure disorders that may possibly be managed through surgical intervention. Barbiturates to a limited degree have also been applied in an experimental fashion, most notably related to characterization of cerebral resuscitation techniques.

Interpretation and Analysis: Phenobarbital (phenobarbitone), the primary anticonvulsant barbiturate, is effective for partial, complex partial, and secondarily generalized seizures. While no longer the drug of choice for all types of seizures, it remains an important and useful agent. Mysoline has been shown to be useful in the treatment of essential tremor and several other movement disorders and as an anticonvulsant. Barbiturates are also used for their sedative-hypnotic properties. Preparations of methohexital and amobarbital (amylobarbitone) are examples of barbiturates that have potential as diagnostic agents for certain seizure disorders.

The clinical use of barbiturates in neurological disorders. Smith MC, Riskin BJ. Drugs 1991 Sep; 42 (3), pp. 365–78.

Seizures: Neonatal/Prediction of Seizure Burden/Prediction of Treatment Regiment Efficacy
Phenobarbitone/Phenytoin/Lignocaine (Lidocaine)/Pharmaco-Electroencephalography

Cellular/Molecular Mechanism of Action: Phenytoin and lignocaine (lidocaine) block cell membrane sodium channels.
Physiological Mechanism of Action: Phenobarbitone is a barbiturate analog that has a depressant effect on central nervous system (CNS) activity.
Application: Prolonged electroencephalographic recordings in the presence of pharmaceutical agents reveal information that challenges the merit and justification of certain established treatment regimens. In this context, indications can be gained regarding the prognosis for future disability and severity of dysfunction attributable to CNS seizure activity.
Considerations: Evidence exists that suggests that seizure episodes are damaging to the neonatal brain. Neonatal seizures can permanently disrupt neuronal development, induce synaptic reorganization, alter plasticity, and "prime" the brain to increased damage from seizures later in life.
Implementation: Evaluation of seizure potential and prediction of future seizure burden.
Interpretation and Analysis: Electroencephalography remains the most useful strategy for determing diagnosis and future prognosis. Alternatively, phenobarbitone remains the mainstay of treatment and is effective in about one-third of cases; babies who respond tend to have a smaller seizure burden and a relatively normal background electroencephalogram. Their prognosis is better than in those who require 2nd-line treatment strategies. Phenytoin and lignocaine (membrane stabilizing drugs) are probably more effective than any of the benzodiazepines as 2nd line treatment strategies , but very few evaluation studies have been reported. Babies who require 2nd-line treatment strategies are more likely to have hypoxic ischemic encephalopathy, an abnormal background electroencephalogram, and a large seizure burden, and to have a worse prognosis than do those who respond to a single agent; most have significant disability at follow-up.
Instrumentation: Computer-integrated electroencephalogram instrumentation.

Neonatal seizures and their treatment. Rennie JM, Boylan GB. Curr Opin Neurol 2003 Apr; 16 (2), pp. 177–81.

Seizures: Non-Epileptic Attacks (NEAs)
Saline Infusion/[Verbal Suggestion]

Background Information: Non-epileptic attacks (NEA) pose as diagnostic and therapeutic delemmas and can be associated with a poor prognosis. Activation procedures like saline infusion have been used for diagnostic purposes.
Application: Induction of NEA episodes.
Implementation: Evaluation of NEA conditions applying EEG analyses. Attempts to induce attacks can be initiated with verbal suggestion and saline infusion. In clinical trials, cases can be monitored with a postal or telephone questionnaire 4.5 years after the EEG.
Human Model: Case with a probable diagnosis of NEA (n = 66).
Interpretation and Analysis: Saline infusion with verbal suggestion can induce an attack in a relatively large percentage of NEA cases (e.g., n = 41/66 cases). In follow-up monitoring, approximately 66% display a reduction in the number of attacks (e.g., n = 20/31 reduction, n = 12/20 attack free). More cases are attack free at follow-up after a positive (e.g., n = 7/16) in comparison to a negative (n = 5/15) test. A provocative test can be diagnostic in some cases with NEA. An early and firm diagnosis may lead to appropriate treatment and better outcome.

Saline infusion: A diagnostic and therapeutic tool in nonepileptic attacks? Wassmer E, Wassmer SR, Donati F. Epilepsy Behav 2003 Oct; 4 (5), pp. 500–6.

Seizures/Pesticide (Neurotoxic): Rotenone/(Dopaminergic System of the CNS)
1-Methyl-1,2,3,4-Tetrahydroisoquinoline (1MeTIQ)

Physiological Mechanism of Action: Counteracts the promotion of elevated concentrations of dopamine within the central nervous system induced by rotenone (neurotoxic pesticide).
Application: 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) can function as a neuroprotective agent against exposure to rotenone (neurotoxic pesticide). In this context, 1MeTIQ could serve as a molecular targeting agent for the purpose of diagnostic imaging and/or as a diagnostic option in the differential diagnosis of acute-onset neurologic seizures.
Implementation: Evaluation of neurologic seizures associated with rotenone intoxication.
Animal Model: Rats. (Wistar strain with rats subjected to the effect of single and multiple administrations of rotenone (neurotoxic pesticide) in concert with the potentially neuroprotective compound 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ).
Interpretation and Analysis: The neurotoxic pesticide rotenone administered in a single dose (12 mg/kg s.c.) does not produce evidence of behavioral or biochemical alterations. In contrast, repeated administration of rotenone doses (12–15 mg/kg) causes abnormalities in general behavior while also producing considerable mortality and dramatic increases in dopamine metabolism that may be ascribed to increases in the oxidative pathway. Interestingly, 1MeTIQ depresses the concentration of the extracellular dopamine metabolite 3-MT. These behavioral and biochemical changes are effectively counteracted by administration of 1MeTIQ before each dose of rotenone. Based on findings from several laboratories, multiple systemic rotenone injections are highly toxic and induce alterations of cerebral dopamine metabolism. The compound 1MeTIQ may be considered as a potential diagnostic and protective agent against environmental factors affecting the function of the dopaminergic system.
Instrumentation: High-performance liquid chromatography (HPLC) and electrochemical monitoring equipment.
Laboratory Methodologies: Concentrations of dopamine and its metabolites (homovanillic acid-HVA, 3,4-dihydroxyphenylacetic acid-DOPAC, and 3-methoxytyramine-3-MT) are measured in 3 regions of the brain applying HPLC in concert with electrochemical detection/monitoring instrumentation. The rate of dopamine catabolism in the striatum along the N-oxidative and O-methylation pathways is assessed through the calculation of the ratio of dopamine metabolites to dopamine. In addition, the effect of rotenone on rat mortality and general behavior is recorded.

1-methyl-1,2,3,4-tetrahydroisoquinoline protects against rotenone-induced mortality and biochemical changes in rat brain. Antkiewicz-Michaluk L, Karolewicz B, Romanska I, Michaluk J, Bojarski AJ, Vetulani J. Eur J Pharmacol 2003 Apr 18; 466 (3), pp. 263–9.

Serotonin (5-HT) and Adrenergic Systems Evaluation/Myoclonic Twitches Evoked by L-5-Hydroxy-Tryptophan, Quipazine, and Fenfluramine.
Quipazine/L-5-Hydroxy-Tryptophan/Cyproheptadine/Cirazoline/Clonidine/Yohimbine/L-dopa/Salbutamol

Cellular/Molecular Mechanism of Action: Quipazine is a serotonin (5-HT) receptor agonist; L-5-hydroxy-tryptophan is a serotonin precursor molecule; fenfluramine is a 5-HT releasing agent; cyproheptadine is a 5-HT receptor antagonist; cirazoline is an alpha 1-adrenoceptor agonist; clonidine is an alpha 2-adrenoceptor agonist; yohimbine is an alpha 2-adrenoceptor antagonist; L-dopa recognizes and physically binds to dopamine receptor complexes (functions as an agonist); and salbutamol is a beta 2-adrenoceptor agonist.

Applications: Potential utilization as a test model for investigating the action of compounds on central 5-HT and adrenergic systems. Clinical relevance could pertain to characterizing exposure to agents that promote myoclonic activity.

Route of Administration: Quipazine (5-HT intravenous); L-5-hydroxy-tryptophan (L-5-HTP intravenous); fenfluramine (intravenous); cirazoline (intravenous); clonidine (intravenous); L-dopa (intraperitoneal); salbutamol (intravenous).

Dosage: Quipazine (intravenous); L-5-hydroxy-tryptophan (L-5-HTP, 50–100 mg/kg); cirazoline (0.3–3 mg/kg); clonidine (0.2 and 0.4 mg/kg); L-dopa (100 mg/kg); salbutamol (0.01–1 mg/kg).

Implementation: Evaluation of evoked myoclonic twitches of the anterior digastricus muscle created by L-5-hydroxy-tryptophan, quipazine, and fenfluramine.

Animal Model: Urethane-anesthetized rats (including decerebrate and artificially respired).

Interpretation and Analysis: The effect of L-5-HTP or quipazine on the frequency of twitches is inhibited by cyproheptadine. In addition, L-dopa (100 mg/kg intraperitoneal) or the alpha 1-adrenoceptor agonist cirazoline (0.3–3 mg/kg i.v.) evokes twitches of the muscle that are inhibited by the alpha 1-adrenoceptor antagonist, prazosin. In decerebrate, artificially respired *in vivo* models, neither L-5-HTP nor L-dopa evoke twitches. The frequency of twitches evoked by fenfluramine but not by L-dopa is increased by clonidine. The effect of clonidine is abolished by yohimbine. Salbutamol has no effect on fenfluramine-induced twitches. Therefore, (i) activation of 5-HT receptors or alpha 1-adrenoceptors in the brain of urethane-anesthetized rats evokes twitches of the anterior digastricus muscle, and (ii) this preparation can be utilized as a test to study the action of compounds on central 5-HT and adrenergic systems.

A novel *in vivo test* for drugs affecting central serotonergic and adrenergic systems. Rawlow A, King RG. Eur J Pharmacol 1990 Dec 4; 191 (3), pp. 263–72.

Serotonin and Dopamine Balance
Substituted Amphetamine Para-methoxyamphetamine (PMA)/3,4-methylenedioxymethamphetamine (MDMA)

Background Information: A number of deaths have occurred related to the recreational administration of PMA (substituted amphetamine). Relatively little is known about the mechanism(s) of action for PMA and its effects on physiological, behavioral, and neurochemical parameters.

Application: Potentially, substituted amphetamine PMA or related analogs may be valuable in evaluating serotonin physiology serotonin/dopamine balance within the central nervous system. Caution: Appropriate studies have not been performed to validate the use of PMA for such purposes.

Implementation: Compare the physiological properties of substituted amphetamine PMA with the well-known effects of MDMA. *In vivo* chronoamperometry can be used to measure the effects of MDMA and PMA on release, and inhibition of uptake, of serotonin (5-HT) and dopamine (DA) in the dorsal striatum.

Animal Model: Rats (anesthetized).

Interpretation and Analysis: At both 20° and 30°C, MDMA stimulates locomotor activity, whereas PMA has a modest effect and then only at high doses. Equivalent doses of substituted amphetamine PMA produce greater hypothermia than MDMA at an ambient temperature of 20°C. At 30°C, PMA continues to evoke hypothermia except at the highest dose, where hyperthermia ensues. MDMA alters body temperature only at the highest dose, where hyperthermia also results.

At the molecular level, MDMA evokes release of dopamine (DA) and inhibits uptake of both dopamine (DA) and serotonin (5-HT). By contrast, PMA is a relatively weak releasing agent and does not inhibit dopamine (DA) uptake. However, PMA potentially inhibits serotonin (5-HT) uptake. Collectively, these observations suggest that the acute adverse effects of PMA are more likely to be asso–ciated with alterations in serotonergic rather than dopaminergic neurotransmission.

Differential behavioural and neurochemical effects of para-methoxyamphetamine and 3,4-methylenedioxymethamphetamine in the rat. Daws LC, Irvine RJ, Callaghan PD, Toop NP, White JM, Bochner F. Prog Neuropsychopharmacol Biol Psychiatry 2000 Aug; 24 (6), pp. 955–77.

Serotonin (5-HT) Production: Central Nervous System/Physiological Processes Mediated by Alterations in Serotonin Production/Over-Active Bladder Conditions
Clomipramine/Fluoxetine

Cellular/Molecular Mechanism of Action: Clomipramine modifies (reduces) concentrations of serotonin (5-HT) within the central nervous system (CNS). Fluoxetine is a selective CNS serotonin uptake inhibitor.

Physiological Mechanism of Action: Clomipramine promotes endogenous CNS depression while fluoxetine can reverse these CNS properties induced by clomipramine.

Background Information: Serotonin (5-HT) in the CNS may inhibit bladder activity. Based on this consideration, it has been postulated that CNS depression associated with altered 5-HT production may be associated with or be the cause of conditions such as over-active bladder (OAB) activity.

Application: Detection and evaluation of physiological functions or conditions (e.g. OAB) that may be associated with alterations in serotonin (5-HT) production within the CNS.

Dosage: Clomipramine hydrochloride (22.5 mg/kg body weight) compared to an equal volume of sterile physiological saline administered by injection to negative reference controls. Fluoxetine (20 mg/kg daily).

Implementation: Evaluation of the influence of clomipramine hydrochloride on urinary voiding frequency at 10 and 15 weeks. Behavioral correlates of depression are assessed using a forced-swim challenge. At age 15 weeks cystometry (CMG) analysis is performed. Fluoxetine is then co-administered with clomipramine or saline followed by the measurement of void frequency (VF) and reevaluation of CMG analyses.

Animal Model: Rat (Wistar pups, postnatal days 8 to 21) endogenous model for depression promoted by lowering serotonin (5-HT) concentrations.
Interpretation and Analysis: Clomipramine increases immobility when combined with forced-swim challenge testing (indication of depression). Clomipramine promotes more frequent voiding compared to negative saline controls at 10 and 15 weeks. Interestingly, the difference is significant only in females. Cystometry of clomipramine/females reveals decreased bladder capacity, micturition volume, and inter-micturition contractions compared with negative saline reference controls. Fluoxetine reverses these changes (comprable to saline negative reference controls). Such observations support the hypothesis that OAB may be associated with altered 5-HT function and may explain previous descriptions of an association between depression and OAB.

Alterations in voiding frequency and cystometry in the clomipramine induced model of endogenous depression and reversal with fluoxetine. Lee KS, Na YG, Dean-McKinney T, Klausner AP, Tuttle JB, Steers WD. J Urol 2003 Nov; 170 (5), pp. 2067–71.

Serotonin 5-HT_2 Receptor Complexes: Spinal Cord/(Spasms: Neuromuscular)
Amitriptyline/Cyclobenzaprine/Cyproheptadine/Ketanserin

Cellular/Molecular Mechanism of Action: In the brain, 5-HT receptors exist in 2 major different classes referred to as 5-HT1 and 5-HT2. Methysergide and metergoline are non specific in their mechanism of action and function as antagonists at both 5-HT1 and 5-HT2 receptor complexes. Buspiron and 8-OH-DPAT function as 5-HT_{1A} agonists that exert antihypertensive properties, while pindolol and WAY100635 are antagonists of 5-$HT_{1A/1B}$ and 5-HT_{1A} receptor complexes respectively. Alternatively, cyclobenzaprine, amitriptyline, cyproheptadine, ketanserin (5-$HT_{2A/2C}$), N-desmethylclozapine (5-HT_{2C}), and spiperone (5-HT_{2A}) interact relatively selectively with 5-HT_2 receptor complexes within the spinal cord, where they all function as 5-HT antagonists. Interestingly, ketanserin also binds to α_1-adrenergic, histamine H_1, and to a lesser exent dopamine receptor complexes.
Physiological Mechanism of Action: Centrally acting muscle relaxant with its action mediated through inhibition of facilitatory descending serotonergic systems that regulate monosynaptic and polysynaptic reflex potentials within the spinal cord. Although 5-HT_2 receptors have relatively weak binding avidity for 5-HT, they are most often associated with functional responses.

The physiological effects evoked by ketanserin include vasodilatory responses (lowering of blood pressure), bronchial relaxation, antiplatelet aggregatory agent, and inhibition of sympathetic outflow from the brain. Cyproheptadine blocks smooth muscle contractions mediated by 5-HT and has weak anticholinergic activity.
Application: Diagnostic aid in determining cause of neuromuscular spasticity. Potential biological carrier systems for imaging spinal cord 5-HT_2 receptor complexes.

Other applications associated with this class of pharmaceutical agents (e.g., cyproheptadine) include its use in postgastrectomy dumping syndrome, and intestinal hypermotility of carcinoid syndrome, but these functions are not mediated by 5-HT concentrations.
Considerations: Interestingly, the structure of cyclobenzaprine is very similar to that of amitriptyline and cyproheptadine.
Interpretation and Analysis: Cyclobenzaprine, amitriptyline, cyproheptadine, and ketanserin significantly inhibit the facilitatory effects of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) on flexor reflexes and mono- and polysynaptic spinal reflex potentials in spinalized *in vivo* models. In intact *in vivo* models, these drugs significantly reduce the mono- and polysynaptic reflex potentials. Depletion of 5-HT significantly prevents depression of spinal reflex potentials induced by cyclobenzaprine, amitriptyline, cyproheptadine, and ketanserin.

Tricyclic analogs cyclobenzaprine, amitriptyline and cyproheptadine inhibit the spinal reflex transmission through 5-HT_2 receptors. Honda M, Nishida T, Ono H. Eur J Pharmacol 2003 Jan 1; 458 (1–2), pp. 91–9.

Serotonin 5-HT_4-Receptor Distribution/Intestinal Motility: Colon
Mosapride Citrate/Cisapride

Cellular/Molecular Mechanism of Action: Function as 5-HT_4-receptor agonists.
Route of Administration: Mosapride and cisapride (intragastric).
Dosage: Mosapride and cisapride (3–30 mg/kg).
Considerations: The 5-HT_4 receptors in the colon of guinea pigs show a distribution similar to that found in humans.
Interpretation and Analysis: The enhancing effect of mosapride on colon motility is antagonized by atropine or 5-HT_4-receptor antagonist (GR113808), but not by methysergide (5-HT_1- and 5-HT_2-receptor antagonist); ondansetron (5-HT_3-receptor antagonist); or CP-99994 [tachykinin NK(1)-receptor antagonist]. *In vitro* receptor autoradiography has shown that mosapride and cisapride inhibit the specific binding of [^{125}I]-SB207710, a selective radioligand of 5-HT_4 receptors, in the colon of guinea pigs. Mosapride therefore enhances colonic motility through 5-HT_4-receptor activation in guinea pigs and may be useful for treating constipation in cases with colonic motility dysfunction.

Effects of mosapride citrate, a 5-HT_4 receptor agonist, on colonic motility in conscious guinea pigs. Inui A, Yoshikawa T, Nagai R, Yoshida N, Ito T. Jpn J Pharmacol 2002 Dec; 90 (4), pp. 313–20.

Serotonergic Systems/Serotonin Transporter (SERT)
Diphenyl Sulfide Derivatives/[^{11}C]-DASB

Application: Characterization of serotonin transporter expression and function (e.g. positron emission tomography imaging of serotonergic systems).
Semi-Synthetic Methods: Molecular modeling techniques have been used to design prototype serotonergic ligands. Predictions of binding affinity are based on models generated by Comparative Molecular Field Analysis (CoMFA) and Comparative Molecular Similarity Indices Analysis (CoMSIA). Applying this investigative approach, a large number of candidate compounds have been designed for screening purposes (n = 100). Diphenyl sulfide derivatives like [^{11}C]-DASB have proven to be promising PET ligands and therefore can serve as a diphenyl sulfide scaffold for rational modification strategies. The novel compounds designed through this approach have a predictably high selective affinity for serotonin transporter (SERT) complexes. Chemical groups that have proven effective include the introduction of fluoroethyl-oxycarbonyl (ester) and fluorethyl-carbonyl (ketone), as well as aldehyde and its corresponding oxime and imine. Another molecular modification that has proven effective is the replacement of the sulfur bridge with a cyanamide group and a fluoroethylamino group.
Interpretation and Analysis: The proposed candidate compounds possess features providing new possibilities for carbon-11 or fluorine-18 labeling. Synthesis, biological testing, and screening for

PET suitability are reasonable and potentially productive future areas of investigation.

Molecular modeling of potential new and selective PET radiotracers for the serotonin transporter. Wellsow J, Kovar KA, Machulla HJ. J Pharm Pharm Sci 2002 Sep–Dec; 5 (3), pp. 245–57.

Serotonin Uptake/$GABA_A$ Receptor Complexes/ Cerebral Cortex Function/Hypocampal Electroencephalogram Profiles/Antidepressants

Pharmaco-Electroencephalogram Analyses/ Serotoninertic Agents/ Pentylenetetrazol/ Imipramine/ Fluoxetine/Trazodone/Desipramine/Nortriptyline

Cellular/Molecular Mechanism of Action: The antidepressants imipramine, fluoxetine, or trazodone inhibit serotonin uptake. Conversely, the antidepressants desipramine and nortriptyline inhibit norepinephrine uptake.

Application: Evaluate the influence of antidepressants with serotonergic and adrenergic effects on the function of $GABA_A$ receptor complexes.

Route of Administration: Pentylenetetrazol, imipramine, fluoxetine, or trazodone (intraperitoneal in rat *in vivo* model).

Dosage: Pentylenetetrazol (PTZ; 27.5 mg/kg) and imipramine, fluoxetine, or trazodone (7 days at 10 mg/kg given twice a day).

Implementation: Evaluate power spectrum changes induced by PTZ (chloride ionophore antagonist) in hippocampal electroencephalograms in order to better delineate the antidepressant drug effects on $GABA_A$ receptor complexes (e.g. $GABA_A$ receptor, chloride ionophore, and benzodiazepine receptor).

Animal Model: Rat (hippocampal EEG).

Interpretation and Analysis: In control recording, PTZ increases EEG power at frequencies under 12 Hz up to 5 times. After *in vivo* models are pretreated with imipramine, fluoxetine, or trazodone for 7 days (10 mg/kg i.p. twice a day), PTZ does not increase EEG power to more than 3 times the power before injection; this effect is not observed after 3 days of pretreatment. These 3 antidepressants inhibit serotonin uptake, while antidepressants that inhibit norepinephrine uptake (desipramine, nortriptyline) fail to counter the effect of PTZ. Antidepressants with serotonergic effects enhance the function of the $GABA_A$ receptor complex.

Electrophysiologic analysis of antidepressant drug effects on the $GABA_A$ receptor complex based upon antagonist-induced encephalographic power spectrum changes. Matsubara M, Suzuki S, Miura K, Terashima M, Sugita S, Kimura H, Hatsuda S, Mori T, Murakami H, Hayashi T, Ohta T, Ohara M. Neuropsychobiology 2000; 42 (3), pp. 149–57.

Sleep Disorders: Application Potential

Melatonin/(Flumazenil)/(Benzodiazepine)/ (Picrotoxin)/Bicuculline Methiodide

Cellular/Molecular Mechanism of Action: Melatonin binds to the $GABA_A$ receptor mediated through the benzodiazepine recognition site, the picrotoxin site on the $GABA_A$ receptor, and partially to the GABA binding site on the $GABA_A$ receptor.

Physiological Mechanism of Action: Melatonin induces a significant sleep-promoting effect (*in vivo* models).

Background Information: The hypnotic activity of melatonin appears to be associated with $GABA_A$ receptors and mediated through the benzodiazepine recognition site on the $GABA_A$ receptor and partially through the GABA binding site on the $GABA_A$ receptor.

Application: Melatonin in concert with electroencephalogram monitoring could be applied in sleep research, based on its sleep-promoting properties when administered to *in vivo* models.

Route of Administration: Melatonin (intraperitoneal injection).

Dosage: Melatonin (10 mg/kg in rats); flumazenil (3.5 and 7 mg/kg); picrotoxin (2 and 4 mg/kg); bicuculline methiodide (2 and 4 mg/kg).

Implementation: Evaluation of sleeping disorders.

Animal Model: Rat.

Interpretation and Analysis: Melatonin induces a sleep-promoting effect that can be monitored through the use of electroencephalography (EEG). Flumazenil (3.5 and 7 mg/kg), a specific antagonist of the benzodiazepine (BZP) recognition site on the $GABA_A$ receptor, and picrotoxin, the ligand of the picrotoxin site on the $GABA_A$ receptor, seem to be devoid of an intrinsic influence on each sleep parameter when used alone, but they significantly antagonize the melatonin-induced increase in total sleep time (TS), slow-wave sleep time (SWS), paradoxical sleep time (PS), and the decrease in time to sleep onset (TSO) and wakefulness time (W). A significant interaction exists between melatonin and flumazenil or picrotoxin. When bicuculline methiodide, (a specific antagonist of the GABA binding site on the $GABA_A$ receptor), is used together with melatonin, then melatonin-induced increases in TS, SWS, and PS, along with decreases in W, are abolished. However, there is no apparent interaction between melatonin and bicuculline methiodide on sleep parameters except PS.

The $GABA_A$ receptor mediates the hypnotic activity of melatonin in rats. Wang F, Li J, Wu C, Yang J, Xu F, Zhao Q. Pharmacol Biochem Behav 2003 Feb; 74 (3), pp. 573–8.

Sleep Quality: Cerebral Function

Pharmaco-Electroencephalogram (EEG) Mapping/ d-Fenfluramine (d-FEN)/l-Fenfluramine (l-FEN)/ Chlorpromazine (CPZ)/d-Amphetamine (AMPH)/Prolactin

Route of Administration: Single oral dose of each d-fenfluramine, l-fenfluramine, chlorpromazine, and d-amphetamine (AMPH @ 50 mg).

Dosage: Single dose of d-fenfluramine (d-FEN at 15 and 30 mg); l-fenfluramine (l-FEN at 30 mg); chlorpromazine (CPZ at 50 mg); and d-amphetamine (AMPH at 50 mg).

Implementation: Pharmaco-EEG mapping of the pharmacodynamic properties of d-FEN, l-FEN compared to CPZ, and AMPH as well as prolactin and safety assessments. Blood samples are collected to determine concentrations of AMPH, FEN and its main metabolite norFEN, as well as plasma prolactin levels; EEG recordings and evaluation of hemodynamics and spontaneous side effects are performed at 0, 2, 4, 6, and 8 hours after drug administration. Before and 24 hours after drug intake, morning subjective sleep quality is then also assessed.

Human Model: Cases of young healthy volunteers (n = 18) receiving pharmaceutical agents at weekly intervals.

Interpretation and Analysis: Blood concentration levels measured after d-FEN demonstrate a dose-dependent blood concentrations peaking after 2–4 hours and slowly declining thereafter. NorFEN, the main metabolite, exhibits a steady increase in plasma concentrations up to the 8th hour. After l-FEN a similar time course is observed, but the concentrations of the parent compound are higher and those of the metabolite lower in comparison with the d-isomer, suggesting stereoselective kinetics. d-FEN induces significant and dose-dependent pharmaco-EEG changes characterized by a decrease in total power and combined delta/theta activity; an increase in beta activity; and an acceleration of the centroid of the total activity with no changes in alpha power that are very different from those of the

reference drugs. l-FEN produces only minimal changes (of the same type as d-FEN). CPZ (50 mg) and d-AMPH (20 mg) produce significant changes compared to placebo but with quite opposite effect profiles; the former exhibits a typical sedative-neuroleptic pattern while the latter is typical of psychostimulants. Dose/treatment and time-efficacy calculations based on all variables show in the V-EEG that only d-FEN (30 mg) and CPZ (50 mg) are different from placebo (with peak effects in the 8th and 4th hour, respectively), while in the resting condition (R-EEG) all drugs/dosages but l-FEN (30 mg) differ from placebo (with the peak effect occurring in the 4th–6th hour after d-FEN and l-FEN; in the 2nd hour after CPZ; and in the 4th–6th hour after AMPH). Plasma prolactin levels are only modified by CPZ (maximal increase in the 2nd hour).

Clinical-pharmacological study with the two isomers (d-, l-) of fenfluramine and its comparison with chlorpromazine and d-amphetamine: Blood levels, EEG mapping and safety evaluation. Saletu B, Barbanoj MJ, Anderer P, Sieghart W, Grünberger J. Methods Find Exp Clin Pharmacol 1993 Jun; 15 (5), pp. 291–312.

Sleep Disorders/Gamma-Hydroxybutyric Acid Receptors/$GABA_A$ and $GABA_B$ Receptors
Gamma-Hydroxybutyric Acid (GHB)/UMB68/UMB75

Cellular/Molecular Mechanism of Action: The exact mechanism of action of GHB remains to be delineated, but it is known to interact with specific GHB binding sites or receptors and that GHB undergoes metabolic conversion to GABA. The tertiary alcohol analog of GHB (UMB68) binds to GHB receptors. In drug discrimination studies, $GABA_B$ agonists, and to a lesser extent $GABA_A$ -positive modulators, substitute for GHB.

Physiological Mechanism of Action: Gamma-hydroxybutyric acid acts as a weak agonist at $GABA_B$ receptors. The tertiary alcohol analog of gamma-hydroxybutyric acid (UMB68) does not induce GABA receptor-mediated effects *in vivo* normally associated with gamma-hydroxybutyric acid.

Background Information: In drug discrimination studies, $GABA_B$ agonists, and to a lesser extent $GABA_A$-positive modulators, can substitute for GHB. To delineate the relative contributions of each receptor system to the profile of GHB, tertiary alcohol analogs of GHB and its homolog, 5-hydroxypentanoic acid (UMB58), referred to as UMB68 and UMB75, respectively, cannot be metabolized to GABA-active compounds but can be used as investigative probes.

Application: Gamma-hydroxybutyric acid (GHB) shows great promise as a treatment for sleeping disorders but is also increasingly being abused. The tertiary alcohol analog of gamma-hydroxybutyric acid (UMB68) could provide a novel tool for studying the pharmacology of the GHB receptor in the absence of complicating GABAergic effects.

Route of Administration: Gamma-hydroxybutyric acid.

Dosage: Gamma-hydroxybutyric acid.

Interpretation and Analysis: Binding studies against [^{3}H]NCS-382 [(2E)-(5-hydroxy-5,7,8,9-tetrahydro-6H-benzo[a][7] annulen-6-ylidene) ethanoic acid] show that the tertiary alcohol analog of GHB (UMB68) has similar affinity for GHB, with the longer chain analogs possessing lower levels of affinity. Against [^{3}H]GABA, UMB68 shows no affinity ($IC_{50} > 100$ μM) at $GABA_A$ or $GABA_B$ receptors. *In vivo* studies show that, at behaviorally active doses, *in vivo* models trained to discriminate GHB do not recognize the novel ligands as GHB. Thus, UMB68 is a selective GHB receptor ligand in binding assays, does not undergo metabolism to GABA-active compounds, and does not show the same effects as GHB *in vivo*. Such data suggests that, although UMB68 binds to the GHB receptor, it does not have the observed GABA receptor-mediated effects of GHB *in vivo* and could provide a novel tool for studying the pharmacology of the GHB receptor in the absence of complicating GABAergic effects.

A tertiary alcohol analog of gamma-hydroxybutyric acid as a specific gamma-hydroxybutyric acid receptor ligand. Wu H, Zink N, Carter LP, Mehta AK, Hernandez RJ, Ticku MK, Lamb R, France CP, Coop A. J Pharmacol Exp Ther 2003 May; 305 (2), pp. 675–9.

Syncope Differentiation
ATP/Isoproterenol (Tilt Test)

Cellular/Molecular Mechanism of Action: Isoproterenol is a non-selective β-adrenergic receptor agonist.

Background Information: In patients with vasovagal syndrome, head-up tilt testing may reproduce symptoms generally associated with vasodepression. Recent research suggests ATP testing can identify cases with abnormal vagal cardiac inhibition.

Application: The tilt and ATP tests individually and jointly can be utilized to determine the underlying mechanisms of vasovagal symptoms in most cases of syncope.

Route of Administration: ATP (20 mg).

Dosage: ATP (intravenous).

Implementation: Evaluation of isoproterenol and ATP provocative tests performed in random order during one session and outside of predominant sympathetic periods in cases related to syncope. In passive and isoproterenol-provocative tilt testing by standard protocols, reproduction of symptoms is defined as a positive test. The ATP test consists of administering injectable ATP (at bedside) while continuously monitoring ECG and blood pressure with a vagal cardiac pause > 10 seconds defined a positive test result. For most cases (64%), ≥ 1 test will be positive.

Human Model: Cases suffering from syncope ($n = 56$); cases of presyncope ($n = 56$) for whom no cardiac or extracardiac etiologies can be identified.

Interpretation and Analysis: In 57% of cases (e.g., $n = 41/72$) with a positive tilt test (either passive or provoked by isoproterenol), approximately 32% have cardiac disease, while a very low percentage have significant bradycardia (e.g., $n = 0$; < 50 bpm). In cases with positive ATP test findings (e.g., $n = 8$ or 11%), approximately two-thirds or 62% have cardiac disease. The probability of a positive result increases with age ($p = 0.015$). Both tests are positive in a small number of cases (e.g., $n = 3$) while a relatively larger number have a negative responses to both evaluation procedures (e.g., $n = 26$). Results from tilt and ATP test procedures appear to be uncorrelated ($p = 0.28$). Therefore, tilt and ATP tests individually and jointly can assist in determining the mechanism of vasovagal symptoms in most cases of syncope. Vagal cardiac inhibition appears to increase with age.

Contribution of head-up tilt testing and ATP testing in assessing the mechanisms of vasovagal syndrome: Preliminary results and potential therapeutic implications. Flammang D, Erickson M, McCarville S, Church T, Hamani D, Donal E. Circulation 1999 May 11; 99 (18), pp. 2427–33.

Syncope/Hypoxia: Sudden/Bradycardia: Sudden/Vasovagal Syncope: Unexplained/[Vascular Graft Patency]
Nitroglycerin/Isoproterenol

Cellular/Molecular Mechanism of Action: Isoproterenol recognizes, physically binds to, and activates sympathetic/adrenergic receptor complexes.

Physiological Mechanism of Action: Nitroglycerin induces both venous and arterial vasodilation. Relatively selective venous vasodilation can be achieved at the lower dosage ranges of nitroglycerin, thereby promoting declines in end-diastolic left and right ventricular pressures. Such changes are more pronounced than are the changes in peripheral arterial pressure. Cardiac output is decreased in normal subjects and in cases with coronary artery disease when nitroglycerin is given by the sublingual route of administration. High-dose nitroglycerin administered rapidly causes declines in both systolic and diastolic pressures accompanied by lower blood pressure levels, pallor, weakness, dizziness, and initiation of reflex sympathetic-mediated responses (tachycardia, arteriolar vasoconstriction). Transient increases in coronary blood flow (coronary vasodilation) and declines in arterial blood pressure contribute to decreases in cardiac output. Sublingual administration can therefore occasionally produce a significant hypotensive episode.

Application: Diagnosis of vasovagal syncope and it can be utilized in combination with quantitative angiography for the evaluation of vascular blood flow integrity (vasodilation vs vasoconstriction) and patency of vascular grafts. Injectable nitroglycerin (TNG) is employed during cardiac catheterizations primarily in cases undergoing provocative tests for coronary spasm with ergonovine maleate where it has demonstrated considerable efficacy.

Route of Administration: Nitroglycerin (intravascular).

Dosage: Nitroglycerin can be given at progressively increasing infusion rates (increments of 0.86 μg/kg/hr every stage).

Implementation: Evaluation of unexplained syncope conditions through analysis of provoked changes in coronary artery vascular blood flow. A maximum of 5 successive stages of 5 minutes in the supine position are performed plus 10 minutes of 80-degree upright tilt at progressively increasing infusion rates (increments of 0.86 μg/kg/hr every stage).

Human Model: Cases of unexplained syncope (n = 40; n = 23/40 women, mean age 47 years; n = 25 asymptomatic controls with negative baseline head-up tilt undergoing 2 other tilting tests, 1 during nitroglycerin infusion and 1 during isoproterenol infusion).

Interpretation and Analysis: During the nitroglycerin test a positive response (syncope in association with sudden hypotension and bradycardia) can occur [i] in approximately 53% (e.g., n = 21) of cases with unexplained syncope; [ii] as an exaggerated response (minor symptoms in association with slowly increasing hypotension alone) in about 25% (e.g., n = 10/40); [iii] as a negative response in 22% of cases (e.g. n = 9/40); and [iv] drug intolerance in a very small percentage (e.g., n = 0 or 0%). During the isoproterenol test these percentages are 25%, 25%, 32%, and 18%, respectively. Only a small percentage of controls have a positive response to the nitroglycerin test and to the isoproterenol test (e.g., n = 2 or 8% for each test). Thus the nitroglycerin test seems to be a useful alternative tool for the diagnosis of vasovagal syncope and it is equally specific but more sensitive and feasible than the isoproterenol test.

Nitroglycerin infusion during upright tilt: a new test for the diagnosis of vasovagal *syncope*. Raviele A; Gasparini G; Di Pede F; Menozzi C; Brignole M; Dinelli M; Alboni P; Piccolo E. Am Heart J] 1994 Jan; Vol. 127 (1), pp. 103-11.

Syncope: Neurocardiogenic
Isosorbide Dinitrate/Isoproterenol

Cellular/Molecular Mechanism of Action: Isosorbide dinitrate (active metabolite: isosorbide-2-mononitrate; isosorbide-5-mononitrate) is believed to activate guanylate cyclase and increase the synthesis of guanosine 3′,5′ monophophate (cGMP) in smooth muscle and other tissues. Formation of reactive free radical nitric oxide (NO) occurs, which interacts with and activates guanylate cyclase. Isoproterenol binds to and potently (positively) activates β-adrenergic receptor complexes (β-adrenergic receptor agonist).

Physiological Mechanism of Action: Isosorbide dinitrate is an arterial and venous dilating agent. Isoproterenol (sympathomimetic) effects are mainly directed at the heart and smooth muscle tissues of the bronchioles, skeletal muscle vasculature, and alimentary tract. Physiologically, isoproterenol promotes declines in peripheral vascular resistance primarily at the level of the vascular beds within skeletal muscle.

Application: Isosorbide dinitrate and isoproterenol can be applied to identify and characterize cases of suspected cardiogenic syncope.

Route of Administration: Isosorbide dinitrate (sublingual), isoproterenol (intravenous).

Dosage: Isosorbide dinitrate (5 mg), isoproterenol (4 μg/min).

Implementation: In cases of suspected neurocardiogenic syncope the response to a head-up tilt test (HUT) can be divided into subjects that either do or do not develop syncope/presyncope during a passive HUT test for 30 minutes. Cases that do not develop symptoms can be further evaluated using repeated HUT tests with isoproterenol infusions (4 μg/min; 10 minutes) and then tilted after a sublingual administration of isosorbide dinitrate (another 12 minutes). The HUT/isosorbide dinitrate test is usually performed after the HUT/isoproterenol test.

Human Model: Cases of suspected neurocardiogenic syncopy (e.g. n = 120; 15–77 years).

Interpretation and Analysis: Sensitivity and specificity of passive HUT is 41% (95% C.I. 32.9% to 51%) and 100%, respectively. Sensitivity of isoproterenol HUT is 51.4% (95% C.I. 39.2% to 63.6%) and specificity 70% (95% C.I. 55.4% to 82.1%), and for isosorbide dinitrate HUT is 70% (95% C.I. 57.9% to 80.4%) and 88% (95% C.I. 75.7% to 95.5%), respectively. The accuracy of the isosorbide HUT is significantly higher than the accuracy of the isoproterenol HUT 77.5% (95% C.I. 68.9% to 84.6%). Sublingual isosorbide dinitrate is at least as sensitive as isoproterenol for the assessment of suspected cases of neurocardiogenic syncope that display a negative tilt test without provocation. The low rate of side effects and the higher accuracy of the idosrobide dinitrate HUT, along with the simplicity of this challenge test compared to the isoproterenol HUT test, suggest that sublingual isosorbide dinitrate should be preferred as a provocative agent for evaluating neurocardiogenic syncope after a negative passive tilt test.

Tilt testing in neurocardiogenic syncope: Isosorbide vs isoproterenol. Hermosillo AG, Marquez MF, Jauregui-Renaud K, Falcon JC, Casanova JM, Guevara M, Cardenas M. Acta Cardiol 2000 Dec; 55 (6), pp. 351–5.

Syncope: Neurocardiogenic
Glyceryl Trinitrate (Nitroglycerin)/Isoprenaline (Isoproterenol)

Cellular/Molecular Mechanism of Action: Glyceryl trinitrate (nitroglycerin) activates guanylate cyclase and increases the synthesis of guanosine 3′,5′ monophophate (cGMP) in smooth muscle and other tissues. Formation of reactive free radical nitric oxide (NO) occurs, which interacts with and activates guanylate cyclase. Isoprenaline (isoproterenol) binds to and potently activates β-adrenergic receptor complexes (β-adrenergic receptor agonist).

Physiological Mechanism of Action: Glyceryl trinitrate (nitroglyerin) is an arterial and venous dilating agent. Isoprenaline (isoproterenol: sympathomimetic) exerts effects mainly directed at the heart and smooth muscle tissues of the bronchioles, skeletal muscle vasculature, and alimentary tract. Physiologically isoprenaline

(isoproterenol) promotes declines in peripheral vascular resistance primarily within the vascular network of skeletal muscle.

Application: Glyceryl trinitrate (nitroglycerin) and isoprenaline (isoproterenol) can be used to detect and characterize conditions of neurocardiogenic syncope.

Implementation: In unexplained cases of syncope with a negative passive head-up tilt at 70 degrees (40 minutes), the glyceryl trinitrate head-up tilt and isoprenaline head-up tilt tests can be of diagnostic value (performed 1 week apart). Diagnostic criteria include induced symptoms (syncope, presyncope) in concert with the development of hypotension.

Interpretation and Analysis: In cases with negative passive head-up tilt, the sensitivity of glyceryl trinitrate in diagnosing vasovagal syncope is approximately 48% with a 71% level of specificity. Isoprenaline sensitivity is 21% with a 64% level of specificity. There is a rather drastic difference in the incidence of undesirable side effects observed with glyceryl trinitrate compared to isoprenaline. Glyceryl trinitrate is well tolerated. In contrast, a large percentage of cases (68%) display side effects with isoprenaline that are severe enough to require termination of the testing procedure. Commonest adverse events are the development of hypertension or tachycardia and intolerable flushing or nausea. Based on comparison evaluations, glyceryl trinitrate (nitroglycerin) head-up tilt is as effective as isoprenaline (isoproterenol) head-up tilt as a provocative agent for vasovagal syncope and has a lower incidence of adverse events.

Comparison of provocative tests for unexplained syncope: Isoprenaline and glyceryl trinitrate for diagnosing vasovagal syncope. Graham LA, Gray JC, Kenny RA. Eur Heart J 2001 Mar; 22 (6), pp. 497–503.

Syncope: Vasovagal
Nitroglycerin/[Tilt-Table Test]

Cellular/Molecular Mechanism of Action: Nitroglycerin has a direct action on vascular smooth muscle and its effects are not directly mediated through modifications of the autonomic nervous system.

Physiological Mechanism of Action: Induces both venous and arterial vasodilation. Relatively selective venous vasodilation can be achieved at the lower dosage ranges of nitroglycerin, thereby promoting declines in end-diastolic left and right ventricular pressures. Such changes are more pronounced than are the changes in peripheral arterial pressure. Cardiac output is decreased in normal subjects and patients with coronary artery disease when nitroglycerin is given by the sublingual route of administration. High-dose nitroglycerin administered rapidly causes declines in both systolic and diastolic pressures accompanied by lower blood pressure levels, pallor, weakness, dizziness, and initiation of reflex sympathetic-mediated responses (tachycardia, arteriolar vasoconstriction). Transient increases in coronary blood flow (coronary vasodilation) and declines in arterial blood pressure contribute to decreases in cardiac output. Sublingual administration can therefore occasionally produce significant episodes of hypotension.

Background Information: Vasovagal syncope can occur in any individual, given sufficient provocation, and probably half the population suffers at least 1 episode during life. Often it occurs in youth and may occur in clusters. Usually there is a history of a previous episode. Prodromal symptoms include nausea, sweatiness, and a sensation of warmth.

Diagnostic Application: Patients suffering from syncope of unknown origin that may be due to vasovagal syncope.

Considerations: A classical history and a positive tilt test obviate the need for further investigation in clinical practice, but in the context of aviation (pilots), it is wise to determine if there is a small possibility of intermittent rhythm and/or conduction disturbance as an alternative explanation for the episode.

Implementation: Diagnostic detection of vasovagal syncope can be achieved by obtaining a careful history and performing a tilt test in concert with nitroglycerin administration. In instances where intermittent rhythm and/or conduction disturbance are thought to be important as an alternative explanation for episodes, it then becomes reasonable to carry out a Holter recording and exercise electrocardiogram, perhaps also echocardiography.

Interpretation and Analysis: Patients suffering from syncope of unknown origin display a 53% rate of positive response (hypotension and bradycardia) when subjected to a tilt-table test and nitroglycerin administration. An exaggerated response (hypotension only) is observed in 20% of cases presented for diagnosis (suffering from vasovagal syncope). Other reports have described a 1.3% false positive rate for passive tilt test, and the true positive rate being about 70% when a nitroglycerin challenge is included. No treatment is of much benefit, although many agents, including beta-blocking drugs, have been used. Some cases have undergone permanent dual chamber pacing with some favorable results. Explanation and reassurance is important. From a licensing point of view (e.g. pilots), following investigation after an attack, consideration may be given to providing restricted certification with regular follow-up examinations. Case review with diagnostic investigations after an event-free interval, (arbitrarily set for 2 years), may permit full certification. Malignant vasovagal syncope with no warning of impending attack should warrant disbarment.

Drugs Used for the Treatment of Angina: Organic Nitrates, Calcium Channel Blockers, and β-Adrenergic Antagonists. Needleman P, Corr PB, Johnson EM (Chapter 33: pp. 806–26). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

Vasovagal syncope: Prevalence and presentation. An algorithm of management in the aviation environment. Sutton R. Eur Heart J Suppl 1999 Apr; 1 Suppl D, pp. D109–13.

Syncope: Vasovagal/Recurrent Syncopy of Unknown Cause
Edrophonium

Cellular/Molecular Mechanism of Action: Edrophonium functions as an inhibitor of acetylcholinesterase biochemical activity.

Physiological Mechanism of Action: Edrophonium through inhibition of acetycholine esterase, effectively promotes accumulation of acetylecholine neurotransmitter at postsynaptic membrane cholinergic receptor complexes.

Background Information: Vasovagal syncope after head-up tilting is thought to be secondary to a complex, neurally-mediated reflex with both vasodepressor and cardioinhibitory efferent components.

Application: Edrophonium has been utilized as a provocative agent for triggering syncope during head-up tilt testing protocols of benefit in detecting cases of vasovagal syncope. The edrophonium/head-up tilt test (i) is currently considered to significantly increase the sensitivity of detecting cases of vasovagal syncope; (ii) may be particularly useful when provocation with isoproterenol is undesirable; and (iii) may be an effective method to help differentiate cases with a significant reflex cardioinhibitory component from those with a predominantly reflex vasodepressor component.

Route of Administration: Edrophonium (intravenous).

Dosage: Edrophonium (10 mg).

Implementation: Evaluation of the efficacy of edrophonium as a provocative agent for "triggering" syncope during head-up tilt testing procedures for cases suffering from recurrent unexplained syncope episodes. Edrophonium is administered after 30 minutes of 60 degrees head-up tilting alone.

Human Model: Cases suffering from recurrent unexplained syncope (e.g., n = 45 affected; n = 20 negative reference controls).

Interpretation and Analysis: Syncope can be induced in a little less than 50% of cases (e.g., n = 19/45), with some cases (e.g. n = 9/19) developing syncope with head-up tilt along with the predominant hemodynamic finding being marked vasodepression. Conversely, other cases will develop syncope only after head-up tilting in combination with edrophonium (e.g., n = 10/19), with the predominant hemodynamic findings being marked vasodepression and bradycardia. Syncope can occasionally be induced in normal reference controls (e.g., n = 1/20) following head-up tilting and edrophonium. There is no long-term complication from using edrophonium.

Evaluation of edrophonium as a provocative agent for vasovagal syncope during head-up tilt-table testing. Lurie KG, Dutton J, Mangat R, Newman D, Eisenberg S, Scheinman M. Am J Cardiol 1993 Dec 1; 72 (17), pp. 1286–90.

Syncope of Unknown Origin
Isoproterenol/[Tilt-Table Test]

Cellular/Molecular Mechanism of Action: Isoproterenol binds to and positively activates both β_1 and β_2 adrenergic membrane-associated receptor complexes.

Due to a relatively minimal influence on α-adrenergic receptor complexes, cardiac function, bronchial smooth muscle, skeletal muscle vasculature, and the gastrointestinal tract are the primary organ systems influenced by the physiological properties exerted by isoproterenol administration. Cardiovascular influences of isoproterenol include diminished peripheral vascular resistance, especially within the vascular networks that perfuse musculoskeletal structures. The increases in heart rate and contractility induced by isoproterenol promote declines in diastolic pressure and elevations in cardiac output sufficient to maintain or elevate systolic pressures (mean blood pressure is lowered).

Background Information: Isoproterenol is widely used as a provocative medium for vasovagal responses during tilt test procedures.

Diagnostic Application: Multi-state isoproterenol tilt-table (MSITT) test.

Route of Administration: Intravenous.

Dosage: Isoproterenol given as constant infusion (3 μg/min). Dose of isoproterenol infusion is generally titrated empirically based on increases in resting heart rate before tilt up test. Administration can involved the use of a 2-stage isoproterenol-tilt protocol, with doses of 0.01 and 0.02 μg/kg per minute for 10 minutes each, or 1-stage isoproterenol-tilt protocol, with a dose of 1 or 2 μg/min for 10 minutes.

Implementation: Evaluation of the optimal increase in resting heart rate with isoproterenol for tilt-induced vasovagal responses. After the end of a negative baseline tilt (80 degrees for 30 minutes), the isoproterenol tilt is performed using 1 of 2 protocols: 2-stage isoproterenol-tilt protocol, or 1-stage isoproterenol-tilt protocol. Resting heart rate increases are defined as a percentage increase in the resting heart rate after isoproterenol infusion, compared to baseline heart rates before initiation of the tilt test.

Human Model: Cases of unexplained syncope (n = 97 consecutive patients).

Interpretation and Analysis: Combining the use of a tilt-table and isoproterenol diagnostic challenge increases the sensitivity of detecting cases suffering from vasovagal mediated syncope (95% by 5 minutes). Conversely, the application of the MSITT alone without the use of isoproterenol results in an 80% positive response rate. Cases that are suffering from bradycardia show a lower rate of positive response rate compared to the MSITT test (10%).

Ninety-three percent (93%) of positive tilt-table procedures (e.g. n = 28/30) occur with a resting heart rate increases of ≥ 21%. With a resting heart rate increase of 60% and 100%, 18 (60%) and 27 (90%) positive responses can be observed, respectively. In conclusion, a minimum resting heart rate increase of ≥ 21% is required to provoke a vasovagal response during a subsequent isoproterenol tilt (80 degrees for 10 minutes). Preferably, heart rate should be increased by 60–100% by isoproterenol titration before tilting.

Norepinephrine, Epinephrine, and the Sympathomimetic Amines. Weiner N (Chapter 8: pp. 145–80). Goodman and Gilman's The Pharmacological Basis of Therapeutics, eds Gilman AG, Goodman LS, Rall TW, Murad F. MacMillan Publishing Company, New York (7th edition: copyright 1985).

What is the optimal increase in resting heart rate with low-dose isoproterenol infusion for tilt-induced vasovagal response? Sumiyoshi M, Abe H, Mineda Y, Tokano T, Yasuda M, Nakazato K, Nakazato Y, Nakata Y, Daida H. J Cardiovasc Pharmacol 2003 Dec; 42 Suppl 1, pp. S19–22.

Thalamic Tectorotundal Pathway/Nucleus Rotundus (Cross-Tectal Projection Tracing)/Embryonic Pathway Development (Embryonic)
1,1′-Dioctadecyl-3,3,3′,3′-Tetramethylinodocarbocyanine (Carbocyanine)/Cholera Toxin B Subunit

Application: Tracing of crossed-tectal projections to the nucleus rotundus of the thalamus (tectorotundal pathways) of paraformaldehyde-fixed and living chick embryos.

Implementation: Molecular tracers are injected into the optic tectum under 3 experimental conditions (carbocyanine postfix, carbocyanine *in vivo*, and cholera toxin B subunit *in vivo*), and the anterograde transport by the nucleus rotundus monitored and compared. In the carbocyanine postfix method, small crystals of carbocyanine dye are inserted into the tectum of paraformaldehyde-fixed embryos. A 6-month postinsertion period is required to label the crossed tectorotundal pathway. In the carbocyanine *in vivo* method, a small volume of carbocyanine dye solution is injected into the tectum of living embryos. Similarly, in the cholera toxin B subunit *in vivo* method, a small volume of cholera toxin B subunit solution is injected into the tectum of living embryos.

Animal Model: Avian (paraformaldehyde-fixed and living chick embryos).

Interpretation and Analysis: Tectal neurons do not begin to innervate the ipsilateral nucleus rotundus until embryonic day 9 and the contralateral nucleus rotundus until embryonic day 17. This slow progression of labeling through the crossed-tectal projection results in significant contrast of "labeling" between the ipsilateral and contralateral nuclei rotundus. In the carbocyanine *in vivo* method, an 8- to 12-hour survival period is sufficient for *in vivo* labeling of the tectorotundal pathway. By embryonic day 8, labeled axons terminate in the ipsilateral nucleus rotundus and crossed tectorotundal projections can be first detected by embryonic day 10. Applying the cholera toxin B subunit *in vivo* method, heavily labeled axons can be found to bilaterally innervate the nucleus rotundus by embryonic day 8 after an initial 6- to 10-hour survival period. This appears to be the earliest time frame for detecting crossed-tectorotundal projection, compared with that of both the postfix and *in vivo* methods of employing carbocyanine dye. Based on the differences in the detectability of the crossed-tectorotundal projection between the

postfix and *in vivo* methods, such findings suggest that the former method is of limited benefit for labeling tectal collaterals during embryogenesis. Moreover, given the rapid transport rate and absence of photobleaching, which is often seen when using carbocyanine dye, the cholera toxin B subunit *in vivo* method appears to be the tracer of choice for investigating embryonic pathways.

Tracing developing pathways in the brain: A comparison of carbocyanine dyes and cholera toxin b subunit. Wu CC, Russell RM, Nguyen RT, Karten HJ. Neuroscience 2003; 117 (4), pp. 831–45.

Tourette's Syndrome (Tic Disorders) *Amphetamine/Dextroamphetamine/Haloperidol/Levamfetamine Succinate/Apomorphine/Piribedil/[^{11}C]-Raclopride*

Cellular/Molecular Mechanism of Action: Apomorphine is postulated to interact with presynaptic inhibitory dopamine receptors.

Background Information: Dopaminergic abnormalities in frontal-subcortical circuits have been hypothesized as the underlying pathophysiological mechanism involved in Tourette's syndrome.

Application: Administration of the agent is based on the hypothesis that presynaptic dopamine/catecholamine release (e.g. striatum) is abnormal in adults with Tourette's syndrome.

Implementation: Evaluation of conditions classified as Tourette's syndrome utilizing 2 positron emission tomography (PET) scans in combination with high specific activity [^{11}C]-raclopride. The first scan is performed after an intravenous injection of saline, while the second scan is made after intravenous amphetamine. The relative dopamine release is estimated as the percentage difference in binding potential between the postsaline and postamphetamine scans.

Interpretation and Analysis:

Nuclear Medicine Studies: Binding potential determined after initial [^{11}C]-raclopride scans do not significantly differ between Tourette's syndrome and control subjects. After amphetamine challenge, the mean value of intrasynaptic dopamine in the putamen (as determined by true equilibrium bolus estimations) increases by 21% in conditions of Tourette's syndrome and does not change in control subjects; the mean values increase by 16.9% and decrease by 1.8%, respectively (constrained method of measurement). Dopamine release in the caudate region is not significantly different with Tourette's syndrome and comparison subjects. Greater putamen dopamine release is seen in adults with Tourette's syndrome than in comparison subjects after a pharmacologic challenge with amphetamine. These results suggest that the underlying pathobiology in Tourette's syndrome is a phasic dysfunction of dopamine transmission.

Behavioral Studies: Both dextroamphetamine and levamfetamine increase the severity of the symptoms with dextroamphetamine being more potent in this regard. Haloperidol controls the symptoms and also antagonizes the effect of dextroamphetamine. Apomorphine injections reduce the severity of symptoms, even in the presence of dextroamphetamine. Therefore, dopamine rather than norepinephrine is the principal catecholamine responsible for observed symptoms. The effect of apomorphine may be understood through its action on postulated presynaptic inhibitory dopamine receptors or other presynaptic mechanisms of action.

Effects of dopamine agonists and antagonists in Tourette's disease. Feinberg M, Carroll BJ. Arch Gen Psychiatry 1979 Aug; 36 (9), pp. 979–85.

Trauma: Cell Reaction in Cerebral Cortex/ Chronological Staging of Traumatic CNS Injury *Beta-Amyloid Precursor Protein IgG/Microglia CD68 IgG*

Cellular/Molecular Mechanism of Action: Antigen-specific immunoglobulin physically binds in a selective manner to biological protein fractions they are designed to recognize.

Application: Detection and characterization of neuronal injury in conditions of brain trauma.

Route of Administration: Intravenous.

Implementation: Evaluate the potential to determine whether axonal injury (AI) induces a microglial reaction within 15 days after brain trauma. One investigative approach involves delineation of the topographical relation of AI and microglial reactions assessed using an immunohistochemical double-labeling technique for simultaneous demonstration of (i) axonal injury using beta-amyloid precursor protein (beta-APP) antibody; and (ii) microglia infiltration using CD68 antibody.

Human Model: Cases of confirmed axonal injury (n = 40).

Interpretation and Analysis: Although traumatic injury is usually followed by a moderate early and diffuse rise in the number of CD68-reactive cells in the white matter, increases in macrophages in areas of AI accumulation are only sporadic and do not occur until after 4 days. At survival intervals of 5 to 15 days a moderate microglial reaction in regions of beta-APP-positive injured axons can be detected, at maximum, in half of biological samples evaluated. During this interval AI-associated satellitosis-like clusters (described as stars in some reports) after a survival time of more than 7 weeks appear as isolated phenomenon. A prolonged microglial reaction as well as a reduction of beta-APP-positive AI during longer survival periods supports the hypothesis that AI is not primarily chemotactically attractive and that the injury to a portion of beta-APP-stained axons may be partly reversible. Most cases clearly require a prolonged interval of more than 15 days before initiation of the final scavenger reaction. For forensic purposes the increase in the number of microglial cells within the region of AI accumulation after a survival time of more than 5 days and the multiple and distinct demonstration of star-like microglial reactions within the white matter after survival times exceeding 7 weeks may provide valuable postmortem information on the timing of a traumatic event.

Is traumatic axonal injury (AI) associated with an early microglial activation? Application of a double-labeling technique for simultaneous detection of microglia and AI. Oehmichen M, Theuerkauf I, Meissner C. Acta Neuropathol (Berl) 1999 May; 97 (5), pp. 491–4.

Trauma: Cerebral Cortex Function Post Trauma/Stress Ulceration/Hypothalamic Injury *Thyrotropin-Releasing Hormone (TRH)/Gonadotropin-Releasing Hormone (GnRH)*

Background Information: In cases with severe head injury, hypothalamohypophysial impairment with subsequent hormone abnormalities has been well documented. Stress ulcers are another commonly encountered problem in such cases. However, little has been reported in the literature about the alterations of pituitary hormones in acute head-injured cases with stress ulcer.

Application: Detection and characterization of alteration in hypothalamic function secondary to cranial trauma reflected by modified responses to GnRH and TRH.

Implementation: Evaluation of basal serum levels of pituitary hormones measured before and in response to TRH and GnRH in order to measure pituitary function in conditions of cranial injury.

Human Model: Cases of cranial injury (n = 40 males). Candidate criteria for eligibility include (i) Glasgow coma scale 4 to 10; (ii) presentation within 24 hours following head injury; (iii) absence of shock or sepsis; and (iv) no past history of peptic ulcer. Stress ulcer is confirmed by endoscopic examination.

Interpretation and Analysis: Approximately two-thirds of cases show evidence of stress ulcer by endoscope examination (e.g., n = 27/40 or 67.5%). In the absence of stress ulcer, basal serum levels of thyroid-stimulating hormone (TSH), prolactin (PRL), growth hormone (GH), luetinizing hormone (LH), and follicle-stimulating hormone (FSH) are within normal range. However, the basal levels of PRL in conditions of stress ulcer are abnormally elevated and significantly higher than in the absence of stress ulcers ($p < 0.001$). Basal levels of TSH and GH are significantly lower with stress ulcer than in the absence of stress ulcers ($p < 0.001$). In conditions of stress ulcer, significant increases ($p < 0.001$) in serum TSH, PRL, LH, and FSH levels after TRH and GnRH provocation can be detected. Hypothalamohypophysial dysfunction and stress ulceration may occur in cases of severe cranial trauma. In the presence of stress ulcers, the abnormalities of pituitary hormones and provocative response of the pituitary to TRH and GnRH reveal normal pituitary function with hypothalamic insufficiency. Therefore, stress ulcers in acute head-injury cases is closely associated with hypothalamic damage.

Hypothalamic dysfunction in acute head-injured cases with stress ulcer. Hwang SL, Lieu AS, Howng SL, Hsieh JS, Huang TJ. Kaohsiung J Med Sci 1998 Sep; 14 (9), pp. 554–60.

Trauma/Intracranial Pressure Response/Cerebral Oxygen Consumption ($CMRO_2$) Response/ Arteriovenous Oxygen Difference ($AVDO_2$) Response/Neurologic Outcomes Prediction
Pentobarbital (Coma)

Physiological Mechanism of Action: Pentobarbital is a barbiturate that promotes depression of the central nervous system.

Application: Assessment and prediction of neurological outcome in cases of severe cerebral trauma.

Route of Administration: Pentobarbital (intravenous).

Implementation: Evaluate changes in cerebral hemodynamics in conditions of head-injury being managed with a barbiturate for refractory intracranial hypertension. Cerebral blood flow (CBF) and metabolism variables are measured at the following time periods: (i) before the loading dose of pentobarbital; (ii) after the loading dose of pentobarbital (average pentobarbital level 28.1 ± 8.3 μg/mL); and (iii) 3 days later, when the peak pentobarbital level averages 42.5 ± 17.2 μg/mL.

Human Model: Cases of severe head injury (n = 67).

Interpretation and Analysis: Intracranial pressure (ICP) and mean arterial blood pressure (MAP) decrease with loading doses of pentobarbital by an average of 12 and 9 mm Hg, respectively. Cerebral perfusion pressure (CPP) is usually unchanged following analysis of an entire group of cases. CBF, $CMRO_2$, and arteriovenous oxygen difference ($AVDO_2$ O_2) are significantly decreased after a loading dose of pentobarbital, by 20%, 31%, and 11%, respectively. The average cerebrovascular resistance (CVR) is increased by 20%. The change in $CMRO_2$ with loading doses of pentobarbital is closely related to pretreatment measurements ($n = 67$, $r^2 = 0.65$, $p < .001$). Slightly less than half of cases have a "good ICP response," with a reduction in ICP from 34 ± 9 to 15 ± 5 mm Hg after an initial loading dose of pentobarbital (n = 30/67 or 45%). In addition, a relatively large percentage of cases will have a "partial ICP response," with ICP decreasing but still remaining above 20 mm Hg after a loading dose of pentobarbital (n = 27/67 or 40%). Approximately 15% of cases have an ICP that does not change or even increase after pentobarbital (n = 10/67). In situations of a good ICP response, pretreatment $CMRO_2$ and $AVDO_2$ are usually greater before administration of pentobarbital, while $CMRO_2$ and $AVDO_2$ decrease more with loading doses of pentobarbital (n = 30/67 cases) than in conditions with partial or no ICP response. Outcomes are significantly better when a good or partial ICP response is observed following pentobarbital, with 21% of these cases having a good recovery or moderate disability at 3 months after injury, compared with 100% persistent vegetative state or death in cases classified as non-responders. Therefore, barbiturate coma can be a useful treatment modality for acutely reducing ICP in selected cases. Conditions with overwhelmingly severe injuries are not likely to benefit, partly because their $CMRO_2$ is already markedly reduced by injury and partly because their outcome is already predetermined by the injury. Cases with systemic hypotension are not likely to have a good response because hypotension limits the amount of barbiturates that can be given.

Cerebral hemodynamic effects of pentobarbital coma in head-injured cases. Cormio M, Gopinath SP, Valadka A, Robertson CS. J Neurotrauma 1999 Oct; 16 (10), pp. 927–36.

Trauma: Neuroemergency Procedures/Growth Hormone Response to TSH/Prolactin Response to GHRH/Prediction of Unfavorable Neurologic Outcome (Prognosis)
Hypothalamic Releasing Hormones/Thyroid-Stimulating Hormone (TSH)/Growth Hormone-Releasing Hormone (GHRH)

Application: Evaluate the effect of severe head injury on both the secretion of basal pituitary hormones and the response to exogenous synthetic hypothalamic releasing factors administration.

Route of Administration: Intravenous.

Implementation: Evaluation of daily basal concentrations of growth hormone (GH), prolactin (PRL), TSH, triiodothyronine, and thyroxine during the first week following CNS trauma and then again on days 15 and 16 posttrauma. Plasma insulin-like growth factor-I and cortisol can also be determined on days 2, 7, and 15. Administration of TRH (TRH test) can be applied to evaluate PRL, TSH, and GH responses on days 1 and 16 after a traumatic incident and a GHRH test for the evaluation of GH and PRL responses on days 2, 7, and 15 posttraumatic incident. Outcomes are evaluated at 6 months with the GOS.

Human Model: Cases of head-injury-induced coma (n = 22) all requiring intubation and mechanical ventilation in addition to invasive monitoring. Each case has no previous endocrinologic problems or substitutive therapies.

Interpretation and Analysis: Triiodothyronine shows low values, even if they are within the normal range, while thyroxine remains within the normal range. Significant increases in insulin-like growth factor-I concentrations are both observed on days 7 and 15 compared with day 2 ($p = .024$ and $p = .034$, respectively). The GH response to GHRH is significantly greater on days 7 and 15 than in the very acute phase ($p < 0.01$ comparing days 7 and 15 vs day 2). Higher GH responses to GHRH can be observed on day 7 in Case Group 1 compared to Case Group 2 (pertaining to both peak and

area-under-the-curve values, $p = .018$ and $p = .015$, respectively). No difference in GH response can be detected on days 2 and 15. A "paradoxical" response of GH to TRH can be observed on the day following head trauma (basal vs peak, $p = .002$) but not on day 16. The GH peak response to TRH is greater on day 1 in cases with an unfavorable clinical course (Group 1 vs Group 2, $p < 0.05$). The TSH response to TRH does not significantly correlate to the severity of trauma, but is significantly ($p < 0.04$) higher in Group 1 cases than in Group 2 cases. Finally, a "paradoxical" PRL response to GHRH administration is present on day 2 (basal vs peak, $p = 0.0003$), day 7 (basal vs peak, $p = .01$), and on day 15 after the trauma (basal vs. peak, $p = 0.04$). Some of the responses to provocative tests have been identified as "paradoxical" and seem to have a great importance in the definition of prognosis in severe head-injury cases, specifically the GH response to TRH and the PRL response to GHRH that are significantly correlated with outcome.

Provocative hypothalamopituitary axis tests in severe head injury: Correlations with severity and prognosis. Della Corte F, Mancini A, Valle D, Gallizzi F, Carducci P, Mignani V, De Marinis L. Crit Care Med 1998 Aug; 26 (8), pp. 1419–26.

Trophic Dependencies (Corticospinal Neuron)/ Response to Pathological Lesions and Therapeutic Agents
Fast Blue

Application: Evaluation of the response of corticospinal neurons (CSN) of the sensory motor cortex to lesions and therapeutic drugs.
Implementation: Before induction of lesions, CSN are retrograde-labeled with fast blue (FB). A stereotaxic cut lesion through the entire internal capsule (ICL) is used to axotomize the CSN. The extent of axotomy is determined by the application of a control tracer.

Animal Model: Rat and mouse (lesion model for the induction of death of analogous populations of CSN in rodent species).
Interpretation and Analysis: In both rodent species, FB-labeled CSN are localized in 3 major areas: (i) the sensory motor cortex; (ii) the supplementary motor and medial prefrontal cortex; and (iii) the somatosensory cortex. The ICL does not lead to complete axotomy of CSN of the rat and mouse somatosensory cortex. In rats, ICL results in complete axotomy of the CSN of the sensory motor cortex and incomplete axotomy of the caudal portion of the supplementary motor and medial prefrontal cortex. In mice, the area of axotomized CSN extends significantly further frontally. In both species, axotomy-induced death of CSN is observed in the center of the sensory motor cortex. This lesion model is useful for investigations on the response of CSN of the sensory motor cortex to pathological lesion and therapeutic drugs.

An axotomy model for the induction of death of rat and mouse corticospinal neurons *in vivo*. Bonatz H, Rohrig S, Mestres P, Meyer M, Giehl KM. J Neurosci Methods 2000 Jul 31; 100 (1–2), pp. 105–15.

Vascular Perfusion/Arteriovenous Malformation: Anterior Spinal Artery/Neurologic Function Evaluation: Arteriovenous Malformation Pre-Embolization/Embolization of Vessels: Ivalon and Polyvinyl Alcohol
Xylocaine

Physiological Mechanism of Action: Xylocaine functions as a local anesthetic.
Background Information: Spinal arteriovenous malformations (spinal AVMs) are now treated using microcatheters and various embolic materials. Interventional techniques of this type are thought to be the 1st choice for treatment of spinal AVMs.
Application: Xylocaine can be applied to preassess the potential development of neurological/cognitive deficits that may be created during arteriovenous malformation embolization procedures.
Route of Administration: Xylocaine (selective intravascular).
Dosage: Xylocaine (20 mg in bolus).
Implementation: Evaluation of CNS arteriovenous malformations using xylocaine and intermittent intravascular pressure monitoring within the anterior spinal artery conducted during embolization procedures of intramedullary arteriovenous malformations ($n = 5$). Intravascular pressure monitoring can be performed via a microcatheter located within the anterior spinal artery. Vascular embolization procedures may utilize Tracker vascular access systems and MAGIC catheters for intravascular treatment of spinal AVMs in order to avoid proximal occlusion. Effective embolic materials designed for this purpose include polyvinyl alcohol (PVA) particles (ivalon: 150–350 microns) and/or PVA solutions. Vital signs and neurologic functions are carefully monitored during and after the procedure.

Human Model: Cases of intramedullary arteriovenous malformations ($n = 5$ AVMs).
Interpretation and Analysis: In many cases, it is possible to totally to obliterate the nidus or markedly reduce its size while preserving the anterior spinal artery. The xylocaine test can be conducted on multiple occasions during embolization procedures (e.g., $n = 2/6$). In cases treated with ivalon and PVA solutions, the final provocation test may become positive, thereby necessitating termination of embolization procedures (e.g., $n = 2/5$ cases). In the remaining cases, a positive result may be appreciated on the first xylocaine test (e.g., $n = 3/5$), thereby allowing treatment with ivalon alone. Measurements of the intravascular systolic pressure prior to embolization are approximately 71.6 ± 14.1 mm Hg and they gradually increase during the procedure, reaching 99.6 ± 12.6 mm Hg (90% of the systemic systolic blood pressure) by the conclusion of embolization procedures. Serial xylocaine tests and intravascular pressure monitoring may be useful for the treatment of spinal AVMs fed mainly by the anterior spinal artery, and embolization with liquid embolic material should be terminated when the provocation test becomes positive and intravascular pressure increases to 90% of the systemic blood pressure.

Intravascular treatment of spinal arteriovenous malformations using a microcatheter—with special reference to serial xylocaine tests and intravascular pressure monitoring. Touho H, Karasawa J, Ohnishi H, Yamada K, Ito M, Kinoshita A. Surg Neurol 1994 Aug; 42 (2), pp. 148–56.

Vascular Perfusion: Arteriovenous Malformations (Cerebral/Spinal)/Safety Evaluation Prior to Vascular Embolization
Amobarbital

Physiological Mechanism of Action: Amobarbital is a central nervous system depressant (barbiturate agent).
Background Information: Transarterial embolization of cerebral arteriovenous malformations (AVMs) has been associated with postprocedural neurologic complications in 7 to 39% of cases.
Application: Assessement of the potential for creating premature neurological or cognitive deficits as a sequelae of selective AVM embolization.
Route of Administration: Sodium amobarbital (superselectively delivered into an intravascular microcatheter position within AVM lesions).

Dosage: Sodium amobarbital (50-mg bolus).
Implementation: Evaluation of a method for assessing the potential neurologic and cognitive deficits that may develop as a consequence of permanent AVM embolization procedures in order to reduce the incidence of undesirable side effects. In this context, the clinical profile of AVM conditions are reviewed prior to and after vascular embolization procedures. Each case is assessed applying a battery of tests individually tailored to each of their specific capabilities using stimuli selected from standard and experimental cognitive tests to probe specific brain regions related to the location of CNS AVM lesion. In each feeder vessel to be embolized, a sodium amobarbital bolus is superselectively administered through a microcatheter, followed immediately by neurologic and cognitive testing with the tailored battery of test evaluations. After testing, the position of the microcatheter tip is checked by fluoroscopy. If the provocative test results are negative, the vascular feeder branch of interest is subsequently embolized with N-butyl cyanoacrylate glue.

Human Model: Cases of AVM (n = 12).
Interpretation and Analysis: A relatively large percentage of provocative amobarbital injections may be negative (e.g., n = 27/29). During the course of some injection trials, deficits in cognitive function may be appreciated during tailored provocative testing (e.g., n = 2/29, n = 2 injections in n = 2 individuals). In the vast majority of these cases, evoked cognitive deficits resolve with dissipation of the amobarbital effect (e.g., n = 2/29), thereby negating the necessity for embolizing of the feeder vessels. In cases determined to have no cognitive deficits after superselective amobarbital delivery, there are almost invariably no major or minor neurologic or cognitive deficits after vascular embolization (e.g., n = 0/27). Therefore, provocative amobarbital testing prior to AVM embolization appears to be helpful in identifying vascular territories where embolization may lead to neurologic and cognitive deficits.

Tailored cognitive testing with provocative amobarbital injection preceding AVM embolization. Moo LR, Murphy KJ, Gailloud P, Tesoro M, Hart J. AJNR Am J Neuroradiol 2002 Mar; 23 (3), pp. 416–21.

Vascular Perfusion: Arteriovenous Malformations (Cerebral/Spinal)/Safety Evaluation Prior to Vascular Embolization
Lidocaine/Amobarbital

Cellular/Molecular Mechanism of Action: Lidocaine disrupts the Na^+/K^+ pump in a manner that inhibits membrane depolarization events.
Physiological Mechanism of Action: Lidocaine functions as a local anesthetic. Amobarbital functions as a central nervous system depressant.
Application: A modified provocative test for assessing the safety of embolization of cerebral and spinal arteriovenous malformations.
Route of Administration: Intravascular (selective catheterization for delivery).
Implementation: The modified test uses successive amobarbital and lidocaine injections to elicit any possible neurological deficit, both mixed with radiopaque material to visualize the distribution of the anesthetic within the lumen of the vascular tree.

Human Model: Cases classified as candidates for embolization of arteriovenous malformations (n = 27).
Interpretation and Analysis: The modified provocative test causes a very low rate of false negative results (e.g., n = 0/11) compared to approximately 23% false negatives when the unmodified method is applied (e.g., n = 6/27).

Improved provocative test for the embolization of arteriovenous malformations—technical note. Sadato A, Taki W, Nakahara I, Nishi S, Yamashita K, Matsumoto K, Tanaka M, Kikuchi H. Neurol Med Chir (Tokyo) 1994 Mar; 34 (3), pp. 187–90.

Vascular Perfusion (Cerebral): Carotid Stenosis (Asymptomatic)/Cerebrovascular Reserve Capacity/Hemispheric Ischemia/Vertebrobasilar Ischemia
Acetazolamide

Background Information: Regional cerebral blood flow reduction can be measured with cerebrovascular imaging (CVI) using [^{99m}Tc]-HMPAO-SPECT.
Application: Acetazolamide can be used as a provocative test agent to improve the diagnostic accuracy of cerebral vascular imaging procedures.
Dosage: Acetazolamide (1 gram).
Implementation: Evaluation of cerebral vascular blood flow abnormalities.

Human Model: Cases with suspected regional cerebral blood flow alterations (n = 84).
Interpretation and Analysis: Acetazolamide can facilitate differentiation between hemispheric (87%) and vertebrobasilar ischemia (64%) by revealing marked increases in sensitivity to provocative tests with acetazolamide. In approximately 62% of high grade but asymptomatic carotid stenoses, a hemodynamic effect can be demonstrated with acetazolamide, thereby justifying correction of vascular anomalies by surgical intervention.

Correlative assessment of hemodynamic parameters obtained with T2*-weighted perfusion MR imaging and SPECT in symptomatic carotid artery occlusion. Kim JH, Lee SJ, Shin T, Kang KH, Choi PY, Kim JH, Gong JC, Choi NC, Lim BH. AJNR Am J Neuroradiol 2000 Sep; 21 (8), pp. 1450–6.

A study of acetazolamide-induced changes in cerebral blood flow using ^{99m}Tc HMPAO SPECT in cases with cerebrovascular disease. Asenbaum S, Reinprecht A, Brücke T, Wenger S, Podreka I, Deecke L. Neuroradiology 1995 Jan; 37 (1), pp. 13–9.

Vascular Perfusion: Cerebral Cortex/Induced Left Temporal Cerebral Blood Flow/Induced Left Frontal Cortex Cerebral Blood Flow/Induced Right Hippocampus and Adjacent Cortex: Glucose Metabolic Rate
N-(4-Acetyl-1-Piperazinyl)-p-Fluorobenzamide

Cellular/Molecular Mechanism of Action: *N*-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide monohydrate (FK960) selectively increases somatostatin release from the hippocampus. Some reports have also described an ability for FK960 (100nM) to increase mRNA and protein levels of glial cell line-derived neurotrophic factor (GDNF).
Physiological Mechanism of Action: *N*-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide monohydrate (FK960) is a putative antidementia drug that modifies regional cerebral blood flow (rCBF) and regional cerebral metabolic rate of glucose (rCMRglc). Additional functions include an ability to augment long-term potentiation (LTP) in the CA3 area of the hippocampus.
Application: Anti-dementia agent.
Route of Administration: N-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide monohydrate (FK960: intramuscular).
Dosage: N-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide monohydrate (FK960: dose = 0, 0.01, 0.1, or 1 mg/kg for 7 consecutive days).

Implementation: Evaluation of the effect of N-(4-acetyl-1-piperazinyl)-*p*-fluorobenzamide monohydrate (FK960) on rCBF and rCMRglc facilitated by the use of positron emission tomography. Each subject is scanned a total of 4 times at ≥ 3-week intervals, after treatment with saline or 3 doses of FK960.

Animal Model: Rhesus macaques (n = 7 aged males; 21.6 ± 2.7 years).

Interpretation and Analysis: Significant increases in rCBF within the left temporal and left frontal cortex, and in rCMRglc within the right hippocampus with adjacent cortex, can be detected for FK960 at the dosage of 1 mg/kg, without affecting any other cardiovascular and respiratory variables. No statistically significant change in any region can be observed at doses of 0.01 or 0.1 mg/kg. These results suggest that FK960 restores rCBF and rCMRglc deficits in brain areas responsible for cognitive functioning (aged rhesus macaques).

Effect of N-(4-Acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate (FK960), an antidementia drug with a novel mechanism of action, on regional cerebral blood flow and glucose metabolism in aged rhesus macaques studied with positron emission tomography. Noda A, Takamatsu H, Matsuoka N, Koyama S, Tsukada H, Nishimura S. J Pharmacol Exp Ther 2003 Jul; 306 (1), pp. 213–7. Epub 2003 Apr 3.

Vascular Perfusion/ Cerebral Cortex: Dural Arteriovenous Fistulas/ Altered Vascular Integrity/ Dural Arteriovenous Fistulas/ Preserved Vasoreactivity: Good Prognosis/SPECT/ Acetazolamide: Hypoperfusion/Venous Infarction/Persistent Symptoms
Acetazolamide

Cellular/Molecular Mechanism of Action: Acetazolamide functions as a carbonic anhydrase inhibitor.

Physiological Mechanism of Action: Acetazolamide can promote antiseizure activity and prevent absence seizures.

Background Information: Dural arteriovenous fistulas (DAVFs) with disturbed regional cerebral blood flow (rCBF) including retrograde leptomeningeal venous drainage (RLVD).

Application: Acetazolamide can be used as an adjuct diagnostic probe applied in conjuction with SPECT to charactize conditions of dural arteriovenous fistulas in a manner that can detect preservation or alterations in vasoreactivity that can correspond with either a good prognosis or evidence of venous infarction and persistence of symptoms.

Implementation: Characterization of pretreatment MRI/SPECT and posttreatment MRI/SPECT symptoms and response to therapeutic intervention in conditions of DAVFs with disturbed rCBF.

Human Model: Cases with DAVFs, disturbed rCBF, and various symptoms (n = 22). Case Group 1 = retrograde leptomeningeal venous drainage (RLVD) into more than 1 venous sinus (n = 11/22 cases); and Case Group 2 = RLVD into a single venous sinus (n = 11/22 cases).

Interpretation and Analysis: In Case Group 1, preoperative T2-weighted MR images show no hyperintense areas, and angiographic images display evidence of blood flow into more than 1 venous sinus. Case Group 2 cases display preoperative SPECT with hypoperfused areas that coincide with hyperintense areas on T2-weighted MR images. Following treatment, hyperintense areas disappear, and symptoms improve in a large number of these cases (e.g., n = 7/11 = Case Group 2a), and their preoperative SPECT studies demonstrate preservation of vasoreactivity after acetazolamide challenge. In Case Group 2b, the hyperintense areas and symptoms persist in response to acetazolamide challenge (n = 4/11 cases) and reoperative SPECT studies reveal marked disturbances of vasoreactivity. Hyperintense MR images are consistently observed in cases with drainage into a single venous sinus. Hypoperfusion on SPECT scans apparently reflects venous congestion, whereas unpreserved vasoreactivity after an acetazolamide challenge on SPECT scans reflects venous infarction. The preservation of vasoreactivity after the challenge appears to be a good prognostic indicator.

Pre- and posttreatment MR imaging and single photon emission CT in cases with dural arteriovenous fistulas and retrograde leptomeningeal venous drainage. Kai Y, Hamada J, Morioka M, Todaka T, Mizuno T, Ushio Y. AJNR Am J Neuroradiol 2003 Apr; 24 (4), pp. 619–25.

Vascular Perfusion/Cerebral Cortex/Arterial Resistance and Dilation
Adenosine

Cellular/Molecular Mechanism of Action: Intracarotid infusion of short-acting vasodilators, such as adenosine and nitroprusside, in doses that lack significant systemic side effects, may permit controlled manipulation of cerebrovascular resistance. In this experiment we assessed changes in cerebral blood flow (CBF) after intracarotid infusion of nitroprusside and adenosine.

Application: Increase arterial dilation in conditions of excessive vasoconstriction of the cerebral arteries.

Implementation: Evaluation of CBF using intra-arterial [^{133}Xe] 4 times during infusions of (i) saline (intravenous); (ii) phenylephrine (intravenous 0.2 μg/kg/min^{-1}) with the intent of increasing mean arterial pressure by 10–15 mm Hg; (iii) phenylephrine intravenous and intracarotid nitroprusside (0.5 μg/kg/min); and (iv) intracarotid adenosine (1 mg/min). Note: Hypotension during nitroprusside injection is avoided by delivery through the intracarotid route of administration (tests for autoregulatory vasoconstriction).

Animal Model: Non-human primate (n = 6 adult baboons) anesthetized under isoflurane anesthesia and controlled ventilation.

Interpretation and Analysis: Intravenous phenylephrine increases mean arterial pressure (69 ± 8 to 91 ± 9 mm Hg, $p < 0.0001$, n = 6), and concurrent infusion of intracarotid nitroprusside reverses such alterations. However, compared with baseline, CBF does not change with phenylephrine (IV) or with concurrent infusions of phenylephrine (IV) and nitroprusside (intracarotid). Intracarotid adenosine profoundly increases CBF (from 29 ± 8 to 75 ± 32 mL/100 g/min; $p < 0.0001$). In non-human primates, intracarotid adenosine increases CBF in doses that lack significant systemic side effects, whereas intracarotid nitroprusside has no effect. Intracarotid adenosine may be useful for manipulating cerebrovascular resistance and augmenting CBF during conditions of cerebral ischemia. Intra-arterial [^{133}Xe] CBF measurements suggest that intracarotid adenosine, in a dose that lacks significant systemic side effects, profoundly increases CBF, whereas nitroprusside has no effect.

In nonhuman primates intracarotid adenosine, but not sodium nitroprusside, increases cerebral blood flow. Joshi S, Duong H, Mangla S, Wang M, Libow AD, Popilskis SJ, Ostapkovich ND, Wang TS, Young WL, Pile-Spellman J. Anesth Analg 2002 Feb; 94 (2), pp. 393–9.

BRAINSTEM, SPINAL CORD, AND PERIPHERAL NERVOUS SYSTEM

Myasthenia Gravis (MG)
Edrophonium (Tensilon)

Cellular/Molecular Mechanism of Action: Exerts a direct inhibitory effect on cholinesterase activity somewhat analogous to organophosphate insecticides. As a result, concentrations of acetyl-

choline begin to accumulate within the synaptic cleft thereby enhancing the amount of the neurotransmitter binding to it's corresponding receptor complex located on the post-synaptic membrane (e.g. motor end-plate) In MG cases, increased availability of acetylcholine neurotransmitter created by inhibitors of acetylcholine esterase compensates for the competitive inhibition of acetylcholine receptor complexes by bound IgG antibodies produced by aberrant autoimmune humoral immune response.

Considerations: Since the response is highly variable, signs of weakness in addition to over-stimulation of parasympathetic muscarinic receptor sites may develop, resulting in the appreciation of clinical signs including salivation, miosis, vomiting, and diarrhea (cholinergic crisis).

Diagnostic Application: Congenital and acquired MG.

Route of Administration: Edrophonium (intravenous)

Dosage: Edrophonium (0.5 to 5.0 mg).

Implementation: Evaluation and characterization of suspected cases of congenital or acquired MG.

Interpretation and Analysis: Correction of exercise intolerance conditions that last a duration of 2 to 3 minutes supports the diagnosis of MG. Response is widely variable, which prevents recommendation of a specific dose regimen. If the capacity to perform physical activity is not restored, a higher dosage can be administered in a matter of just a few minutes.

Approach to generalized weakness and peripheral neuromuscular disease. LoVecchio F, Jacobson S. Emerg Med Clin North Am 1997 Aug; 15 (3), pp. 605–23.

Myasthenia Gravis (MG)
Edrophonium/Neostigmine/Pyridostigmine/ Vecuronium

Cellular/Molecular Mechanism of Action: Edrophonium (acetylcholinesterase inhibitor that binds selectively to the biochemically active site of the enzyme). Alternatively neostigmine (cholinesterase inhibitor) and pyridostigmine (carbamate cholinesterase inhibitor: charged quaternary compound) contain carbamyl ester linkages that are directly hydrolyzed by acetylcholine esterase. Vecuronium (congener of pancuronium), is a *bis*-quaternary ammonium steroid that is 5 times more potent than *d*-tubocurarine as a neuromuscular blocking agent but inhibits plasma butyrylcholinesterase to a lesser degree than pancuronium so that less cardiovascular side effects are produced.

Application: Detection and characterization of cases affected by MG that are hypersensitive to nondepolarizing neuromuscular blocking drugs. Vecuronium can be applied in ocular tests for the detection of cases suffering from myasthenia gravis.

Edrophonium: Edrophonium chloride 2 mg IV followed in 45 seconds by another dose of 8 mg if no improvement is observed. Excessive doses should be avoided in order to avoid weakness from generalized depolarization of motor end plates.

Oral Test: Neostigmine (7.5 mg); pyridostigmine (30 mg); or ambenonium (2.5 mg). Signs for improvement in neuromuscular strength are monitored. In an hour, the dose is then increased 1.5-fold and repeated until a desired effect is achieved. Results can be confirmed with the edrophonium test.

Intravenous Test: Neostigmine (0.125 mg) or pyridostigmine (0.5 mg) administered at intervals of several minutes. Atropine (0.4 to 0.6) is given prior to the initiation of the test to prevent muscarinic side effects. The optimal oral dose is equivalent to 30-fold the intravenous dose that achieved the desired level of effect.

Vecuronium and General Anesthesia: [10 μg/kg intravenously under general anesthesia with sevoflurane (2.5%) and nitrous oxide (60%) in oxygen]. Neuromuscular transmission is measured as twitch tension of the adductor pollicis muscle with supramaximal stimulation measurements serving as an evaluation parameter. Following the recording of baseline measurements, incremental doses of vecuronium (10 μg/kg IV) are administered sequentially until a 90% blockade is exceeded. The degree of blockade and onset time after the initial 10 μg/kg dose of vecuronium are assessed, and the dose required to exceed 90% blockade recorded. In addition, effective doses of 50% and 95% for vecuronium are calculated from a single data point.

Considerations: A diagnosis can usually be obtained from history, presenting complaint, and physical examination, but its differentiation from neurasthenic, infectious, endocrine, neoplastic, or degenerative neuromuscular disease states may be difficult. Provocative testing with an anticholinesterase pharmaceutical agent results in a drastic improvement in conditions of myasthenia gravis.

Although antibodies to the acetylcholine receptor (AChR) often are observed in MG cases, 10% to 30% of cases do not show an anti-AChR antibody.

In childhood cases of MG, the majority (e.g., 92%, n = 25/27) have localized ocular myasthenia with a median onset of symptoms appreciated at 33 months of age. Approximately 24% of cases progress to generalized myasthenia gravis. A few (8%) may present with respiratory failure that require ventilatory support with an onset of symptoms being appreciated at about 22 months. Thymectomy (n = 10/27 cases) has been used as a atherapeutic intervention strategy which is associated with approximately 70% complete, and a 26% partial remission rates when applied in combinatinon with immunosuppressant therapy (e.g. azathioprine), and a cholinesterase inhibitor (e.g. pyridostigmine). The development of a myasthenic crisis is relatively infrequent when cases are properly managed with appropriate therapy and follow-up monitoring.

Interpretation and Analysis: Agents should produce a relatively immediate (dramatic) improvement in clinical signs. Seronegative and seropositive MG cases are equally sensitive to vecuronium compared with unaffected negative reference control cases.

The effect of use of pyridostigmine and requirement of vecuronium in cases with myasthenia gravis. Tripathi M, Kaushik S, Dubey P. J Postgrad Med 2003 Oct–Dec; 49 (4), pp. 311–4; discussion 314–5.

Myasthenia Gravis (MG)
Edrophonium/[Esophogeal Function Screening Test]

Cellular/Molecular Mechanism of Action: Edrophonium is a short-acting acetylcholinesterase inhibitor.

Background Information: Myasthenia gravis is an autoimmune disease in which impairment of neuromuscular transmission results in pathological fatigability of striated muscles. Dysphagia is a common MG symptom. It is caused by a weakness of the striated muscles in the pharynx and esophagus.

Dosage: Edrophonium chloride (EC; 10 mg).

Implementation: Evaluate the role of esophageal scintigraphy in the assessment of esophageal function in MG aided by provocative testing with edrophonium. In this context, esophageal transit time is measured in concert with the use of a multiple swallow test protocol. Cases are studied first under baseline conditions, and then immediately after pharmacological stimulation with edrophonium.

Human Model: Cases of clinically proven myasthenia gravis (n = 15; n = 6/15 oculopharyngeal manifestations; n = 9/15 generalized weakness; n = 10/15 history of dysphagia.)

Interpretation and Analysis: Under baseline conditions all MG cases display impaired esophageal function (emptying = 58% ± 21; normal range > 85%). In a large percentage of cases (e.g. n =

14/15) esophageal transit improves after the administration of EC (emptying = 75% ± 18; $p < 0.01$), with measurements reaching the normal range in approximately 40% of cases (e.g. n = 6/15). Some cases may show no effect attributable to EC (e.g., n = 1/15). Esophageal transit is often compromised in MG. Functional abnormalities may also be present in cases without a history of dysphagia. Inhibition of cholinesterase positively affects striated muscles in the pharynx and upper esophagus, thus improving esophageal transit. Esophageal scintigraphy may be a simple, non-invasive method for diagnosing impairment of esophageal function in MG and to monitor changes under pharmacological stimulation.

Assessment of esophageal function in cases with myasthenia gravis. Linke R, Witt TN, Tatsch K. J Neurol 2003 May; 250 (5), pp. 601–6.

Myasthenia Gravis (MG)
Vecuronium

Cellular/Molecular Mechanism of Action: Vecuronium is an acetylcholinesterase enzyme inhibitor which promotes the accumulation of acetylcholine neurotransmitter within the synaptic cleft thereby increasing binding to and activation of acetylcholine receptor complexes.
Background Information: Conditions of MG are hypersensitive to non-depolarizing neuromuscular blocking drugs. Although antibodies to the acetylcholine receptor (AChR) often are observed in MG, 10% to 30% of affected cases do not show an anti-AChR antibody. Little is known about differences in sensitivity to non-depolarizing neuromuscular blocking drugs between MG cases with and without anti-AChR antibody.
Application: Determination if seronegative MG cases are as sensitive to vecuronium as seropositive MG cases.
Route of Administration: Vecuronium (intravenous).
Dosage: Incremental doses of vecuronium (10 μg/kg) during anesthesia with sevoflurane (2.5%) and nitrous oxide (60%) in oxygen.
Implementation: Evaluation of the potential differential effect of vercuronium between sero-positive and sero-negative MG conditions. Measurement and monitoring of neuromuscular transmission can be achieved by assessing twitch tension of the adductor pollicis muscle with supramaximal stimulation. After baseline measurements, 10 μg/kg IV dose increments of vecuronium are administered sequentially until blockade exceeds 90%. The degree of blockade and onset time after the initial 10 μg/kg of vecuronium is assessed, and doses required to exceed 90% blockade recorded. In addition, effective doses of 50% and 95% for vecuronium are calculated from a single data point.
Interpretation and Analysis: Both types of MG cases show increased sensitivity to vecuronium compared with non-MG cases. Therefore, seronegative and seropostive MG conditions are equally sensitive to vecuronium administration.

Vecuronium in the myasthenic patient. Hunter JM, Bell CF, Florence AM, Jones RS, Utting JE. Anaesthesia 1985 Sep; 40 (9), pp. 848–53.

Myasthenia Gravis/Ileus: Postoperative
Neostigmine

Cellular/Molecular Mechanism of Action: Inhibits biochemical activity of acetylecholine esterase, resulting in accumulation of acetylcholine neutrotransmitter. As a result, an elevated level of parasympathetic activity is created within the autonomic nervous system.
Diagnostic Application: (i) Verification of the presence of normal gastrointestinal function pertaining to parasympathetic innervation and smooth muscle integrity that are usually intact in conditions such as postoperative and pain-induced ileus. (ii) Diagnostic detection of myasthenia gravis conditions.
Route of Administration and Dosage: Subcutaneous injection is preferred.
Considerations: Rapid intravenous injection can promote spastic (painful) contractions of gastrointestinal smooth muscle. Administration in cases suffering from myasthenia gravis can exacerbate the severity of the condition through depletion of axonal acetylcholine stores.
Interpretation and Analysis: Alleviation of gastrointestinal ileus based upon detection of auscultatable borborygmi, palpable contractions, and visualization of fecal production. Myasthenia gravis cases will temporarily demonstrate an improvement in generalized motor function and a reduction in the severity of apparent paresis.

Respiratory Center Depression/Brain Stem Suppression vs Primary Pulmonary Dysfunction
Doxopram (Dopram)

Cellular/Molecular Mechanism of Action: Doxopram directly stimulates the central respiratory centers located in the reticular formation of the brainstem.
Diagnostic Application: Increases respiratory function (respiration) through direct stimulation of the brainstem respiratory center.
Route of Administration and Dosage: Intravenous injection.
Application: Differentiation between reversible suppression of central brainstem respiratory center function and primary respiratory (pulmonary) function or pathology as a cause for subnormal arterial blood oxygen levels (e.g., blood gas analysis of premature neonates).
Interpretation and Analysis: Subnormal blood oxygen concentrations attributed to primary pulmonary dysfunction may not be fully corrected by coxopram administration.
Laboratory Instrumentation: Evaluation of respiratory rate, in concert with measurement of arterial blood oxygen and carbon dioxide concentrations.

Spinal Cord/Characterization of Anatomical Morphology
Ipomiadol/Metrizamide/Renagrafin

Cellular/Molecular Mechanism of Action: The active moiety of radiographic contrast dyes is iodine because it strongly absorbs x-ray photons (radiodense properties).
Diagnostic Application: Radiographic contrast dyes are injected directly into the cerebral spinal fluid (CSF) space for the purpose of imaging anatomical features of the vertebral spinal cord.

Additional diagnostic applications include infiltration of chronic draining tracts in an effort to detect their extent, and/or presence, location, or position of foreign body objects.
Route of Administration and Dosage: One of the most common sites for injection is the atlanto-occipital (AO) space. The cerebral spinal needle is directed toward the lower mandible while the head and neck are flexed ventrally. Advancing the cerebral spinal needle with the head and neck held in such a position avoids or minimizes the risk of directing the spinal needle through the foramen magnum and into neural tissues of the CNS (e.g., cerebellum). To promote flow of radiographic contrast dyes distally along the spinal

cord, the head, neck, and cranial thorax can be held on a raised (flat but not horizontal) plane.

Techniques have been described for the injection of radiographic contrast dyes into the CSF at the level of the lumbosacral space, and less frequently the vertebral foramen. The latter method is technically difficult to perform in large animal species and may require careful interpretation of specialized imaging modalities (e.g. fluoroscopy, NMR, or CT-scan).

Direct infusion or catherization techniques are applied for the instillation of radiographic dyes into chronic draining tract lesions.

Considerations: Metrizamide-based contrast dye reagents can potentially induce seizure activity due to their ability to competitively inhibit glucose metabolic pathways that are CNS associated. Based upon the known mechanism of action, this complication can be avoided by preadministering intravenous glucose (dextrose 5%), in addition to maintaining the head and neck in a flat elevated plane in order to promote flow to more distal segments of the vertebral spinal cord. Newer generation radiographic contrast dye reagents such as ipomiadol are much less likely to interfere with CNS glucose metabolism compared to older generation contrast dye reagents such as metrizamide.

Interpretation and Analysis: Radiographic contrast dyes assist in detecting space-occupying lesions that may physically be impinging on the cervical, thoracic, lumbar, and sacral segments of the spinal cord. Lesions of this type are detected by appreciating a narrowing or obstruction of radiographic dye "column" within the CSF space surrounding the spinal cord.

Environmental Implications: Iodinated contrast media belong to the most frequently applied compounds in medicine. They exhibit a high polarity and are very persistent against metabolism by the organism and environmental degradation. A sensitive method for the determination of 5 iodinated contrast media in aqueous matrices has been described. Solid phase extraction utilizing isolute ENV+ material has been used for sample enrichment. The contrast media are partially separated on an RP-C18 column, and detection achieved using electrospray-tandem MS (API III plus and API 365), allowing the sensitive quantitation of these compounds down to the lower ng/L range. The recovery rates generally exceeded 70%, for spiked surface water as well as tap water. The analytes can be detected in native samples such as municipal sewage treatment plant effluent, the river Rhine, and even in tap water.

SPECIAL SENSES

Cataracts and Idiopathic Macular Hole/Circular Curvilinear Capsulorhexis/Internal Limiting Membrane Peeling
Indocyanine Green (ICG)

Cellular/Molecular Mechanism of Action: Indocyanine green emits fluorescent color when excited by certain wavelengths of visible light energy.

Application: Determine the elimination kinetics of ICG after intraocular operations using ICG staining and a monitoring indicator.

Implementation: Evaluation of the applicability of ICG mediated intraocular fluorescence (ICG) in concert with the use of a scanning laser ophthalmoscope (*in vivo: ICG angiographic mode*), and fluorescent microscopy (*in vitro*) to facilitate circular curvilinear capsulorhexis for cataract lesions, and internal limiting membrane (ILM) peeling for macular hole surgery.

Human Model: White cataract (n = 7 cases and n = 9 eyes) and idiopathic macular holes (n = 14 cases; n = 14 eyes).

Interpretation and Analysis: Scanning laser ophthalmoscopy can reveal fluorescence in the anterior segment in cases with cataracts on the first postoperative day, and fluorescence persists for a mean of 6.0 ± 2.2 days. Scanning laser ophthalmoscopy also reveals fluorescence in the posterior pole of cases with macular holes, and it remains detectable for a mean of 2.7 ± 1.4 months postoperatively. Fluorescence microscopy shows fluorescence of the entire tissues, suggesting that ICG stains not only the surface of membranes but also penetrates these structures. In both operations, visual outcomes are not significantly different from results obtained without ICG. Because entire tissues are stained, the differences in ICG kinetics may also be caused by factors other than differences in stainability, such as the environment surrounding tissue structures or molecular structural differences between the lens capsule and the ILM. Although complete disappearance of fluorescence and good functional recovery can be appreciated, the longer resident time of the dye after macular hole surgery may suggest a potential risk to intraocular tissues.

Instrumentation: Fluorescence microscopy (*in vitro*) and scanning laser ophthalmoscope (*in vivo*).

Kinetics of indocyanine green dye after intraocular surgeries using indocyanine green staining. Horiguchi M, Nagata S, Yamamoto N, Kojima Y, Shimada Y. Arch Ophthalmol 2003 Mar; 121 (3), pp. 327–31.

Corneal Trauma
Fluorescein Dye

Cellular/Molecular Mechanism of Action: Fluorescein dye is an agent that emits colored visible light upon exposure to white light energy.

Diagnostic Application: Detectable dye marker system that is compatible with biological systems.

Dosage: Applied in a topical fashion to the moist surface of the corneal epithelium.

Implementation: Detection of surface defects affecting the corneal epithelium.

Interpretation and Analysis: Fluorescein dye does not bind to intact surfaces of the corneal epithelium. Alternatively, fluorescein dye has a profound molecular affinity for subcorneal epithelial (stromal) collagen. Upon exposure, the fluorescent fluorescein dye reacts with visible light resulting in the emission of a fluorescent green color to facilitate localization of defects in the corneal epithelium.

Laboratory Instrumentation: Visual observation.

Cycloplegia Induction for Retinoscopy
Cyclopentolate/Tropicamide

Cellular/Molecular Mechanism of Action: Cyclopentolate and tropocamide are both synthetic tertiary amines that inhibit acetylcholine at muscarinic receptor complexes (competitive).

Physiological Mechanism of Action: Cyclopentolate and tropocamide inhibit motor and sensory nerve transmission (e.g., paralysis of intraoccular smooth muscle tissues). Note: cycloplegics cause ciliary muscle paralysis while mydriatics are agents that promote dilation of the pupil.

Application: Induction of ocular mydriasis and cycloplegia to facilitate improved diagnostic accuracy of ophthalmoscopic/retinoscopy procedures. Tropicamide has a much shorter recovery period compared to cyclopentolate.

Route of Administration: Ophthalmic (topical).

Dosage: Cycloplegia with tropicamide (1%) and cyclopentolate (1%) delivered as 2 drops of cycloplegic agent separated by a time span of 5 minutes. Retinoscopy is performed 25 to 30 minutes after the 1st drops are instilled.

Considerations: There were no serious adverse reactions with either agent, although an infant has been reported to have temporarily developed reddened cheeks after instillation of cyclopentolate. Certain iatrogenic side effects should be recognized, including the precipitation of acute angle-closure. A shallow anterior chamber is a fundamental ocular variable that should be evaluated before inducing mydriasis and/or cycloplegia. The technical procedures for the oblique illumination shadow test and the van Herick slit lamp test both provide an accurate estimation of the anterior chamber angle. The risk of precipitating an attack after reasonable circumspection by such preliminary evaluations approaches zero. However, should an attack occur, the procedures to rapidly lower the intraocular pressure and open the chamber angle are available for immediate application.
Interpretation and Analysis: Previous investigations have generated findings that suggest that tropicamide is as effective as cyclopentolate for the measurement of refractive error in most healthy, non-strabismic infants (4–7 months of age; mean = 5.71 months).

On a scale of 0 to 4.9, the median iris grade in infants is 4.0, which corresponds to a brown or darkly pigmented iris. Retinoscopy results reported for the horizontal meridian of the right eye include (i) mean refractive error using non-cycloplegic near retinoscopy is equal to +0.94 D (± 1.19 D); and (ii) mean refractive error +1.81 D (± 1.19 D) with tropicamide and +1.88 D (± 1.45 D) with cyclopentolate.

No statistically or clinically significant difference between the 2 cycloplegic measurements using different diagnostic agents can be appreciated ($t = -0.46$, $p = 0.65$). The mean difference between non-cycloplegic and cycloplegic retinoscopy has been observed to be 0.89 D (+/- 0.66 D) with tropicamide ($t = -6.57$, $p < 0.0001$) and 1.04 D (+/- 0.94 D) with cyclopentolate ($t = -5.38$, $p < 0.0001$; all 2-sided paired t tests).

Glaucoma
Pilocarpine/(Tonography Alternative)

Cellular/Molecular Mechanism of Action: Pilocarpine is a cholinomemitic agents that functions as a cholinergic/parasympathomimetic agonist that binds to and activates acetylcholine receptor complexes.
Physiological Mechanism of Action: Promotes parasympathomimetic-induced pupillary constriction.
Application: Glaucoma.
Route of Administration: Ophthalmic topical (Hollwichs' pilocarpine test).
Interpretation and Analysis: The pilocarpine test performed at the peak of the daily pressure curve gives comparable results to tonography and hence can be useful in suspected cases of glaucoma.
Instrumentation: Ophthalmascope and tonometer.

Pilocarpine as a diagnostic agent for early recognition of a tendency to glaucoma. [Comparable results between Hollwich's pilocarpine test and tonography (author's transl).] Lienert F, Busse H. Klin Monatsbl Augenheilkd 1976 Jul; 169 (1), pp. 116–8.

Glaucoma
Ibopamine

Diagnostic Application: Detection of cases with preclinical and clinical signs of glaucoma.
Implementation: Evaluation of suspected cases of glaucoma.
Interpretation and Analysis: Glaucoma cases show a substantial increase in ocular pressure following ibopamine administration compared to normal unaffected individuals (92% of the known glaucoma cases are positive for the ibopamine test). Many affected cases demonstrate a progressive increase in the ocular pressure increases induced by ibopamine as their disease state progresses in severity.

Glaucoma
Dexamethazone

Route of Administration: Ophthalmic (topical).
Dosage: Dexamethazone ophthalmic topical (0.1%).
Implementation: Evaluation of the responsiveness of glaucoma conditions to topically administered corticosteroids.
Human Model: Retrospective follow-up study (n = 134 cases; n = 84 women). Follow-up time varies from 5 to 15 years (mean 7.1 years).
Interpretation and Analysis: Approximately 25.4% of survey cases have a high response (n = 34/134; intraocular pressure (IOP) rise greater than or equal to 16 mm Hg), while 49.3% (n = 66/134) have an intermediate response (IOP rise 6–15 mm Hg); and 25.4% (n = 34/134) have a low response (IOP rise less than or equal to 5 mm Hg) to topically administered 0.1% dexamethasone eye drops. During follow-up periods, 21.6% (n = 29/134) cases develop glaucoma. Of these, 31% (n = 9/29) are high responders, while 24.0% (n = 7/29) are low responders to corticosteroids. Log-linear analysis shows no correlation between corticosteroid response and development of glaucoma in "study" eyes ($SD = 0.232$, $df = 2$, $p = 0.890$), the control eye ($SD = 1.234$, $df = 2$, $p = 0.540$), or both eyes ($SD = 0.618$, $df = 2$, $p = 0.734$). On final examination 56% (n = 75/134) have an IOP of greater than or equal to 22 mm Hg. Of these ocular hypertensive cases 37.7% (n = 28/75) are high responders, and 20% (n = 15/75) have a low response to corticosteroids. Approximately 33% of cases will have a family history of glaucoma (e.g., n = 44/134). Approximately 25.1% (n = 11/44) of these will be high responders and 31.8% (n = 14/44) will be low responders. Of the cases that develop glaucoma (n = 29) less than 30% (n = 8) have a family history of glaucoma. Of these, some will be high (n = 3/8) or low responders (n = 3/8). Such findings indicate that the dexaprovocative test yields relatively limited value in the screening of cases for glaucoma.

Glaucoma (Related)
Acepromazine and Ketamine/Water Provocative Test

Diagnostic Application: A standard water provocative test for dogs anesthetized with acepromazine-ketamine.
Route of Administration: Oral.
Dosage: Administration of 60 ml/kg of water to anesthetized dogs.
Interpretation and Analysis: Pressure levels decrease close to original levels by 60 minutes. Pressure increases in females and young dogs are greater than those observed in males and adults. An increase in intraocular pressure can be detected that reaches a maximum at 15 minutes postingestion. The exception is young dogs that attained a maximum increase at 10 mintues.

Graves' Ophthamology/Type Th2 Interleukines: IL-4 and IL-10
Methylprednisolone/Prednisone

Cellular/Molecular Mechanism of Action: Corticosteroids inhibit general protein synthesis and stabilize cell membrane structures, in addition to inhibiting the biochemical activity of phospholi-

pase A_2 in a manner that decreases both leukotriene and prostaglandin synthesis.

Physiological Mechanism of Action: Corticosteroids exert anti-inflammatory and immunosuppressive properties.

Application: Elevated expression of serum IL-4 and IL-10 during successful treatment with corticosteroids indicates the possibility of using these cytokines as predictors of the beneficial effect of corticosteroids in Graves' ophthalmopathy.

Route of Administration: Methylprednisolone (MP; intravenous); prednisone (oral).

Dosage: Methylprednisolone (2 series, 3 g dose in each series); prednisone (60 mg per day according to a tapering dose schedule).

Implementation: Evaluation of conditions of Graves' ophthalmology related to the estimated influence of corticosteroids on Th1 and Th2 serum cytokine balance. Cytokines of particular interest include IFN-γ,, TNF-α, IL-4, and IL-10. In addition, complementary analysis involves delineation of the upregulation of Th2 immune response during successful treatment with corticosteroids to determine if there is a correlation with their beneficial effect in Graves' ophthalmopathy. Corticosteroid challenge/exposure consists of intravenous infusions of and subsequent treatment with oral prednisone in a tapering schedule. Serum samples are collected 24 hours before MP, 24 hours after MP, 14 days of treatment with prednisone, and at the end of corticosteroid therapy. Levels of IFN-γ, TNF-α, IL-4, and IL-10 in serum are determined using ELISA. Statistical significance is estimated by the Mann-Whitney U-test.

Human Model: Cases of Graves' disease without ophthalmopathy (e.g. n = 20 Gd); cases with clinical ophthalmopathy symptoms (e.g. n = 16 GO with CAS over 3 points, last consultation record for GO less than a year old); and normal healthy volunteers (e.g. n = 16).

Interpretation and Analysis: Deviations exist pertaining to systemic Th2 cytokine profile in Graves' disease. In GO cases, a significant increase in serum IL-10 concentration can be detected. In corticosteroid-responsive cases, the balance of serum cytokines IL-4/IFN-γ, IL-4/TNF-α, IL-10/IFN-γ, and IL-10/TNF-α increases and remains upregulated. In non-responders, the balance of serum cytokines studied increases after methylprednisolone but declines markedly during continuation of the therapy with prednisone. Therefore, efficient corticosteroid therapy may be related to its influence on cytokine profiles and Th2/Th1 cytokine balance. The upregulation of serum IL-4 and IL-10 during successful treatment with corticosteroids indicates the possibility of using these cytokines as predictors of the beneficial effect of corticosteroids in Graves' ophthalmopathy.

Serum Th1 and Th2 profile cytokine level changes in cases with Graves' ophthalmopathy treated with corticosteroids. Myśliwiec J, Kretowski A, Topolska J, Siewko K, Jakubczyk D, Szelachowska M, Mikita A, Kinalska I. Horm Metab Res 2001 Dec; 33 (12), pp. 739–43.

Glaucoma: Angle-Closure Glaucoma (ACG) Risk *Pilocarpine/Phenylephrine*

Cellular/Molecular Mechanism of Action: Pilocarpine is a cholinomimetic agent that functions as a cholinergic/parasympathomimetic agent (agonist) that binds to and activates acetylcholine receptor complexes. Phenylephrine is an analog of the adrenergic neurtransmitter epinephrine.

Physiological Mechanism of Action: Promotes parasympathomimetic-induced pupillary constriction. Phenylephrine promotes pupil dilation.

Application: The pilocarpine/phenylephrine provocative test (PPPT) has been considered a highly sensitive method for identifying active or "at-risk" conditions of ACG affecting the eye.

Route of Administration: Ophthalmic (topical).

Implementation: The pilocarpine/phenylephrine provocative test (PPPT) has been considered a highly sensitive method for identifying active or "at-risk" conditions of ACG affecting the eye.

Interpretation and Analysis: Over a 10-year time span, reports have described the outcomes assessment for cases of primary acute ACG and subacute ACG in which management was determined by results from the PPPT. A positive result has been interpreted as an indication for a peripheral iridectomy. Conversely, a negative response implies that conservative treatment is required. In spite of a negative PPPT the contralateral eyes of cases of acute ACG treated conservatively have a high rate (40%) of developing ACG. Similarly, in eyes with a history of subacute ACG with a negative PPPT, ACG develops at some stage during the follow-up in 60% of affected cases. Peripheral iridectomy alone results in normal intraocular pressure in 63% of eyes that have suffered an attack of acute ACG. In hypertensive eyes that present with subacute ACG, however, only 1 eye becomes normotensive following peripheral iridectomy alone. In the subacute ACG group a positive PPPT is closely related to the presence of glaucomatous optic disc damage. These results indicate that the PPPT lacks sensitivity in detecting eyes at risk of ACG, and a positive result is likely in eyes with damaged outflow that will not respond to peripheral iridectomy.

Glaucoma: Angle-Closure Glaucoma (ACG) Risk *Pilocarpine/Phenylephrine*

Cellular/Molecular Mechanism of Action: Pilocarpine binds to and activates parasympathetic muscarinic cholinergic receptor complexes thereby exerting cholinomimetic perperties pertaining to action of neurotransmitter, acetylcholine. Phenylephrine binds to powerfully activate sympathetic α1-adrenergic receptor complexes with little to no activation of β adrenergic receptor complexes.

Background Information: Mapstone introduced the term "partial angle closure" to describe how eyes with apparently open angles could be damaged by a covert angle-closing mechanism. Such abnormalities are identifiable by a positive result of the pilocarpine/phenylephrine provocative test (PPPT).

Application: Detection and characterization of ACG.

Route of Administration: Ophthalmic (topical).

Considerations: Clinical review demonstrates that eyes with narrow angles that have positive PPPT results do not benefit from peripheral iridectomy (PI) or laser iridectomy (LI), and therefore a positive PPPT is not indicative of the presence of an angle-closing mechanism.

Interpretation and Analysis: A review over a 10-year period of cases (n = 68) with narrow but open anterior chamber angles that underwent PI or LI based on a positive PPPT reveals that a large percentage with glaucoma or ocular hypertension (62%) have unchanged mean intraocular pressures (IOPs) following iridectomy. Approximately 45% of eyes that show glaucomatous optic disc damage at presentation, and 25.6% of ocular hypertensive eyes subsequently require trabeculectomy to achieve IOP control. In instances of no ocular abnormality other than a narrow angle (e.g. n = 16 cases with n = 19 affected eyes) have positive PPPTs and undergo PI. After a 10-year follow-up, only 1 of the 13 untreated fellow eyes developed ACG. As this is usually bilateral, many of the positive tests in this group of cases may therefore have actually been false positives.

Can the pilocarpine phenylephrine provocative test be used to detect covert angle closure. Wishart PK. Br J Ophthalmol 1991 Oct; Vol. 75 (10), pp. 615–8.

Glaucoma/Angle-Closure Glaucoma: Primary (PACG)
Brimonidine/Pilocarpine

Cellular/Molecular Mechanism of Action: Brimonidine is a selective alpha-2 adrenoceptor agonist. Pilocarpine is a cholinomimetic agent that functions as a cholinergic/parasympathomimetic agonist that binds to and activates acetylcholine receptor complexes.
Physiological Mechanism of Action: Brimonidine tartrate suppresses aqueous humor flow in the human eye and promotes parasympathomimetic-induced pupillary constriction.
Application: Provocative test for characterizing PACG.
Route of Administration: Ophthalmic topical.
Dosage: Brimonidine tartrate 0.2% instilled topically into each eye 2 hours prior to a 2nd dark-prone test.
Implementation: Determination of the capacity for pilocarpine to prevent intraocular pressure (IOP) elevations in the dark-prone provocative test for PACG.
Interpretation and Analysis: An IOP elevation of greater than 8 mm Hg is interpreted as a positive result. The IOP elevations in the first dark-prone test can be in the range of 11.91 $\pm$ 5.17 (5.7 to 27.3) mm Hg in contrast to the frequent increase in IOP (approximately 5.70 $\pm$ 2.96; range: 2.9–12.2 mm Hg) that occurs during a 2nd dark-prone test combined with brimonidine tartrate (0.2%) pretreatment ($p < 0.001$). Significant differences may also be noted in pretest IOP (15.59 $\pm$ 3.86 mm Hg vs 13.33 $\pm$ 3.65 mm Hg, $p = 0.008$) as well as in posttest IOP (27.62 $\pm$ 7.27 mm Hg vs 19.03 $\pm$ 3.50 mm Hg, $p < 0.001$) in two sequential dark-prone tests. A relatively high percentage of initially positive dark-prone tests (86.46%) convert to negative tests after pretreatment with brimonidine. There are often significant effects imposed by brimonidine tartrate (0.2%) in the prevention of IOP elevations in PACG conditions previously found to test positive in the dark-prone provocative test.

The efficacy of brimonidine in preventing intraocular pressure elevation in the provocative test for primary angle-closure glaucoma. Chen YF, Hung PT, Hsieh JW, Shein J, Hsiao CK. J Ocul Pharmacol Ther 2002 Apr; 18 (2), pp. 99–103.

Glaucoma: Closed-Angle Glaucoma (Postperipheral Iridectomy)
Corticosteroid

Application: Identification of closed-angle glaucoma in cases treated by peripheral iridectomy (affected eye) and prophylactic peripheral iridectomy (unaffected eye).
Route of Administration: Ophthalmic (topical).
Considerations: An acquired form of corticosteroid-induced ocular hypertension appears to exist as a clinical entity. The possible pathogenisis of the condition has been tentatively described, as has its clinical significance.
Interpretation and Analysis: In cases of unilateral closed-angle glaucoma treated by peripheral iridectomy (e.g. $n = 35$), ophthalmic corticosteroid in both eyes results in the detection of increased intraoccular pressure in 51% of the affected eyes and 11% of the unaffected eyes (positive corticosteroid pressure test). The closed-angle glaucoma eyes had their fellow eyes respond differently, as shown by frequency distribution graphs and also by differences between the corticosteroid-induced change in pressure (Wilcoxon test, $z = -4.80$, $p < 0.0001$).

Corticosteroid-induced ocular hypertension. II. An acquired form. Akinbehin AO. Br J Ophthalmol] 1982 Aug; Vol. 66 (8), pp. 541-5.

Glaucoma/Predictive Risk of Developing Ocular Hypertension and Perimetric Defects
Ibopamine

Application: Ibopamine can be applied as a provocative test due to its pharmacological property of increasing ocular pressure in eyes with outflow system impairment.
Route of Administration: Ophthalmic (topical).
Implementation: Evalution of glaucoma conditions applying the ibopamine provocative test.

Human Model: Cases suspected of having glaucoma ($n = 175$ [250 eyes] POAG; $n = 101$ [190 eyes] mild ocular hypertension; $n = 39$ [64 eyes] NTG; and $n = 163$ [326 eyes] healthy volunteers). Among cases suspected of POAG and glaucoma, some may be included that, starting with performing the ibopamine test, have at least 1 year of perimetric follow-up (e.g., $n = 49$ [92 eyes] and $n = 20$ [38 eyes], respectively).
Interpretation and Analysis: The ibopamine test is positive in approximately 92% of the glaucomatous cases, 61% of glaucoma suspect cases, 52% of NTG cases, and 0% of the healthy volunteers. Of the ibopamine-positive glaucoma suspects, about 28% show a perimetric deterioration within a 2.5-year follow-up period. Perimetric deterioration is rarely appreciated in ibopamine-negative glaucoma suspects (Fisher's exact test: $p = 0.038$). Among glaucomatous cases, 46% of those that are positive will show a progressive trend of the perimetric defect, compared to about 8% of glaucomatous test-negative cases (Fisher's exact test: $p = 0.003$). In this context, ibopamine can potentially be applied as a provocative test, especially in epidemiological screening studies to identify cases that might develop ocular hypertension or glaucoma and in the follow-up of glaucoma suspects, to identify cases that have a greater risk of developing perimetric defects.

Horner's Syndrome
Guanethidine/Pholedrine/[Hydroxyamphetamine]

Cellular/Molecular Mechanism of Action: Amphetamines function as indirectly acting sympathomimetic agents by promoting the release of biogenic amines from storage sites within nerve presynaptic terminals. (Note: Pholedrine is *n*-methyl derivative of hydroxyamphetamine.)
Physiological Mechanism of Action: Guanethidine's major effect is inhibition of responses to stimulation of sympathetic nerves and indirectly-acting sympathomimetic amines (e.g., tyramine, amphetamines). Pholedrine (4′-hydroxymethamphetamine) is a cardiovascular agent that exerts hypertensive and adrenergic effects.
Application: Diagnosis of Horner's syndrome.
Route of Administration: Ophthalmic (topical).
Dosage: Pholedrine (1%) yields a biological effect roughly equivalent to hydroxyamphetamine (0.5%).
Considerations: Hydroxyamphetamine (0.5% ophthalmic) is no longer available through commercial vendors.
Implementation: Evaluation of suspected conditions of Horner's syndrome.
Interpretation and Analysis: Pretreatment with topical guanethidine attenuates the response to pholedrine. Preganglionic

Horner's syndrome can be distinguished clearly when pholedrine is combined with guanethidine pretreatment.

Horner's Syndrome
Dextroamphetamine/Phenylephrine

Cellular/Molecular Mechanism of Action: Phenylephrine is an adrenergic neurotransmitter analog of epinephrine. Dextroamphetamine promotes the release of adrenergic neurotransmitter across the presynaptic membrane.
Application: Identification of the anatomical location of a deficit/lesion affecting adrenergic/sympathetic autonomic nervous system that provide input to smooth muscle ophthalmic structures (e.g., Horner's syndrome).
Route of Administration: Ophthalmic (topical).
Implementation: Differentiation between alterations in preganglionic and postganglionic adrenergic/sympathetic (autonomic nervous system) innervation to smooth muscle ophthalmic structures.
Interpretation and Analysis: Topical application of dextroamphetamine to the corneal surface when Horner's syndrome is caused by a lesion located above the terminal lower motor neuron of the sympathetic (adrenergic) autonomic nervous system results in a hyperactive pupillary light reflex response. Detection of a positive response suggests that the postganglionic adrenergic/sympathetic neuron is viable and entirely functional.

Such responses in the eye is neuroanatomically analogous to the detection of a hyperactive limb reflex with an upper motor neuron lesion affecting the brain or spinal cord. Topical application of phenylephrine to the corneal surface of cases with Horner's syndrome caused by a lesion affecting the terminal lower motor neutron of the sympathetic (adrenergic) segment of the autonomic nervous system that results in a hyperactive pupil response is due to chronic catecholamine neurotransmitter depravation of visceral (ocular) smooth muscle tissue.
Instrumentation: Ophthalmascope.

Microvasculature/Retina/Estimation of Microvasculature Blood Flow Velocity/Leukocyte Dynamics in the Choroidal Microcirculation
Indocyanine Green (ICG) Dye-Stained Leukocytes

Cellular/Molecular Mechanism of Action: Indocyanine green is a dye marker molecule that can be used *in vivo* as a detection or indicator molecule.
Application: Visualization of leukocytes moving through the choroidal vessels of pigmented animals. Enables the evaluation of leukocyte dynamics in the choroidal microcirculation.
Route of Administration: Intravascular.
Implementation: Leukocytes are collected following centrifugal separation of autologous blood and then stained with ICG dye. The ICG-stained leukocyte suspension is then injected intravenously, and fundus images obtained with a scanning laser ophthalmoscope. Images are recorded on videotape and analyzed with a personal computer-based image analysis system.
Animal Model: Pigmented rabbits and monkeys.
Interpretation and Analysis: In pigmented rabbits, fluorescent leukocytes moving in the choroidal circulation are clearly visible for a period of more than 1 hour. In monkeys, distinct fluorescent dots are seen moving approximately 50–200 μm in the foveal avascular zone for more than 30 minutes after injection of ICG-stained leukocyte fluid. Dim fluorescent dots can be seen moving in the fundus. Although the movement of the dim dots is difficult to trace, they seemed to be moving in the choroidal vessels. In rabbits, the mean flow velocity of leukocytes moving without plugging is 0.48 ± 0.14 mm/sec in the peripheral choriocapillaris. In monkeys, the mean flow velocity of distinct fluorescent leukocytes without plugging is 2.45 ± 0.48 mm/sec in the posterior choroid. In pigmented rabbits and monkeys, this method allows visualization of leukocytes passing through the choroidal vessels and provides a new way to investigate, non-invasively and *in vivo*, leukocyte dynamics in the choroidal microcirculation.
Instrumentation: Scanning laser ophthalmoscope.

Evaluation of leukocyte dynamics in choroidal circulation with indocyanine green-stained leukocytes. Takasu I, Shiraga F, Okanouchi T, Tsuchida Y, Ohtsuki H. Invest Ophthalmol Vis Sci 2000 Sep; 41 (10), pp. 2844–8.

Neurons: *In Vivo* Antegrade Staining/Visual Pathway Staining/Retinal Ganglion Staining/ Vitreoretinal Surgery: Intraoperative Probe
Indocyanine Green (ICG)

Cellular/Molecular Mechanism of Action: Indocyanine green emits fluorescent color when excited by certain wavelengths of light energy.
Background Information: It has been shown that the application of ICG over the retinal surface is followed by a prolonged staining of the optic disc.
Application: Indocyanine green can function as a bidirectional axonal tracer for *in vivo* (research) to stain the visual pathway and retinal ganglion.
Implementation: Evaluate the diffusion of ICG in the optic tract. More specifically ICG can be characterized for it's ability to undergo; (i) anterograde diffusion after injection into the vitreous; and (ii) retrograde diffusion after microinjection into the lateral geniculate nucleus.
Animal Model: Rabbit (vitreous ICG injection) and rats (lateral geniculate nucleus injection).
Interpretation and Analysis: Anterograde and retrograde diffusion occurs along axons at a rate of about 2 mm per hour following ICG injection. Anterograde staining of the visual pathway persists for several weeks. After injection into the lateral geniculate nucleus, fluorescent retinal ganglion cells can be visualized for at least 7 days in conscious rats. Microscopic examination reveals evidence of retrograde-labeled retinas and the detection of ICG vesicles inside the axons, cytoplasm, and dendrites of retinal ganglion cells. Evidence of toxic effects are usually not observed when applying optical microscopy. Injection of ICG into normal vitreous results in long-term staining of the visual pathway. *In vivo* counting of ICG-labeled retinal ganglion cells in rats can be performed for several days after injection. Indocyanine green is therefore potentially of interest for use in experimental neurophysiological studies. It is speculated that in humans, the epiretinal application of ICG results in prolonged staining of the visual pathway. Additional studies of long-term toxic effects of ICG on neural cells are warranted before recommending its use in humans as an intraoperative tool for vitreoretinal surgery.
Instrumentation: Conventional infrared photography.

Axon-tracing properties of indocyanine green. Paques M, Genevois O, Regnier A, Tadayoni R, Sercombe R, Gaudric A, Vicaut E. Arch Ophthalmol 2003 Mar; 121 (3), pp. 367–70.

Ophthalmic Artery Dependent Vision/ Pre-Evaluation Prior to Ophthalmic Artery Embolization/[Arteriovenous Malformations of the Orbit/Dural Arteriovenous Fistulae]/ [Orbital Meningiomas/Planum Sphenoidale Meningioma/Juvenile Nasal Angiofibroma] *Lidocaine/Amytal*

Background Information: A number of anteriorly located cranial base and extracranial lesions receive their vascular supply wholly or in part from the ophthalmic artery. Embolization of the ophthalmic artery or its branches can be helpful in the management of these lesions, either as the primary treatment or as an adjunct to surgery.
Application: Determination of the dependency of visual function on patency of the ophthalmic artery.
Route of Administration: Lidocaine/amytal (intra-arterial catheterization).
Dosage: Lidocaine/amytal.
Implementation: Evaluation of the degree visual function is impared by local delivery of anesthetic agents into branches of the ophthalmic artery that supply intra-occular and extra-occular lesions. Such preliminary examinations can be used to predict to what extent visual sight will be affected if selective embolization therapy is implemented for partial or total resolution of lesions. In this context, lidocaine and amytal can be used as a provocative test prior to embolization procedures to assess the role of the ophthalmic artery in vision. In each case, a Tracker No. 18 microcatheter (Target Therapeutics, Inc. Fremont, CA) is navigated into the ophthalmic artery using a steerable guidewire and digital road mapping. Embolic agents include polyvinyl alcohol particles ranging from 350 to 1500 μm in diameter, 2 mm platinum microcoils, and n-butyl-cyanoacrylate. Examples of clinical conditions that can benefit from lidocaine/amytal diagnostic procedures include (i) arteriovenous malformations of the orbit; (ii) dural arteriovenous fistulae; (iii) orbital meningioma; (iv) planum sphenoidale meningioma; and (v) juvenile nasal angiofibroma.
Interpretation and Analysis: In conditions where provocative testing with lidocaine and amytal indicate that the ophthalmic artery has a substantial effect on vision, then embolization procedures are not performed. Applying this protocol, proper case selection, judicious use of embolic agents, and use of provocative testing agents can result in safe embolization of lesions supplied by the ophthalmic artery.

Embolization of neurosurgical lesions involving the ophthalmic artery. Lefkowitz M, Giannotta SL, Hieshima G, Higashida R, Halbach V, Dowd C, Teitelbaum GP. Neurosurgery 1998 Dec; 43 (6), pp. 1298–303.

Parasympathetic Innervation: Ophthalmic *Physostigmine*

Cellular/Molecular Mechanism of Action: Exerts parasympathomimetic activity dependent on the existence of an intact second neuron capable of releasing endogenous stores of neurotransmitter (indirect parasympathomimetic mechanism of action).
Diagnostic Application: Diagnostic evaluation of compromised parasympathetic innervation to the pupillary constrictor muscle.
Dosage: Physostigmine (0.5%) ophthalmic solution applied as a single drop topically to the eye.
Considerations: In normal individuals or those who have lost function of either the upper motor neuron or first lower motor neuron (preganglionic), topical physostigmine in the eye causes relatively rapid pupillary constriction.
Interpretation and Analysis: Lack of rapid pupillary constriction following topical ophthalmic application implies loss of 2nd lower motor neuron function. Normal eyes constrict within 40 to 60 minutes in contrast to eyes with a UMN or preganglionic lesions.

Parasympathetic Innervation: Ophthalmic *Pilocarpine*

Cellular/Molecular Mechanism of Action: Pilocarpine is a cholinomimetic agent that functions as a cholinergic/parasympathomimetic agonist that binds to and activates acetylcholine receptor complexes.
Diagnostic Application: Diagnostic evaluation of compromised parasympathetic innervation to the pupillary constrictor muscle. Pilocarpine has been used to facilitate the examination of conditions such as plateau iris syndrome.
Route of Administration and Dosage: Pilocarpine (2.0%) ophthalmic solution applied as a single drop topically to the eye.
Interpretation and Analysis: Topical pilocarpine will cause a relatively rapid constriction of the denervated pupil that is more extensive and of longer duration than the response observed in a normal eye. An exaggerated response is noted when a lower motor neuron deficit exists. Therefore, lower motor neuron deficits affecting either preganglionic or postganglionic axons will produce the same response to topical pilocarpine. In the normal eye, topical pilocarpine will result in constriction of the pupil in approximately 20 minutes.
Instrumentation: Ophthalmascope.

Parasympathetic Innervation: Ophthalmic *Atropine*

Cellular/Molecular Mechanism of Action: Direct blockade of the muscarinic subclass of acetycholine membrane receptor sites resulting in parasympatholytic activity due to competitive inhibition of the neurotransmitter, acetylcholine at postsynaptic receptor sites.
Diagnostic Application: Dilate the pupil of the eye for diagnostic procedures and to evaluate the integrity of smooth muscle tissue function within the pupil.
Route of Administration: Atropine is effective applied in a topical fashion to the corneal surface of the eye.
Considerations: In neonates of species known to be sensitive to the effects of atropine (e.g. equine), cases may demonstrate clinical signs of gastrointestinal ileus following topical application.

Phenylephrine, Proparacaine, Tropicamide/ Antidote Reversal *Dapiprazole*

Application: Dapiprazole can be used to reverse topical ophthalmic proparacaine 0.5%, tropicamide 1.0%, and phenylephrine 2.5% (1 drop each).
Dosage: Proparacaine 0.5%; tropicamide 1.0%; and phenylephrine 2.5% (1 drop each).
Consideration: The portion of accommodation recovery if any is attributable to a ciliary muscle effect, and what portion results from an increased depth of field attending pupillary constriction is uncertain.
Implementation: Dapiprazole given 30 minutes after dilation with 1 drop each of proparacaine 0.5%, tropicamide 1.0%, and then 5 minutes later another drop of tropicamide 1.0%. Pupil diameter, amplitude of accommodation, conjunctival injection, and intraocular pressure are evaluated. Each of these variables is measured (i) before

instillation of the diagnostic agents; (ii) before the instillation of dapiprazole; and (iii) at 30, 60, 120, and 180 minutes after the final application of dapiprazole.

Human Model: Cases administered topical agents (n = 30 subjects with findings reported from a random study design, masked with placebo, and one eye of each subject used as a control).

Interpretation and Analysis: The average pupillary recovery time for dapiprazole-treated eyes is significantly less than for non-treated eyes. The average pupillary recovery time for dapiprazole-treated eyes is similar to previously published data. Accommodation also shows significantly faster recovery, with comfortable reading ability returning after approximately 30–43 minutes, compared to 66 minutes without dapiprazole. The vast majority of cases exhibit conjunctival hyperemia after dapiprazole administration that persists through the entire 180-minute observation period following dapiprazole administration.

Pupillary Constriction Responsiveness: Normal/ [Anterior Synechia]
Pilocarpine

Cellular/Molecular Mechanism of Action: Pilocarpine is a cholinomimetic agent that functions as a cholinergic/parasympathomimetic agonist that binds to and activates acetylcholine receptor complexes.

Physiological Mechanism of Action: Promotes parasympathomimetic-induced pupillary constriction.

Application: Evaluate the degree of pupillary constriction.

Route of Administration: Ophthalmic (topical).

Dosage: Pilocarpine (4% topical).

Interpretation and Analysis: Slit-image photography before and 30 minutes after the instillation of pilocarpine 4% into one eye demonstrates the range of response of the anterior segment to pilocarpine. Those eyes whose axial anterior chamber depth that becomes more shallow by $\geq$ 0.1 mm reflects shallowing of the periphery of the anterior chamber, while those eyes with axial anterior chamber depth that decreases by $<$ 0.1 mm reflects deepening of the peripheral anterior chamber. The relevance of these findings pertains to the development of peripheral anterior synechiae.

Neoplasia/Ophthalmic Artery Dependent Vision/ Preoperative Risk Evaluation Prior to Selective Ophthalmic Artery Embolization/ [Arteriovenous Malformations of the Orbit/Dural Arteriovenous Fistulae]/[Orbital Meningiomas/ Planum Sphenoidale Meningioma/Juvenile Nasal Angiofibroma]
Lidocaine/Amytal

Background Information: A number of anteriorly located cranial base and extracranial lesions receive their vascular supply wholly or in part from the ophthalmic artery, and embolization of the ophthalmic artery can be helpful in the management of these lesions, either as the primary treatment or as an adjunct to surgery.

Application: Determination of the dependency of visual function on patency of the ophthalmic artery.

Route of Administration: Lidocaine/amytal (intra-arterial catheterization).

Dosage: Lidocaine/amytal.

Implementation: Evaluation of the degree visual function is impared by local delivery of anesthetic agents into branches of the ophthalmic artery that supply intra-occular and extra-occular lesions. Such preliminary examinations can be used to predict to what extent visual sight will be affected if selective embolization therapy is implemented for partial or total resolution of lesions. In this context, lidocaine and amytal can be used as a provocative test prior to embolization procedures to assess the role of the ophthalmic artery in vision. In each case, a Tracker No. 18 microcatheter (Target Therapeutics, Inc. Fremont, CA) is navigated into the ophthalmic artery using a steerable guidewire and digital road mapping. Embolic agents include polyvinyl alcohol particles ranging from 350 to 1500 μm in diameter, 2 mm platinum microcoils, and *n*-butyl-cyanoacrylate. Examples of clinical conditions that can benefit from lidocaine/amytal diagnostic procedures include (i) arteriovenous malformations of the orbit; (ii) dural arteriovenous fistulae; (iii) orbital meningiomas; (iv) planum sphenoidale meningioma; and (v) juvenile nasal angiofibroma.

Interpretation and Analysis: In conditions where provocative testing with lidocaine and amytal indicate that the ophthalmic artery has a substantial effect on vision, then embolization procedures are not performed. Applying this protocol, proper case selection, judicious use of embolic agents, and use of provocative testing agents can result in safe embolization of lesions supplied by the ophthalmic artery.

Embolization of neurosurgical lesions involving the ophthalmic artery. Lefkowitz M, Giannotta SL, Hieshima G, Higashida R, Halbach V, Dowd C, Teitelbaum GP. Neurosurgery 1998 Dec; 43 (6), pp. 1298–303.

Retinal Angiography/Choroid/Retinal Pigment Epithelium/[Premortem Diagnostic Agent]
Rose Bengal/Aluminum Phthalocyanine Tetrasulfonate/Chlorin e6

Cellular/Molecular Mechanism of Action: Rose bengal, aluminum phthalocyanine tetrasulfonate, and chlorin e6 when exposed to certain wavelengths of light can emit visible light energy at different wavelengths.

Physiological Mechanism of Action: Rose bengal, aluminum phthalocyanine tetrasulfonate, and chlorin e6 can emit light energy at different wavelengths that can be cytotoxic to various cell types.

Application: Localization and site of action of photosensitizers in the eye may be important for photodynamic therapy of fundus disorders but remain poorly understood.

Route of Administration: Rose bengal (intravenous), aluminum phthalocyanine tetrasulfonate (intravenous), chlorin e6 (intravenous).

Dosage: Rose bengal (40 mg/kg), aluminum phthalocyanine tetrasulfonate (CASPc) (5 mg/kg), or chlorin e6 (2 mg/kg). Digital fundus fluorescence angiography of rose bengal (2–4 mg/kg), CASPc (2 mg/kg), and chlorin e6 (2 mg/kg).

Implementation: Evaluate the ability of Rose Bengal, aluminum phthalocyanine tetrasulfonate (CASPc), and chlorin e6 to localize intraocular xanthene, phthalocyanine, and chlorin photosensitizers through the application of fluorescence microscopy and digital fundus fluorescence angiography.

The eyes are then enucleated and examined by means of fluorescence microscopy 5, 20, 60, and 120 minutes and 24 hours after dye injection. *In vivo* digital fundus fluorescence angiography can be performed with rose bengal, CASPc, or chlorin e6.

Animal Model: Rabbit (albino).

Interpretation and Analysis: Pathologically, all agents displayed moderate fluorescence from the choroid and retinal pigment epithelium 5 minutes after dye injection. Mild fluorescence detected from the photoreceptor outer segments at 5 minutes is increased by

20 minutes. Angiographic studies with the use of rose bengal, CASPc, and chlorin e6 reveal differences in the pattern and rate of photosensitizer accumulation. Rose bengal, CASPc, and chlorin e6 accumulate rapidly in the choroid and retinal pigment epithelium and less rapidly in the outer retina. Differences in ocular localization of these photosensitizers can be demonstrated. The significance of these findings for potential models for the development of diagnostic agents and photodynamic therapy will require further investigation.
Instrumentation: Fluorescence microscopy.

Localization of rose bengal, aluminum phthalocyanine tetrasulfonate, and chlorin e6 in the rabbit eye. Haimovici R, Ciulla TA, Miller JW, Hasan T, Flotte TJ, Kenney AG, Schomacker KT, Gragoudas ES. Retina 2002 Feb; 22 (1), pp. 65–74.

Retinal Microglial Cell Development: Postnatal/ Optic Nerve Crush Injury
Fluorogold (FG)/4-[4-Didecylaminostyryl]-N-Methylpyridinium Iodide (4Di-10ASP)

Cellular/Molecular Mechanism of Action: Fluorogold (FG) and (4-[4-didecylaminostyryl]-N-methylpyridinium iodide (4Di-10ASP) function as fluorescent dye reagents.
Application: Characterization of retinal microglial cell populations and their development in the postnatal period.
Route of Administration: Injected into the superior colliculus (SC) at postnatal day 0.
Implementation: Fluorogold (FG) or (4-[4-didecylaminostyryl]-N-methylpyridinium iodide (4Di-10ASP) can be injected into the superior colliculus (SC) at postnatal day 0. Retinas can then be examined to quantify microglial cells from P5 to adulthood. Complementary studies can evaluate the reaction of microglia to optic nerve crush in adult animals.
Animal Model: Mouse (postnatal day 0 [P0] to adulthood).
Interpretation and Analysis: Fluorogold (FG) or (4-[4-didecylaminostyryl]-N-methylpyridinium iodide (4Di-10ASP) are transported retrogradely to the RGC soma when either dye is injected into the SC at P0. Some of the labeled RGCs die due to natural apoptosis during this stage of development and are phagocytosed by microglial cells that move into the site of RGC death and become labeled with the same dye. Both dye reagents label RGCs in the contralateral retina and a few RGCs in the retina ipsilateral to the injected SC. The density of labeled RGCs decreases by 22% between P5 and P7. During this phase, microglial cells become visible as they ingest fluorescent detritus of the dying RGCs. Microglial cells are evenly distributed across the entire retinal surface and migrate to the outer plexiform layer. Migrating microglia consecutively alter their morphology from the amoeboid to the ramified form. In terms of intracellular storage of the dyes, resident microglial cells retain the fluorescent dye 4Di-10ASP over a period of 12 months. In contrast, FG is completely transferred from the RGCs and microglial cells to intramural cells (pericytes) of the retinal capillaries after 10 months. This results in delineation of the entire intraretinal vascular network. Finally, resident retinal microglial cells are also activated by injury to the adult optic nerve and actively phagocytose degenerating neurons. Retinal microglial cells can be monitored with vital fluorescent dyes while they migrate across the retina and establish their intraretinal network. It is possible to label microglia with lipophilic dyes, and they remain labeled for an extended period of time. In addition, intramural pericytes can be labeled by slow release of FG from RGCs and microglial cells. Such observations suggest that ingested fluorescent dyes having different properties can be used *in vivo* to study different cell populations of the retina.

Migration of phagocytotic cells and development of the murine intraretinal microglial network: An *in vivo* study using fluorescent dyes. Bodeutsch N, Thanos S. Glia 2000 Oct; 32 (1), pp. 91–101.

Retinal Microvasculature: Pathology/Platelet Endothelium Interactions
Carboxyfluorescein Diacetate Succinimidyl Ester/ Platelets (Fluorescent Labeled)

Cellular/Molecular Mechanism of Action: Carboxyfluorescein diacetate succinimidyl ester interacts with light and functions as a fluorescent indicator/marker molecule suitable for "tracing" the migration of different cell types.
Application: Visualization of *in vivo* platelet behavior in the retinal microcirculation. The methodology allows for quantitative evaluation of platelet dynamics and platelet-endothelial interactions in retinal pathologic conditions.
Route of Administration: Intravascular.
Considerations: Represents the development of a new method for evaluating the *in vivo* dynamics of platelets in the retinal microcirculation and the quantitative investigation of platelet-endothelial interactions.
Implementation: Isolated platelet samples are labeled with carboxyfluorescein diacetate succinimidyl ester. After intravenous administration, platelet behavior in the retinal microcirculation can be evaluated with a scanning laser ophthalmoscope. Images are recorded on S-VHS videotape and analyzed with a computer-assisted image analysis system. The platelet-endothelial interactions in the retinal microcirculation can also be investigated employing lipopolysaccharide-stimulated endothelium or platelets activated with thrombin.
Animal Model: Rat retina.
Interpretation and Analysis: Fluorescent platelets are recognized as distinct dots in the retinal microcirculation that can be traced frame by frame. The velocity of platelets in the retinal arteries, capillaries, and veins is approximately 26.1 ± 6.4, 1.6 ± 0.4, and 19.9 ± 8.2 mm/sec, respectively. In control *in vivo* models, even activated platelets show minimal interaction with retinal endothelial cells. Alternatively, stimulated retinal endothelium shows active platelet-endothelial interactions, with many platelets observed rolling and adhering along the major retinal veins. The interactions between platelets and stimulated endothelial cells are substantially inhibited with the injection of P-selectin monoclonal antibody.

In vivo evaluation of platelet-endothelial interactions in retinal microcirculation of rats. Tsujikawa A, Kiryu J, Nonaka A, Yamashiro K, Nishiwaki H, Tojo SJ, Ogura Y, Honda Y. Invest Ophthalmol Vis Sci 1999 Nov; 40 (12), pp. 2918–24.

Retinal Neuron Visualization
Retrogradely Transportable Fluorescent Dyes

Cellular/Molecular Mechanism of Action: Fluorescent reagents emit color when exposed to certain wavelengths of light energy.
Background Information: Post-traumatic death of mature retinal neurons occurs in glaucoma and after optic nerve injury.
Application: Characterization of dynamic processes associated with cell death applying analytical methods capable of monitoring changes over time.
Implementation: Coupling the development of retrograde-transported fluorescent dyes with modification of conventional epi-

fluorescence microscopy employed to visualize and manipulate retinal neurons applicable to *in vivo* models.

Interpretation and Analysis: Retinal conditions can be effectively monitored, including glaucoma or optic nerve transection, and for evaluating the effectiveness of neuroprotective strategies.

In vivo FM: Using conventional fluorescence microscopy to monitor retinal neuronal death *in vivo*. Thanos S, Indorf L, Naskar R. Trends Neurosci 2002 Sep; 25 (9), pp. 441–4.

Retina: Postischemia/Platelet–Endothelium Interactions
Anti-P-Selectin Immunogloblin/Carboxyfluorescein Diacetate Succinimidyl Ester-Labeled Platelets

Cellular/Molecular Mechanism of Action: Carboxyfluorescein diacetate succinimidyl ester can function as a signal or indicator molecular. Anti-P-selectin monoclonal antibody recognizes and physically binds to membrane-associated P-selectin complexes.

Background Information: Accumulating evidence suggests that platelets play an important role in ischemia-reperfusion injury. To fulfill that role, platelets flowing in the bloodstream would have to interact with retinal endothelial cells and to accumulate in the postischemic retina.

Application: Carboxyfluorescein diacetate succinimidyl ester can be used to label cell populations (e.g. platelets) for the purpose of quantitatively characterizing platelet-endothelial interactions in postischemic retinal structures after transient retinal ischemia.

Route of Administration: Intravenous (labeled platelets).

Implementation: Isolated and partially purified platelet populations are labeled with carboxyfluorescein diacetate succinimidyl ester and administered intravenously after various scheduled reperfusion periods. Platelet-endothelial interactions in postischemic retina are evaluated *in vivo* with a scanning laser ophthalmoscope. Anti-P-selectin monoclonal antibody (mAb) is administered 5 minutes before the injection of labeled platelets. P-selectin gene expression in the postischemic retina is then analyzed by semiquantitative polymerase chain reaction (PCR) analyses.

Animal Model: Rats (Long-Evans strain). Transient retinal ischemia can be induced for 60 minutes by temporal ligation of the ophthalmic nerve.

Interpretation and Analysis: Under basal conditions, infused platelets show minimal interactions with retinal endothelial cells. Conversely, postischemic retinas show active platelet-endothelial interactions. Many platelets can be observed rolling along and adhering to the major retinal veins. The number of rolling and adhering platelets reaches a peak (555 ± 65/mm per minute and 25.8 ± 3.2/mm^2) 12 hours after reperfusion. However, interactions between platelets and postischemic retinal endothelial cells is substantially inhibited by neutralizing P-selectin expressed on endothelial cells using monoclonal antibody. In addition, P-selectin gene expression in postischemic retina corresponds with the time course of platelet-endothelial interactions during the reperfusion period. Platelets actively interact with retinal endothelial cells in the postischemic retina through P-selectin complexes expressed on the retinal endothelial cells.

Instrumentation: Scanning laser ophthalmoscope.

In vivo evaluation of platelet—endothelial interactions after transient retinal ischemia. Nishijima K, Kiryu J, Tsujikawa A, Honjo M, Nonaka A, Yamashiro K, Tanihara H, Tojo SJ, Ogura Y, Honda Y. Invest Ophthalmol Vis Sci 2001 Aug; 42 (9), pp. 2102–9.

Retinal Vascular Permeability Monitoring/ Minimally-Invasive Diagnostic Strategy [Diabetes]
Sodium Fluorescein

Background Information: Diabetic retinopathy is the leading cause of blindness in working-age individuals in the United States. Breakdown of the blood-retinal barrier is one of the earliest events in the progression of diabetic retinopathy. Ideally, therapeutic measures sould be directed at events occurring during this early stage, but there are few sensitive, quantitative methods to assess *in vivo* the retinal vascular barrier.

Application: Quantitative assessment of the retinal vascular barrier.

Implementation: Utilization of 2 fluorescent tracers in combination with fluorescence microangiography to quantitatively assess retinal vascular barrier. A second concept of interest is delineation of the influence of hyperosmolar mannitol (1.6 M) infusions for 5 minutes on the diffusion/penetration of fluorescent tracers.

Animal Model: Rat (Long-Evans strain).

Interpretation and Analysis: Permeability $\times$ surface area/flow (PS/F) describes the characteristics of the retinal vasculature (approximately 0.086 ± 0.031 for $n = 13$). Based on estimates of flow and surface area, estimated permeability of sodium fluorescein is approximately 1.2×10^{-5} cm/sec. Infusion of a hyperosmolar mannitol solution significantly increases PS/F within individual veins and significantly increases flow-weighted PS/F from 0.073 ± 0.028 to 0.16 ± 0.034 ($n = 3$). Quantitative *in vivo* assessment of retinal vasculature can be delineated applying an adapted indicator dilution technique. Dual-tracer fluorescence angiography appears to be a sensitive indicator for detecting increases in the blood-retinal barrier produced by hyperosmolar mannitol. The methodology may be a promising new minimally-invasive strategy that may be adapted to quantitatively track retinal vascular permeability.

Instrumentation: Fluorescence microangiography.

Retinal vascular permeability determined by dual-tracer fluorescence angiography. Russ PK, Gaylord GM, Haselton FR. Ann Biomed Eng 2001 Aug; 29 (8), pp. 638–47.

Retinal Vascular Reperfusion Injury/Leukocyte Rolling Phenomenon Following Ischemic Insult
Acridine Orange

Cellular/Molecular Mechanism of Action: Acridine orange emits a fluorescent color when exposed to certain wavelengths of light energy.

Background Information: Recent reports have shown that ischemic preconditioning induces strong protection against retinal damage from prolonged episodes of ischemia and that such protection is mediated by mechanisms involving the adenosine A1 receptor.

Application: Evaluate quantitatively the effects of ischemic preconditioning on leukocyte-mediated reperfusion injury after transient retinal ischemia and to define the role of the adenosine A1 receptor in these effects.

Implementation: The adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) is administered intramuscularly immediately after ischemic preconditioning. Leukocyte behavior in the retina after 60 minutes of ischemia can then be evaluated *in vivo* with acridine orange digital fluorography.

Animal Model: Rats/male. (Transient retinal ischemia created by temporary ligation of the optic nerve. Ischemic preconditioning [5 minutes of ischemia] is induced 24 hours before 60 minutes of ischemia.)
Interpretation and Analysis: Ischemic preconditioning inhibits leukocyte rolling. The maximum number of rolling leukocytes is reduced to 3.0% at 12 hours after reperfusion ($p < 0.01$). Subsequent leukocyte accumulation is also decreased with ischemic preconditioning. The maximum number of accumulated leukocytes is reduced to 22.6% at 24 hours after reperfusion ($p < 0.01$). These inhibitory effects are suppressed by the administration of DPCPX ($p < 0.0001$). The numbers of rolling leukocytes at 12 hours after reperfusion and accumulated leukocytes at 24 hours after reperfusion is approximately 102.7% (NS) and 83.4% ($p < 0.01$), respectively, compared with numbers without ischemic preconditioning. Ischemic preconditioning, therefore, has an inhibitory effect on leukocyte rolling and subsequent leukocyte accumulation during retinal ischemia-reperfusion injury. Furthermore, the adenosine A1 receptor may play an important role in these inhibitory effects.
Instrumentation: Digital fluorography.

Inhibitory effect of ischemic preconditioning on leukocyte participation in retinal ischemia-reperfusion injury. Nonaka A, Kiryu J, Tsujikawa A, Yamashiro K, Nishijima K, Miyamoto K, Nishiwaki H, Honda Y, Ogura Y. Invest Ophthalmol Vis Sci 2001 Sep; 42 (10), pp. 2380–5.

Sympathetic Innervation: Ophthalmic
Phenylephrine

Cellular/Molecular Mechanism of Action: Adrenergic receptor agonist activity, thereby causing increased sympathetic tone within the autonomic nervous system.
Diagnostic Application: Detect absent or impaired sympathetic (autonomic) nervous system innervation to smooth muscle structures of the pupil (iris).
Route of Administration and Dosage: Ophthalmic (topical) application.
Implementation: Detection of the presence or absence of a dilated pupilary response following topical application to the cornea as a means of detecting and characterizing alterations in adrenergic/sympathetic innervation to ocular anatomical structures.
Interpretation and Analysis: Phenylephrine promotes a hyperactive response in ocular smooth muscles that have lost postganglionic neuron innervation. A normal response analogous to that appreciated in the unaffected contralateral eye is appreciated in instances where postganglionic neuron function is intact. Smooth muscle tissues deprived of lower motor neuron innervation respond in a hyperactive manner when exposed to exogenous (topical) adrenergic neurotransmitter substances or their equivalent pharmacological agonists. Therefore, exposure of ocular structures to phenylephrine after they have lost a substantial amount of sympathetic (autonomic) innervation results in an excessively dilated pupil in contrast to reference (normal) controls.

Sympathetic Innervation: Ophthalmic
Amphetamine

Cellular/Molecular Mechanism of Action: Promotes release of the neuromuscular transmitter norepinephrine from the postganglionic, presynaptic sympathetic lower motor neuron.
Diagnostic Application: Detection and characterization of compromised upper or lower motor neuron dysfunction associated with sympathetic axons innervating ocular structures.
Dosage: Ocular therapeutic preparations formulated for topical application.
Implementation: Differentiation of alterations in preganglionic versus postganglionic adrenergic/sympathetic autonomic nervous system innervation to smooth muscle ophthalmic structures.
Interpretation and Analysis: Detection of the presence or absence of pupillary dilation following topical application. Damage to lower motor neurons results in an absence of functional sympathetic neurotransmitter at the neuromuscular junction. Pupillary smooth muscle tissue that has been deprived of sympathetic (adrenergic) lower motor neuron innervation (autonomic nervous system) is relatively unresponsive to topical amphetamine administration. Explanation for this observation is based upon the concept that amphetamine is incapable of inducing the release of adrenergic neurotransmitter (norepinephrine) if the integrity of the sympathetic lower motor neuron has been seriously compromised or is nonexistent. Alternatively, in the presence of a viable and intact sympathetic lower motor neurons that have been deprived of upper motor neuron innervation, they still can synthesize and release adrenergic neurotransmitter (norepinephrine) from the presynaptic membrane (neuromuscular cleft).

Sympathetic Innervation: Ophthalmic
Dextroamphetamine

Cellular/Molecular Mechanism of Action: Promotes release of adrenergic (catecholamine) neuromuscular transmitter from the sympathetic lower motor neuron.
Application: Identification of the anatomical location of a deficit/lesion affecting input from the adrenergic/sympathetic autonomic nervous system to smooth muscle ophthalmic structures.
Route of Administration: Ophthalmic (topical).
Dosage: Topical application of ophthalmic formulations.
Implementation: Differentiation of alterations in preganglionic versus postganglionic adrenergic/sympathetic autonomic nervous system innervation of smooth muscle ophthalmic structures.
Interpretation and Analysis: Lack of a response can be appreciated when there has been a loss of sympathetic lower moter neuron function. The explanation for this observation is based on the mechanism of dextroamphetamine which only promotes the release of adrenergic neurotransmitter from intact/viable lower motor neurons.

Topical application of dextroamphetamine to the corneal surface when Horner's syndrome is caused by a lesion located above the terminal lower motor neuron of the sympathetic (adrenergic) autonomic nervous system results in a hyperactive pupillary light reflex response. Such a phenomenon is functionally and neuroanatomically analogous to a hyperactive reflex recognized with an upper motor neuron lesion. Detection of a positive response suggests that the postganglionic adrenergic/sympathetic lower motor neuron is viable.
Instrumentation: Ophthalmascope.

SPECIAL SENSES: NON-OPHTHALMIC

Vestibular-Cochlea/Noise-Related Injury/Damage
Gd-DTPA-BMA

Cellular/Molecular Mechanism of Action: The element gandolinium (Gd) is a paramagnetic ion that has its spacial orientation altered within a magnetic fields.
Application: Detection and characterization of normal and noise-damaged cochlea where it can be used as a T1 contrast agent with MRI modalities.

Route of Administration: Gd-DTPA-BMA (intravenous).

Implementation: Evaluate the time course and distribution of the *in vivo* uptake of the gadodiamide chelate bound paramagnetic Gd ion (Gd-DTPA-BMA) throughout the membranous labyrinth of normal and impulse noise-damaged guinea pig cochleae utilizing MRI at 4.7T.

Interpretation and Analysis: Simultaneous signal enhancement of the basal, medial, and apical scala tympani (ST) and scala vestibuli (SV) can be observed within 10 minutes following injection with maximal levels reached at around 100 minutes. Evaluation by ANOVA and post hoc paired t tests reveals statistically significant differences in the levels and rates of Gd uptake-enhancement between the scalae. The basal, medial, and apical ST reveal the most rapid and extensive enhancement throughout the period of active Gd uptake, while the SV show comparatively slower and less enhancement, and the intact scala media (SM) indicate insignificant enhancement. The *in vivo* Gd penetration and enhancement of the membranous SM increases significantly in the noise-damaged cochlea, suggesting lesioning of the cochlear membranes.

Magnetic resonance imaging of the membranous labyrinth during *in vivo* gadolinium (Gd-DTPA-BMA) uptake in the normal and lesioned cochlea. Counter SA, Bjelke B, Borg E, Klason T, Chen Z, Duan ML. Neuroreport 2000 Dec 18; 11 (18), pp. 3979–83.

Vestibular-Cochlear Disease/Ménière's Disease (MD): Predictive Risk and Prediction of Disease Progression/Endolymphatic Hydrops (ELH)
Furosemide and Glycerol/Electrochochleography (EcochG)

Application: Utilization of ECochG and glycerol and furosemide tests to predict the probability that atypical MD will progress to definite MD.

Implementation: Evaluation of vestibular disease conditions utilizing ECochG in combination with glycerol and furosemide tests as a potential avenue for predicting MD.

Human Model: Cases of vestibular disease (n = 1569). The vestibular condition can be preclassified as definite MD, atypical MD, syphilitic labyrinthitis, delayed ELH, sudden hearing loss, cochleovestibulopathy, and sensorineural hearing loss. Cases with atypical MD are divided into 5 categories based on their symptoms.

Interpretation and Analysis: Approximately 97% of cases (e.g. n = 115/118) with definite MD that undergo all 3 tests show a positive result in at least 1 test. In cases that do not satisfy the diagnostic criteria of definite MD but initially have vertigo and/or hearing loss subsequently progress to definite MD. Approximately 92% of cases show at least 1 positive finding in these 3 tests at the initial stage. In those patients that show a negative test result in either ECochG or the glycerol test, the possibility of progression to definite MD is apparently low. Therefore, the combination of ECochG and the glycerol and furosemide tests can assist in diagnosing endolymphatic hydrops (ELH). In this context, ECochG and the glycerol test are effective agents for predicting the progression to definite MD in cases with atypical MD, sudden hearing loss and other cochleovestibular diseases. Such findings also indicate that the pathological state of atypical MD includes both non-ELH and ELH.

Prediction of progression from atypical to definite Ménière's disease using electrocochleography and glycerol and furosemide tests. Kimura H, Aso S, Watanabe Y. Acta Otolaryngol 2003 Apr; 123 (3), pp. 388–95.

3 Endocrine System

GASTROINTESTINAL AND PANCREATIC SYSTEMS

Gastrinoma/Insulinoma/Intestinal Carcinoids
Calcium Ion Infusion with Pentagastrin/Adjunct Secretin Test

Cellular/Molecular Mechanism of Action: Induces the release of gastrin from gastrinoma in response to alterations in serum calcium concentrations.
Application: Detection of endocrine tumors affecting the pancrease and gastrointestinal tract (gastrinoma, insulinoma, intestinal carcinoids)
Route of Administration: Intravenous.
Interpretation and Analysis: Calcium infusion is recommended for the diagnosis of Zollinger-Ellison syndrome when the secretin stimulation test is equivocal. The calcium provocative test in the detection of insulinoma and carcinoid tumors is less reliable than other safer and simpler procedures. Intravenous injection of calcium followed by pentagastrin stimulates the release of somatostatin in patients with somatostatinoma and offers a reliable means for establishing the diagnosis of this tumor. Calcium administration has not proven to be useful in the diagnosis of other endocrine tumors of the digestive system.

Use of calcium provocative test in the diagnosis of gastroenteropancreatic endocrine tumors. Vezzadini C, Poggioli R, Casoni I, Vezzadini P. Panminerva Med 1996 Dec; 38 (4), pp. 255–8.

Gastrinoma/Insulinoma/Intestinal Carcinoids/ Zollinger Ellison Syndrome
Calcium Infusion

Cellular/Molecular Mechanism of Action: Induces release of certain endogenous hormones.
Diagnostic Application: (i) Gastrinoma—gastrin assay; (ii) Zollinger-Ellison syndrome—secretin assay; (iii) somatistatinoma—somatostatin (calcium/pentagastrin combination); (iv) insulinoma—diagnostically not reliable; (v) intestinal carcinoid—diagnostically not reliable.
Interpretation and Analysis: Elevations in gastrin, secretin, and somatostatin following calcium admnistration when above normal reference control values implies the existence of a gastrinoma, Zollinger-Ellison syndrome, or somatistatinoma respectively.

Pancreatic Function/Diabetes (Insulin-Dependent)/ Obesity/Cushing's Syndrome/Acromegaly
Arginine/Glucose Suppression

Physiological Mechanism of Action: Glucose suppresses pancreatic glucagon secretion and increases insulin secretion by beta cells residing within the islets of Langerhans of the pancreas.
Application: Characterization of endocrine/metabolism function in diabetics and conditions of obesity.
Semi-Synthetic Methods: Glucagon can be radioiodinated by the chloramine T technique and purified on QAE-Sephadex A 25 to a specific activity of 225 μCi/μg for use in a glucagon radioimmunoassay method. The standard curve reveals that it is possible to measure (detect) from 12 to 500 pg/ml with sensitivity level of 17.5 pg/ml, precision of 6.3 to 14.9% (CV, within-assay) and 5.6 to 10.7% (CV, between-assay). Recovery is between 82 and 112%.
Implementation: Evaluation of glucagon levels in conditions of diabetes, obesity, acromegaly, and Cushing's syndrome.
Interpretation and Analysis: Fasting plasma glucagon levels in diabetics, obese subjects, acromegalics, and patients with Cushing's syndrome are greater than in normal subjects (22.0 ± 91 pg/ml; mean ± SD). Very low glucagon levels after oral glucose suppression (15.2 ± 3.1 pg/ml) can be detected in normal subjects. Conversely, greatly increased values after arginine can be measured in insulin-dependent diabetics (271.0 ± 132.3 pg/ml).

Evaluation of a sensitive and specific radioimmunoassay for pancreatic glucagon in human plasma and its clinical application. Borghi VC, Wajchenberg BL, Albuquerque RH. Clin Chim Acta 1984 Jan 16; 136 (1), pp. 39–48.

Pancreatic Function in Obesity/Insulin/Glucagon
Glucose

Physiological Mechanism of Action: Glucose suppresses pancreatic glucagon secretion and increases insulin secretion by beta cells residing within the islets of Langerhans of the pancreas.
Route of Administration and Dosage: Glucose: 2-hour glucose infusion (12 mg/kg/min), primed by an initial injection of 0.33 g/kg glucose.
Implementation: Evaluation of pancreatic glucagon (IRG) and insulin (IRI) secretion patterns in conditions of obesity.
Human Model: Obese subjects with normal (n = 7), borderline (n = 5), and pathological carbohydrate tolerance (n = 11), in addition to non-obese healthy controls (n = 19) without a family history of diabetes.
Interpretation and Analysis: Insulin secretion in a very high percentage of obese subjects can be characterized by a significant hyperinsulinemia during the late secretion phase (n = 18/18 or 100%).

The early insulin response (delta IRI-area 0–5 minutes) is significantly reduced in obesity with pathological carbohydrate tolerance. There is no significant difference in fasting IRG levels among controls (29.7 ± 6.1 pmol/l) and pathological glucose tolerance (31.2 ± 4.6 pmol/l). In addition, absolute IRG levels and the IRG concentration pattern during glucose infusion are comparable in all groups confirming no alpha-cell resistance to glucose suppression in obesity, irrespective of normal or impaired carbohydrate tolerance. The molar IRI-IRG ratio is significantly increased during glucose infusion in the vast majority of obese individuals (n = 18/18 or 100%) reflecting a relative anabolic state. There are no correlations between IRG secretion and relative body weight, glucose tolerance, or insulin response to glucose.

Pancreatic glucagon response to glucose in obesity with normal and impaired carbohydrate tolerance. Ratzmann KP, Schulz B, Witt S, Ziegler M. Int J Obes 1981; 5 (2), pp. 163–9.

Pancreatic Neoplasia: Insulin (Islet Cell) Adenoma *Calcium*

Application: Diagnosis of insulin-secreting tumors including islet cell adenoma that are undetectable during the course of preoperative morphology-based imaging modalities.
Route of Administration: calcium test (selective intra-arterial catheterization).
Dosage: calcium gluconate (4 mg/kg/hour) is administered over a 2-hour infusion period.
Considerations: calcium ion *in vitro* plays a fundamental role in regulating insulin secretion but the influence of calcium excess on insulin release *in vivo* has not been clearly defined. The described test is not of diagnostic value for this particular purpose.
Implementation: Evaluate the efficacy of the intra-arterial calcium test for the purpose of localizing sources of hyperinsulinism that remain undetectable with preoperative morphologic studies (e.g., ultrasound, computed tomography, magnetic resonance imaging, endoscopic US, abdominal arteriography). The calcium infusion test, is performed in conjuction with the assay of insulin concentrations in hepatic venous blood after selective intraarterial calcium stimulation.

Human Model: Cases of clinically proven endogenous hyperinsulinism due to tumorous insulin production (e.g., n = 24) Of these cases, approximately 30% (n = 7/24) will have negative morphologic findings (n = 5/7 famales; n = 2/7 males; age range, 30–66 years; mean age, 47 years).
Interpretation and Analysis: Approximately 30% of affected cases will have negative results from morphological analyses (e.g., n = 7/24). Surgical intervention may lead to the detection of solitary insulinomas (e.g., n = 6 = mean diameter, 0.73 cm) and nodular hyperplasia (e.g., n = 1). Calcium tests allow accurate localization of the pathologic source of insulin secretion (e.g., n = 7/7). In < 50% of cases, results of arterial calcium stimulation with hepatic venous sampling (ASVS) may affect intraoperative management (e.g., n = 3/7). Lack of an increase in insulin concentrations after stimulation in the hepatic artery suggests that hepatic metastases is unlikely. ASVS, which is procedurally simpler than transhepatic pancreatic venous sampling, is effective for localizing sources of hyperinsulinism not detected with preoperative morphology-based studies.

Other clinical trials have reported mean calcium plasma levels can increase from 9.6 + 0.4 to 11.6 + 0.8 mg/100 ml. During calcium infusion, blood glucose and plasma insulin concentrations can remain unchanged. Observations noted in these case-based studies suggests that calcium fails to stimulate basal insulin secretion even in cases of organic hyperinsulinism, and that calcium infusion is not helpful as a provocative test in the diagnosis of insulinoma.

Insulinoma and islet cell hyperplasia: value of the calcium intraarterial stimulation test when findings of other preoperative studies are negative. Pereira PL, Roche AJ, Maier GW, Huppert PE, Dammann F, Farnsworth CT, Duda SH, Claussen CD. Radiology 1998 Mar; 206 (3), pp. 703–9.

Pancreatic Neoplasia: VIP Secreting/VIPoma Syndrome: Vasoactive Intestinal Peptide/Pancreatic Peptide/Neurotensin/Enhanced Immunohistopathology Accuracy *Calcium,/Secretin/Pentagastrin*

Diagnostic Application: Detection of patients suffering from pancreatic tumors consistent with VIPoma syndrome.

Background Information: Pancreatic endocrine tumors (PETs) may secrete a variety of peptide hormones, either alone or in combination. Intravenously administered provocative agents have been used to stimulate hormone release to aid in the diagnosis and localization in suspected cases.
Route of Administration: Intravenous injection.
Dosage: Pentagastrin (0.5 mg/kg/5 sec or 0.5 mg/kg/min); rapid calcium infusion (2 mg/kg/min).
Semi-Synthetic Methods: Plasma hormone radioimmunoassays and immunohistochemical studies are performed for a panel of peptide hormones, including VIP, neurotensin, and pancreatic polypeptide (PP). Acid alcohol extracts of tumor specimens are analyzed for the peptide hormones as well.
Implementation: Before surgical intervention, 4 provocative test regimens are administered intravenously after an overnight fast: (i) pentagastrin (0.5 mg/kg/5 sec); (ii) rapid calcium infusion (2 mg/kg/min); (iii) a combination of calcium (2 mg/kg/min) followed by pentagastrin (0.5 μg/kg/min); and (iv) secretin (2 clinical units/kg bolus). Blood samples are collected before each test and 1, 2, 3, 5, and 10 minutes after the infusions.

Case Example: Detailed biochemical investigations and provocative testing in a 26-year-old man with a 5 cm vasoactive intestinal peptide (VIP)-secreting tumor of the head of the pancreas.
Interpretation and Analysis: Increases in the production/release of vasoactive intestinal peptide, neurotensin, and pancreatic polypeptide (PP) can be detected in both serum samples and within tumor biopsies by immunohistochemical analysis. Coexpression of VIP and neurotensin can be seen immunohistochemically within some individual tumor cells. Provocative testing results in maximal stimulation of VIP and neurotensin secretion with pentagastrin administration, which produces increases in plasma levels of VIP and neurotensin over basal levels of 81% and 87%, respectively. Following surgical intervention, plasma levels of VIP, neurotensin, and PP are undetectable before and after administration of pentagastrin. Such observations emphasize the importance of comprehensive biochemical evaluation in VIPoma syndrome cases to detect production of a range of peptide hormones. Administration of intravenous pentagastrin appears to stimulate release of VIP and NT and should be evaluated further as a provocative agent for the diagnosis and follow-up of cases with these tumors.

Stimulation of vasoactive intestinal peptide and neurotensin secretion by pentagastrin in a patient with VIPoma syndrome. Brunt LM, Mazoujian G, O'Dorisio TM, Wells SA Jr. Surgery 1994 Mar; 115 (3), pp. 362–9.

HYPOTHALAMUS AND PITUITARY: GENERAL AND MISCELLANEOUS

Note: Hypothalamic/pituitary functional tests can also be applied to evaluate conditions such as neonatal prematurity and severe cranial trauma.

Anterior Pituitary Function: Combined *Corticotropin-Releasing Factor (CRF)/Growth Hormone-Releasing Factor (GHRF)/Gonadotropin-Releasing Hormone (GnRH)/Thyrotropin-Releasing Hormone (TRH)*

Cellular/Molecular Mechanism of Action: Ovine corticotropin-releasing factor (oCRF) stimulates increased plasma immunoreactive adrenocorticotropin (IR-ACTH) and IR-cortisol.
Dosage: Threshold, half-maximal, and maximal doses that have been applied include 0.01–0.03, 0.3–1, and 3–10 μg/kg, respectively.

Considerations: Cushing's disease patients respond variably, suggesting that oCRF may not be a very useful diagnostic agent in Cushing's syndrome.
Interpretation and Analysis: Side effects occur with increasing frequency, severity, and duration at doses above 1 μg/kg. Ovine CRF has a prolonged duration of action, at least in part because of the long circulating half-life of intact oCRF in plasma. Plasma IR-lipotropins and IR-beta-endorphin rise and fall concomitantly with IR-ACTH after oCRF injection. Arginine vasopressin increases the IR-ACTH response to oCRF 4-fold when given simultaneously with oCRF.

Increasing doses of oCRF given in late afternoon progressively diminish the next morning's circadian rise in plasma IR-ACTH in normal subjects, but not in Addisonian patients or subjects receiving metyrapone, indicating that prolonged oCRF-induced hypercortisolemia is the cause. The combination of oCRF with GHRF, GnRH, and TRH appears to provide a rapid and useful test of combined anterior pituitary function.

Clinical studies with synthetic ovine corticotropin-releasing factor. Orth DN, DeBold CR, DeCherney GS, Jackson RV, Sheldon WR Jr, Nicholson WE, Uderman H, Alexander AN, Island DP, Rivier J, et al. Fed Proc 1985 Jan; 44 (1 Pt 2), pp. 197–202.

Chorea-Acanthocytosis (Familial)/Dopaminergic System
Levodopa/Thyroid-Stimulating Hormone (TSH)/ Thyrotropin-Releasing Hormone (TRH)/Glucose Tolerance

Physiological Mechanism of Action: Levodopa promotes the release of growth hormone-releasing hormone (GHRH).
Application: Assessment of hypothalamic/pituitary function (acromegaly, gigantism, anterior secretion, somatropin secretion). L-dopa can be used to assess growth hormone-releasing hormone synthesis/release. Thyroid-stimulating hormone can be used to evaluate thyroid function and TRH can serve to assess plasma prolactin responses.
Dosage: Glucose (oral tolerance test 75 g).
Implementation: Hypothalamic/pituitary interactions and functionality can be assessed by the collective administration of L-dopa, TSH, TRH, and oral glucose tolerance test. Evaluation of the endocrinology of dopaminergic regulation of the hypothalamic-pituitary axis in conditions of familial chorea-acanthocytosis.

Human Model: Cases of familial chorea-acanthocytosis (n = 3 females, 38–47 years of age).
Interpretation and Analysis: Many cases of familial chorea-acanthocytosis (e.g., n = 3/3) will exhibit low basal levels of triiodothyronine (T-3), in addition to slightly elevated baseline plasma prolactin levels (e.g., n = 2/3). Cases will have delayed plasma TSH responses and plasma prolactin (PRL) excessive responses to TRH, and a low plasma growth hormone-releasing hormone (GRF) response to L-dopa. Such TSH, PRL, and GRF responses represent a secretion pattern due to a hypothalamic disorder that suggests the presence of impaired regulation of hormone secretion by the dopaminergic system, primarily in the hypothalamus. In addition, an increase in growth hormone (GH) response secretion following TRH (paradoxical response) can also be observed in some cases (e.g., n = 2/3), suggesting pituitary involvement. The vast majority of affected cases may display a diabetic profile when evaluated using an oral glucose tolerance test.

Disturbance of hypothalamic-pituitary hormone secretion in familial chorea-acanthocytosis. Terao S, Sobue G, Takahashi M, Miura N, Mitsuma T, Takeda A, Sakakibara T. No To Shinkei 1995 Jan; 47 (1), pp. 57–61.

Cranial Trauma: Hypothalmic/Pituitary Function/Neuroemergency Procedures/Prediction of Unfavorable Neurologic Outcome (Prognosis)/Prolactin (PRL)/Thyroid-Stimulating Hormone (TSH)/Growth Hormone (GH)/Triiodothyronine (T-3)/Thyroxine (T4)
Hypothalamic-Releasing Hormones/Thyrotropin-Releasing Hormone (TRH)

Application: Evaluate the effect of severe head injury on both the basal secretion of pituitary hormones and the response to exogenous synthetic hypothalamic-releasing factor administration.
Route of Administration: Intravenous.
Implementation: Evaluation of daily basal concentrations of GH, PRL, TSH, T-3, and thyroxine during the 1st week following CNS trauma and then again on days 15 and 16 posttrauma. Plasma insulin-like growth factor-I and cortisol are also determined on days 2, 7, and 15. Administration of TRH test can be applied for the evaluation of PRL, TSH, and GH responses on days 1 and 16 after a traumatic incident and a growth hormone-releasing hormone (GHRH) test for the evaluation of GH and PRL responses on days 2, 7, and 15 posttraumatic incident. Outcomes are evaluated at 6 months with the GOS. Triiodothyronine shows low values, even if they are within the normal range; thyroxine remains in the normal range. Significant increases in insulin-like growth factor-I concentrations are observed on both days 7 and 15 compared with day 2 ($p = 0.024$ and $p = 0.034$, respectively).

Human Model: Cases of head injury–induced coma (n = 22) all requiring intubation and mechanical ventilation in addition to invasive monitoring and without previous endocrinologic problems and substitutive therapies.
Interpretation and Analysis: The GH response to GHRH is significantly greater on days 7 and 15 than in the very acute phase ($p < 0.01$ comparing days 7 and 15 vs day 2). Higher GH responses to GHRH can be observed on day 7 in Case Group 1 compared to Case Group 2 (pertaining to both peak and area-under-the-curve values, $p = .018$ and $p = .015$, respectively). No difference in GH response can be detected on days 2 and 15. A "paradoxical" response of GH to TRH can be observed on the day following head trauma (basal vs peak, $p = .002$) but not on day 16. The GH peak response to TRH is greater on day 1 in cases with an unfavorable clinical course (Group 1 vs Group 2, $p < .05$). The TSH response to TRH does not significantly correlate with the severity of trauma but is significantly ($p < .04$) higher in Group 1 cases than in Group 2 cases. Finally, a "paradoxical" PRL response to GHRH administration is present on day 2 (basal vs peak, $p = .0003$), day 7 (basal vs peak, $p = .01$), and on day 15 after the trauma (basal vs peak, $p = .04$). Some of the responses to provocative tests have been identified as "paradoxical" and seem to have a great importance in the definition of prognosis in severe head-injured patients, specifically the GH response to TRH and the PRL response to GHRH that are significantly correlated with outcome.

Provocative hypothalamopituitary axis tests in severe head injury: Correlations with severity and prognosis. Della Corte F, Mancini A, Valle D, Gallizzi F, Carducci P, Mignani V, De Marinis L. Crit Care Med 1998 Aug; 26 (8), pp. 1419–26.

Cranial Trauma: Hypothalamic/Pituitary/Stress Ulceration/Hypothalamic Injury
Thyrotropin-Releasing Hormone (TRH)/Gonadotropin-Releasing Hormone (GnRH)

Background Information: In patients with severe head injury, hypothalamohypophyseal impairment with subsequent hormone

abnormalities has been well documented. Stress ulcer is another commonly encountered problem in such patients. However, little has been reported in the literature about the alterations of pituitary hormones in acute head-injured patients with stress ulcer.

Application: Detection and characterization of alterations in hypothalamic function secondary to detected cranial trauma as modified responses to GnRH and TRH.

Implementation: Evaluate the severity of cranial injury based pituitary function measured by assessment of basal serum levels of pituitary hormones measured before, and in response to TRH, and GnRH.

Human Model: Cases of cranial injury (n = 40 males). Candidate criteria for eligibility include (i) Glasgow coma scale 4–10; (ii) presentation within 24 hours following head injury; (iii) absence of shock or sepsis; and (iv) no past history of peptic ulcer. Stress ulcer is confirmed by endoscopic examination.

Interpretation and Analysis: Approximately two-thirds of cases will show evidence of stress ulcer based upon endoscopic examination (e.g., n = 27/40 or 67.5%). In the absence of stress ulcer, basal serum levels of thyroid-stimulating hormone (TSH), prolactin (PRL), growth hormone (GH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) are within normal range. However, basal levels of PRL in conditions of stress ulcer are abnormally elevated and significantly higher than in the absence of stress ulcers ($p < 0.001$). Basal levels of TSH and GH are significantly lower with stress ulcer than in the absence of stress ulcers ($p < 0.001$). In conditions of stress ulcer, significant increases ($p < 0.001$) in serum TSH, PRL, LH, and FSH levels after thyrotropin-releasing hormone (TRH) and gonadotropin-releasing hormone (GnRH) provocation can be detected. Hypothalamohypophyseal dysfunction and stress ulceration may occur in cases of severe cranial trauma. In the presence of stress ulcers, the abnormalities of pituitary hormones and provocative response of the pituitary with TRH and GnRH reveal normal pituitary function with hypothalamic insufficiency. Therefore, stress ulcers in acute head-injured patients are closely associated with hypothalamic damage.

Hypothalamic dysfunction in acute head-injured patients with stress ulcer. Hwang SL, Lieu AS, Howng SL, Hsieh JS, Huang TJ. Kaohsiung J Med Sci 1998 Sep; 14 (9), pp. 554–60.

Hypopituitary Conditions/Hallerman-Streiff Syndrome (HSS)/Hypothalamic/Pituitary Axis Function
Thyrotropin-Releasing Hormone (TRH)/Adenocorticotropic Hormone (ACTH)

Cellular/Molecular Mechanism of Action: Metyrapone inhibits enzymatic activity of 11β-hydroxylase thereby reducing cortisol production

Application: Evaluation of anterior hypopituitarism in HSS in conditions of ketotic hypoglycemia, micro-genitalia, and short stature.

Interpretation and Analysis: Endocrine evaluation may show low levels of T4 and TSH levels, suggesting hypothalamic hypothyroidism that can be confirmed by TRH stimulation. Performing a metyrapone test can confirm ACTH deficiency as a contributing factor to the ketotic hypoglycemia. A superagonist GnRH test can reveal concominant hypothalamic GnRH deficiency. A growth hormone provocative test in such instances can conclusively demonstrate a complete deficiency of growth hormone production. Imaging studies of the brain using MRI methodologies can detect evidence supportive of hypopituitarism in such cases. Although facial findings may not be completely classical for HSS, it has been suggested racial background may influence such phenotypic characteristics. Endocrine evaluation of HSS patients with clinical signs representative of hypopituitarism provides an opportunity to treat this condition in a manner that will substantially improve the quality of life of these patients.

Hallermann-Streiff syndrome with hypopituitarism contributing to growth failure. Pivnick EK, Burstein S, Wilroy RS, Kaufman RA, Ward JC. Am J Med Genet 1991 Dec 15; 41 (4), pp. 503–7.

Hypopituitary Conditions/Adenocorticotropic Hormone (ACTH) Deficiency/ACTH Reserve Status
Corticotropin-Releasing Hormone (CRH)/Adrenocorticotropin-Releasing Hormone/Metyrapone/Urine 17-OH Corticosteroids/Serum Deoxycorticosterone

Cellular/Molecular Mechanism of Action: Corticotropin-releasing hormone from the hypothalamus promotes release/synthesis of ACTH from the pituitary, which in turn promotes glucocorticoid release/synthesis from the adrenal cortex.

Diagnostic Application: Detection of conditions associated with pituitary dysfunction or developmental abnormalities.

Route of Administration: Corticotropin-releasing hormone (intravenous bolus).

Dosage: Metyrapone administration (450 mg/m^2/dose every 4 hours × 7 doses); ovine CRH (1 μg/kg).

Implementation: Evaluation of hypopituitary conditions as a function of comparative responses of (i) ACTH and cortisol (F) to (ii) CRH administration and its effect on urinary 17-OH corticosteroids (17-OHCS); and (iii) metyrapone-induced alterations in serum deoxycorticosterone (DOC). The CRH test is performed 3 days before or 3 weeks after the metyrapone test for the purpose of establishing ACTH reserve status.

Human Model: Cases of hypopituitary conditions (n = 16 with glucocorticoid therapy withheld for a minimum of 3 weeks before testing.)

Diagnostic Interpretation: In ACTH-intact hypopituitary conditions (e.g., n = 9 cases postmetyrapone 17-OHCS > 12.2 mumol/m^2/d; DOC ≥ 11.5 nmol/L), the peak F (497–773 nmol/L) and ACTH (5.2–22 pmol/L) responses to CRH stimulation are similar to those of normal subjects (F peak = 554–993 nmol/L and ACTH peak = 6–25 pmol/L at 15 to 60 minutes). In cases with partial ACTH deficiency (e.g., n = 1 case: postmetyrapone 17-OHCS = 10.5 mumol/m^2/d; DOC = 6 nmol/L), the peak F response is low and delayed (246 nmol/L at 180 minutes), while the peak ACTH response is normal (7 pmol/L). Severely ACTH-deficient conditions (e.g., n = 6 cases: postmetyrapone 17-OHCS < 5.4 mumol/m^2/d; DOC ≤ 3.4 nmol/L) have a low F response at 15 to 90 minutes in all, with a delayed rise at 120 to 180 minutes in response to CRH administration (n = 3/6), whereas ACTH responses are variable: absent or low, normal, delayed, or persistently exaggerated. Therefore, the CRH-stimulated F response pattern in hypopituitary patients is comparable to urinary 17-OHCS and serum DOC response to metyrapone administration.

Laboratory Instrumentation: ELISA-based immunodiagnostic assay of serum samples.

Comparison of adrenocorticotropin and adrenal steroid responses to corticotropin-releasing hormone versus metyrapone testing in patients with hypopituitarism. Riddick L, Chrousos GP, Jeffries S, Pang S. Pediatr Res 1994 Aug; 36 (2), pp. 215–20.

Multiple Sclerosis (MS): Hyperprolactinemia
Thyrotropin-Releasing Hormone (TRH) Stimulation/Bromocriptine

Cellular/Molecular Mechanism of Action: Thyrotropin-releasing hormone promotes prolactin (PRL) secretion.
Background Information: Before the onset and during experimental allergic encephalomyelitis (EAE; animal counterpart of MS), the peripheral concentrations of PRL have been found to be elevated and bromocriptine has been shown to attenuate such attacks. Prolactin may play a role in the immunology of MS.
Application: Multiple sclerosis cases reportedly display elevations in plasma PRL concentrations.
Interpretation and Analysis: Multiple sclerosis cases have slightly but significantly higher PRL levels at baseline (10.2 ± 1.6 vs 6.4 4 ± 0.57 ng/ml, p = 0.042) than do matched reference control subjects, however, values are within the normal range. Prolactin levels post-TRH are significantly higher in patients with MS with peak PRL levels higher than negative reference controls (57.08 ± 6.144 vs 32.94 ± 4.92 ng/ml, p = 0.006). The area under the curve for PRL is also higher in affected patients than in controls (3421.87 ± 394.53 vs 2317.62 ± 257.22 ng/ml, p = 0.030).

Prolactin secretion is increased in patients with multiple sclerosis. Azar ST, Yamout B. Endocr Res 1999 May; 25 (2), pp. 207–14.

Pituitary: Anterior Pituitary Function/Postop Transsphenoidal Hypophysectomy
Thyrotropin-Releasing Hormone (TRH)/Corticotropin-Releasing Hormone (CRH)/Growth Hormone-Releasing Hormone (GHRH)/Gonadotropin-Releasing Hormone (GnRH)

Physiological Mechanism of Action: Thyrotropin-releasing hormone is produced in the hypothalamus and promotes the release of thyroid-stimulating hormone (TSH) from the anterior pituitary (adenohypophysis). Pituitary adenomas that secrete excessive adenocorticotropic hormone (ACTH) are also responsive to exogenous TRH administration.
Considerations: Much safer than exogenous administration of either ACTH or dexamethasone in instances of pre-existing laminitis in equine patients suffering from Cushing's disease.
Application: Detection and characterization of conditions associated with pituitary-dependent hyperadrenal corticism.
Implementation: Evaluation of anterior pituitary function can be assessed using several hypophysiotropic-releasing hormones including CRH, GHRH, GnRH, and TRH. These agents can be applied simultaneously (combined test) or administered individually.
Following administration, serum concentrations of ACTH, cortisol, growth hormone (GH), luteinizing hormone (LH), and prolactin (PRL) are measured. Complementary analyses can include the determination of TSH pars intermedia function established by the measurement of basal plasma alpha-MSH concentrations, in concert with the assessment of adrenocortical function by measuring baseline urinary corticoid/creatinine ratios.
Interpretation and Assessment: In cases of pituitary-dependent hyperadrenocorticism, excessive elevations in serum (systemic) cortisol can be detected. In normal individuals, exogenous TRH administration does not result in elevated concentrations of systemic cortisol.

In dogs, 8 weeks after hypophysectomy (n = 39), basal plasma ACTH, cortisol, GH, LH, PRL, and TSH concentrations are significantly lower than prior to surgery. In dogs with elevated alpha-MSH concentrations (e.g., n = 7/39), values return to the normal level after surgery. In the combined anterior pituitary function test there are no plasma GH, LH, PRL, and TSH responses to stimulation, whereas plasma ACTH and cortisol responses are usually small but significant. Remission of hyperadrenocorticism may be expected in most dogs (e.g., n = 35/39) compared to a small number of recurrences (e.g., n = 3/39 within 16 months postoperatively). At 8 weeks posthypophysectomy, such dogs are not discernible, with respect to residual pituitary and adrenocortical function, from dogs (e.g., n = 32) with persistent remission. Urinary corticoid/creatinine ratios in the latter group of dogs usually do not increase during the 22-month period after hypophysectomy. In contrast to presurgical findings, at 8 weeks after hypophysectomy there are significant positive correlations between baseline urinary corticoid/creatinine ratios and basal levels and responses for ACTH, indicating return to normal function of the pituitary-adrenocortical axis. Therefore, among the adenohypophyseal cells present in the sella turcica after hypophysectomy, the corticotropes have a distinct behavior. Much more so than the other cell types, the unaffected corticotropes tend to remain functional, or a repressed reserve fraction of corticotropes may become functional. This may be due to the removal of the hypothalamic influence of a postulated corticotropin-release inhibiting factor or a diminished inhibitory influence of a postulated paracrine factor. The corticotropes may maintain normocorticism but may also lead to mild recurrence after relatively long periods of remission.

Residual pituitary function after transsphenoidal hypophysectomy in dogs with pituitary-dependent hyperadrenocorticism. Meij BP, Mol JA, Bevers MM, Rijnberk A. J Endocrinol 1997 Dec; 155 (3), pp. 531–9.

Pituitary Tumors (Non-Functional)/Hypothalamic/Pituitary Function
Corticotropin-Releasing Hormone (CRH)/Growth-Hormone Releasing Hormone (GHRH)/Thyrotropin-Releasing Hormone (TRH)/Luteinizing Hormone-Releasing Hormone (LHRH)/ Lysine Vasopressin (LVP)

Cellular/Molecular Mechanism of Action: Corticotropin-releasing hormone, GHRH, TRH, and LHRH are produced in the hypothalamus and are selectively transported internally to the pituitary gland, where they promote the release of their corresponding stimulating hormone or hormone mediator.
Physiological Mechanism of Action: Corticotropin-releasing hormone promotes the release/synthesis of pituitary adenocorticotropic hormone (ACTH); GHRH promotes the synthesis/release of pituitary growth hormone; TRH promotes the synthesis/release of pituitary thyroid-stimulating hormone (TSH); LHRH promotes the synthesis/release of pituitary leutinizing hormone (LH); LVP exerts properties similar to pituitary antidiuretic hormone (ADH).
Application: Combined anterior pituitary tests can be utilized to evaluate pituitary function in conditions of non-functioning pituitary tumors.
Dosage: Hypothalamic-releasing hormones (CRH: 1 μg/kg; GHRH: 1 μg/kg; TRH: 500 μg; LHRH: 100 μg; and LVP: 5 mU/kg = relatively small dose).
Implementation: Evaluation of non-functioning pituitary tumors utilizing a combined anterior pituitary test consisting of CRH, GHRH, TRH, LHRH, and LVP.
Human Model: Cases of non-functioning pituitary tumors (n = 16; and n = 8 normal healthy reference control volunteers).
Interpretation and Analysis: Non-functional pituitary tumors can be due to a number of conditions, including chromophobe adenoma (e.g., n = 9/16); craniopharyngioma (e.g., n = 3/16); Rathke's

cleft cyst (e.g., n = 2/16); intrasellar cyst (e.g., n = 1/16); and tuberculum sella meningioma (e.g., n = 1/16). In normal subjects, LVP enhances the secretion of ACTH induced by CRH, but has no significant effect on the secretion of other anterior pituitary hormones. In the combined test with 5 releasing hormones, the plasma ACTH and cortisol responses are not impaired in the majority of cases before pituitary surgery. Serum TSH, prolactin (PRL), and follicle-stimulating hormone (FSH) responses are not impaired in approximately 82%, 70%, and 67% of these cases, respectively, while serum LH and GH responses are impaired in 67% and 73% of cases, respectively. Following pituitary surgery, responses of these hormones to combined testing are similarly impaired in more than 75% of patients. These results indicate that plasma ACTH, cortisol, and serum TSH responses are fairly good before pituitary surgery but are impaired significantly after surgery. Few subjects experience any serious adverse effects related to diagnostic testing procedures. Therefore, the combined testing protocol with hypothalamic hormones is a convenient and useful method for evaluating pituitary function.

Combined anterior pituitary function test using CRH, GRH, LH-RH, TRH and vasopressin in patients with non-functioning pituitary tumors. Hashimoto K, Makino S, Hirasawa R, Takao T, Kageyama J, Ogasa T, Ota Z. Acta Med Okayama 1990 Jun; 44 (3), pp. 141–7.

HYPOTHALAMUS, PITUITARY, AND ADRENAL AXIS

Addison's Disease: Adrenal Dysfunction/Primary Adrenocortical Insufficiency (PAI)/Severe Secondary Adrenocortical Insufficiency (SAI)
Adenocorticotropic Hormone (ACTH)/Corticotropin-Releasing Hormone (CRH)/Insulin

Background Information: The rapid ACTH injection test is an indirect screening test for adrenocortical insufficiency. As a supplement to this test, the practicability of single measurements of plasma cortisol, ACTH, aldosterone, and plasma renin activity (PRA) has been evaluated as a definitive diagnostic test of PAI.
Application: Diagnosis and therapeutic surveillance in Addison's disease.
Implementation: Evaluation of the value of PRA measurements during treatment with hydro- and fludrocortisone (HC and FC) as a guide for correct mineralocorticoid substitution utilizing a rapid ACTH test and single measurements of 4 hormones (all tests between 0800–0900 hours) compared. Studies can be complemented by acquiring single-hormone measurements in conditions of pituitary disease (most having mild to severe SAI).

Human Model: Cases with PAI (n = 45); pituitary disease (n = 46; cortisol and ACTH only), most having mild to severe SAI; and normal healthy volunteers (n = 55).
Interpretation and Analysis: The rapid ACTH test is abnormal in the vast majority of case with PAI (e.g., n = 41/41 or 100% PAI). Plasma ACTH, PRA, and the ratios of ACTH/cortisol and PRA/plasma or urinary aldosterone are clearly elevated in the vast majority of cases with PAI (e.g., 100% PAI). The ACTH/cortisol ratio also can distinguish a high percentage of cases with PAI from those with severe SAI (100%), but not always control subjects from those with SAI. Dynamic tests (CRH or insulin tests) are indicated if severe SAI is suspected. Primary adrenocortical insufficiency and involvement of zona fasciculata and glomerulosa function can be diagnosed with high reliability by measuring cortisol, ACTH, aldosterone, and PRA either together with the rapid ACTH test or later, after a short interval of steroid substitution. PRA measurements during treatment with HC and FC correlate better with the mineralocorticoid dose than plasma potassium and sodium levels. PRA measurement is a valuable guide for FC replacement therapy. It should be titrated into the upper normal range to avoid undertreatment and overtreatment.

Diagnosis and therapy surveillance in Addison's disease: rapid adrenocorticotropin (ACTH) *test* and measurement of plasma ACTH, renin activity, and aldosterone. Oelkers W, Diederich S, Bähr V. J Clin Endocrinol Metab 1992 Jul; 75 (1), pp. 259–64.

Addison's Disease: Primary Adrenal Dysfunction/ Adrenocortical Dysfunction/Preclinical Adrenal Dysfunction
Adenocorticotropic Hormone (ACTH) (High- vs Low-Dose)

Background Information: The presence of 21-hydroxylase autoantibodies (21OHAb) is a marker of adrenal autoimmunity and can be used to identify subjects with preclinical Addison's disease. The low-dose (1 μg) ACTH test (LDT) is more sensitive than the high-dose (250 μg) test (HDT) for the diagnosis of pituitary adrenal insufficiency, but no information is available on the use of an LDT in subjects with autoimmune adrenalitis and primary adrenal insufficiency.
Application: Detection and characterization of primary adrenal insufficiency and accurate identification of preclinical adrenal dysfunction conditions.
Implementation: Evaluation of cortisol responses to both an LDT and an HDT preparation for the diagnosis of early adrenocortical dysfunction in cases with adrenal autoantibodies. In all cases evaluated, the LDT is followed by a sequential HDT that can be used as a control test of the sensitivity and specificity of the LDT.

Human Model: Cases with a normal health status (n = 12 volunteers), and cases positive for 21OHAb fractions but without clinical signs of Addison's disease (n = 11 subjected to an LDT) identified by screening 920 patients with one or more organ-specific autoimmune diseases.
Interpretation and Analysis: In healthy subjects, the peak cortisol levels after the LDT are similar to those after the classical HDT. In 21OHAb-positive subjects, the LDT shows a pathological response in approximately 45% of cases (e.g., n = 5/11) and the diagnostic concordance between the results of the LDT and those of the HDT is 100%. The majority of cases with pathological LDT are also positive for adrenal cortex autoantibodies (ACA; n = 5/5), and the majority of these have high levels of basal ACTH (n = 4/5). A limited number of cases with pathological LDT develop clinical Addison's disease within 4 months after performing diagnostic testing procedures (e.g., n = 1). Low-dose ACTH test has a high diagnostic sensitivity and specificity for primary adrenal insufficiency and suggests that it can accurately identify subjects with preclinical adrenal dysfunction.

Low-dose (1 μg) ACTH test in the evaluation of adrenal dysfunction in preclinical Addison's disease. Laureti S, Arvat E, Candeloro P, Di Vito L, Ghigo E, Santeusanio F, Falorni A. Clin Endocrinol (Oxf) 2000 Jul; 53 (1), pp. 107–15.

Adrenal Insufficiency
Basal Cortisol vs Corticotropin-Releasing Hormone (CRH)

Cellular/Molecular Mechanism of Action: Corticotropin-releasing hormone promotes the release of pituitary adrenocorticotropic hormone (ACTH) that in turn promotes the synthesis/release of cortisol from the adrenal cortex.

Application: Detection and evaluation of adrenal insufficiency conditions (comparison of basal cortisol measurements to CRH stimulation tests).
Implementation: Putative peak cortisol cut points for the CRH test and basal cortisol cut points can be determined by receiver operating characteristic (ROC) analysis with the insulin tolerance test as reference test.

Human Model: Cases of suspected hypothalamic-pituitary-adrenal disease (n = 54; n = 20 normal healthy reference controls).
Interpretation and Analysis: In healthy controls, CRH leads to a mean peak cortisol of 594.8 ± 21.7 nmol/liter. The lower limit of a normal response is calculated as approximately 400 nmol/liter. Receiver operating characteristic analysis of peak cortisol levels during CRH testing of patients with suspected hypothalamic-pituitary-adrenal disease suggests an optimal peak cortisol cut point of ≤ 377 nmol/liter for the diagnosis of adrenal insufficiency and a 96% specificity but poor sensitivity of 76%. The baseline cortisol in the healthy control group shows a mean of 439.3 ± 24.9 nmol/liter, resulting in a lower limit of 267 nmol/liter. Receiver operating characteristic analysis of cases suggests the highest accuracy for basal cortisol levels of 285 nmol/liter or more for the diagnosis of adrenal insufficiency (100% sensitivity and 61% specificity). Within such a patient group, a cortisol of more than 98 nmol/liter excludes adrenal insufficiency among those without the disorder, yielding 100% specificity. Using these criteria of upper (285 nmol/liter) and lower (98 nmol/liter) cutoff points with high sensitivity and specificity can reduce the number of individuals who need provocative testing. Basal cortisol is less expensive and is therefore suggested for use as a 1st-line test of adrenal insufficiency. Because of the low sensitivity of the human CRH test, it has not been recommended as a 2nd test procedure.

Diagnosis of adrenal insufficiency: Evaluation of the corticotropin-releasing hormone test and Basal serum cortisol in comparison to the insulin tolerance test in patients with hypothalamic-pituitary-adrenal disease. Schmidt IL, Lahner H, Mann K, Petersenn S. J Clin Endocrinol Metab 2003 Sep; 88 (9), pp. 4193–8.

Adrenal Mineral Corticoid Function: Aldosterone-Producing Adenoma (APA)/Dopamine Excretion: Urinary
Metoclopramide/High-Salt Intake/Low-Salt Intake

Physiological Mechanism of Action: Metoclopramide enhances cholinergic activity at muscarinic synapses and suppresses/antagonizes CNS dopaminergic activity. Normally, dopaminergic input inhibits aldosterone secretion.
Background Information: Impairment of renal dopamine excretion on a high-salt diet (HS) may account for increased blood pressure (BP) in hypertensive subjects. Whether such impairment of dopaminergic activity exists in the adrenal gland is unknown.
Application: Differentiate degrees of dopaminergic inhibition of aldosterone secretion in cases of aldosterone-producing adenomas.
Route of Administration: Metoclopramide (intravenous).
Dosage: Metoclopramide (10 mg).
Implementation: Evaluation of APA conditions on diets of different salt levels at the end of which a 24-hour ambulatory BP recording is obtained in concert with the measurement of daily urine catecholamine and the performance of a metoclopramide test.

Human Model: Cases of unilateral APA (n = 15 APA) fed a low-salt diet (LS) for 1 week, followed by another week on an HS diet. At the end of each diet period, 24-hour ambulatory BP recording, daily urine catecholamine measurement, and a metoclopramide test is performed.

Interpretation and Analysis: A high-salt diet increases both daytime and nighttime blood pressure (BP at $p < 0.001$), and urine dopamine excretion ($p < 0.01$). Intravenous metoclopramide injection increases plasma aldosterone concentrations (PAC) on both diets. The area under the curve for PAC between LS and HS is not different, but the area under the curve for PAC increment is greater on HS ($p < 0.05$). Less than half of affected cases with increment areas on HS greater than those on LS by 50% are termed "suppressible" (n = 6/15) and the remainder are referred to as "non-suppressible." On a high-salt diet, so-called "suppressible" cases have greater urine dopamine and less urine norepinephrine excretions ($p < 0.05$). The "non-suppressible" cases have a greater percentage increase of nighttime BP by HS than the suppressible subjects (for systolic BP, 13.1% vs 4.5%, $p < 0.01$; for mean BP, 12.0% vs 5.1%, $p < 0.01$, respectively), but no difference in daytime BP.

Two subtypes of APA can be defined according to their responses to metoclopramide during salt manipulation. On an HS diet, the non-suppressible subjects, with less dopaminergic inhibition of aldosterone secretion, have less urinary DA excretion and greater BP elevation. The renal and adrenal dopaminergic activities are regulated in a parallel fashion.

Dopaminergic modulation of aldosterone secretions on changes of sodium intake in aldosterone-producing adenoma. Wu KD, Chen YM, Chu TS, Chueh SC, Tsai GC, Tseng YZ, Hsieh BS. Am J Hypertens 2002 Jul; 15 (7 Pt 1), pp. 609–14.

Adrenalcortical Axis/Hypothalamic-Pituitary-Adrenal Function/Influence of Age and Gender
Synthetic Human hCRH (MCI-028)

Cellular/Molecular Mechanism of Action: The synthetic human corticotropin-releasing hormone (hCRH) MCI-028 induces increases in plasma adenocorticotropic hormone (ACTH) concentrations that in turn promote elevations in plasma cortisol.
Application: Evaluation of the integrity and function of anterior pituitary function.
Route of Administration: Synthetic human corticotropin-releasing hormone (IV).
Dosage: Synthetic human corticotropin-releasing hormone (MCI-028 100 μg).
Interpretation and Analysis: Mean maximum concentrations of plasma ACTH and cortisol after intravenous administration of MCI-028 are 3.0 and 2.0 times their basal concentrations, respectively, and there are no significant age or sex differences in responses. Good reproducibility can be observed in responses when a 2nd dose is administered 1–2 weeks later. Although slight adverse reactions such as mild and transient hot flushing may be observed, these sequellae are usually not serious in nature.

Human corticotropin-releasing hormone (hCRH) test: Sex and age differences in plasma ACTH and cortisol responses and their reproducibility in healthy adults. Tanaka K, Shimizu N, Imura H, Fukata J, Hibi I, Tanaka T, Nakagawa S, Fujieda K, Takebe K, Yoshinaga K, et al. Endocr J 1993 Oct; 40 (5), pp. 571–9.

Adrenocortical Insufficiency
Theophylline

Cellular/Molecular Mechanism of Action: In contrast to most processes involved in the activation of endocrine processes, theophylline circumvents binding to receptor complexes and instead directly increases cAMP by inhibiting phosphodiesterase-mediated hydrolysis.

Physiological Mechanism of Action: Theophylline is a nonspecific stimulus of several endocrine processes.
Application: Used for determination of the potential adrenocortical reserve, and also as a diagnostic indicator of pathogenesis.
Route of Administration: Theophylline (intravenous, 4-hour infusion).
Examples of Clinical Implementation: In conditions of documented adrenal insufficiency (lack of cortisol secretion in response to exogenous adenocorticotropic hormone [ACTH]), an infusion of theophylline can stimulate significant increases in serum cortisol in a large percentage of patients. Types of conditions where theophylline infusion has been of diagnostic and therapeutic value include ACTH insensitivity, ACTH deficiency, and idiopathic primary adrenal failure. Conditions that may not respond to theophylline infusion include autoimmune-mediated adrenalitis. Theophylline can also promote renin release, and in situations where an intact zona glomerulosa exists, theophylline can cause a secondary rise in aldosterone equivalent to that produced by diuresis and upright poster.
Interpretation and Analysis: The preservation of cortisol responsiveness to theophylline, after the loss of sensitivity to ACTH, may be related to either the duration of the adrenal insufficiency or to the etiological mechanism. Patients with autoimmune adrenalitis may undergo more rapid and complete adrenocortical destruction, thereby losing sensitivity to both ACTH and theophylline. In contrast, in cases of insufficient or ineffective ACTH stimulation there may be a condition of receptor failure before the loss of intracellular function. Thus, responsiveness to intravenous theophylline may serve not only as a probe of potential adrenocortical reserve but also as an indicator of pathogenesis.

The use of theophylline as an *in vivo* probe of adrenocortical function. Geffner ME, Lippe BM, Kaplan SA, Itami RM. J Clin Endocrinol Metab 1982 Jul; 55 (1), pp. 56–60.

Cushing's Disease: Corticotropin-Releasing Hormone (CRH) Test Failure/Catecholamines (Adrenal)/11β-Hydroxylase/Tyrosine Hydrolase/ Phenylethanolamide/Pituitary Adenocorticotropic Hormone (ACTH)-Dependent Cushing's Syndrome Differential
Metyrapone/Dexamethasone/[ACTH]/CRH

Cellular/Molecular Mechanism of Action: Metyrapone inhibits enzymatic activity of 11β-hydroxylase, thereby reducing cortisol production.
Application: Characterize the role of glucocorticoids on activity of the adrenal gland.
Considerations: The hypothalamic CRH system and the sympathetic nervous system are anatomically and functionally interconnected and hormones of the hypothalamic-pituitary-adrenocortical axis contribute to the regulation of catecholaminergic systems.
Implementation: Evaluation of plasma and adrenal catecholamines, tyrosine hydroxylase (TH), and phenylethanolamine N-methyltransferase (PNMT) mRNA expression following injections of metyrapone or dexamethasone.
Interpretation and Analysis: Metyrapone-induced corticosterone depletion elicits transsynaptic TH activation, implying the occurrence of non-cholinergic neurotransmission. This may involve neuropeptides other than pituitary adenylate cyclase activating polypeptide (PACAP).

The CRH test may sometimes be useful in the differential diagnosis of Cushing's syndrome, because most patients with pituitary ACTH-dependent Cushing's syndrome (Cushing's disease) respond to CRH, but those with other causes of Cushing's syndrome usually do not. However, about 10% of Cushing's disease patients fail to respond to CRH.

Metyrapone-treated rats have significantly lower epinephrine and higher norepinephrine production than control rats. Metyrapone increases TH protein synthesis and TH mRNA expression, whereas its administration does not affect PNMT mRNA expression. Dexamethasone restores plasma and adrenal epinephrine concentrations and increases PNMT mRNA levels, which is consistent with an absolute requirement of glucocorticoids for PNMT expression. Adrenal denervation completely abolishes metyrapone-induced TH mRNA expression. Blockage of cholinergic neurotransmission by nicotinic or muscarinic receptor antagonists does not prevent the metyrapone-induced rise in TH mRNA. Finally, PACAP adrenal content is not affected by metyrapone.

Cushing's Disease/Differentiation: Normal/ Pituitary-Dependent Hyperadrenocorticism (PDH) vs Adrenocortical Tumors
Corticotropin-Releasing Hormone

Application: Differentiation between normal dogs and those suffering from pituitary-dependent hyperadrenocorticism from those with adrenocortical tumors.
Route of Administration: Intravenous (single injection).
Considerations: Corticotropin-releasing hormone is of ovine origin.
Interpretation and Analysis: Normal cases and those suffering from pituitary-dependent hyperadrenocorticism display elevations in plasma adenocorticotropic hormone (ACTH) and cortisol. Conversely, in conditions of adrenocortical tumors, no marked changes in plasma ACTH or cortisol can be detected.

Cushing's Disease/Pituitary vs Adrenal/ Treatment Monitoring
Dexamethasone (Low-Dose)/Dexamethasone (High-Dose)/Adenocorticotropic Hormone (ACTH) (Treatment Monitoring)

Cellular/Molecular Mechanism of Action: Dexamethasone suppresses release/synthesis of CRF, thereby suppressing ACTH release/synthesis. Pituitary ACTH promotes the release of glucocorticoid from the adrenal gland cortex.
Background Information: Some pituitary tumors will have some residual cortisol receptor complexes.
Application: Detection of hyperadrenocorticism and differentiation between primary adrenocortical tumors and pituitary-dependent hyperadrenocorticism.
Route of Administration: Intravenous.
Dosage: Low-dose dexamethasone suppression test; high-dose dexamethasone suppression test (0.1 mg/kg).
Considerations: Adrenal tumors, like many endocrine-related neoplasias, are usually unilateral and are for the most part not responsive to dexamethasone suppression tests. In cases of iatrogenic Cushing's syndrome, cases will have low-normal baseline cortical levels and will not respond to exogenous ACTH administration. Adrenocortical tumors function autonomously.
Implementation: The low-dose dexamethasone suppression test can be applied as an initial screening test in dogs suspected of suffering from Cushing's disease. The high-dose dexamethasone suppression test can be used as a discriminating test for distinguishing between primary adrenal vs primary pituitary conditions. Plasma cortisol concentrations are measured at baseline ($< T = 0$) and then at 4 hours and 8 hours post–dexamethasone administration.

Interpretation and Analysis: Plasma cortisol levels should be below 1.4 μg/dl at 8 hours postinjection. In contrast, following high-dose dexamethasone administration, plasma cortisol concentrations should normally be below 50% of resting values at 4 and 8 hours postadministration. Following the low-dose dexamethasone suppression test, if the observed 4-hour and 8-hour plasma cortisol concentrations are both elevated, then either a primary adrenal or primary pituitary lesion may be the cause of the Cushing's condition. Abdominal ultrasound can then be beneficial in identifying abnormalities in adrenal gland morphology. In situations when the adrenal gland can be imaged with ultrasound (or nuclear scintigraphy) and no abnormalities are detected, then a primary pituitary lesion should be suspected. In situations where abnormal adrenal gland morphology is detected, then an endogenous ACTH or high-dose dexamethasone suppression test can be performed. In scenarios where the ACTH level is normal or elevated and there is evidence of a positive response to the high-dose dexamethasone suppression test (50% suppresion), then the condition is classified as being pituitary-dependent hyperadrenocorticism.

In scenarios where the 4-hour plasma cortisol concentration after the low-dose dexamethasone suppression test is low, but the 8-hour concentration is relatively high, then a primary pituitary lesion is the most likely etiological cause for the condition. In dogs, 85% of Cushing's cases are primary pituitary conditions in contrast to 15% of cases that are primary adrenal cases (often with locally invasive lesions).

Canine and Feline Endocrinology and Reproduction. Feldman EC, Nelson RW. W.B. Saunders Co., Philadelphia (2nd edition: copyright 1996).

Cushing's Disease/Differentiation: Normal/ Pituitary-Dependent Hyperadrenocorticism (PDH) vs Adrenocortical Tumors
Corticotropin-Releasing Hormone

Application: Differentiation between normal dogs and those suffering from PDH from those with adrenocortical tumors.
Route of Administration: Intravenous (single injection).
Considerations: Corticotropin-releasing hormone is frequently of ovine origin.
Interpretation and Analysis: Normal cases and those suffering from pituitary-dependent hyperadrenocorticism display elevations in plasma adenocorticotropic hormone (ACTH) and cortisol. Conversely, in conditions of adrenocortical tumors, no marked changes in plasma ACTH or cortisol can be detected.

Cushing's Disease/Syndrome: Hypothalamic/Pituitary Function/Adenoma: Corticotropic/[Ectopic Adenocorticotropic Hormone (ACTH) Syndrome Differential]
Desmopressin (DDAVP)

Cellular/Molecular Mechanism of Action: Desmopressin promotes pituitary ACTH release.
Application: Utilization of the DDAVP test for the diagnosis of ACTH-dependent Cushing's syndrome. In this context, the DDAVP stimulation test, involving the determination of plasma ACTH levels after DDAVP injection, seems useful for discriminating Cushing's disease from normality, and may serve to facilitate the differentiation between Cushing's disease and ectopic ACTH syndrome.
Route of Administration: DDAVP (intravenous).
Dosage: DDAVP (5 μg).
Implementation: Evaluation of ACTH levels in conditions of Cushing's disease in response to DDAVP administration.

Human Model: Cases of Cushing's disease (e.g., n = 10/10; n = 3 Addison's disease cases; n = 5 Cushing's disease cases in remission; n = 3 cases with ectopic ACTH syndrome).
Interpretation and Analysis: Injection of DDAVP promotes increases in plasma ACTH levels more than 200% of the basal levels in most cases of Cushing's disease (e.g., n = 10/10), but remain < 150% in all normal subjects (n = 11/11); Addison's disease (n = 3/3); Cushing's disease in remission (n = 5/5); and ectopic ACTH syndrome (n = 3/3). Peak levels of plasma cortisol after the DDAVP stimulation are 159 ± 14% in cases with Cushing's disease, and < 150% of the basal levels in other case types (n = 5 case groups). Some cases of Cushing's disease periodically responded to DDAVP only in the active stage. In controlled *in vitro* investigations, DDAVP directly stimulates ACTH release from corticotropic adenoma cells through V1b but not V2 vasopressin receptors.

The DDAVP stimulation test, involving the determination of plasma ACTH levels after 5 μg DDAVP injection, seems useful for discriminating Cushing's disease from normality, and may serve to facilitate the differentiation between Cushing's disease and ectopic ACTH syndrome.

Desmopressin stimulation *test* for diagnosis of ACTH-dependent Cushing's syndrome. Sakai Y, Horiba N, Tozawa F, Sakai K, Kuwayama A, Demura H, Suda T. Endocr J 1997 Oct; 44 (5), pp. 687–95.

Cushing's Disease: Pituitary-Dependent/Thyroid Function
Thyrotropin-Releasing Hormone (TRH)/Corticotropin-Releasing Hormone (CRH) / Growth Hormone-Releasing Hormone (GHRH)/Gonadotropin-Releasing Hormone (GnRH)

Physiological Mechanism of Action: Thyrotropin-releasing hormone is produced in the hypothalamus and promotes the release of thyroid-stimulating hormone (TSH) from the anterior pituitary (adenohypophysis). Pituitary adenomas that secrete excessive adenocorticotropic hormone (ACTH) are also responsive to exogenous TRH administration.
Considerations: Much safer than exogenous administration of either ACTH or dexamethasone in instances of pre-existing laminitis in equine patients suffering from Cushing's disease.
Application: Detection and characterization of conditions associated with pituitary-dependent hyperadrenal corticism.
Implementation: Evaluation of anterior pituitary function can be assessed using several hypophysiotropic releasing hormones including CRH, GHRH, GnRH, and TRH. These agents can be applied simultaneously (combined test) or administered individually.

Following administration, serum concentrations of ACTH, cortisol, growth hormone (GH), luteinizing hormone (LH), and prolactin (PRL) are measured. Complementary analyses can include the determination of TSH pars intermedia function established by the measurement of basal plasma alpha-MSH concentrations, in concert with the assessment of adrenocortical function by measuring baseline urinary corticoid/creatinine ratios.
Interpretation and Assessment: In cases of pituitary-dependent hyperadrenocorticism, excessive elevations in serum (systemic) cortisol can be detected. In normal individuals, exogenous TRH administration does not result in elevated concentrations of systemic cortisol.

In dogs, 8 weeks after hypophysectomy (n = 39), basal plasma ACTH, cortisol, GH, LH, PRL, and TSH concentrations are significantly lower than prior to surgery. In dogs with elevated alpha-MSH

concentrations (e.g., n = 7/39), values return to the normal level after surgery. In the combined anterior pituitary function test there are no plasma GH, LH, PRL, and TSH responses to stimulation, whereas plasma ACTH and cortisol responses are usually small but significant. Remission of hyperadrenocorticism may be expected in most dogs (e.g., n = 35/39) compared to a small number of recurrences (e.g., n = 3/39 within 16 months postoperatively). At 8 weeks posthypophysectomy, such dogs are not discernible, with respect to residual pituitary and adrenocortical function, from dogs (e.g., n = 32) with persistent remission. Urinary corticoid/creatinine ratios in the latter group of dogs usually do not increase during the 22-month period after hypophysectomy. In contrast to presurgical findings, at 8 weeks after hypophysectomy there are significant positive correlations between baseline urinary corticoid/creatinine ratios and basal levels and responses for ACTH, indicating return to normal function of the pituitary-adrenocortical axis. Therefore, among the adenohypophyseal cells present in the sella turcica after hypophysectomy, the corticotropes have a distinct behavior. Much more so than the other cell types, the unaffected corticotropes tend to remain functional, or a repressed reserve fraction of corticotropes may become functional. This may be due to the removal of the hypothalamic influence of a postulated corticotropin-release inhibiting factor or a diminished inhibitory influence of a postulated paracrine factor. The corticotropes may maintain normocorticism but may also lead to mild recurrence after relatively long periods of remission.

Residual pituitary function after transsphenoidal hypophysectomy in dogs with pituitary-dependent hyperadrenocorticism. Meij BP, Mol JA, Bevers MM, Rijnberk A. J Endocrinol 1997 Dec; 155 (3), pp. 531–9.

Cushing's Disease/Syndrome: Hypothalamic/Pituitary Function
Desmopressin/[Corticotropin-Releasing Hormone (CRH) Testing]

Cellular/Molecular Mechanism of Action: Desmopressin stimulates adenocorticotropic hormone (ACTH) and hence cortisol release.
Background Information: To assess the ability of desmopressin administration to stimulate ACTH/cortisol secretion in cases with Cushing's disease, either before or after surgery, and in patients with other states characterized by ACTH hypersecretion, and to compare the results with those obtained after CRH testing.
Application: Desmopressin stimulates ACTH and hence cortisol release in Cushing's disease. It may be a useful test in cases with doubtful responses to corticotropin-releasing hormone (CRH) test, in those exhibiting responses to CRH indistinguishable from those of normal subjects and in the postoperative follow-up of Cushing's disease. In some cases with abolished or reduced cortisol feedback at the hypothalamic-pituitary level, the sensitivity of normal corticotrophs to desmopressin is enhanced.
Route of Administration: Desmopressin (intravenous), CRH intravenous.
Dosage: Desmopressin (10 μg), CRH (1 μg/kg).
Implementation: Evaluation of plasma ACTH and serum cortisol levels following the administration of desmopressin, CRH or saline in conditions of Cushing's disease.

Human Model: Cases of Cushing's disease (n = 17; n = 1/17 occult ectopic ACTH syndrome; n = 5/17 Addison's disease; n = 3/17 bilaterally adrenalectomy for Cushing's syndrome; n = 4/17 normal subjects; n = 13 pituitary adenomectomy for Cushing's disease).

Interpretation and Analysis: In cases of Cushing's disease with microadenoma (e.g., n = 16) a positive ACTH/cortisol rise can occur in a relatively large number of cases after both CRH and desmopressin (e.g., n = 11/16). A small percentage of these cases are responsive only to desmopressin (e.g., n = 2/16) or only to CRH (e.g., n = 2/16) or demonstrate equivocal response (e.g., n = 1/16). The persistence of a hormonal response to desmopressin after pituitary adenomectomy for Cushing's disease correlates with unsuccessful surgery, while, contrary to CRH, absent ACTH/cortisol rises are found in cured cases 1 and 12 months after operation. In some cases of suspected of ectopic ACTH hypersecretion, desmopressin and CRH administration do not cause any ACTH/cortisol rise (e.g., n = 1/1). Significant ACTH rises can occur after both desmopressin and CRH testing in cases of Addison's disease. Majority of cases that have been adrenalectomized for Cushing's syndrome show rises in ACTH levels after CRH (n = 3/3), while a similar response after desmopressin occurs in only a fraction of these cases (e.g., n = 1/3).

Effect of desmopressin on ACTH and cortisol secretion in states of ACTH excess. Colombo P, Passini E, Re T, Faglia G, Ambrosi B. Clin Endocrinol (Oxf) 1997 Jun; 46 (6), pp. 661–8.

Cushing's Disease/Syndrome
Adenocorticotropic Hormone (ACTH) Stimulation/Dexamethasone Suppression/Dexamethasone Clearance

Cellular/Molecular Mechanism of Action: Exposure to pituitary ACTH promotes the synthesis/release of adrenal cortisol. Dexamethasone suppresses hypothalamic corticotropin-releasing hormone (CRH) release, thereby diminishing pituitary ACTH synthesis/release.
Application: Detection and characterization of Cushing's syndrome (canine).
Implementation: Evaluation of cortisol concentrations in conditions of Cushing's syndrome applying dexamethasone-suppression and ACTH-stimulation testing protocols. Complementary analyses can include determination of the clearance of exogenous dexamethasone used in the dexamethasone-suppression test (normal and cushingoid dogs).

Animal Model: Healthy, mixed-breed dogs and dogs with clinical signs of Cushing's syndrome.
Interpretation and Analysis: Absolute concentration of cortisol is less in resting, dexamethasone-suppressed, and ACTH-stimulated plasma samples, when measured by high-performance liquid chromatography (HPLC) relative to cortisol concentrations determined by radioimmunoassay. Diagnosis of Cushing's syndrome can be made using either method. Cortisone and corticosterone concentrations alone are not diagnostic of Cushing's syndrome, but when used in conjunction with cortisol determinations, they provide additional criteria for laboratory confirmation of the clinical diagnosis of Cushing's syndrome. Plasma concentrations of exogenous dexamethasone are constant during the 3-hour test in normal dogs. However, in 75% of dogs with Cushing's syndrome, exogenous dexamethasone is cleared completely in the 3-hour test. Thus, dexamethasone clearance rates are excessively fast in most dogs with Cushing's syndrome.
Instrumentation: High-performance liquid chromatography (cortisone and corticosterone).

Diagnosis of canine Cushing's syndrome based on multiple steroid analysis and dexamethasone turnover kinetics. Lothrop CD Jr, Oliver JW. Am J Vet Res 1984 Nov; 45 (11), pp. 2304–9.

Cushing's Disease/Syndrome/Adrenocortical Tumor: Hyperadrenocorticism/Heat-Resistant Isoenzyme of Alkaline Phosphatase (SIAP)
Dexamethasone/[High-Dose vs Low-Dose]/Alkaline Phosphatase Activity Levels/Cortisol/Creatinine (Urine C/C)

Physiological Mechanism of Action: Dexamethasone suppresses the production/release of hypothalamic corticotropin-releasing hormone that in turn results in suppression of the production/release of pituitary adenocorticotropic hormone (ACTH) release/production, thereby reducing adrenalcorticoid release/synthesis in healthy subjects.
Background Information: Spontaneous hyperadrenocorticism is a common and well-recognized endocrine disorder occurring in dogs. The biochemical diagnosis of hyperadrenocorticism rests on the documentation of excessive glucocorticoid levels or metabolites in urine or blood. The diagnosis of canine hyperadrenocorticism is historically developed from human methods and based on non-dynamic and dynamic tests and visualization. In some cases the different ranges of endocrine parameters or metabolites necessitate the modification of human protocols. Elevation of the activity of alkaline phosphatase (AP) and its heat-resistant isoenzyme (SIAP) induced by endogenous or exogenous glucocorticoid excess raises the suspicion of hyperadrenocorticism. Cortisol values in morning urine are related to creatinine concentrations to correct for differences in urine concentration.
Application: Characterization of hyperadrenocorticism conditions.
Dosage: Dexamethasone (low-dose 0.01 mg/kg of body weight).
Implementation: Evaluation of Cushing's disease/syndrome utilizing the dexamethasone suppression test.
Animal Model: Canine (dogs suffering from hyperadrenocorticism).
Interpretation and Analysis: Theoretically, the administration of dexamethasone (DX) at a relatively "low-dose" level can inhibit the pituitary secretion of ACTH and, in turn, decreasing endogenous cortisol secretion for as long as 24 to 48 hours. Dexamethasone administration to dogs with a functioning adrenocortical tumor does not affect the plasma cortisol concentration at any time following its administration.

The high-dose dexamethasone suppression test (HDDS) is based on the observation that the function of an adrenocortical tumor is independent of pituitary ACTH regulation and they completely suppress ACTH secretion through the autologous production of cortisol; therefore, regardless of dose, dexamethasone is never able to suppress cortisol secretion. HDDS can be combined with the measurement of urinary cortisol/creatinine (c/c) ratio from morning urine samples on 3 consecutive days. In cases of non-suppressible urinary c/c ratios, one has to speculate on differentiating adrenal tumor (AT) from non-suppressible pituitary-dependent hyperadrenocorticism (PDH) due to a pituitary tumor arising from the intermediate lobe. Radiocholesterol scintigraphy is a less frequently used technique in the diagnosis of canine Cushing's syndrome (CCS); however, it has the same advantages in the localization and characterization of adrenocortical diseases as in humans.

Diagnosis of hyperadrenocorticism in dogs as compared to human diagnostic methods: a review. Thuróczy J; Balogh L; Huszenicza G; Jánoki GA; Kulcsár M. Acta Vet Hung 1998; 46 (2), pp. 157–73.

Cushing's Disease/Syndrome
Adrenocorticotropic Hormone (ACTH)

Cellular/Molecular Mechanism of Action: Promotes glucocorticoid production/release from the adrenal cortex.
Diagnostic Application: (i) Apply systemically in an attempt to differentiate between adrenal and pituitary lesions. (ii) Determine the relative degree of neonatal prematurity by establishing the functional integrity of the thalamic-pituitary-adrenal triad.
Route of Administration and Dosage: Porcine aqueous gelatin ACTH (2.2 IU/kg), or synthetic ACTH (0.25 mg/dog or 0.5 mg/dog of synthetic tetracosactrin) injected intramuscularly.
Considerations: Administration of ACTH has been proposed to be a simple test of practical value for the diagnosis of juvenile adrenocortical function. In equine patients, TSH has been used successfully to diagnosis Cushing's syndrome. Since corticosteroids readily promote equine laminitis, provocative testing with TSH is considered to be much safer.
Implementation: Approximately 30 minutes before, and then 30-, 60- (dog), and 120-minute blood samples are collected following ACTH injection for the measurement of plasma cortisol concentrations.
Diagnostic Interpretation: Excessively high concentrations of cortisol measured following an ACTH administration are diagnostic for Cushing's disease/syndrome. In this context, mean plasma cortisol levels are approximately 184% higher than baseline values after administration and there are no significant differences related to age or sex. Increased diagnostic value of ACTH can be achieved when applied in combination as the dexamethasone suppression/ACTH stimulation test (CDS/AST). In dogs, approximately 86% of pituitary-dependent Cushing's cases have an abnormal cortisol concentration following ACTH administration.
Laboratory Instrumentation: Assay of cortisol concentrations by either RIA or ELISA.

Cushing's Disease/Syndrome
Dexamethasone Suppression and Adenocorticotropic Hormone (ACTH) Stimulation Test

Cellular/Molecular Mechanism of Action: Depresses synthesis and release of ACTH at the level of the hypothalamus, which is mediated through a negative feedback mechanism.
Diagnostic Application: Differentiation between pituitary and adrenal lesions. The normal cortisol negative feedback mechanism becomes dysfunctional in patients with ACTH-secreting pituitary adenomas. In cases with an intact pituitary adrenal axis, cortisol suppresses pituitary ACTH secretion.
Route of Administration and Dosage: Dexamethasone is administered by intravenous injection at a low and high dosage level of 0.010 to 0.015, and 0.1 mg/kg body weight, respectively.
Implementation: Dexamethasone at any dosage fails to suppress cortisol concentrations in conditions of pituitary adenoma. Alternatively, high dexamethasone doses can suppress ACTH secretion in most cases (canine) of pituitary-dependent hyperadrenal corticism.
Laboratory Instrumentation: Assay of cortisol concentrations by either RIA or ELISA.

Cushing's Disease/Syndrome
Metyrapone

Cellular/Molecular Mechanism of Action: Metyrapone inhibits the enzymatic activity of 11β-hydroxylase, thereby reducing normal cortisol production.
Diagnostic Application: Differentiation between pituitary-dependent hyperadrenocorticism (PDH) and adrenal tumor (AT).
Route of Administration: Metyrapone (oral).
Dosage: Metyrapone (25 mg/kg q6 hours for 4 consecutive treatments begining at noon).
Considerations: Diagnostic results may be considered invalid if excessively high premetyrapone cortisol concentrations are detected. Another test method (e.g., adenocorticotropic hormone [ACTH] assay, high-dose DST) or repeating the procedure is recommended if postmetyrapone 11-DOC is between 1.2 and 2.0 μg/dl, or inadequate postmetyrapone cortisol suppression occurs with and 11-DOC < 2.0 μg/dl, or if the premetyrapone cortisol value is > 13 μg/dl. Sequelae that may be observed while performing this provocative test relate to the increased risk for developing acute adrenal insufficiency. If signs of weakness develop, immediately discontinue administration, collect an appropriate blood sample, and administer a glucocorticoid (IM, IV).

The corticotropin-releasing hormone (CRH) test may sometimes be useful in the differential diagnosis of Cushing's syndrome, because most patients with pituitary ACTH-dependent Cushing's syndrome (Cushing's disease) respond to CRH, but those with other causes of Cushing's syndrome usually do not. However, about 10% of Cushing's disease patients fail to respond to CRH.
Implementation: Metyrapone is administered (4 consecutive treatments) and postmetyrapone blood samples are collected into EDTA at 6 hours for measurement of cortisol and 11-DOC following administration of the last dose.
Interpretation and Analysis: Patients (e.g., canine) that are suffering from PDH display an accumulation of 11-deoxycortisol (11-DOC, precortisol metabolite), which occurs simultaneously with a decline in systemic cortisol concentrations. In contrast, patients suffering from AT do not significantly increase 11-DOC concentrations but do reflect a decline in cortosol levels. Advantage of this test and the endogenous plasma ACTH assay is their ability to positively identify AT patients.

Postmetyrapone cortisol concentrations should be $\leq$ 50% of preadministration levels. Patients should be suspected of suffering from PDH when postmetyrapone 11-DOC concentrations are ≥ 2 μg/dl (2–12.5 μg/dl), while values ≤ 1.2 μg/dl (0.1–1.2 μg/dl) strongly suggest a diagnosis of AT.
Laboratory Instrumentation: Assay techniques require the application of either ELISA or RIA methodologies.

Cushing's Disease/Syndrome/Cortisol/Growth Hormone
Metyrapone

Cellular/Molecular Mechanism of Action: Inhibits enzymatic activity of 11β-hydroxylase, thereby reducing cortisol production.
Background Information: Transsphenoidal surgery (TSS) is considered the primary treatment for Cushing's disease (CD). Options for treatment of postoperative persisting hypercortisolemia are pituitary radiotherapy (RT), repeat TSS, or bilateral adrenalectomy.
Application: Delineation of the effect of the early morning rise in plasma cortisol concentration on plasma GH concentrations. Metyrapone is administered early in the morning to reduce plasma cortisol concentrations.
Dosage: Metyrapone (750 mg, 4 hourly from 3.00 am). Plasma GH and cortisol concentrations are measured every 30 and 60 minutes, respectively, from 22.00 until 14.00.
Considerations: Exogenous steroids affect plasma growth hormone (GH) concentrations and hypothalamic somatostatin levels either directly, by stimulating the anterior pituitary gland, or indirectly by partially inhibiting the response of the pituitary gland to stimuli. The effect of the circadian rhythm of cortisol on plasma GH concentrations is unknown. In normal subjects, highest plasma GH concentrations are recorded within the 1st 2 hours of onset of sleep. During the early morning the plasma GH concentration decreases and the plasma cortisol concentration increases.
Interpretation and Analysis: Metyrapone causes a significant reduction in the rise of plasma cortisol concentration, but the GH concentration is identical on both occasions. The morning rise in cortisol concentration has no acute regulatory effect on the plasma GH concentration.

Cushing's Disease/Syndrome
Metyrapone/Corticotropin-Releasing Hormone (CRH)/Arginine Vasopressin (AVP)

Cellular/Molecular Mechanism of Action: Metyrapone inhibits enzymatic activity of 11β-hydroxylase, thereby reducing cortisol production.
Application: Metyrapone can be exploited for its additive or synergistic effect as an adenocorticotropic hormone (ACTH) secretagogue (reduces glucocorticoid inhibition of CRH → ACTH releasing effect).
Route of Administration: (i) Ovine CRH (intravenous) alone; (ii) AVP (intramuscular) alone; (iii) metyrapone (oral).
Dosage: (i) Ovine CRH (1 μg/kg BW) alone; (ii) AVP (10 U) alone; (iii) combinations of CRH and AVP; and (iv) CRH after pretreatment with metyrapone (1 g every 4 hours for 3 doses; CRH + MET).
Considerations: The CRH test may sometimes be useful in the differential diagnosis of Cushing's syndrome, because most patients with pituitary ACTH-dependent Cushing's syndrome (Cushing's disease) respond to CRH, but those with other causes of Cushing's syndrome usually do not. However, about 10% of Cushing's disease patients fail to respond to CRH.

In normal subjects, plasma pregnenolone sulfate (PS) levels are high at birth but are decreased during the 1st year of life in relation to the pattern of involution of the fetal adrenal zone. Thereafter, PS levels, in contrast with those of DHAS, do not show the abrupt rise characteristic of the adrenarche but increase very progressively till adulthood. The response of PS to various provocative tests of adrenal and pituitary function (ACTH and metyrapone stimulation, dexamethasone suppression) has been established in normal subjects. The measurement of plasma PS levels in basal conditions as well as in response to dynamic tests is very useful in the diagnosis of various adrenal and pituitary diseases in children.

In pediatric patients, endogenous Cushing's syndrome is an infrequent condition almost always due to either adrenal gland tumors or Cushing's disease. Adrenal gland tumors account for 70% of Cushing's syndromes in young pediatric patients. They cause rapidly progressive hypercorticism not due to increased ACTH production (elevated plasma and urine cortisol levels, very low ACTH and LPH levels unchanged by dexamethasone, metyrapone, or CRH). Imaging techniques determine the size and spread of the tumor and look for

metastases. Following surgical removal, patients with indicators of malignant disease (tumor weight above 30 g, extracapsular spread or metastases, independently from pathological data) are given op'DDD. The 2nd etiology in pediatric patients is attributed to Cushing's disease and in peripubertal patients causes overweight with delayed statural gain. Due to the possibility that elevated ACTH production (based on interpretation of a positive dexamethasone suppression test and provocative metyrapone and CRH tests) may be due to pituitary adenoma, the existence and characteristics of such lesions should be further pursued by magnetic resonance imaging methodologies. Resolution by removal of such lesions results in recovery in approximately 50% of affected cases. Other therapeutic tools include (i) op'DDD; (ii) radiation therapy delivered to the pituitary; and (iii) bilateral adrenalectomy as the last resort given the high risk of post-adrenalectomy pituitary tumor (50% of pediatric patients). Other causes are exceedingly rare: primary nodular hyperplasia of the adrenal glands and production of ACTH by a non-pituitary tumor. Corticosteroid treatment is the most common cause of Cushing's syndrome in children.

Interpretation and Analysis: The rates of non-response (ACTH increment, $< 35\%$; cortisol increment, $< 20\%$) to AVP and CRH alone are 26% and 8%, respectively. Essentially all patients respond to CRH applied in combination with AVP. The lack of response is not due to improper administration or rapid metabolism of the agonist, because plasma CRH and AVP concentrations are similar in responder and non-responder patients. A synergistic ACTH response to the combination of CRH and AVP occurs in 65% of the patients. Pretreatment with metyrapone increases basal plasma ACTH levels in most patients and induces the greatest mean peak ACTH response to CRH. However, approximately 8% of patients do not respond to the combination of CRH and metyrapone with an ACTH increment of 35% or more. Because all Cushing's disease patients in previous reports responded to CRH in combination with AVP, whereas 8% failed to respond to CRH alone, it has been proposed that CRH in combination with the administration of AVP may provide a more reliable test in the differential diagnosis of ACTH-dependent Cushing's syndrome than administration of CRH alone. Whether this improved sensitivity is accompanied by unaltered specificity for Cushing's disease must be tested in patients with chronic ectopic ACTH syndrome.

Plasma corticotropin and cortisol responses to ovine corticotropin-releasing hormone (CRH), arginine vasopressin (AVP), CRH plus AVP, and CRH plus metyrapone in patients with Cushing's disease. Dickstein G, DeBold CR, Gaitan D, DeCherney GS, Jackson RV, Sheldon WR Jr, Nicholson WE, Orth DN. J Clin Endocrinol Metab 1996 Aug; 81 (8), pp. 2934–41.

Cushing's Disease/Syndrome
Exogenous T_3

Cellular/Molecular Mechanism of Action: Thyroid hormone (T_3) functions in a negative feedback mechanism to inhibit the release of thyrotropin-releasing hormone (TRH) from the hypothalamus, which in turn regulates the secretion/release of thyroid-stimulating hormone (TSH) from the pituitary. Ultimately, elevations in T_3 result in decreased release of endogenous thyroid hormone.

Diagnostic Application: #1: Utilized in the T_3 suppression test based upon the concept that exogenous thyroid hormone inhibits secretion of TSH in normal individuals. Conversely, in instances when endogenous thyroid hormone secretion occurs in an autonomous fashion (independent of TSH regulation), then exogeneous T_3 has little influence on the secretion of thyroid hormone. The T_3 suppression test is of particular diagnostic benefit in patients (e.g., feline) with high-normal T_3 and T_4 concentrations while simultaneously displaying signs of hyperthyroidism.

Diagnostic Application: #2: Patients suffering from Cushing's syndrome may suffer from secondary hypothyroidisms caused by corticosteroid suppression of pituitary TSH secretion. In addition, corticosteroids decrease peripheral deiodination of T_4 to T_3, and binding of thyroid hormone to plasma proteins that enhance metabolism. Dogs with Cushing's syndrome will display an elevation in T_4 following a TSH stimulation test, but both baseline levels and post-TSH serum concentrations of T_4 are well below normal.

Route of Administration and Dosage: In feline conditions, the T_3 suppression test can be performed by determining basal serum concentrations of T_4, administering exogenous T_3 at a dosage of 25 μg every 8 hours for 2 days with the last and 7th dose administered on the morning of the 3rd day. Serum is collected 96 hours after the last T_3 injection and T_4 concentrations measured.

Interpretation and Analysis: Suppression of the T_4 concentration in a blood sample collected at this time period is approximately $\geq 50\%$ depressed in a normal feline patient (≤ 1.5 μg/ml).

Laboratory Instrumentation: Assay of T_3/T_4 concentrations by either RIA or ELISA.

Cushing's Disease/Syndrome: Hypothalamic-Pituitary Dysfunction
Dexamethasone/[Cortisone, Corticosterone, and Cortisol]/[High-Performance Liquid Chromatography (HPLC) vs Radioimmunoassay]

Cellular/Molecular Mechanism of Action: Dexamethasone suppresses hypothalamic corticotropin-releasing hormone production, thereby decreasing the release/production of pituitary adenocorticotropic hormone (ACTH), which in turn then decreases production/release of cortisol from the adrenal cortex under normal conditions.

Background Information: Previous investigations have compared cortisol concentrations measured by HPLC and radioimmunoassay using samples collected from healthy, mixed-breed dogs and in dogs with clinical signs of Cushing's syndrome. Absolute concentrations of cortisol are less in resting, dexamethasone-suppressed, and ACTH-stimulated plasma samples, when measured by HPLC, relative to cortisol concentrations determined by radioimmunoassay. Diagnosis of Cushing's syndrome can be made using either method. Plasma concentrations of cortisone and corticosterone can be determined by HPLC. Cortisone and corticosterone concentrations alone are not diagnostic of Cushing's syndrome, but when used in conjunction with cortisol determinations, they provide additional criteria for laboratory confirmation of the clinical diagnosis of Cushing's syndrome.

Application: Dexamethasone can be applied to detect and characterize conditions of Cushing's disease/syndrome.

Implementation: Evaluation of Cushing's syndrome by measuring the clearance of exogenous dexamethasone used in the dexamethasone-suppression test.

Animal Model: Canine (cases suffering from Cushing's disease).

Interpretation and Analysis: Plasma concentrations of exogenous dexamethasone are constant during 3-hour tests in normal dogs. However, in 75% of the dogs with Cushing's syndrome, exogenous dexamethasone is cleared completely in 3-hour test protocols. Thus, dexamethasone clearance rates are excessively fast in most dogs with Cushing's syndrome.

Diagnosis of canine Cushing's syndrome based on multiple steroid analysis and dexamethasone turnover kinetics. Lothrop CD Jr, Oliver JW. Am J Vet Res 1984 Nov; 45 (11), pp. 2304–9.

Cushing's Disease/Syndrome: Hypothalamic/Pituitary Dysfunction/Hyperadrenocorticism: Pituitary Dependent/Evaluation of Therapeutic Efficacy: Trilostane

Dexamethasone/Thyrotropin-Releasing Hormone (TRH)

Cellular/Molecular Mechanism of Action: Trilostane is a competitive 3-beta hydroxysteroid dehydrogenase inhibitor.

Physiological Mechanism of Action: Dexamethasone suppresses the release of hypothalamic corticotropin-releasing hormone, thereby suppressing pituitary production/release of adenocorticotropic hormone (ACTH).

Background Information: Trilostane has been used successfully to control clinical signs and cortisol excess in canine pituitary dependent hyperadrenocorticism.

Implementation: Evaluation of equine cases of Cushing's syndrome treated with trilostane utilizing the combined dexamethasone suppression and TRH stimulation tests. Horses affected by equine Cushing's disease/syndrome receive 0.4–1 mg/kg (mean 0.5 mg/kg) trilostane once daily.

Animal Model: Equine (n = 20 horses; mean age = 21 years) diagnosed with equine Cushing's disease syndrome followed over a 1- or 2-year period.

Interpretation and Analysis: Clinical signs assessed over 1 or 2 years reveal a reduction in lethargy in all horses post–trilostane treatment. Similarly, polyuria and/or polydipsia, if originally present, become significantly reduced (e.g., n = 11/11).

Recurrent or chronic laminitis, if originally present, improves in a large percentage of cases (e.g., n = 13/16 or 81%). Combined dexamethasone suppression and TRH stimulation tests detect significant differences before and 30 days after initiation of therapy. There is a significant reduction ($p = 0.01$) of cortisol following TRH administration before (160 ± 53.0 nmol/l) and after (130 ± 46.1 nmol/l) trilostane. Trilostane causes improvement in clinical signs in horses, without side effects, and a corresponding decrease in cortisol response to TRH administration. Trilostane may be a useful therapy for the treatment of ECS. Further work comparing the effects of trilostane and pergolide is warranted.

Efficacy of trilostane for the treatment of equine Cushing's syndrome. McGowan CM, Neiger R. Equine Vet J 2003 Jun; 35 (4), pp. 414–8.

Cushing's Disease/Syndrome: Hypothalamic/Pituitary Dysfunction/Pituitary Pars Intermedia Dysfunction/Plasma Cortisol: Diurinal Variation

Dexamethasone/Adenocorticotropic Hormone (ACTH) (Natural)/ACTH (Synthetic)

Physiological Mechanism of Action: Adenocorticotropic hormone is produced in the pituitary and, upon its release, it promotes cortisol synthesis/release at the level of the adrenal cortex.

Background Information: Pituitary pars intermedia dysfunction is a slowly progressive disorder that afflicts most breeds of horses. Because it shares features with human Cushing's disease, it has been referred to as equine Cushing's disease. A variety of tests of pituitary-adrenocortical function can be performed on horses with evidence of pituitary pars intermediate dysfunction, and results are then compared with those observed in healthy control horses.

Route of Administration: Adenocorticotropic hormone (intramuscular); dexamethasone (intramuscular); synthetic ACTH (intravenous).

Dosage: Adenocorticotropic hormone (1 U of natural ACTH gel/kg); dexamethasone (10 mg and 5, 10, 20, and 40 μg/kg); synthetic ACTH (100 mg IV).

Implementation: Evaluation of diurnal variations in plasma cortisol concentration in equine conditions of pituitary pars intermedia dysfunction can be determined through the use of an ACTH stimulation test. Alternatively, a combined dexamethasone suppression test and synthetic ACTH stimulation test can also been applied in attempts to distinguish between normal reference control horses and those affected by dysfunction of the pituitary pars intermedia.

Interpretation and Analysis: Diurnal variations in plasma cortisol concentration are not statistically different between control horses and those with pituitary pars intermedia dysfunction. An ACTH stimulation test or a combined dexamethasone suppression test and ACTH stimulation test fail to distinguish between horses with pituitary pars intermedia dysfunction and control unaffected horses.

A significant ($p < 0.001$) dose-related suppression of cortisol concentration in response to increasing doses (5, 10, 20, and 40 μg/kg) of dexamethasone can be detected in control horses but not in those with pituitary pars intermedia dysfunction. On the basis of plasma cortisol concentration, the dexamethasone suppression test, using 40 μg/kg—whether initiated at 5 P.M. with sample collection at 15 (8 A.M.) and 19 (12 P.M.) hours after dexamethasone administration, or initiated at 12 A.M. with sample collection at 8 (8 A.M.), 12 (12 P.M.), 16 (4 P.M.), 20 (8 P.M.), and 24 (12 A.M.) hours after dexamethasone administration]—is capable of reliably distinguishing between control horses and those with pituitary pars intermedia dysfunction.

Diagnostic testing for pituitary pars intermedia dysfunction in horses. Dybdal NO, Hargreaves KM, Madigan JE, Gribble DH, Kennedy PC, Stabenfeldt GH. J Am Vet Med Assoc 1994 Feb 15; 204 (4), pp. 627–32.

Cushing's Syndrome (Preclinical): Adrenal Incidentaloma/21-Hydroxylase and 11β-Hydroxylase Biochemical Activity/Tetrahydrocortisol, Tetrahydrocortisone, Tetrahydroaldosterone/Potential to Distinguish Between NFI and PCS

1-24 Adenocorticotropic Hormone (ACTH) Stimulation Test

Background Information: Adrenal incidentalomas are mostly non-functioning adrenocortical adenomas (NFI). However, in 5 to 12% of the patients a preclinical Cushing's syndrome (PCS) with autonomous cortisol production by the tumor is present. Since urinary-free cortisol excretion is not sensitive enough to determine subclinical hypercortisolism, in the study described in the referenced paper, more sensitive indicators of daily cortisol production are measured.

A limited amount of information is known about daily aldosterone production.

Route of Administration: 1-24ACTH stimulation test (intravenous).

Dosage: 1-24ACTH stimulation test (250 μg).

Implementation: Evaluation of tetrahydrocortisol, tetrahydrocortisone, urinary-free cortisone together with urinary-free cortisol concentration in conditions of adrenal incidentalomas. Complementary analyses involve the measurement of excreted aldosterone metabolites. The types of steroidogenic enzymes that have altered

activities are assessed with the aid of the 1-24ACTH stimulation test combined with the determination of multiple steroids. In situations where it is assumed that a 21-hydroxylase deficiency, or even an 11β-hydroxylase deficiency, may be involved in adrenal tumorigenesis, the prevalence of germline CYP21B and CYP11B1 mutations can be studied in the same cases that have been evaluated applying the ACTH stimulation test.

Note: The ACTH stimulation test can reveal increased response of precursors of the glucocorticoid and mineralocorticoid pathway.

Human Model: Cases of adrenal incidentalomas (n = 35; n = 29 NFI; n = 6 PCS; n = 35 healthy controls). Performance of the 1-24ACTH stimulation test (n = 25 cases; n = 18 healthy controls).
Interpretation and Analysis: Glucocorticoid metabolites are within the normal range in a small percentage of NFI cases (e.g., n = 3/29). As a group, cases have subtle alterations in cortisol metabolism. Tetrahydrocortisol excretion is elevated in NFI and PCS compared to normal subjects (2.1 ± 0.2 and 2.5 ± 0.5 vs 1.5 ± 0.1 mg/24 h; $p < 0.05$). Accordingly, a 2-fold elevation of the tetrahydrocortisol/free cortisol ratio indicates increases in 5β-reduction of cortisol in the liver. Tetrahydroaldosterone and aldosterone-18-glucuronide excretions are not different relative to controls. In incidentalomas cases, an increased response to ACTH can be appreciated for 17-hydroxyprogesterone (595 ± 133 vs 160 ± 25 ng/dL), 21-desoxycortisol (105 ± 25 vs 29 ± 9 ng/dL) and 11-desoxycortisol (401 ± 40 vs 293 ± 17 ng/dL).

In a small percentage of cases (n = 1/25 or 4%), a heterozygous deletion in exon 3 of the CYP21B gene can be detected. Therefore, even the excretion of the main glucocorticoid metabolites appears not to be a marker that is sensitive enough to distinguish between NFI and PCS. However, it is also possible that alterations in cortisol secretion are qualitative rather than quantitative. Zona glomerulosa function is not influenced. The elevation of 21-desoxycortisol serves to argue against an impairment of 11β-hydroxylase and is instead better explained by a decrease in 21-hydroxylase activity. The remainder of cases evaluated will have wild-type sequences of both genes. In conclusion, neither 21-hydroxylase deficiency nor 11β-hydroxylase deficiency are predisposing factors for adrenal tumorigenesis.

Metabolism of glucocorticoids and mineralocorticoids in patients with adrenal incidentalomas. Maser-Gluth C, Reincke M, Allolio B, Schulze E. Eur J Clin Invest 2000 Dec; 30 Suppl 3, pp. 83–6.

Cushing's Syndrome: Human vs Canine/Adrenal Tumor vs Pituitary-Dependent Hyperadrenocorticism (PDH)
Dexamethasone/Dexame

Application: Spontaneous hyperadrenocorticism is a common and well-recognized endocrine disorder occurring in dogs. The biochemical diagnosis of hyperadrenocorticism rests on the documentation of excessive glucocorticoid levels or metabolites in urine or blood. The diagnosis of canine hyperadrenocorticism is historically derived from human methods and based on non-dynamic and dynamic tests and visualization. In some cases the different ranges of endocrine parameters or metabolites necessitate the modification of human protocols. Elevation of the activity of alkaline phosphatase (AP) and its heat-resistant isoenzyme (SIAP) induced by endogenous or exogenous glucocorticoid excess raises the suspicion of hyperadrenocorticism. Cortisol values in morning urine are related to creatinine concentrations to correct for differences in urine concentration. Theoretically, the administration of dexamethasone (DX) at a relatively low dose (0.01 mg/kg of body weight) can inhibit the pituitary secretion of adenocorticotropic hormone (ACTH) and, in turn, decrease endogenous cortisol secretion for as long as 24 to 48 hours. Therefore, DX administration to dogs with a functioning adrenocortical tumor would not affect the plasma cortisol concentration at any time following its administration. The high-dose dexamethasone suppression test (HDDS) is based on the observation that the function of adrenocortical tumors is independent of pituitary ACTH and they completely suppress ACTH secretion; therefore, regardless of its dose, DX is never able to suppress cortisol secretion. The HDDS can be combined with the measurement of urinary cortisol/creatinine (c/c) ratio from morning urine samples on 3 consecutive days. In case of non-suppressible urinary c/c ratio, there must be speculation on differentiating adrenal tumor (AT) from non-suppressible PDH due to a pituitary tumor arising from the intermediate lobe. Radiocholesterol scintigraphy is a less frequently used technique in the diagnosis of canine Cushing's syndrome (CCS); however, it has the same advantages in the localization and characterization of adrenocortical diseases as in humans.

Diagnosis of hyperadrenocorticism in dogs as compared to human diagnostic methods: A review. Thuróczy J, Balogh L, Huszenicza G, Jánoki GA, Kulcsár M. Acta Vet Hung 1998; 46 (2), pp. 157–73.

Cushing's Syndrome/Hypercorticism: Adrenal vs Pituitary/Urinary 17-Ketogenic Steroids (17-KGS)/Tetrahydrocortisol/Tetrahydrocortisone (THF/THE) Ratio/Cortisol Production Rate
Adenocorticotropic Hormone (ACTH) Stimulation/Dexamethasone Suppression/Insulin Tolerance Test/Lysine-Vasopressin (LVP) Test/Metopirone (Metyrapone) Test

Physiological Mechanism of Action: Increases in exogenous ACTH promote the release/synthesis of adrenal glucocorticoid while dexamethasone suppresses hypothalamic corticotropin releasing hormone synthesis/release thereby reducing pituitary ACTH synthesis/release. Insulin promotes intracellular transfer of glucose. Metopirone (metyrapone) reduces cortisol production by inhibiting 11β-hydroxylation reactions in addition to inhibition of side-chain cleavage to some degree, but the latter effect is largely offset by ACTH stimulation of the adrenal gland. The biosynthetic pathway is terminated at 11-desoxycortisol, which has essentially no inhibitory influence on ACTH secretion.
Background Information: (i) Arginine vasopressin increases the immunoreactive (IR) ACTH response to corticotropin-releasing factor (CRF) 4-fold when given simultaneously with CRF. (ii) The combination of ovine-CRF with growth hormone-releasing factor (GHRF), gonadotropin-releasing hormone (GnRH), and thyrotropin-releasing hormone (TRH) appears to provide a rapid and useful test of combined anterior pituitary function. (iii) The desmopressin (DDAVP) stimulation test, involving the determination of plasma ACTH levels after 5 μg DDAVP injection, seems useful for discriminating Cushing's disease from normality, and may serve to facilitate the differentiation between Cushing's disease and ectopic ACTH syndrome.
Application: Detection and characterization Cushing's syndrome (dexamethasone suppression test) in addition to delineating the cause of the condition (LVP test and metyrapone test).
Implementation: Evaluation of hypercorticism in conditions of Cushing's syndrome as a function of estimated urinary neutral steroid metabolites, plasma cortisol, percent unbound-cortisol, cortisol production rate, and plasma ACTH. Assessment of these measurements can be facilitated by the utilization of several provocative response procedures including the (i) dexamethasone suppression test;

(ii) ACTH stimulation test; (iii) metyrapone test; (iv) LVP test; and (v) insulin tolerance test.

Human Model: Cases affected by Cushing's syndrome (n = 16).

Interpretation and Analysis: Measurements of plasma cortisol late in the day and single-dose dexamethasone suppression test appear to be the most useful procedures for the diagnosis of hypercorticism because of their reliability and simplicity. Urinary 17-ketogenic steroids (17-KGS), tetrahydrocortisol/ tetrahydrocortisone (THF/THE) ratio, cortisol production rate, and low-dose dexamethasone suppression test are also useful, whereas the insulin test and LVP test are less valuable for this purpose. Identification of the cause of Cushing's syndrome can most reliably be determined applying the LVP test and metyrapone test. Plasma ACTH is also useful for this purpose, while the insulin test and ACTH stimulation test are less valuable.

The diagnostic value of hypothalamic-pituitary-adrenocortical function tests in patients with Cushing's syndrome. Hashimoto K. Acta Med Okayama 1975 Apr; 29 (2), pp. 111–26.

Cushing's Syndrome: Hypertension Mechanism/ Epinephrine/Norepinephrine/Adrenomedullary Phenylethanolamine N-Methyltransferase
Glucagon

Cellular/Molecular Mechanism of Action: Stimulates hepatic glycogenolysis and liberation of glucose into the intravascular compartment.

Diagnostic Application: Glucagon tolerance test for screening Cushing's syndrome.

Considerations: The glucagon tolerance test can not distinguish between diabetic patients (dogs), and those with both overt diabetes meletus and Cushing's syndrome; patients (dogs) with mild secondary diabetes due to Cushing's syndrome and those with type III diabetes mellitus; iatrogenic Cushing's syndrome, and spontaneous Cushing's syndrome; and lack of appreciation for Cushing's syndrome patients that do not have significant insulin antagonism. Value of the test remains to be further established.

Implementation: Delineate if chronic endogenous hypercortisolism might alter adrenomedullary phenylethanolamine N-methyltransferase activity, by measuring epinephrine/norepinephrine (E/NE) ratios in the adrenal venous blood. Complementary investigations can include (i) evaluation of adrenomedullary secretory activity in Cushing's syndrome by measuring basal E plasma levels; (ii) characterizing adrenomedullary response to glucagons; and (iii) determination if chronic endogenous hypercortisolism modifies E plasma levels through a modification of E metabolism involving the measurement of E MCR (e.g., n = 4 cases; n = 4 age-matched controls).

Human Model: Cases with Cushing's syndrome undergoing surgery (n = 8 and n = 12 control subjects undergoing surgery for left kidney diseases) employed for measurements of epinephrine/norepinephrine in adrenal venous blood. Cases of Cushing's syndrome evaluated for adrenomedullary secretory activity (n = 24 cases; n = 32 age- and sex-matched normal controls). Adrenomedullary response to glucagons (n = 9 cases; n = 22 age /sex-matched reference controls).

Interpretation and Analysis: Mean (± SEM) E/NE ratio in adrenal venous blood is similar in patients with Cushing's syndrome (4.61 ± 0.78) and in the control group (4.71 ± 0.74). Mean (± SEM) basal plasma E is significantly lower in patients with Cushing's syndrome (98.2 ± 10.9 vs 184 ± 25.1 pmol/L, $p < 0.01$) than in controls. Similarly, plasma NE is also reduced (0.75 ± 0.09 vs 1.10 ± 0.07 nmol/L, $p < 0.01$). In patients with Cushing's syndrome the E response to glucagon is significantly reduced ($p < 0.01$). E MCR is almost identical in patients with Cushing's syndrome (1.48 ± 0.10 L/min.m^2) and in control subjects (1.51 ± 0.10 L/min.m^2). Such data demonstrates that (i) chronic endogenous hypercortisolism is not able to change adrenomedullary phenylethanolamine N-methyltransferase activity and therefore the quality of adrenomedullary secretion; and (ii) chronic endogenous hypercortisolism causes a decrease in basal and stimulated adrenomedullary activity without altering E MCR significantly. Therefore the adrenal medulla does not seem to play a pathogenetic role in the hypertension of Cushing's syndrome.

Laboratory Instrumentation: Standard clinical pathology laboratory methodology for measuring plasma glucose concentrations.

Adrenal medulla secretion in Cushing's syndrome. Mannelli M, Lanzillotti R, Pupilli C, Ianni L, Conti A, Serio M. J Clin Endocrinol Metab 1994 Jun; 78 (6), pp. 1331–5.

Cushing's Syndrome (Iatrogenic): Hypothalamic/ Pituitary Function/ Electroacupuncture (EA) Efficacy Evaluation
Adenocorticotropic Hormone (ACTH) Stimulation Test

Cellular/Molecular Mechanism of Action: Increases in exogenous ACTH mimic pituitary ACTH in a manner that promotes the synthesis/release of adrenal glucocorticoids.

Application: The ACTH stimulation test can be applied as a validation technique to evaluate the effectiveness of EA to modulate adrenocortical function in conditions of canine iatrogenic Cushing's syndrome (ICS).

Implementation: Evaluation of the potential efficacy of acupuncture for the treatment of Cushing's syndrome utilizing ACTH stimulation tests (selection of the most effective acupuncture point to treat ICS). Dogs are treated bilaterally with EA for 15 minutes at loci BL22 + BL23 + BL24, ST36, or a non-locus control point on M. brachialis. Complementary investigations involve treatment of the ST36 site bilaterally with EA for 30 minutes, 3 times per week, for 3 consecutive weeks. For the control, a non-locus point on M. brachialis is treated bilaterally with the same protocol. Each dog is tested at all 3 sites in rotation. Blood samples are collected before and 0, 15, and 60 minutes after EA, and serum cortisol levels are measured applying radioimmunoassay techniques.

Animal Model: Canine (n = 6 healthy adult female dogs; n = 8 dogs given prednisolone acetate [2 mg/kg/day IM × 21 days] and then randomly divided into ST36 and control groups of 4 dogs each).

Interpretation and Analysis: Electroacupuncture at ST36 results in the highest response of serum cortisol levels among the 3 treatments. Evaluation of the effectiveness of EA at ST36 in the treatment of ICS in dogs reveals that after the 1st week of EA, serum cortisol levels of the ST36 and control groups are 0.9 ± 0.1 and 0.5 ± 0.1 μg/dl ($p < 0.005$) baseline and 2.5 ± 0.2 and 1.4 ± 0.4 μg/dl ($p < 0.05$) and after ACTH stimulation test, respectively. After the 3rd week of EA treatment, the results are 1.0 ± 0.1 and 0.6 ± 0.2 μg/dl ($p < 0.05$) baseline and 4.0 ± 0.5 and 1.7 ± 0.5 μg/dl ($p < 0.001$) after ACTH stimulation, respectively. Electroacupuncture at ST36 therefore can restore the adrenocortical hypofunction resulting from ICS in dogs.

Treatment of iatrogenic Cushing's syndrome in dogs with electroacupuncture stimulation of stomach 36. Lin JH, Su HL, Chang SH, Shien YS, Wu LS. Am J Chin Med 1991; 19 (1), pp. 9–15.

Cushing's Syndrome: Hypothalamic/Pituitary Function/Pars Intermedia Pituitary Adenoma
Dexamethasone Suppression/Thyrotropin-Releasing Hormone (TRH)

Cellular/Molecular Mechanism of Action: Dexamethasone suppresses hypothalamic corticotropin-releasing hormone (CRH) synthesis/release, which in turn decreases adenocorticotropic hormone (ACTH) synthesis.
Application: Detection and characterization of horses affected by pars intermedia pituitary adenoma. To evaluate results of a combined dexamethasone suppression/TRH stimulation test in horses clinically suspected of having a pars intermedia pituitary adenoma (PIPA).
Route of Administration: Dexamethasone (intravenous); TRH (intravenous).
Dosage: Dexamethasone (40 μg/kg [18 μg/lb] of body weight); TRH (1.1 mg).
Implementation: Evaluation of changes in cortisol concentrations in conditions of PIPA induced by dexamethasone administration. Blood samples are collected at baseline prior to dexamethasone suppression and then at 3 hours postinjection. A dose of TRH is then administered, followed by the serial collection of blood samples at 15, 30, 45, 60, and 90 minutes and 21 hours after TRH administration (24 hours after dexamethasone injection). Cortisol concentrations are measured in all blood samples collected for analysis.

Animal Model: Equine (n = 5 horses suspected of having PIPA; n = 7 healthy adult horses).
Interpretation and Analysis: Baseline cortisol concentrations are significantly lower in horses suspected to have a PIPA than in healthy horses. Cortisol concentrations are suppressed by dexamethasone in both groups; however, after TRH administration, cortisol concentration returns to baseline values in horses suspected to have a PIPA, but not in healthy horses. Concentrations are still less than baseline values 24 hours after dexamethasone administration in healthy horses.

The combined dexamethasone suppression/TRH stimulation test may be a useful diagnostic test in horses suspected of having PIPA. For clinical application, collection of a blood sample 30 minutes after TRH administration is recommended.

Results of a combined dexamethasone suppression/thyrotropin-releasing hormone stimulation test in healthy horses and horses suspected to have a pars intermedia pituitary adenoma. Eiler H, Oliver JW, Andrews FM, Fecteau KA, Green EM, McCracken M. J Am Vet Med Assoc 1997 Jul 1; 211 (1), pp. 79–81.

Cushing's Syndrome: Hypothalamic/Pituitary/Adrenalcortical Axis Function
Metyrapone/Adenocorticotropic Hormone (ACTH) and Compound-S (CMP-S)

Cellular/Molecular Mechanism of Action: Metyrapone inhibits enzymatic activity of 11β-hydroxylase, thereby reducing cortisol production.
Application: Assess the diagnostic accuracy of combined stimulation of ACTH and CMP-S.
Dosage: Metyrapone (2 g).
Considerations: The corticotropin-releasing hormone (CRH) test may sometimes be useful in the differential diagnosis of Cushing's syndrome because most patients with pituitary ACTH-dependent Cushing's syndrome (Cushing's disease) respond to CRH, but those with other causes of Cushing's syndrome usually do not. However, about 10% of Cushing's disease patients fail to respond to CRH.
Implementation: Evaluation of conditions of pituitary/adrenal dysfunction as a function of response to metyrapone tests and determination of the safety and practicality of such tests for use as an outpatient procedure. Example: Performance of 327 metyrapone tests analyzed retrospectively (e.g., n = 113 cases with 1 test; n = 72 cases with 2 and 6 tests over 1 to 3 years). Metyrapone is given at 2400 hours (outpatient procedure). Blood samples for analysis of ACTH, CMP-S, and cortisol are collected at 0730 hours.

Human Model: Cases of pituitary/adrenal dysfunction (n = 185, with mean age = 50.3 ± 15.2 years with most patients suffering from pituitary adenomas [60 macroadenomas, 63 microadenomas] or other pituitary lesions [n = 29]).
Interpretation and Analysis: Stimulation of adrenal CMP-S and cortisol by pituitary ACTH demonstrates a dose-response curve with the shape of half a geometric parabola. Compound-S reaches a plateau when ACTH rises above 175 ng/liter ($r = 0.661$, $p < 0.0001$ for ACTH < 175 ng/liter; $r = 0.083$, p = ns for ACTH > 175 ng/liter), cortisol "flattens" at ACTH levels above 230 ng/liter ($r = 0.633$; $p < 0.0001$ for ACTH < 230 ng/liter; p = ns for ACTH > 230 ng/liter). Alternatively, the sum of CMP-S plus cortisol also "flattens" when ACTH rises above 230 ng/liter ($r = 0.696$; $p < 0.0001$ for ACTH < 230; p = ns for ACTH > 230 ng/liter). Receiver operating curve analysis defining a cutoff for ACTH at 150 ng/liter demonstrates a sensitivity of 47% and 67% at a cutoff level for CMP-S at 200 or 260 nmol/liter, respectively. The respective specificity is 82% and 68% for CMP-S. This compares with a sensitivity of 71% and specificity of 69% if the sum of CMP-S plus cortisol of 450 nmol/liter can be used as a cutoff. The response curve between CMP-S and ACTH implies a maximally stimulated adrenal cortex at circulating ACTH levels above 175 ng/liter. Single measurement of CMP-S using the cutoff at 200 nmol/liter, as suggested in the literature, yields a poor sensitivity of only 47% compared with ACTH.

Despite the relatively high cross-reactivity of CMP-S in the cortisol assay, the sum of CMP-S and cortisol levels with a cutoff value of 450 nmol/liter yields a better diagnostic accuracy compared with CMP-S alone.
Instrumentation: The development and validation of a direct-injection high-performance liquid chromatography (HPLC) method, with column switching, for the determination of metyrapol enantiomers and metyrapone in human plasma has been described. The system used in this work is composed of a restricted access media (RAM) bovine serum albumin (BSA) octyl column coupled to an amylose tris(3,5-dimethoxyphenylcarbamate) chiral column. Water is used as eluent for the 1st 5 minutes at a flow rate of 1.0 ml/min for the elution of the plasma proteins and then acetonitrile-water (30:70 v/v) for the transfer and analysis of metyrapol enantiomers and metyrapone, which can be detected by UV at lambda = 260 nm. The total analysis time is about 32 minutes. The calibration curves for each enantiomer and for the metyrapone are linear in the ranges 0.075–0.75 μg/ml and 0.150–1.50 μg/ml, respectively. Recoveries, intra- and interday precision, and accuracy can be determined using 3 quality controls, 1 low (0.18 μg/ml), 1 medium (0.75 μg/ml), and 1 high (1.35 μg/ml) plasma concentration. Quantitative recoveries and good precision and accuracy can be obtained. The limit of quantitation is 0.045 μg/ml for both enantiomers and for metyrapone.

Combined stimulation of adrenocorticotropin and compound-S by single dose metyrapone test as an outpatient procedure to assess hypothalamic-pituitary-adrenal function. Berneis K, Staub JJ, Gessler A, Meier C, Girard J, Müller B. J Clin Endocrinol Metab 2002 Dec; 87 (12), pp. 5470–5.

Hyperadrenalcorticism: Stress-Induced/ Hemodialysis-Induced Dysfunction/Chronic Stress: Depression/Anorexia Nervosa
Corticotropin-Releasing Hormone (CRH)/Adenocorticotropic Hormone (ACTH)

Physiological Mechanism of Action: Corticotropin-releasing hormone is produced by the hypothalamus and modulates the secretion of pituitary ACTH.
Background Information: A state reminiscent of Cushing's syndrome has been reported to be precipated by hemodialysis procedures.
Application: Corticotropin-releasing hormone has been shown to be useful as a provocative test for evaluationg HPA axis function.
Route of Administration: Ovine CRH (oCRH) (intravenous bolus).
Dosage: oCRH.
Implementation: Evaluation of the influence of hemodialysis on hypothalamic-pituitary-adrenal (HPA) axis function by determining responses in plasma ACTH and cortisol concentrations following administration of exogenous ovine corticotropin-releasing hormone (oCRH).

Human Model: Cases subjected to hemodialysis (n = 13).
Interpretation and Analysis: The plasma concentrations of immunoreactive CRH following oCRH administration are similar in cases subjected to hemodialysis and controls. In the vast majority of cases, oCRH given intravenously as a bolus injection causes a further increase in already elevated levels of cortisol (n = 13/13). The mean basal plasma levels of ACTH are within the normal range. There is, however, a blunted ACTH response to oCRH. The HPA axis in chronic HD conditions retains the ability to respond to exogenous oCRH. The patterns of the ACTH and cortisol responses to this peptide resemble patterns observed with chronic stress (depression, anorexia nervosa). In addition, the kinetics for the disappearance of oCRH indicates that the kidney may not be the major organ that metabolizes oCRH.

Ovine corticotropin-releasing hormone stimulation test in patients with chronic renal failure: Pharmacokinetic properties, and plasma adrenocorticotropic hormone and serum cortisol responses. Siamopoulos KC, Eleftheriades EG, Pappas M, Sferopoulos G, Tsolas O. Horm Res 1988; 30 (1), pp. 17–21.

Hypopituitary Conditions/Adenocorticotropic Hormone (ACTH) Deficiency/ACTH Reserve Status
Corticotropin-Releasing Hormone (CRH)/ Adrenocorticotropin-Releasing Hormone/ Metyrapone/Urine 17-OH Corticosteroids/Serum Deoxycorticosterone

Cellular/Molecular Mechanism of Action: Corticotropin-releasing hormone from the hypothalamus promotes release/synthesis of ACTH from the pituitary, which in turn promotes glucocorticoid release/synthesis from the adrenal cortex.
Diagnostic Application: Detection of conditions associated with pituitary dysfunction or developmental abnormalities.
Route of Administration: Corticotropin-releasing hormone (intravenous bolus).
Dosage: Metyrapone administration (450 mg/m^2/dose every 4 hours $\times$ 7 doses); ovine CRH (1 μg/kg).
Implementation: Evaluation of hypopituitary conditions as a function of comparative responses of (i) ACTH and cortisol (F) to (ii) CRH administration and its effect on urinary 17-OH corticosteroids (17-OHCS); and (iii) metyrapone-induced alterations in serum deoxycorticosterone (DOC). The CRH test is performed 3 days before or 3 weeks after the metyrapone test for the purpose of establishing ACTH reserve status.

Human Model: Cases of hypopituitary conditions (n = 16 with glucocorticoid therapy withheld for a minimum of 3 weeks before testing).
Diagnostic Interpretation: In ACTH-intact hypopituitary conditions (e.g., n = 9 cases postmetyrapone 17-OHCS $>$ 12.2 mumol/m^2/d; DOC $\geq$ 11.5 nmol/L), the peak F (497–773 nmol/L) and ACTH (5.2–22 pmol/L) responses to CRH stimulation are similar to those of normal subjects (F peak = 554–993 nmol/L and ACTH peak = 6–25 pmol/L at 15 to 60 minutes). In cases with partial ACTH deficiency (e.g., n = 1 case: postmetyrapone 17-OHCS = 10.5 mumol/m2/d; DOC = 6 nmol/L), the peak F response is low and delayed (246 nmol/L at 180 minutes), while the peak ACTH response is normal (7 pmol/L). Severely ACTH-deficient conditions (e.g., n = 6 cases: postmetyrapone 17-OHCS $<$ 5.4 mumol/m^2/d; DOC $\leq$ 3.4 nmol/L) have a low F response at 15 to 90 minutes in all, with a delayed rise at 120 to 180 minutes in response to CRH administration (n = 3/6), whereas ACTH responses are variable: absent or low, normal, delayed, or persistently exaggerated. Therefore, the CRH-stimulated F response pattern in hypopituitary patients is comparable to urinary 17-OHCS and serum DOC response to metyrapone administration.
Laboratory Instrumentation: ELISA-based immunodiagnostic assay of serum samples.

Comparison of adrenocorticotropin and adrenal steroid responses to corticotropin-releasing hormone versus metyrapone testing in patients with hypopituitarism. Riddick L, Chrousos GP, Jeffries S, Pang S. Pediatr Res 1994 Aug; 36 (2), pp. 215–20.

Pheochromocytoma
Clonidine/[^{131}I]-MIBG

Pheochromocytomas are rare tumors of catecholamine-producing chromaffin cells leading to hypertension and symptoms of catecholamine excess. They can be benign or malignant, sporadic or familial tumors. Genetic syndromes associated with pheochromocytoma are MEN II, VHL disease, and neurofibromatosis type 1. Usually, pheochromocytomas occur in the adrenal medulla. Clinical manifestations include hypertension (which can be intermittent, stable, or in the form of hypertensive peaks) and symptoms related to catecholamine excess such as headache, palpitations and tachycardia, pallor, anxiety and nervousness, nausea, vomiting, and weight loss. This clinical syndrome can be mimicked by various hyperkinetic and hyperadrenergic states. When pheochromocytoma is suspected, the first diagnostic step is represented by the measurement of catecholamines and their metabolites (metanephrines) in urine and plasma. Chro-mogranin A quantification can be useful. The clonidine suppression test may be helpful in ruling out other conditions that may elevate catecholamines and metanephrines. Localization and staging of pheochromocytoma is based on MRI, which is more sensitive than CT scan, and [^{131}I]-MIBG scintiscan. The best therapeutic option for pheochromocytoma is surgery with a laparoscopic approach. An appropriate pre-, intra- and postoperative medical management of the patient is mandatory. In the absence of optimal medical treatment, intraoperative mortality reaches 50%.

Recent advances in diagnosis and treatment of pheochromocytoma. Veglio F, Morello F, Morra Di Cella S, Del Colle S, Rabbia F, Mulatero P. Minerva Med 2003 Aug; 94 (4), pp. 267–71.

Pheochromocytoma/Urinary Vanillylmandelic Acid, Metanephrine, Normetanephrine
Glucagon/[^{125}I]-Thyr10-Glucagon/ Des-His1[Glu9]Glucagon Amide

Background Information: Pheochromocytoma cases frequently have elevated plasma concentrations of adrenaline and noradrenaline in addition to increases in urinary vanillylmandelic acid, metanephrine, and/or normetanephrine.
Application: Detection and characterization of pheochromocytoma in addition to conditions of essential hypertension, thyroid disease, diabetes mellitus, and acromegaly.
Route of Administration: Pheochromocytoma and chromaffin granules of the pig adrenal medulla in experimental *ex vivo*/*in vitro* scenarios.
Dosage: Glucagon (10^{-4}) infusion for 10 minutes following 2 hours of preincubation.
Considerations: Most patients suffering from pheochromocytoma are diagnosed by measuring urine concentrations of vanillylmandelic acid, metanephrine, and/or normetanephrine in addition to plasma catecholamine concentrations. However, a subset of patients suffering from the condition will not have elevated concentrations of the metabolites in the plasma or urine.

Administration of phenotolamine (blocking agent) prior to histamine challenge test in order to protect against hypotension or catecholamine-induced arrythmias.
Implementation (Clinical): Evaluation of conditions of suspected pheochromocytoma as a function of catecholamine and blood pressure response to glucagon challenge. Examples: (i) Study of pig adrenal medulla or human pheochromocytoma to determine the influence of glucagon infusion. Given this objective, a continuous-flow incubation system can be employed in which the secretory response to these drugs is characterized by a serial fluorimetric assay of catecholamines in the effluent medium. (ii) Characterization of the *in vivo* effect of glucagon on blood pressure and urinary catecholamine levels of healthy control subjects, for comparison to cases of essential hypertension, thyroid disease, diabetes mellitus, and acromegaly.

Human Model: Extra-adrenal pheochromocytoma.
Interpretation and Analysis: During *in vitro* preparations, glucagons (10^{-4}) for 10 minutes following a 2-hour incubation period result in abrupt increases in adrenaline and noradrenaline (epinephrine to 0.08 μg/mg, and norepinephrine to 0.07 μg/mg). Systemically (*in vivo*), there is initially a massive release of catecholamines that declines to basal levels (0.02 μg/mg) after 1.5 hours. During the course of *in vivo* administration trials, glucagon induces a slight but constant increase in blood pressure. By contrast, no significant urinary catecholamine elevation may be evoked. Adjunct diagnostics can include detection using [^{131}I]-metaiodobenzylguanidine (MIBG) scintigraphy.

Pheochromocytoma: Adrenal Incidentaloma
Metoclopramide

Background Information: Pheochromocytoma cases occasionally are identified incidentally on examination.

Case Example: Left adrenal mass (3.0 cm) detected by abdominal computed tomography.
Considerations: Cases may have to be observed and treated conservatively due to the possible occurrence of arterial hypotension after tumor resection.

Human Example: Pheochromocytoma detected by abdominal computed tomography as a left adrenal mass (3.0 cm). (Signalment: 52-year-old male patient with weight loss of 3 kg over a 3-month period.)
Implementation: Measurement of plasma adrenaline and noradrenaline levels; metaclopramide provocative test; [^{123}I]-metaiodobenzylguanidine (MIBG) scintiscan.
Interpretation and Analysis: Plasma adrenaline and noradrenaline in cases of pheochromocytoma may increase to 521 pg/ml and 1,341 pg/ml, respectively. Metoclopramide provocative tests are usually positive, while scintiscan using [^{123}I]-MIBG will demonstrate accumulation of the radionuclide in the adrenal tumor region. Patients may need to undergo observation and conservative treatment due to the possible occurrence of arterial hypotension after resection of a tumor.

Pheochromocytoma in a long-term hemodialysis patient, discovered as an adrenal incidentaloma. Asaka S, Takayama Y, Tagawa S, Ito Y, Yoshimura A, Masunaga T, Oiwake H, Shinozaki K, Takeda R. Intern Med 1997 Jun; 36 (6), pp. 403–7.

Pheochromocytoma/[Elevated Glucagon-Receptor Expression]
Des-His(1)[Glu$_9$]Glucagon Amide/ [^{125}I-Thyr$_{10}$]Glucagon

Cellular/Molecular Mechanism of Action: [^{125}I-Thyr$_{10}$]-glucagon is a radiolabeled analog of glucagon. Des-His(1)[Glu$_9$] glucagon amide is a glucagon receptor antagonist.
Background Information: Pheochromocytoma lesions expressed glucagon receptor complexes at elevated membrane densities.
Application: Potential diagnostic parameter for detecting conditions of pheochromocytoma. Des-His(1)[Glu$_9$]glucagon amide (GR-A) abolishes the secretory response to glucagon by pheochromocytomas.
Route of Administration: Experimental (*in vitro*/*ex vivo*).
Dosage: Experimental (*in vitro*).
Implementation: Evaluation of glucagon mRNA expression and glucagon receptor expression in pheochromocytoma lesions.

Human *Ex Vivo* Model: Pheochromocytoma lesions (n = 3 and n = 4 normal adrenal medullas).
Interpretation and Analysis: Glucagon-receptor mRNA can be detected by reverse transcription-polymerase chain reaction in human pheochromocytomas, but not in normal adrenal medullas. Quantitative autoradiography demonstrates the presence of abundant [^{125}I-Thyr$_{10}$]glucagon binding sites in pheochromocytomas, which can be displaced by both cold glucagon and the glucagon receptor antagonist Des-His(1)[Glu$_9$]glucagon amide (GR-A). The adrenal medulla is weakly labeled, and binding is not displaced by GR-A. Glucagon enhances epinephrine and norepinephrine release by pheochromocytoma slices, minimal and maximal effective concentrations being 10^{-8} M and 10^{-6} M. Adrenomedullary slices evoke a weak catecholamine response only to 10^{-5} M glucagon.

GR-A abolished the secretory response to glucagon by pheochromocytomas, but not of adrenal medullas. Collectively, these findings indicate that human pheochromocytomas, but not adrenal medulla, express glucagon receptors and possess a marked secretory response to glucagon, thereby providing the rationale to explain the specificity of the glucagon provocative test in the diagnosis of pheochromocytoma.

Human pheochromocytomas, but not adrenal medulla, express glucagon-receptor gene and possess an *in vitro* secretory response to glucagon. Albertin G, Aragona F, Gottardo L, Malendowicz LK, Nussdorfer GG. Peptides 2001 Apr; 22 (4), pp. 597–600.

Pheochromocytoma
Metoclopramide

Cellular/Molecular Mechanism of Action: Metoclopramide functions as a dopamine antagonist agent.
Route of Administration: Metoclopramide (intravenous).
Dosage: Metoclopramide (5 mg).
Application: Detection and characterization of the condition of pheochromocytoma.
Implementation: Evaluation of the cardiovascular and plasma catecholamine responses to metoclopramide in cases of pheochromocytoma (e.g., n = 5), essential hypertension (e.g., n = 12); and normotensive subjects (e.g., n = 9) that display symptoms suggestive of pheochromocytoma and on a constant daily intake of sodium (100 mEq) and potassium (80 mEq).
Instrumentation: Significant pressor responses to metaclopramide (producing no serious pressor episodes and no other undesirable side effects) are found only in cases of pheochromocytoma. In contrast, cases of essential hypertension and normotensive individuals experience a slight depressor response.

Following curative surgery for pheochromocytoma, metoclopramide-induced pressor effects return to normal. Furthermore, the enhanced pressor effects of metaclopramide in patients with pheochromocytoma are associated with increases in plasma norepinephrine (NE) concentrations. However, the plasma epinephrine (E) concentrations remain unchanged after metaclopramide IV injection. In brief, the 5 mg dose of MCP appears to be a more suitable vasopressor provocative agent in the pharmacological diagnosis of pheochromocytoma compared to currently used agents.

Metoclopramide in the diagnosis of pheochromocytoma. Kawabe H, Itaya Y, Suzuki H, Kondo K, Saruta T. Jpn Heart J 1985 Jul; 26 (4), pp. 557–66.

Pheochromocytoma
Histamine/Methacholine/Glucagon/Phentolamine

Background Information: Pheochromocytoma accounts for about 0.1% of patients with diastolic hyperstension. It mimics many diseases varying from anxiety psychoneurosis to intracranial tumors. Cardinal symptoms include severe headache (72–92%), sweating (60–70%), palpitations (51–73%), and hypertension (> 90%) of which 50% is sustained, 50% paroxysmal. Many drugs (phenothiazines, Saralasin, antiemetics, steroids, etc.) have been reported as precipitating factors.
Diagnostic Application: Provocative testing for pheochromocytoma.
Implementation: Provocative agents (histamine, glucagon, tyramine) are needed rarely. Administration of phenotolamine (blocking agent) prior to histamine challenge test is sometimes applied in order to protect against hypotension or catecholamine-induced arrythmias. Preoperative localization of the tumor can be done with nephrotomography IVP, computerized axial tomography, ultrasound, [^{131}I]-19-iodocholesterol scan, arteriography, and venography.

Human Model: Clinical signs suggestive of pheochromocytoma include (i) all symptomatic patients with sustained or paroxysmal hypertension; (ii) asymptomatic hypertension; (iii) all patients with MEA 2a,b (hyperparathyroidism, medullary carcinoma of the thyroid, neurocutaneous lesions) and their 1st-degree relatives, even if the latter are asymptomatic and normotensive; (iv) hypertension plus diabetes mellitis or hypermetabolism; (v) hypertensive episode during induction of anesthesia or radiologic procedure; and (vi) hypertensive response during histamine administration, (i.e., gastric analysis).
Interpretation and Analysis: Most patients suffering from pheochromocytoma are diagnosed by measuring urine concentrations of vanillylmandelic acid, metanephrine, and/or normetanephrine in addition to plasma catecholamine concentrations. Urinary metanephrine is the single best screening test. However, a subset of patients suffering from the condition will not have elevated concentrations of the metabolites in the plasma or urine. Plasma catecholamine determination is particularly helpful when collected before and immediately after an attack.

Pheochromocytoma: Clinical manifestations and diagnostic tests. Juon D. Urology 1981 Jan; 17 (1), pp. 1–12.

Pheochromocytoma: Asymptomatic/Multiple Endocrine Neoplasia Type 2 (MEN-2 Carriers)
Exercise Test

Cellular/Molecular Mechanism of Action: Exercise-induced release of plasma adrenaline, noradrenaline, and dopamine.
Application: Early detection of asymptomatic pheochromocytoma.

Plasma catecholamines before and after submaximal exercise measured in MEN-2 gene carriers (e.g., n = 26; n = 8/26 asymptomatic pheochromocytomas; n = 9/26 medullary thyroid carcinoma; n = 10/26 following uni- or bilateral adrenalectomy). Comparisons can be made to normal healthy controls (e.g., n = 17) and cases of neurovegitative lability with symptoms mimicking pheochromocytoma (e.g., n = 11).
Considerations: Asymptomatic pheochromocytoma is a major risk for patients with MEN-2.
Implementation: Characterization of pheochromocytoma conditions utilizing a physiological test that has a high sensitivity and specificity. It is practical and well suited for repeated examinations and seems to be of value for the detection of early pheochromocytoma in MEN-2 patients. Furthermore, the test could be used in the differential diagnosis between pheochromocytoma and neurovegetative lability. An example of the diagnostic application of the test can involve the measurement of plasma catecholamine levels before and after submaximal exercise.

Human Model: Cases suffering from pheochromocytoma (MEN-2 gene carriers [e.g., n = 26; n = 8/26 asymptomatic pheochromocytomas; n = 9/26 medullary thyroid carcinoma; n = 10/26 following uni- or bilateral adrenalectomy]). Comparisons can be made to normal healthy controls (e.g., n = 17) and cases of neurovegitative lability with symptoms mimicking pheochromocytoma (e.g., n = 11).
Interpretation and Analysis: Plasma adrenaline, noradrenaline, and dopamine increase after exercise except for adrenaline after bilateral adrenalectomy. The postexercise levels of adrenaline and the adrenaline/dopamine ratio are significantly higher in the pheochromocytoma patients compared to the healthy controls and the patients with neurovegetative lability, while the patients with medullary thyroid carcinoma represented an intermediate group with a high probability of developing adrenal tumors.

Pheochromocytoma
Clonidine/Glucagon/Metoclopramide/Naloxone

Cellular/Molecular Mechanism of Action: Clonidine (central acting α_2-adrenergic agonist); glucagon (promotes hyperglycemia); naloxone (competitive antagonist at μ-, δ-, κ- and σ-opioid receptor complexes); and metoclopramide (enhances the action of acetylcholine at muscarinic receptor complexes and in the central nervous system antagonizes dopamine).
Physiological Mechanism of Action: Clonidine (reduced heart rate, reduced stroke volume, reduced peripheral vascular resistance that parallels reductions in systemic norepinephrine concentrations); glucagon (most significantly regulated by plasma glucose concentrations and primarily antagonizes the hormonal effects of insulin); naloxone (reverses or attenuates vascular hypertension); and metoclopramide (parasympathomemitic).
Application: Clonidine, glucagons, naloxone, and metoclopramide have been applied in the detection and monitoring of pheochromocytoma disease states.
Background Information: Some clinical investigations have shown that clonidine, which is widely used in clonidine suppression test, is sometimes fallible for the diagnosis of pheochromocytoma.
Route of Administration: Clonidine (oral); glucagons (intravenous); naloxone (intravenous); and metoclopramide (intravenous).
Dosage: Clonidine (150 μg); glucagons (1 mg); naloxone (10 mg); and metoclopramide (5 mg).
Implementation: Distinguishing pheochromocytoma positive cases from negative pheochromocytoma suspect cases through the measurement of plasma norepinephrine and epinephrine concentrations.

Human Model: Cases of pheochromocytoma (n = 6); and suspect but non-pheochromocytoma (n = 19).
Interpretation and Analysis: In comparisons between the diagnostic tests employing suppression (clonidine) and provocative agents (glucagon, metoclopramide, naloxone) performed in cases of pheochromocytoma (n = 6) and suspect but non-pheochromocytoma patients (n = 19), the blood pressure response does not predict well the presence of pheochromocytoma applying any of the tests. Plasma norepinephrine (NE) concentrations measured at 120 and 180 minutes following clonidine (150 μg per os) produces false negative results in approximately 2 out of 5 cases with pheochromocytoma. Both plasma norepinephrine and epinephrine concentrations measured before and sequentially after each provocative agent reveal that neither of the 2 agents respond to glucagons (1 mg IV) in some cases with pheochromocytoma (e.g., n = 2/4 or 50%). Determination of the peak level, peak increment, and % peak increment of norepinephrine and epinephrine following naloxone (10 mg IV) does not aid in distinguishing between the 2 groups. The % peak increments of both norepinephrine and epinephrine in all cases of pheochromocytoma (e.g., n = 4) given metoclopramide (5 mg IV) exceed the mean + 3 SD values for pheochromocytoma negative cases (norepinephrine = 25 ± 28%; epinephrine = 25 ± 42%). These results suggest that, when performed with judicious patient selection (ambiguous plasma or urinary catecholamine levels), the measurement of plasma catecholamines in response to metoclopramide can be a useful adjunctive tool in the diagnosis of pheochromocytoma.

Evaluation of clonidine suppression and various provocation tests in the diagnosis of pheochromocytoma. Koshida H, Miyamori I, Soma R, Matsubara T, Okamoto S, Ikeda M, Takeda R. J Endocrinol Invest 1990 Nov; 13 (10), pp. 807–15.

Pheochromocytoma (Silent/Subclinical)
Clonidine/Glucagon/[^{131}I]-Metaiodobenzylguanidine

Cellular/Molecular Mechanism of Action: Glucagon promotes catecholamine production/secretion.
Background Information: Pheochromocytomas have elevated membrane expression densities for glucagons receptor complexes.
Application: Diagnosis of subclinical/silent pheochromocytoma.
Considerations: Cases with pheochromocytoma present with clinical signs of hypertension, elevated serum catecholamine levels, elevated urinary norepinephrine concentrations, and/or unexpected detection of an abdominal mass.
Implementation: The glucagon provocation test promotes increased norepinephrine production/release while clonidine-suppression tests reduce norepinephrine production/secretion in normal individuals.
Interpretation and Analysis: A glucagon provocative test and [^{131}I]-metaiodobenzylguanidine scintigram can be useful in diagnosing conditions of atypical pheochromocytoma. The clonidine suppression test can be helpful in ruling out other conditions that may elevate catecholamines and metanephrines, or when catecholamines are only moderately elevated, or for any other reason when a diagnosis of pheochromocytoma is still uncertain.

A case of metastatic extra-adrenal pheochromocytoma 12 years after surgery. Mori S, Okura T, Kitami Y, Takata Y, Nakamura M, Watanabe S, Iwata T, Hiwada K. Hypertens Res 2002 Jan; 25 (1), pp. 141–4.

Cervical pheochromocytoma: A rare localization and a difficult diagnosis. Becker G, Jockenhövel F, Bauer R, Lederbogen S, Lange R, Reinwein D. J Endocrinol Invest 1992 Nov; 15 (10), pp. 767–70.

Pituitary Macroadenomas (Adenocorticotropic Hormone [ACTH]-Secreting)/Pituitary Surgery Response Evaluation
Dexamethasone/Corticotropin-Releasing Hormone (CRH)

Cellular/Molecular Mechanism of Action: Exposure to pituitary ACTH promotes the synthesis/release or adrenal cortisol. Dexamethasone suppresses hypothalamic CRH release, thereby diminishing pituitary ACTH synthesis/release.
Background Information: Cushing's disease due to large pituitary tumors is rare.
Appliation: Evaluation of pituitary/adrenal function in suspected cases of pituitary ademoma.
Implementation: Evaluation of conditions of ACTH-secreting pituitary macroadenomas through the measurement of plasma ACTH, serum cortisol, and 24-hour urinary free cortisol (UFC) levels. Complementary analyses include performing a high-dose dexamethasone (dexa) suppression test. Cases are then reevaluated following surgical intervention and, if not cured, a 2nd surgical procedure is performed in addition to , radiotherapy, and/or ketoconazole treatment. The follow-up periods can range over a time frame of 78 ± 10 months. In this manner, it is possible to establish certain biochemical characteristics at the time of clinical presentation and profile both results of 1st surgical approach, and adjuvant therapeutic strategies during a long-term follow-up period.

Human Model: Cases of ACTH-secreting pituitary macroadenoma (n = 26 cases; n = 9/26 male, n = 17/26 female; 42.5 ± 12.7 years, mean ± s.e.; tumor dimensions = 11–40 mm diameter).

Cases of ACTH-secreting pituitary macroadenomas evaluated with a high-dose dexamethasone (dexa) suppression test (n = 22); or CRH (n = 20 cases).

Interpretation and Analysis: Before surgery, dexamethasone decreases ACTH (> 50% of baseline) in approximately 50% of cases (e.g., n = 14/26). The CRH-stimulated ACTH/cortisol response is normal in < 25% of cases (e.g., n = 6/26); impaired in slightly < 25% of cases (e.g., n = 6/26), and exaggerated in < 33% of cases (e.g., n = 8/26). Following surgical intervention, < 33% are cured (e.g., n = 8/26), slightly > 33% have normalized cortisol levels (n = 9/26), and > 33% are not cured (e.g., n = 9/26).

Presurgery, mean ACTH values are significantly higher in cases not curred of the condition than in those normalized ($p < 0.05$) and cured ($p < 0.01$); the ACTH response to CRH can be impaired in some cases not curred (e.g., n = 6). Tumor diameter is significantly less in cured patients ($p < 0.02$) and in normalized patients ($p < 0.05$) compared to cases not cured of the condition. Magnetic resonance imaging (MRI) shows invasion of the cavernous sinus in some normalized cases (e.g., n = 2/9), and in not-cured cases (e.g., n = 6/9). After surgery, ACTH, cortisol and UFC are significantly lower than at entry in cured and in normalized cases, but not in not-cured cases. In the cured group, the disease can reoccur in some cases (e.g., n = 1 unsuccessfully treated with ketoconazole). In normalized case types, a relapse can occur (e.g., n = 8); in addition to potential outcomes including radiotherapy and ketoconazole induced cortisol normalization (e.g., n = 1), hypoadrenalism (e.g., n = 1); ineffective (e.g., n = 1); or lost to follow-up (e.g., n = 5). In not-cured case types, some may require a 2nd attempt at surgical intervention, radiotherapy and/or ketoconazole (e.g., n = 8), while a small number may be lost to follow-up (e.g., n = 1). Therapies can induce cortisol normalization (e.g., n = 2) or hypoadrenalism (e.g., n = 1). A subset of patients with ACTH-secreting pituitary macroadenoma show low sensitivity to high doses of dexamethasone and to CRH. Pituitary surgery cures Cushing's disease in a minority of patients. High baseline ACTH levels, impaired ACTH responses to CRH, increases in tumor size or invasion of the cavernous sinus are unfavorable prognostic factors for surgical intervention. A 2nd attempt at surgical intervention, radiotherapy, and/or ketaconazole can cure or normalized hypercortisolism in approximately half of cases with recurrence or not cured.

Long-term results of treatment in patients with ACTH-secreting pituitary macroadenomas. Cannavò S, Almoto B, Dall'Asta C, Corsello S, Lovicu RM, De Menis E, Trimarchi F, Ambrosi B. Eur J Endocrinol 2003 Sep; 149 (3), pp. 195–200.

HYPOTHALAMUS AND PITUITARY: GROWTH HORMONE

Acromeglia/Pituitary Dwarfism
Clonidine

Cellular/Molecular Mechanism of Action: Clonidine is an α-adrenergic agonist (xylazine is a structural analog). Systemically, it promotes the release of growth hormone (GH) mediated through induced secretion or liberation of growth hormone-releasing factor.

Diagnostic Application: Diagnosis of acromeglia and pituitary dwarfism (e.g., canine) in scenarios that fail to display resting elevations in growth hormone concentrations.

Route of Administration and Dosage: Intramuscular injection at a dosage of 10 μg/kg. Alternatively human growth hormone-releasing factor (hGHRF) can be administered at a dosage of 1 to 5 μg/kg.

Considerations: Clonidine can cause bradycardia and profound hypotension. Atropine can be administered to prevent or correct the induction of bradycardia. Antagonists such as phentolamine and yohimbine (α-adrenergic antagonists) can be applied to reverse hypotensive sequela.

Interpretation and Analysis: Blood samples (2 ml) are collected at intervals of 15, 30, 45, 60, and 120 minutes into EDTA anticoagulant tubes.

Laboratory Instrumentation: Assay of either growth hormone (GH) concentrations by either RIA or ELISA methodologies.

Acromegaly: Postoperative Growth Hormone (GH) Function/Hypothalamic/Pituitary Dysfunction
Thyrotropin-Releasing Hormone (TRH)/Glucose Tolerance Test (GTT)/Lanreotide

Cellular/Molecular Mechanism of Action: Exogenous or endogenous TRH and glucose can promote the release of pituitary GH in conditions of acromegaly.

Route of Administration: Thyrotropin-releasing hormone test (intravenous); glucose (oral).

Dosage: TRH test (400 μg); glucose (75 g).

Implementation: Evaluation of active postoperative acromegaly before and after long-term slow-release (SR) lanreotide therapy (30 mg IM every 10–14 days) achieved by measuring alterations in plasma GH in response to the oral glucose tolerance test (OGTT) and TRH stimulation test. The OGTT and TRH test are repeated before and after 6, 12, 24, and 36 months.

Human Model: Cases of active postoperative acromegaly (n = 10, of which n = 7/10; n = 2/7 males; age 29–71 years; cases that during therapy maintained plasma GH and insulin-like growth factor-I (IGF-I) concentrations under 5 μg/l and 450 μg/l, respectively, are considered as responders and studied for 24 [n = 1 case] to 36 months [n = 6 cases] in addition to n = 3/10 cases; n = 1/3 male; age = 46–61 years with levels of GH and IGF-I above those values studied for 12 months).

Interpretation and Analysis: The GH response to OGTT is abnormal (nadir: > 2 μg/l) at 6 and 12 months in poorly responsive patients. This response is normalized in all responsive patients. Nonetheless, some responsive patients show abnormal GH values after OGTT throughout the 36-month study period (n = 2). The GH response to TRH is characterized by great variability and exhibits unpredictable behavior throughout the study period both in responsive and in poorly responsive cases. Only 2 patients in the responsive group show persistent normal GH levels (peak: ≤ 5 μg/l) after TRH for 3 years. In conclusion, SR lanreotide treatment gives rise to a correct control of GH hypersecretion and to a normalization of GH response to oral glucose in 7 out of 10 patients, although it does not abolish the paradoxical reaction of GH to TRH in all responders. The effect of SR lanreotide on GH response to GTT is not paralleled by GH response to TRH.

Growth hormone responses to oral glucose and intravenous thyrotropin-releasing hormone in acromegalic patients treated by slow-release lanreotide. Díez JJ, Iglesias P, Gómez-Pan A. J Endocrinol Invest 2001 May; 24 (5), pp. 303–9.

Acromegaly/Pituitary Dwarfism
Glucose

Cellular/Molecular Mechanism of Action: Absorbed from the proximal small intestine resulting in elevated synthesis and secretion of pancreatic insulin. Transport of glucose across intact cell

membranes is regulated (promoted) by insulin. When the renal threshold is exceeded, glucose is excreted into the urine.

Diagnostic Application: Detection and characterization of pituitary-based acromegaly.

Route of Administration and Dosage: A Glucose Suppression Test is performed by administering glucose either orally or by intravenous injection.

Dosage: Glucose (1 gm/kg).

Considerations: Glucose given orally in horses can promote the onset of laminitis.

Implementation: Glucose Suppression Test for identification and characterization of acromegaly and pituitary dwarfism conditions.

Animal Model: Feline.

Interpretation and Analysis: Cases suffering from pituitary-based acromegaly display elevated concentrations of growth hormone (GH) within the systemic circulation. Glucose suppresses pituitary GH secretion and this finding can be utilized as a means of verifying a diagnosis of acromegaly. In normal reference control subjects, systemic concentrations of GH are suppressed to concentrations $<$ 5 ng/ml within 60 minutes after glucose infusion. Presistently elevated GH levels following administration of glucose are diagnostically suggestive of acromegaly.

Laboratory Instrumentation: Measurement of spectrophotometric absorbance.

Acromegaly/Short Stature
Growth Hormone-Releasing Hormone (GHRH)/GRF_{1-37}-OH, GRF_{1-40}-OH, GRF_{1-44}-NH_2

Cellular/Molecular Mechanism of Action: Promotes the synthesis/secretion of growth hormone. Control of the GHRH secretion by peptidergic hypothalamic neurons occurs through 4 principal monoaminergic systems such as dopaminergic, noradrenergic, adrenergic, and serotoninergic 1s, and also by cholinergic fibers and by endogenous opiates, all acting to cause the release of GHRH into the hypothalamo-hypophyseal portal circulation.

Application: Diagnosis and characterization of acromegaly and short-stature conditions. Preparations may also be beneficial when applied as a therapeutic agent for children with GH deficiency and may improve current understanding about the hypothalamic pathogenesis of short stature.

Considerations: Beginning in 1982, multiple publications have reported the application of 3 GRF preparations, including GRF_{1-37}-OH, GRF_{1-40}-OH, GRF_{1-44}-NH_2. The later preparations in concert with immunohistochemical methods produced results similar to hypothalamic hGHRH.

Interpretation and Analysis: Some clinical investigators have concluded that GHRH testing has limited diagnostic usefulness in the clinical evaluation of acromegaly but allows discrimination of acromegalic patients with ectopic production of GHRH from those with pituitary tumors.

In patients with short stature, they demonstrate a level of GH deficiency that is detectable through the exogenous administration of GHRH demonstrating a level of hypothalamic disregulation and not a pure pituitary deficiency as has been supposed before GHRH discovery.

Delayed Growth: Constitutional Growth Delay (CGD)/Hypogonadotropic Hypogonadism (HH)
Gonadotropin-Releasing Hormone (GnRH)/Nafarelin

Background Information: Previous investigations have observed nocturnal luteinizing hormone (LH) equal to 5.5 $\pm$ 0.9 IU/I for CGD and 2.7 $\pm$ 0.7 IU/I for HH that represents a statistically significant difference. Mean nocturnal follicle-stimulating hormone (FSH) has been reported to be 5.1 $\pm$ 1.0 and 2.5 $\pm$ 0.2 IU/I, whereas mean nocturnal T concentrations are statistically different at approximately 4.2 $\pm$ 0.8 and 0.7 $\pm$ 0.2 nmol/I for CGD compared to HH, respectively. Peak LH responses to nafarelin are approximately 36.9 $\pm$ 8.9 IU/I for CGD, and 7.0 $\pm$ 2.0 IU/I for HH (statistically significant). Peak FSH released by nafarelin is about 14.2 $\pm$ 2.4 IU/I for CGD and 4.8 $\pm$ 2.0 IU/I for HH (statistically significant). Peak Ts are reached at 24 hours following nafarelin injection and are equivalent to 5.7 $\pm$ 1.7 nmol/I for CGD and 0.3 $\pm$ 0.2 nmol/I for HH (statistically significant).

Application: Distinguish between CGD in boys with Tanner stage I of sexual development and patients with HH.

Route of Administration: Subcutaneous.

Dosage: Nafarelin (1 μg/kg up to 100 μg).

Implementation: Evaluation of hypothalamic/pituitary function based on the interpretation of changes in LH, FSH, T, and E2 concentrations following nafarelin administration. Blood samples are collected at timed intervals every 15 minutes for 24 hours for the measurement of LH, FSH, T, and E2 (e.g., RIA, ELISA). Pretreatment baseline values are obtained by collecting blood samples every 15 minutes during a 12-hour overnight schedule.

Human Model: Cases of suspected CGD from HH.

Interpretation and Analysis: Stimulated release of plasma LH, FSH, and T is significantly higher with nafarelin compared to GnRH, which also takes a longer period of time to reach maximum peak concentrations. Experimental findings indicate that in early stages of puberty (before detectable changes of sexual maturation) the nafarelin test combined with the measurement of LH, FSH, and T in the blood or urine is superior to and more practical than overnight hormonal estimates to clearly distinguish CGD from HH.

Instrumentation: Instrumentation and facilities necessary to perform RIA- or ELISA-based methodologies.

Gonadotropin-releasing hormone agonist analog (nafarelin): A useful diagnostic agent for the distinction of constitutional growth delay from hypogonadotropic hypogonadism. Kletter GB, Rolfes-Curl A, Goodpasture JC, Solish SB, Scott L, Henzl MR, Beitins IZ. J Pediatr Endocrinol Metab 1996 Jan–Feb; 9 (1), pp. 9–19.

Growth Hormone Deficiency (GHD): Insulin-Like Growth Factor
Clonidine

Application: Identification of conditions associated with GHD.

Route of Administration: Clonidine (oral).

Dosage: Clonidine.

Considerations: Serum levels of total insulin-like growth factor I (IGF-I) and IGF-binding protein-3 (IGFBP-3) reflect endogenous GH secretion in healthy children, which makes them good diagnostic markers for screening of GHD in short children, although some controversy still exists. Only a minor fraction of the total IGF-I circulates in "free form", which is believed to be the 1 that exerts the highest level of biological activity. However, our knowledge of the clinical or physiological value of determination of free IGF-I in serum is limited at present. In adults, the diagnostic value of total IGF-I and IGFBP-3 determinations in patients suspected of GHD has only been reported in a few studies, whereas no previous reports on the diagnostic value of free IGF-I levels in adults suspected of GHD exist.

Implementation: Evaluation of serum levels of free IGF-I utilizing a immunoradiometric assay (Diagnostic Systems Laboratories) to establish valid normative data for this analysis. Complementary

investigations can assess the diagnostic value of free IGF-I in relation to total IGF-I and IGFBP-3 determinations in adults suspected of having GHD. A GH provocative test, using oral clonidine, is performed in cases previously treated with GH in childhood.

Human Model: Cases without evidence of disease (n = 1430 children, adolescents, adults; n = 108 previously treated with GH in childhood and received oral clinidine)

Interpretation and Analysis: In healthy subjects, free IGF-I levels increase during childhood, with the highest mean values detected during puberty. After puberty, a subsequent decline in serum levels of free IGF-I occurs. Unmeasurable free IGF-I values may be detected in approximately 3.3% (e.g., n = 34) of prepubertal children. The vast majority of individuals over 8 years of age have measurable free IGF-I levels that amount to approximately 1% of the total IGF-I concentrations (e.g., 100% of cases). Free IGF-I levels are below normal in most GHD cases (e.g., n = 56/79 GHD; sensitivity = 71%) and above 2 SD in most patients with a normal GH response (e.g., n = 24/29, specificity = 83%). Multiple linear regression analyses demonstrate that free IGF-I is significantly dependent on peak GH levels, duration of the disease, and number of other pituitary axes affected. Therefore, free IGF-I serum levels increase during childhood with a peak in puberty, whereafter free IGF-I levels return to prepubertal levels. Three percent of healthy prepubertal children have unmeasurable free IGF-I levels using this assay. Determination of the free IGF-I serum concentration may predict the outcome of a GH provocative test in adults suspected of GHD, but a single determination of free IGF-I offers no significant advantage compared to determination of total IGF-I or IGFBP-3 serum levels.

Free insulin-like growth factor I serum levels in 1430 healthy children and adults, and its diagnostic value in patients suspected of growth hormone deficiency. Juul A, Holm K, Kastrup KW, Pedersen SA, Michaelsen KF, Scheike T, Rasmussen S, Müller J, Skakkebaek NE. J Clin Endocrinol Metab 1997 Aug; 82 (8), pp. 2497–502.

Growth Hormone Deficiency (GHD)
Growth Hormone-Releasing Hormone/Arginine (ARG)

Cellular/Molecular Mechanism of Action: Growth hormone-releasing hormone promotes the release of pituitary GH.

Application: Detection and characterization of GHD including idiopathic inflammatory pituitary stalk thickness, Langerhans cell histiocytosis (LCH) affecting the hypothalamic-pituitary area, and craniopharyngioma.

Implementation: Evaluation of conditions of acquired GHD including idiopathic inflammatory pituitary stalk thickness, Langerhans cell histiocytosis (LCH) affecting the hypothalamic-pituitary area, and craniopharyngioma.

Interpretation and Analysis: A high percentage of patients show GH response < 10 μg/liter after 2 pharmacological stimuli and tested with GHRH + ARG (mean age of 11.2 ± 4.1 years). All cases (mean age, 9.6 ± 3.1 years; range, 5.6–20.8) show GH response < 10 μg/liter after 2 pharmacological stimuli and tested with GHRH + ARG at a mean age of 11.2 ± 4.1 years. Acquired GHD cases will also often display vasopressin deficiency, TSH deficiency, gonadotropin deficiency, and ACTH deficiency. The median peak GH response to the insulin test is approximately 2.1 μg/liter (range, 1.1–2.9) compared to 1.5 μg/liter (range, 1.3–2.4) after arginine. The median peak GH response to insulin is significantly lower in patients with craniopharyngioma (1.4 μg/liter; range, 0.8–1.7) than in the patients with idiopathic pituitary stalk thickness (2.2 μg/liter; range, 1.0–2.4) or with LCH (2.6 μg/liter; range, 2.0–4.3, p = 0.02). The median peak GH response to arginine is significantly lower in patients with idiopathic inflammatory pituitary stalk thickness (1.3 μg/liter; range, 0.8–1.8) in contrast to those with craniopharyngioma (1.5 μg/liter; range, 1.1–1.6) or with LCH (2.8 μg/liter; range, 1.9–3.2, p = 0.00007). The median peak GH response after GHRH + ARG is significantly lower in the overall patient population (8.3 μg/liter; range, 4.4–28.4) than in age-matched controls (49.8 μg/liter; range, 39.9–81.6, p < 0.00001). The median peak GH response is significantly lower in craniopharyngioma patients (4.6 μg/liter; range, 3.6–6.3) than in those with LCH (8.9 μg/liter; range, 4.4–28.4) or with idiopathic pituitary stalk thickness (12.6 μg/liter, range, 6.4–24, p = 0.07). Some affected patients may have a GH response of more than 20 μg/liter after GHRH + ARG. There is often a trend toward a decrease in peak GH response to GHRH + ARG (r = −0.57, p = 0.06) as patient age increases. For cutoff values of 20 μg/liter, the sensitivity of GHRH + ARG is approximately 75% (95% CI, 57.8–87.9%) and the specificity 96.4% (95% CI, 89.9–99.2%); whereas, for cutoff values of 24.2 μg/liter, sensitivity is 86.1% (95% CI, 70.5–95.3%), and specificity 95.2% (95% CI, 88.2–98.7%). The median insulin-like growth factor-I (IGF-I) level does not differ between children with idiopathic pituitary stalk thickness (57 μg/liter; range, 46–68), and those with either LCH (55 μg/liter; range, 34–63), or craniopharyngioma (41 μg/liter; range, 39–49).

Interpretation the clinical findings confirmed the diagnostic potential of the GHRH + ARG test in children with acquired GHD caused by hypothalamic-pituitary lesion. It stimulates GH secretion to a greater extent in those patients with GHD with primary involvement of the hypothalamic area, for example, patients with idiopathic pituitary stalk thickness or LCH, than in those with both hypothalamic and pituitary lesion, as in craniopharyngioma. In some patients, the GHRH + ARG test stimulates GH response to a so-called normal value, suggesting that pituitary responsiveness to GHRH + ARG may fail to recognize acquired GHD. Finally, the number of pituitary hormone deficits and the patient's age affect the GH response to GHRH + ARG.

GHRH + arginine in the diagnosis of acquired GH deficiency of childhood-onset. Maghnie M, Cavigioli F, Tinelli C, Autelli M, Aricò M, Aimaretti G, Ghigo E. J Clin Endocrinol Metab 2002 Jun; 87 (6), pp. 2740–4.

Growth Hormone Deficiency (GHD): Radiation Injury/Somatotropic Axis Function
Arginine/Insulin

Background Information: Growth hormone provocative tests remain the mainstay for the diagnosis of GHD, with the insulin tolerance test (ITT) serving as the gold standard methodology for a number of years. There are, however, a variety of other stimulation tests used in clinical practice. Each necessitates the use of a specific cutoff derived from normative data, but there remains a widely held view that the implications from a "failed" test are independent of the nature of the stimulus.

Application: Assessment of GH production in conditions of suspected injury/damage to the somototropic axis (e.g., radiation exposure).

Implementation: Evaluation of the integrity of the somatostatin axis by assessing plasma somatostatin responses following arginine and insulin administration. Non-acromegalic patients can be identified following the completion of an arginine stimulation test (AST) and an ITT within a 3-month period.

Human Model: Non-acromegalic cases (n = 161). Groups are divided into those tested before (n = 81; n = 48 males) and those tested after (n = 80; 36 males) completion of growth and puberty. Case selection for inclusion is based on a history of cranial irradia-

tion and a GH response to 1 provocative test of < 8 μg/L (suggesting some damage to the GH axis). Reference controls can consist of cases of normal adults (n = 35 adults; n = 18 males) and prepubertal children (n = 16, n = 10 males).

Interpretation and Analysis: The median peak (range) GH response to an ITT is significantly greater ($p < 0.0001$) than that to the AST in adult controls: 24.9 (4.1–76.9) vs 12.2 (0.88–35.0) μg/L, respectively. However, in patients the GH responses are similar ($p = 0.28$): 2.2 (0.2–25.7) vs 1.4 (0.2–12.8) μg/L to the ITT and AST, respectively. In contrast to the pattern seen in the adult controls, responses to an ITT in childhood controls are of a similar magnitude ($p = 0.5$) to AST: 17.5 (8.1–40.0) vs 19.4 (7.3–53.8) μg/L, respectively. However, in patients, the GH response to AST is greater than that to the ITT ($p < 0.0001$): 4.3 (0.7–17.2) vs 3.0 (0.4–18.1) μg/L, respectively. Therefore, the impact of irradiation on GH responsiveness to provocative agents is stimulus dependent. The GH response to an AST appears to be more resistant to the effects of irradiation than to the ITT. When investigating the impact of irradiation on GH secretory status, the GH response to an AST may be a less sensitive guide to the functional ability of the GH axis.

The impact of irradiation on growth hormone responsiveness to provocative agents is stimulus dependent: Results in 161 individuals with radiation damage to the somatotropic axis. Lissett CA, Saleem S, Rahim A, Brennan BM, Shalet SM. J Clin Endocrinol Metab 2001 Feb; 86 (2), pp. 663–8.

Growth Hormone Deficiency (GHD): Spontaneous Hyperadrenocorticism/Mitotane (o,p'DDD) Therapeutic Response
Xylazine

Cellular/Molecular Mechanism of Action: Xylazine is an α-adrenergic agonist (clonidine is a structural analog) that promotes the release of growth hormone (GH) through elevated secretion or liberation of growth hormone-releasing factor (GHRF).

Diagnostic Application: Diagnosis of acromeglia and pituitary dwarfism that fail to display elevations in growth hormone following xylazine administration (e.g. hyperadrenal corticism).

Route of Administration and Dosage: Intramuscular administration at a dosage of 100 to 300 μg/kg. Alternatively human growth hormone-releasing factor (hGHRF) can be administered at a dosage of 1 to 5 μg/kg.

Considerations: Xylazine can cause bradycardia and profound hypotension. Atropine can be administered to prevent or correct the induction of bradycardia. Antagonists such as phentolamine and yohimbine (α-adrenergic antagonists) can be applied to reverse hypotensive sequela.

Implementation: Evaluation of plasma growth hormone response to the xylazine provocative agent before and after therapeutic intervention for hyperadrenocorticism. Blood samples (2 ml) are collected at intervals of 15, 30, 45, 60, and 120 minutes into EDTA anticoagulant tubes.

Animal Model: Canine (n = 4 with spontaneous hyperadrenocorticism), before and after therapy.

Interpretation and Analysis: Before treatment of the hyperadrenocorticism, no significant increase in growth hormone concentration can usually be detected following xylazine administration (e.g., n = 0/4). A significant increase in growth hormone concentrations following xylazine administration may be appreciated in conditions of hypophysis (pituitary)-dependent hyperadrenocorticism after treatment with mitotane (e.g., n = 2/3) or following surgical removal of a hyperfunctional adrenal adenoma (e.g., n = 1/1). In instances where impaired growth hormone response may persist, the condition may respond to xylazine after hyperadrenocorticism as been controlled for a longer period of time. Such findings demonstrate that in dogs, as in humans,, the excessive production of endogenous corticosteroids associated with either hypophysis-dependent hyperadrenocorticism or hyperfunctional adrenal tumor can induce suppression of growth hormone release but is reversible following treatment.

Laboratory Instrumentation: Assay of either growth hormone (GH) concentrations by either RIA or ELISA methodologies.

Suppression of growth hormone secretion in spontaneous canine hyperadrenocorticism and its reversal after treatment. Peterson ME, Altszuler N. Am J Vet Res 1981 Nov; 42 (11), pp. 1881–3.

Growth Hormone Deficiency (GHD)
Hexarelin/Arginine

Physiological Mechanism of Action: Growth hormone secretagogue.

Application: Evaluation of GH endocrinology.

Background Information: Due to persistent qualitative abnormalities in GH secretion following treatment and lack of a sensitive marker of GHD in mid-adult life, it is extremely difficult to diagnose GHD in treated acromegalic patients. The diagnosis of GHD in patients with pituitary disease relies on provocative tests of GH reserve. Arginine releases GH by reducing somatostatin inhibition of GH release, whereas GH secretagogues (GHS) affect GH release by direct stimulation of the GHS receptor, though an intact GH-releasing hormone (GHRH) axis is a prerequisite. The peak GH response to insulin-induced hypoglycemia and arginine in acromegalic patients, in whom basal serum GH levels of < 5 mU/l have been achieved, is greatly diminished in those treated by hypothalamo-pituitary irradiation.

Route of Administration: Arginine (intravascular infusion over 30 minutes); hexarelin (intravenous bolus).

Dosage: Arginine (20 g/m^2); hexarelin (1.5 mcg/kg).

Considerations: Increasing total fat mass results in a blunted GH response following subcutaneous hexarelin.

Implementation: Assess the effect of body composition and gender on GH-releasing capacity (hexarelin-induced) in successfully treated acromegalic cases in view of the different putative mechanism of action of hexarelin, in addition to determining if it has any value in the diagnosis of GHD in case subsets of this type. In an example diagnostic protocol, case subjects undergo 20 minutes of blood sampling to provide a 24-hour GH profile. Serum insulin-like growth factor-I (IGF-I) is measured from a sample drawn between 0900 hours and 1000 hours. On a 2nd occasion, arginine is infused and blood samples collected before infusion and at 30-minute intervals thereafter for 180 minutes. At the final visit hexarelin is administered (t = 0) and blood drawn at 15-minute intervals from −30 to +180 minutes.

Human Model: Cases of acromegaly in whom mean serum GH levels below 5 mU/l have been achieved through treatment (n = 19; n = 8 treated by surgery alone [Case Group Type A]; n = 11 receiving primary or postoperative irradiation [Case Group Type B]).

Interpretation and Analysis: The vast majority of Group Type A cases will show an increment in serum GH following hexarelin (DeltaGHHEX) > 20 mU/l, a normal response to arginine, and a mean 24-hour GH > 0.5 mU/l. In Group Type B cases, less than half (e.g., n = 5/11) will achieve a DeltaGHHEX > 20 mU/l, producing a response of < 2 mU/l (e.g., n = 4/11). Approximately 80% of cases with a DeltaGHHEX < 2 mU/l will also demonstrate a mean 24-hour GH of < 0.5 mU/l and serum IGF-I SDS $< +0.5$ (e.g., n = 4/5). The vast majority of Group Type B cases achieving a DeltaGHHEX > 20 mU/l will show an absent or minimal GH response to

arginine (e.g., n = 4/4). Despite loss of the GH response to arginine, the DeltaGHHEX is retained in a proportion of those patients in whom "safe" GH levels have been achieved following irradiation. From the putative mechanisms of action of these provocative agents a plausible explanation would be that the GHRH axis is more resilient than endogenous somatostatin-secreting neurones to radiation-induced damage. Furthermore, GH secretagogues may have a role, in combination with serum IGF-I levels, in the diagnosis of GHD in treated acromegaly.

Note: Total fat mass appears to be a useful predictor of peak GH response even in normal individuals. This indicates that there is a continuum of effect of fat mass on hexarelin-stimulated GH release. Any impact of gender on the GH response to hexarelin is almost certainly indirect and mediated via differences in body composition. This observation will have an impact on the potential diagnostic and therapeutic uses of hexarelin and related GH secretagogues.

The diagnosis of growth hormone deficiency (GHD) in successfully treated acromegalic patients. Murray RD, Peacey SR, Rahim A, Toogood AA, Thorner MO, Shalet SM. Clin Endocrinol (Oxf) 2001 Jan; 54 (1), pp. 37–44.

Growth Hormone Deficiency (GHD)/ Geriatric
Hexarelin

Cellular/Molecular Mechanism of Action: Hexarelin is a GH-releasing peptide (GHRP) hexapeptide that induces the release of GH.

Background Information: Growth hormone release is influenced by several factors including age, gender, physical exercise, nutritional status, sex steroids, and body composition. The relationship with body composition is complex. Obesity is accompanied by suppression of spontaneous and stimulated GH release. As increasing body fat reduces stimulated GH secretion following a standard provocative test, the potential clinical uses of GHRPs, therapeutically or diagnostically, may be dependent on the relationship between body fat and GHRP-stimulated GH release.

Application: Evaluation of GH production and release.

Route of Administration: Hexarelin (subcutaneous injection).

Dosage: Hexarelin (1.5 μg/kg).

Implementation: Blood samples are collected at −10, 0, 10, 20, 30, 40, 50, 60, 90, 120, 170, and 180 minutes following hexarelin administration.

Human Model: Twenty-one (8 male) healthy elderly subjects with a median (range) age of 68 (60–81) years and BMI of 26 (19–30) kg/m^2.

Interpretation and Analysis: Peak GH responses correlate negatively with fat mass, BMI, percentage body fat, and weight ($r = -0.72$, $p = 0.0001$; $r = -0.56$, $p = 0.009$; $r = -0.63$, $p = 0.002$, and $r = -0.48$, $p = 0.029$, respectively). The area under the curve (AUC) for GH concentrations correlates negatively with fat mass, BMI, and percentage fat mass ($r = -0.58$, $p = 0.006$; $r = -0.51$, $p = 0.019$; and $r = -0.66$, $p = 0.001$, respectively). Using multiple linear regression analyses, fat mass can be found to be the most useful predictor for both peak GH response ($r^2 = 0.61$, $p < 0.0001$) and AUC GH ($r^2 = 0.38$, $p = 0.003$). Gender is not a significant variable. Increasing total fat mass results in a blunted GH response following subcutaneous hexarelin. Total fat mass appears to be a useful predictor of peak GH response even in normal individuals. This indicates that there is a continuum of effect of fat mass on hexarelin-stimulated GH release. Any impact of gender on the GH response to hexarelin is almost certainly indirect and mediated via differences in body composition. This observation will have an impact on the potential diagnostic and therapeutic uses of hexarelin and related GH secretagogues.

Hexarelin enhances the diagnostic value of performing a conventional provocative test for GHD.

Instrumentation: Facilities and instrumentation necessary to perform immunoassay procedures (e.g., RIA or ELISA). Dual-energy X-ray absorptiometry (DEXA) can be used to assess body composition.

The effect of body composition on hexarelin-induced growth hormone release in normal elderly subjects. Rahim A, O'Neill P, Shalet SM. Clin Endocrinol (Oxf) 1998 Nov; 49 (5), pp. 659–64.

Growth Hormone Deficiency (GHD)
GH-Releasing Peptides (GHRPs)/(Hexarelin Alternative)

Cellular/Molecular Mechanism of Action: Growth hormone-releasing peptides and their non-peptidyl mimetics are synthetic molecules that possess marked, dose-related, and reproducible GH-releasing effect even after oral administration. Hexarelin is a GHRP hexapeptide.

Background Information: Clinical investigations imply that GHRPs are a powerful stimulus of GH secretion in pubertal children and young adults only. Alternatively, the age-related variations in the GH response to GHRPs probably limit their reliability for the evaluation of GH-releasable pool in prepubertal children and elderly subjects.

Application: Diagnosis of GHD.

Route of Administration: Hexarelin (IV); hexapeptide GHRH (IV); arginine (IV).

Dosage: Hexarelin (2 μg/kg), and GHRH (1 μg/kg); arginine (0.5 g/kg).

Considerations: The potent stimulatory effect of GHRPs on GH secretion suggests their usefulness as a provocative test for the diagnosis of GHD.

Implementation: Comparison of the GH response to the maximal effective dose of hexarelin with that of GHRH alone and combined with arginine (likely acts via inhibition of hypothalamic somatostatin release).

Interpretation and Analysis: In prepubertal children (n = 6; 4 boys and 2 girls, age 2.6–12.2 years) the GH response to HEX (19.0 ± 4.6 μg/l; 611.5 ± 121.4 μg/l/h) is lower than that to GHRH (27.4 ± 12.7 μg/l; 1209.0 ± 590.9 μg/l/h) but such differences are not statistically significant Both these responses are, in turn, lower ($p < 0.05$) than that to ARG + GHRH (57.9 ± 15.1 μg/l; 2483.6 ± 696.6 μg/l/h). In pubertal children, the GH response to HEX (67.6 ± 12.7 μg/l; 2755.3 ± 547.3 μg/l/h) is higher than that to ARG + GHRH (49.1 ± 8.9 μg/l; 2554.1 ± 356.6 μg/l/h) but this difference does not attain statistical significance; both these responses are, in turn, clearly higher ($p < 0.05$) than that to GHRH alone (23.1 ± 7.9 μg/l; 1004.8 ± 214.3 μg/l/h). In young adults, the GH response to HEX (60.9 ± 8.0 μg/l; 2401.0 ± 376.2 μg/l/h) is similar to ARG + GHRH (68.9 ± 11.7 μg/l; 3035.7 ± 466.6 μg/l/h) and both are clearly higher ($p < 0.001$) than that to GHRH alone (21.6 ± 3.6 μg/l; 790.0 ± 137.0 μg/l/h). In elderly subjects, the GH response to HEX (22.4 ± 4.9; 855.0 ± 199.0 μg/l/h) is higher ($p < 0.01$) than that to GHRH (3.6 ± 0.8 μg/l; 151.8 ± 24.6 μg/l/h) but lower ($p < 0.05$) than to ARG + GHRH (48.1 ± 4.6 μg/l; 1758.2 ± 149.1 μg/l/h).

Instrumentation: Facilities and instrumentation necessary to perform immunoassay procedures (e.g., RIA or ELISA). Dual-energy X-ray absorptiometry (DEXA) can be used to assess body composition.

Hexarelin, a synthetic GH-releasing peptide, is a powerful stimulus of GH secretion in pubertal children and in adults but not in prepubertal children and in elderly subjects. Bellone J, Bartolotta E, Sgattoni C, Aimaretti G, Arvat E, Bellone S, Deghenghi R, Ghigo E. J Endocrinol Invest 1998 Sep; 21 (8), pp. 494–500.

Growth Hormone Deficiency (GHD): (Pituitary Dwarfism): Insulin Resistant Diabetes
Insulin/Glucose

Cellular/Molecular Mechanism of Action: Promotes transport of glucose across cellular membranes into the cytosol.
Diagnostic Application: Pituitary-based (feline) acromegaly.
Route of Administration: Subcutaneous injection.
Dosage: See under interpretation and analysis.
Interpretation and Analysis: Patients (feline) displaying insulin-resistant diabetes mellitus (persistent hyperglycemia) following administration of insulin at a dose $\geq$ 25 U/day should be considered to be possibly suffering from pituitary-based acromegaly. Additional clinical signs that support the diagnosis of acromegaly would include the presence of arthropathy and cardiomyopathy. Acromegaly patients will have excessively high systemic concentrations of GH within the intravascular compartment. Further diagnostic pharmacology strategies can be utilized to verify a diagnosis of acromegaly through the application of a Glucose Suppression Test since glucose suppresses pituitary GH secretion.
Laboratory Instrumentation: Laboratory facilities and instrumentation necessary for performing immunoassay procedures (RIA or ELISA).

Growth Hormone Deficiency (GHD)/Preadult
Estradiol and Arginine/Clonidine

Route of Administration: Aerosolized E2 estradiol followed by arginine/clonidine-induced GH production.
Interpretation: Estradiol enhances GH-insulin-like growth factor (GH-IGF) induced by arginine/clonidine. Enhances the diagnostic value of an existing provocative test for GHD.

Growth Hormone Deficiency (GHD)
Arginine/Clonidine/Glucagon/Insulin

Physiological Mechanisms of Action:
Clonidine: Central acting α_2-adrenergic agonist.
Glucagon: Recognizes and binds to glucagon receptor complexes.
Insulin: Recognizes and binds to insulin receptor complexes resulting in enhanced intracellular glucose uptake.
Arginine: Arginine functions to potentiate the release of GH in response to growth hormone-releasing hormone (GHRH).
Clonidine: Promotes release of GH through elevated secretion or liberation of growth hormone-releasing factor.
Glucagon: Promotes hyperglycemia and is most extensively regulated by systemic blood glucose concentrations.
Insulin: Promotes hypoglycemia and is most extensively regulated by systemic blood glucose concentrations.
Application: Characterization of GH synthesis/secretion capacity in suspect cases of GHD. The most profound GH release is seen after insulin-induced hypoglycemia. Glucagon appears to be more effective at inducing GH release than arginine. Clonidine at a dose of 100 or 200 μg is no more effective than placebo.
Route of Administration: Insulin (intravenous); arginine (intravenous infusion over 30 minutes); clonidine (oral); glucagon (IM).
Dosage: Insulin (0.2 IU/kg); arginine (20 g/m^2) as an IV infusion over 30 minutes; clonidine (either 100 or 200 μg); glucagon (1 mg).
Considerations: In adults, there is limited information regarding GH responses to provocative stimuli other than insulin-induced hypoglycemia.
Implementation: In comparisons of 4 different growth hormone secretagogues, blood samples are collected from an intravenous catheter for GH concentrations at −30, −15, and 0 minutes (e.g., normal healthy adult males post–overnight fast). Provocative agents are then administered in a randomized manner on different days as indicated (insulin 0.2 IU/kg IV; arginine 20 g/m^2 as an IV infusion over 30 minutes; oral clonidine at either 100 or 200 μg; glucagon 1 mg IM). Additional blood samples are taken at 15-minute intervals for 180 minutes for GH estimation.
Interpretation and Analysis: The median (range) GH peak response that can be appreciated for each diagnostic agent includes insulin 107.7 (28.1–200) mU/l; arginine 22.3 (3.1–72.9) mU/l; glucagon 42 (11.8–200) mU/l; 100 μg clonidine 7.2 ($<$ 1–22.2) mU/l; 200 μg clonidine 8.2 (1.1–88) mU/l, and placebo 2.4 ($<$ 1–30.2) mU/l. Peak GH response to insulin-induced hypoglycemia (ITT) is significantly greater than for any other agent ($p < 0.0001$). The peak GH response to glucagon is significantly greater than for arginine ($p < 0.05$), clonidine at 100 and 200 μg ($p < 0.01$), and placebo ($p < 0.01$). The peak GH response following administration of arginine is significantly greater than for clonidine 100 and 200 μg ($p < 0.05$), and placebo ($p < 0.01$). The peak GH response following clonidine 200 μg is not significantly greater than observed with clonidine 100 μg ($p = 0.38$) or placebo. In 1 clinical investigation, n = 2/18; n = 6/18, and n = 15/18 test cases failed to achieve a peak GH level of $>$ 20 mU/l to glucagon, arginine, and clonidine, respectively. In complete contrast only 1 subject achieved a peak response of $<$ 40 mU/l (28.1 mU/l) to ITT.

The assessment of growth hormone status in normal young adult males using a variety of provocative agents. Rahim A, Toogood AA, Shalet SM. Clin Endocrinol (Oxf) 1996 Nov; 45 (5), pp. 557–62.

Growth Hormone Deficiency (GHD)
Growth Hormone-Releasing Hormone (GHRH)/Growth Hormone-Releasing Peptide (GHRP)-6 (Single 30-Minute Assay)

Application: The GHRH + GHRP-6 test biochemically classifies patients into 3 groups; those with a stimulated GH peak $\geq$ 20 μg/l are considered normal and those with peaks at $\leq$ 10 μg/l as GH deficient.
Route of Administration: Growth hormone-releasing hormone (IV) and GHRP-6 (IV).
Dosage: Growth hormone-releasing hormone (1 μg/kg) and GHRP-6 (1 μg/kg).
Considerations: The diagnosis of GHD in adults is based on the provocative testing of GH secretion. When testing a patient with suspected GHD, clinicians assess the whole secretory curve and select the GH peak as an index of secretory capability. This procedure is time consuming and the determination of GH in several samples is necessary. The combined administration of GHRH + GHRP-6 is an effective test of GH secretion, and it has been unambiguously demonstrated that the elicited GH peak is capable of segregating normal GH secretion subjects from GH-deficient patients on an individual basis.

The GHRH + GHRP-6 test is a convenient, safe, and reliable, provocative test of GH reserve in adults, which can be reduced to a single fixed GH determination 30 minutes after stimulus.

Interpretation and Analysis: A high degree of correlation can be observed between GH values at 30 minutes and GH peaks ($r = 0.994$, $p < 0.0001$). Evaluation of 30-minute values instead of the peak in GH-deficient patients in previous clinical trials results in only 2 out of 146 cases being reclassified from uncertain to a diagnosis of GHD. Thus, better diagnostic classification can be provided for patients. Administration of GHRH (1 μg/kg IV) + GHRP-6 (1 μg/kg IV) reveals that GHRP-6 evokes GH peaks in controls and patients and values do not correlate with GH basal values, making this determination useless for test validation.

Instrumentation: Laboratory facilities and instrumentation necessary for performing immunoassay procedures (RIA or ELISA).

Single growth hormone (GH) determination is sufficient for the diagnosis of GH-deficiency in adult patients using the growth hormone-releasing hormone plus growth hormone releasing peptide-6 test. Leal A, Lage M, Popovic V, Torres E, Koppeschaar HP, Paramo C, Micic D, Garcia-Mayor RV, Dieguez C, Casanueva FF. Clin Endocrinol (Oxf) 2002 Sep; 57 (3), pp. 377–84.

Growth Hormone Deficiency (GHD)
Ghrelin/Insulin, Growth Hormone-Releasing Hormone [GHRH]/Arginine Alternative

Cellular/Molecular Mechanism of Action: Ghrelin is a 28 amino acid acylated peptide that is a natural ligand of the GH secretagogues (GHS) receptor (GHS-R) that is specific for synthetic GHS and expressed in the hypothalamus and pituitary. In addition to GH stimulation, ghrelin and synthetic GHSs are reported to stimulate the hypothalamo-pituitary-adrenal (HPA) axis (*in vivo*).

Application: Determination of the influence of ghrelin on GH, in addition to prolactin, adenocorticotropic hormone (ACTH), and cortisol levels in adult cases of isolated severe GHD.

Route of Administration: Systemic intravenous or intracerebroventricular (ghrelin), and intravenous routes of application (ghrelin, insulin, GHRH, arginine).

Dosage: Ghrelin (1 μg/kg), insulin-induced hypoglycemia (ITT, 0.1 IU regular insulin); GHRH (1 μg/kg) + arginine (ARG, 0.5 g/kg). Dosages described in other reports report the administration of ghrelin in an amount between 100 nM and 1,000 nM.

Considerations: Similar to synthetic GHS, ghrelin strongly stimulates GH secretion but also displays significant stimulatory effects on lactotroph and corticotroph secretion. It has been hypothesized that isolated GHD could reflect hypothalamic impairment that would theoretically involve a defect in ghrelin activity. Cases of GHD and normal controls have overlapping prolactin (1 μg/l = 32 mU/l) (10.0 ± 1.4 vs 14.9 ± 2.2 μg/l), ACTH (22.3 ± 5.3 vs 18.7 ± 4.6 pmol/l), and cortisol responses (598.1 ± 52.4 vs 486.9 ± 38.9 nmol/l).

Interpretation and Analysis: Cases assessed for GH response to insulin-induced hypoglycemia (ITT, 0.1 IU regular insulin IV) and GHRH (1 μg/kg IV) + arginine (ARG, 0.5 g/kg IV) reveal that with respect to GH, levels of insulin-like growth factor-I (IGF-I) are markedly lower than in age-matched controls (69.8 ± 11.3 vs 167.9 ± 19.2 μg/l, $p < 0.003$). Ghrelin administration induces significant increases in GH, prolactin (PRL), ACTH, and cortisol levels in the vast majority of GHD-affected cases. The GH response is usually higher with ghrelin compared to GHRH + ARG, which itself is higher ($p < 0.05$) than observed with insulin (9.2 ± 4.1 vs 5.3 ± 1.7 vs 1.4 ± 0.4 μg/l). Given this consideration, GH responses (1 μg/l = 2 mU/l) in GHD are markedly lower ($p < 0.0001$) than those measured in age-matched controls (ghrelin vs GHRH + ARG vs insulin 92.1 ± 16.7 vs 65.3 ± 8.9 vs 17.7 ± 3.5 μg/l). Furthermore, cases of GHD display the highest individual GH peaks in response to ghrelin but are markedly lower than the lowest peak GH response in age-matched controls (28.5 vs 42.9 μg/l).

The findings noted in some preliminary investigations collectively suggest that ghrelin is 1 of the most powerful provocative stimuli of GH secretion, even in those patients with isolated severe GHD. In this condition, however, the somatotroph response is markedly reduced while the lactotroph and corticotroph responsiveness to ghrelin is fully preserved, indicating that this endocrine activity is fully independent of mechanisms underlying the GH-releasing effect. These results do not support the hypothesis that ghrelin deficiency is a major cause of isolated GHD but suggest that ghrelin might represent a reliable provocative test to evaluate the maximal GH secretory capacity provided that appropriate cutoff limits are assumed.

Ghrelin (100 and 1,000 nM) stimulates significant release of GHRH from hypothalamic explants (100 nM: 39.4 ± 8.3 vs basal 18.3 ± 3.5 fmol/explant, $n = 49$, $p < 0.05$) but does not affect either basal or 28 mM KCl-stimulated somatostatin release. Ghrelin (10, 100, and 1,000 nM) stimulates the release of both corticotropin-releasing hormone (CRH) (100 nM: 6.0 ± 0.8 vs basal 4.2 ± 0.5 pmol/explant, $n = 49$, $p < 0.05$) and arginine vasopressin (AVP) (100 nM: 49.2 ± 5.9 vs basal 35.0 ± 3.3 fmol/explant, $n = 48$, $p < 0.05$), while ghrelin (100 and 1,000 nM) also stimulates the release of neuropeptide Y (NPY) (100 nM: 111.4 ± 25.0 vs basal 54.4 ± 9.0 fmol/explant, $n = 26$, $p < 0.05$) from hypothalamic explants (*in vitro*). The HPA axis is stimulated (*in vivo*) following acute intracerebroventricular administration of ghrelin 2 nmol (ACTH 38.2 ± 3.9 vs saline 18.2 ± 2.0 pg/ml, $p < 0.01$; corticosterone 310.1 ± 32.8 ng/ml vs saline 167.4 ± 40.7 ng/ml, $p < 0.05$), but not following intraperitoneal administration of ghrelin 30 nmol. Such findings suggest a hypothalamic site of action and imply that the mechanisms of GH and ACTH regulation by ghrelin may include hypothalamic release of GHRH, CRH, AVP, and NPY.

Instrumentation: Laboratory facilities and instrumentation necessary for performing immunoassay procedures (RIA or ELISA).

Endocrine responses to ghrelin in adult patients with isolated childhood-onset growth hormone deficiency. Aimaretti G, Baffoni C, Broglio F, Janssen JA, Corneli G, Deghenghi R, van der Lely AJ, Ghigo E, Arvat E. Clin Endocrinol (Oxf) 2002 Jun; 56 (6), pp. 765–71.

Growth Hormone Deficiency (GHD)
Growth Hormone-Releasing Hormone (GHRH)/ Arginine (ARG)

Cellular/Molecular Mechanism of Action: Growth hormone-releasing hormone promotes the release/synthesis of growth hormone. The influence of GHRH on GH release/synthesis is potentiated by arginine.

Considerations: The relationship between the hypothalamus-pituitary morphology and the somatotroph responsiveness to maximal provocative tests exploring the GH releasable pool is still unclear. In has been shown in some preliminary investigations that the partial integrity of the hypothalamic pituitary connections is essential for GHRH + arginine to express its GH-releasing activity. Investigations have also shown that this provocative test is able to stimulate GH secretion to a greater extent in those patients with GHD, but with a residual vascular component of the pituitary stalk. This test is reliable in the diagnosis of congenital hypopituitarism in both children and adults when associated with complete pituitary stalk agenesis and MPHD. In younger children with congenital GHD but less severe impairment of the pituitary stalk the GH response to GHRH + ARG may be within the normal range; deterioration of

pituitary GH reserve with a GH response of < 10 μg/L after 20 years of age makes this test very sensitive in the diagnosis of adult GHD.

Interpretation and Analysis: In studies comparing the GH-releasing effect of GHRH + ARG in cases of congenital GHD relative to their pituitary magnetic resonance imaging findings for the anterior pituitary hypoplasia, stalk agenesis (neural and or vascular component), and posterior pituitary ectopia. In children, the median peak GH response to GHRH + ARG (7.6 μg/L; range, 2.4–40.2 μg/L) is significantly higher than that in the adults (1.8 μg/L; range, 0.8–37.4 μg/L; p = 0.0039), and is also significantly higher in the isolated GHD patients (18 μg/L; range, 3.3–40.2 μg/L) than in those with MPHD (1.9 μg/L; range, 0.8–7.6 μg/L; p = 0.00004). In the patients with residual vascular component of the pituitary stalk the median peak GH response to GHRH + ARG (19.1 μg/L; range, 1.6–40.2 μg/L) is significantly higher than that found in cases with complete pituitary stalk agenesis (2.2 μg/L; range, 0.8–8.8 μg/L; p = 0.00005).

A tendency is often noticed toward a decrease with age in peak GH response to GHRH + ARG: Mean serum insulin-like growth factor I (IGF-I) levels are 36 ± 7.1 μg/L in children and 63.5 ± 22.6 μg/L in adults (p = 0.0001). The mean IGF-I levels do not differ between the children with (35.7 ± 4.8 μg/L) and those without (36.3 ± 8.7 μg/L) the pituitary stalk, but are much higher in the adults with residual vascular pituitary stalk (81.1 ± 17.7 μg/L) than in those with complete pituitary stalk agenesis (47.7 ± 12.5 μg/L; p = 0.0002). An IGF-I level of 36.1 ± 6.7 μg/L in the isolated GHD children and 36 ± 8.6 μg/L in those with MPHD has been reported with levels of 82.1 ± 19.4 and 52.7 ± 16.8 μg/L, respectively, described for adults (p = 0.003).

Instrumentation: Laboratory facilities and instrumentation necessary for performing immunoassay procedures (RIA or ELISA).

Relationship between the morphological evaluation of the pituitary and the growth hormone (GH) response to GH-releasing hormone plus arginine in children and adults with congenital hypopituitarism. Maghnie M, Salati B, Bianchi S, Rallo M, Tinelli C, Autelli M, Aimaretti G, Ghigo E. J Clin Endocrinol Metab 2001 Apr; 86 (4), pp. 1574–9.

Growth Hormone Deficiency (GHD)
Estradiol (E2)

Application: Determine the influence of estradiol on GH-insulin-like growth factor (GH-IGF) axis in prepubertal GHD and idiopathic short-stature children.

Dosage: Micronized estradiol E2 (1 or 2 mg) for 3 days followed by a sequential arginine-clonidine test.

Considerations: The growth hormone stimulation tests after E2 priming had the highest diagnostic efficiency. Our findings suggest that the effect of estrogen priming on GH stimulated levels, by reducing the number of false nonresponders, might be useful to better discriminate between normal and abnormal GH status in short-stature children.

Interpretation and Analysis: In short-stature children, GH maximal responses are approximately 27.9 ± 14.5 μg/L with estrogen (17.8 ± 10.9 placebo: p < 0.0001). The lower 95% confidence limits for GH maximal response shifts from 3.7 μg/L (without E2) to 8.3 μg/L (on E2). In GHD children, no significant stimulatory effect of estrogen on GH levels can be appreciated. After placebo, a cutoff limit of 3.7 μg/L (the lower 95% confidence interval limit) results in 73% sensitivity, 95% specificity, and an overall 90% diagnostic efficiency. After E2, a cutoff limit of 8.3 μg/L results in a sensitivity of 87%, a specificity of 98%, and a diagnostic efficiency of 95%. After placebo, short-stature children (68%) show normal IGF-I levels, and the mean does not change on E2 (13.7 ± 6.3 vs 14.3 ± 6.8 nmol/L, not significant). In almost all short stature children (93%) IGF binding protein (IGFBP)-3 levels are normal with placebo evaluation. Estradiol E2 produces mean IGFBP-3 that does not change significantly (2.63 ± 0.70 vs 2.70 ± 0.70 mg/L). In the vast majority of GHD cases (14/15), IGF-I values are below normal with placebo, and the group mean does not change after E2 administration. During placebo, most growth hormone deficient children (e.g., n = 13/15) GHD present with low IGFBP-3 values. Estradiol produces a small significant increase IGFBP-3 values (1.06 ± 0.58 vs 1.20 ± 0.69 mg/L, p < 0.02). The highest diagnostic efficiencies for IGF-I and IGFBP-3 that can be expected to be observed with placebo are approximately 75% and 91%, respectively.

Instrumentation: Laboratory facilities and instrumentation necessary for performing immunoassay procedures (RIA or ELISA).

Estrogen priming effect on growth hormone (GH) provocative test: A useful tool for the diagnosis of GH deficiency. Martínez AS, Domené HM, Ropelato MG, Jasper HG, Pennisi PA, Escobar ME, Heinrich JJ. J Clin Endocrinol Metab 2000 Nov; 85 (11), pp. 4168–72.

Growth Hormone Deficiency (GHD)
Growth Hormone-Releasing Hormone (GHRH) and Arginine/Insulin-Induced Hypoglycemia (ITT) Alternative

Cellular/Molecular Mechanism of Action: Growth hormone-releasing hormone and arginine promote the release of pituitary growth hormone. Arginine functions to potentiate the release of GH in response to GHRH.

Application: Detection of GHD.

Route of Administration: Growth hormone-releasing hormone (IV) and arginine (IV).

Dosage: Growth hormone-releasing hormone (1 μg/kg) and arginine (0.5 g/kg).

Considerations: Patients with childhood-onset GHD need retesting in late adolescence or young adulthood to verify whether they have to continue recombinant human GH treatment. Provocative testing with GHRH + arginine (GHRH + ARG) is the most reliable alternative to the insulin-induced hypoglycemia test (ITT) for the diagnosis of GHD in adulthood, provided that appropriate cutoff limits are assumed (normal limits, 16.5 μg/L as 3rd and 9.0 μg/L as 1st centile).

In conclusion, given appropriate cutoff limits, GHRH + ARG is as reliable as ITT for retesting patients who have undergone GH treatment in childhood. Among these patients, severe GHD in adulthood is generally confirmed in organic hypopituitarism with GHD (oGHD), is frequent in idiopathic isolated GHD (iGHD), but never occurs in GH neurosecretory dysfunction (GHNSD).

Interpretation and Analysis: The GH response to a single GHRH (1 μg/kg iv) + ARG (0.5 g/kg iv) test has been described in young patients that have received GH replacement in childhood, based on the following diagnosis: (i) oGHD with GH peak < 10 μg/L after 2 classical tests; (ii) iGHD: GH peak < 10 μg/L after 2 classical tests; and (iii) GHNSD: GH peak > 10 μg/L after classical test but mGHc < 3 μg/L. Responses in GH synthesis/release in these groups following GHRH + ARG administration reveal insulin-like growth factor I levels in oGHD subjects (61.5 ± 13.7 μg/L) that are lower (p < 0.001) than those in iGHD subjects (117.2 ± 13.1 μg/L); with the latter being lower than those in GHNSD subjects (210.2 ± 12.9 μg/L), which, in turn, are analogous to those in age-matched reference controls (220.9 ± 7.1 μg/L). The mean GH peak after GHRH + ARG in oGHD (2.8 ± 0.8 μg/L) is lower (p < 0.001) than that in

iGHD (18.6 ± 4.7 μg/L), which, in turn is markedly lower ($p < 0.001$) than that in GHNSD (31.3 ± 1.6 μg/L). The GH response in GHNSD is lower than that in age-matched controls (65.9 ± 5.5 μg/L), but this difference does not attain statistical significance. Regarding the 3rd centile limit of GH response in young adults (i.e., 16.5 μg/L), retesting confirms GHD in all oGHD, in 65.2% of iGHD, and in none of the GHNSD subjects. In the 1st centile limit of GH response (i.e., 9.0 μg/L), retesting reveals severe GHD in oGHD (94%) and iGHD (52.1%). All oGHD and iGHD with a GH peak after GHRH + ARG that are lower than 9 μg/L also have GH peaks lower than after ITT (3 μg/L). In cases that have GHD confirmed by retesting, the mean GH peak after GHRH + ARG is higher than values observed after ITT (3.4 ± 0.5 vs 1.9 ± 0.4).

Instrumentation: Laboratory facilities and instrumentation necessary for performing immunoassay procedures (RIA or ELISA).

Retesting young adults with childhood-onset growth hormone (GH) deficiency with GH-releasing-hormone-plus-arginine test. Aimaretti G, Baffoni C, Bellone S, Di Vito L, Corneli G, Arvat E, Benso L, Camanni F, Ghigo E. J Clin Endocrinol Metab 2000 Oct; 85 (10), pp. 3693–9.

Growth Hormone Deficiency (GHD)/ Childhood

Acipimox/Arginine/Growth Hormone-Releasing Hormone (GHRH)/Arginine (ARG)/GHRH

Cellular/Molecular Mechanism of Action: Growth hormone-releasing hormone/arginine induces the release of GH. Acipimox reduces plasma free fatty acids in a manner that enhances GH response after stimulation by GHRH.

Background Information: The hormonal diagnosis of GHD in childhood is conventionally based on the GH response to at least 2 provocative stimuli. Among these, arginine has long been considered a classical, centrally mediated stimulus of GH secretion. Arginine is also able to potentiate the GH response to GHRH, likely inhibiting hypothalamic somatostatin; this combined test is 1 of the most potent to explore the maximal secretory capacity of somatotroph cells.

Application: Diagnosis of childhood GHD (48 normal short children; 35 M and 13 F, 12.0 ± 0.4 years, PS 1: 255 II-IV: 23). Testing with the sequential administration of arginine and ARG + GHRH may allow the single-step evaluation of the somatotroph response to central and pituitary stimuli in short children.

The acipimox/GHRH test is a potential additional tool to detect GHD in patients with pituitary disease, in particular in cases with a perturbation of fatty acid metabolism. The acipimox/GHRH test leads to GH responses similar to those of the GHRH/arginine test, and to higher peak GH values if compared with the insulin tolerance test (ITT) when applied in patients displaying such types of physiological abnormalities.

Route of Administration: Arginine (IV), GHRH (IV).

Dosage: Arginine (0–+30 minutes) followed by a co-administration of arginine (from +120 to 150 minutes) plus GHRH (1 μg/kg at +120 minutes).

Considerations: Reduction of plasma free fatty acids leads to enhanced GH response after stimulation by GHRH.

Implementation: Evaluate the sequential administration of arginine and ARG + GHRH to determine if it can feasibly serve as a single-step provocative test to evaluate the GH-releasable pool in short children.

Interpretation and Analysis: Arginine alone elicits a clear GH response (mean peak vs baseline: 12.1 ± 1.7 vs 2.0 ± 0.4 μg/l, $p < 0.001$, Cmax range 12–51.0 μg/l). Following this GH rise, the hormonal levels at +120 minutes approach baseline levels (4.2 ± 0.8 μg/l) but then show marked response to the co-administration of ARG + GHRH. The GH peak following ARG + GHRH (mean peak: 47.8 ± 3.3 μg/l, $p < 0.001$; Cmax 22.4–150.0 μg/l) is clearly higher ($p < 0.001$) than that recorded after arginine alone. The GH responses to both arginine and ARG + GHRH are independent of gender, puberty, height velocity, body mass index (BMI), and insulin-like growth factor-I (IGF-I) levels. Some normal short children (e.g., n = 9/56 or 16%) have GH peaks lower than 7 μg/l after arginine alone, while a very low number will show GH peaks below 20 μg/l after ARG + GHRH (e.g., n = 0/56). Thus, arginine alone is a good stimulus of GH secretion but false positive responses frequently occur in normal short children. ARG + GHRH is a more potent stimulus giving no false positive responses even after previous challenge with arginine alone.

In cases after pituitary surgery (mean age 53.0 years; mean BMI 26.7 kg/m^2), comparisons of GH responses after acipimox and GHRH with GH responses during an ITT or a GHRH/arginine test reveal that mean (± SEM) peak GH is almost 4-fold higher after acipimox/GHRH (6.94 ± 1.07 μg/l, range, 0.46–23.1; $p < 0.001$) and after GHRH/arginine (8.32 ± 1.23 μg/l, range, 1.1–49.2; $p < 0.001$) than after ITT (1.84 ± 0.46 μg/l, range, 0.01–11.9). According to results from ITT, affected cases usually are found to be severely GH deficient (peak GH < 3.0 μg/l). Peak GH levels after acipimox/GHRH in controls range from 7.5 to 78.4 μg/l (mean 29.3 ± 3.5). Growth hormone peak values during the acipimox/GHRH test significantly correlate with values from ITT ($r = 0.63$, $p < 0.01$) and GHRH/arginine tests ($r = 0.87$, $p < 0.001$). Areas under the curve (AUC) also correlate. According to generally accepted cutoff peak GH levels for the ITT and GHRH/arginine test, a GH peak exceeding 11.2 μg/l excludes severe GH deficiency after acipimox/GHRH. Therefore the cutoff level is lower at older age. The acipimox/GHRH test leads to GH responses similar to those of the GHRH/arginine test, and to higher peak GH values if compared with the ITT. The acipimox/GHRH test is a potential additional tool to detect GHD in patients with pituitary disease, in particular in patients with a perturbation of fatty acid metabolism.

Diagnosis of growth hormone deficiency after pituitary surgery: The combined acipimox/GH-releasing hormone test. van Dam PS, Dieguez C, Cordido F, de Vries WR, Veldhuyzen BF, van Thiel E, Casanueva FF, Koppeschaar HP. Clin Endocrinol (Oxf) 2003 Feb; 58 (2), pp. 156–62.

Growth Hormone Deficiency (GHD)

Arginine/Insulin-Mediated Hypoglycemia/L-dopa/ Clonidine/Growth Hormone-Releasing Hormone (GHRH)

Cellular/Molecular Mechanism of Action: Arginine, insulin hypoglycemia, L-dopa (dopaminergic) and clonidine (α-adrenergic), and GHRH all promote elevations in serum GH normal control subjects.

Application: Determine the integrity of dopaminergic and α-adrenergic neurotransmitter regulation of GH secretion in children with decreased GH secretion. The spectrum of cases (children) that can be evaluated include those with (i) GH neurosecretory dysfunction (GHND; n = 16); (ii) classical GH deficiency (n = 9); and (iii) short but otherwise normal children (n = 12).

Considerations: Growth hormone deficiency can be defined as children with height in the 1st percentile or below, growth velocity of 4 cm/year or less, low plasma somatomedin-C for age, delayed skeletal age by 2 or more years, peak serum GH responses to any 1 (or more) provocative test of 10 ng/ml or more, and mean 24-hour GH concentration below 3 ng/ml.

Implementation: Detection and evaluation of potential GHD cases. Subjects undergo a 24-hour GH study (blood sampling every

20 minutes for 24 hours) and provocative tests using arginine, insulin hypoglycemia, L-dopa (dopaminergic) and clonidine (a-adrenergic), and GHRH.

Interpretation and Analysis: Growth hormone neurosecretory dysfunction and GH-deficient children have reduced endogenous GH secretion, expressed as mean serum 24-hour GH concentration (1.6 ± 0.1 [± SEM] and 2.1 ± 0.1 vs 6.1 ± 0.5 ng/ml [GH-deficient and GHND vs normal, respectively]; $p < 0.01$). The mean peak serum GH levels after arginine (8.2 ± 2.0 vs 20.8 ± 6.6 ng/ml [GHND vs normal]; $p < 0.05$) and insulin (9.3 ± 1.0 vs 16.2 ± 1.7 ng/ml [GHND vs normal]; $p < 0.01$) are lower in GHND children. The mean peak responses after L-dopa (13.4 ± 3.4 vs 14.6 ± 4.7 ng/ml [GHND vs normal]; p = ns) and clonidine (19.0 ± 2.2 vs 23.3 ± 3.8 ng/ml [GHND vs normal]; p = ns) are preserved in GHND children. In GH-deficient children, mean peak serum GH concentrations after all 4 provocative tests are low (arginine, 2.7 ± 0.8; insulin, 2.6 ± 0.8; L-dopa, 3.0 ± 0.9; clonidine, 3.4 ± 1.0 ng/ml; all $p < 0.01$ vs normal). The mean peak serum GH concentration after GHRH is blunted in GH-deficient children (9.1 ± 1.7 ng/ml) compared to those in GHND (32.9 ± 8.5 ng/ml) and normal (43.2 ± 6.4 ng/ml) children ($p < 0.01$). The area under the GH curve after GHRH stimulation is greater for normal than GHND children ($p < 0.05$). Therefore, dopaminergic and α-adrenergic neurotransmitter pathways are preserved in GHND children. Such findings further suggest a defect in the release of pituitary GH secondary to an abnormality in alternative neurotransmitter pathways resulting in decreased GHRH and/or increased somatostatin secretion.

Preservation of dopaminergic and α-adrenergic function in children with growth hormone neurosecretory dysfunction. Bercu BB, Root AW, Shulman DI. J Clin Endocrinol Metab 1986 Oct; 63 (4), pp. 968–73.

Growth Hormone Deficiency (GHD): Adult Onset
GHRH and Arginine/L-dopa and Arginine/Arginine/L-dopa/Insulin (Insulin Tolerance Test [ITT])

Application: Characterization of adult-onset GHD accompanied either (i) with multiple pituitary hormone deficiencies; or (ii) with no more than 1 additional hormone abnormality.

Route of Administration: Arginine test (intravenous); GHRH (intravenous bolus at 0 minutes).

Dosage: Arginine test (0.5 g/kg from 0 to +30 min, up to a maximum of 30 g); GHRH (1 μg/kg at 0 minutes). The protocol for these tests generally includes measurement of GH in blood samples collected every 15 minutes from baseline up to 90 to 120 minutes.

Implementation: Evaluation of the primary end point (peak serum GH responses) to 5 GH stimulation tests administered in random order at 5 separate visits: ITT, arginine (ARG), levodopa (L-dopa), ARG + L-dopa, and ARG + GHRH. Serum IGF-I concentrations can be measured on 2 separate occasions. Three diagnostic cut-points are calculated for each test to provide optimal separation of multiple pituitary hormone deficient and control subjects according to 3 criteria: (i) to minimize misclassification of control subjects and deficient patients (balance between high sensitivity and high specificity); (ii) to provide 95% sensitivity for GHD; and (iii) to provide 95% specificity for GHD.

Human Model: Cases with adult-onset hypothalamic-pituitary disease and multiple pituitary hormone deficiencies studied in comparison with age, sex, and estrogen status (n = 39; n = 26 male, n = 13 female; n = 34 body mass index–matched controls n = 20/34 male, n = 14 female). A 3rd group of patients (n = 21) with adult-onset hypothalamic-pituitary disease have no more than 1 additional pituitary hormone deficiency. For purposes of analysis, cases with multiple pituitary hormone deficiencies are assumed to be GH deficient.

Interpretation and Analysis: The greatest diagnostic accuracy ($p = 0.001$) occurs with the ITT and the ARG + GHRH test (patients prefer the latter). Using peak serum GH cut-points of 5.1 μg/liter for the ITT and 4.1 μg/liter for the ARG + GHRH test, high sensitivity (96 and 95%, respectively) and specificity (92 and 91%, respectively) for GHD have been achieved. To obtain 95% specificity, the peak serum GH cut-points can be lowered to 3.3 μg/liter and 1.5 μg/liter for the ITT and ARG + GHRH test, respectively. There is substantial overlap between patients and control subjects for the ARG + L-dopa, ARG, and L-dopa tests, but test-specific cut-points can be defined for all 3 tests to provide 95% sensitivity for GHD (peak GH cut-points: 1.5, 1.4, and 0.64 μg/liter, respectively). However, 95% specificity can be achieved with the ARG + L-dopa and ARG tests only with very low peak GH cut-points (0.25 and 0.21 μg/liter, respectively) and not at all with the L-dopa test. Although serum IGF-I levels provide less diagnostic discrimination than all 5 GH stimulation tests, a value below 77.2 μg/liter is 95% specific for GHD. In conclusion, the diagnosis of adult GHD can be made without performing an ITT, provided that test-specific cut-points are used. The ARG + GHRH test represents an excellent alternative to the ITT for the diagnosis of GHD in adults. Either in children or in adults, arginine alone and combined with GHRH (GHRH + ARG) are reliable tests for the diagnosis of GHD.

After arginine alone, taking into account data from +15 to +105 minutes, GH values above the 3rd centile limit of arbitrary cutoff (7 or 10 μg/l in C and 5 μg/l in A) occur in 85% or 64% and 94% of the subjects (11.3 ± 0.4 years vs 30.0 ± 0.6 years, respectively). After GHRH + ARG tests, taking into account only data at +30, +45, +60 minutes GH values above the 3rd centile limit (20 μg/l in Group C and 16.5 μg/l in Group A) occurred in 99% of subjects in both groups. Taking into account only these 3 timing points, the percentage of GH peak above the 3rd centile limits after arginine alone is never higher than 60% in Group C and 85% in Group A. Therefore, the single GHRH + ARG test can be reliably performed in a shortened, easier, and cheaper procedure for clinical practice.

Sensitivity and specificity of six tests for the diagnosis of adult GH deficiency. Biller BM, Samuels MH, Zagar A, Cook DM, Arafah BM, Bonert V, Stavrou S, Kleinberg DL, Chipman JJ, Hartman ML. J Clin Endocrinol Metab 2002 May; 87 (5), pp. 2067–79.

Growth Hormone Deficiency (GHD)
Arginine/Growth Hormone-Releasing Hormone (GHRH)

Cellular/Molecular Mechanism of Action: Induces the release of GH.

Application: Either in children or in adults, arginine (ARG) alone and combined with GHRH (GHRH + ARG) are reliable tests for the diagnosis of GHD.

Route of Administration: Arginine (intravenous) and/or GHRH (intravenous bolus).

Dosage: Arginine (0.5 g/kg from 0 to +30 min, up to a maximum of 30 g) and/or GHRH (1 μg/kg bolus at 0 minutes).

Implementation: The procedures for the arginine and the combined arginine/GHRH (GHRH + ARG) tests generally include GH measurement every 15 minutes from baseline up to 90 to 120 minutes.

Human Model: Children (case groups defined as growing children and adolescents (n = 117 M and n = 56 F, age: 11.3 ± 0.4 years), while adult case groups are represented by young and middle-aged normal adults (A, n = 68 M and n = 57 F, age: 30.0 ± 0.6 years).

Interpretation and Analysis: Following arginine alone (taking into account data from +15 to +105 minutes), GH values above the 3rd centile limit of arbitrary cutoff (children = 7 or 10 μg/l, and adult = 5 μg/l) occur in 85% or 64% and 94% of subjects, respectively. After the GHRH + ARG test (taking into account only data at +30, +45, and +60 minutes), GH values above the 3rd centile limit (children = 20 μg/l, and adult = 16.5 μg/l) occur in 99% of subjects in both groups. Taking into account only these 3 timing points, the percentage of GH peak above the 3rd centile limits after ARG alone is never higher than 60% in children and 85% in adults. Therefore, a single GHRH + ARG test can be reliably performed in a shortened procedure, which makes clinical practice easier and further reduces costs.

Growth hormone deficiency in the transition adolescent: Should treatment be continued in adult life? Aimaretti G, Corneli G, Bellone S, Baffoni C, Camanni F, Ghigo E. J Pediatr Endocrinol Metab 2001; 14 Suppl 5, pp. 1233–42; discussion 1261–2.

Growth Hormone Deficiency (GHD) (Adult)
Arginine (ARG)/Hexarelin (HEX)/Growth Hormone-Releasing Hormone (GHRH)/GHRH + ARG vs GHRH + HEX

Application: Validation of the reliability of hexarelin and GHRH for the diagnosis of adult GHD.

Background Information: Growth hormone deficiency in adults must be shown by provocative testing of GH secretion. Insulin-induced hypoglycemia (ITT) is the test of choice, and severe GHD, treated with recombinant human GH replacement, is defined by a GH peak response to ITT of < 3 μg/L. GHRH + ARG is a more provocative test and is as sensitive as ITT provided that appropriate cutoff limits are assumed. GH secretagogues are a family of peptidyl and nonpeptidyl GH-releasing molecules that strongly stimulate GH secretion and, even at low doses, truly synergize with GHRH.

Route of Administration: Hexarelin (IV) and GHRH (IV).

Dosage: Hexarelin (0.25 μg/kg) and GHRH (1 μg/kg).

Considerations: Previous investigations have generated laboratory results that have defined the normal limits for GH responses to stimulation with low-dose HEX + GHRH in normal adults. Observations noted in these clinical trials reveal that the test is as sensitive as ITT for the diagnosis of adult GHD provided that appropriate cutoff limits are considered.

Implementation: Evaluation of GH response to GHRH + HEX administration, and comparison between GHRH + HEX, the ITT test with insulin, and GHRH + ARG normal controls and in conditions of suspected GHD and hypopituitaric adults.

Human Model #1: Cases without any known disease abnormalities (n = 25; 18 men and 7 women; age, 28.5 ± 0.6 years; n = 11 subjected to 2nd verification provocative test).

Human Model #2: Cases without any known disease abnormality (n = 33; 12 men and 21 women; age, 34.1 ± 1.5 years) and hypopituitaric adults with GHD (n = 19; 10 men and 9 women; age, 39.9 ± 2.2 years; GH peak < 5 μg/L after ITT).

Human Model #3: Cases with GHD (n = 19) and normal subjects (n = 77; n = 40 men; n = 37 women; age, 28.1± 0.6 years).

Interpretation and Analysis: The mean GH peak after GHRH + HEX in normal subjects (NS) is 83.6 ± 4.5 μg/L; the 3rd and 1st percentile limits of the normal GH response are 55.5 and 51.2 μg/L, respectively. The GH response to GHRH + HEX in NS shows good intraindividual reproducibility. In GHD the mean GH peak after GHRH + HEX (2.6 ± 0.7 μg/L) is similar to that after GHRH + ARG (3.6 ± 1.0 μg/L), and both are higher ($p < 0.001$) than that after ITT (0.6 ± 0.1 μg/L); the GH responses to GHRH + HEX are positively associated with those to ITT and GHRH + ARG. Analyzing individual GH responses, approximately 100% have severe GHD after ITT (GH peak, < 3 μg/L). After GHRH + HEX all GHD have GH peaks below the 3rd percentile limit of normality appropriate for this test (i.e., 55.5 μg/L). Approximately 68.4% (n = 13/19) of GHD cases have GH peaks below 3 μg/L after GHRH + HEX but the vast majority (e.g., n = 19/19 or 100%) have GH peaks below the 1st percentile limit of normality (i.e., 51.2 μg/L). The GH responses to GHRH + HEX are highly concordant with those after GHRH + ARG.

Low-dose hexarelin and growth hormone (GH)-releasing hormone as a diagnostic tool for the diagnosis of GH deficiency in adults: Comparison with insulin-induced hypoglycemia test. Gasperi M, Aimaretti G, Scarcello G, Corneli G, Cosci C, Arvat E, Martino E, Ghigo E. J Clin Endocrinol Metab 1999 Aug; 84 (8), pp. 2633–7.

Growth Hormone Deficiency (GHD) (Adult and Elderly)
Arginine/Growth Hormone-Releasing Hormone (GHRH)/Pyridostigmine/GHRH/Insulin Tolerance Test Alternatives

Cellular/Molecular Mechanism of Action: Promotes the release of GHRH.

Background Information: Presently, there is no therapeutic approach that can function as an alternative to recombinant human growth hormone (rhGH) for adult and elderly patients with GHD and/or for the somatopause. Growth hormone-releasing substances need the functional integrity of somatotroph cells to induce the release of GH. Probably only patients with childhood-onset, isolated GHD (frequently hypothalamic-dependent) could benefit from treatment with GHRH or GH secretagogues (GHS). Whenever restoration of the activity of the GH/insulin-like growth factor-I (IGF-I) axis in the elderly would be of use, GHRH and/or GHSs would be good candidates. In fact, the existence of a considerable pool of releasable growth hormones has been demonstrated in the elderly.

Application: Detection and evaluation of GH endocrinology (e.g., deficiency and overproduction).

Implementation: Determine if there are any alternative provocative tests equal to, or even better than, the insulin tolerance test (ITT), the so-called gold standard, for the diagnosis of GHD in adults and the elderly.

Interpretation and Analysis: Growth hormone-releasing hormone, when combined with arginine or pyridostigmine, becomes 1 of the most potent and reproducible tests for distinguishing patients with severe GHD from normal subjects. Owing to its tolerability and its suitability for use in the elderly, the GHRH + arginine test is the best alternative choice and is at least as sensitive as the ITT provided that appropriate cutoff limits are given.

Diagnostic and therapeutic uses of growth hormone-releasing substances in adult and elderly subjects. Ghigo E, Arvat E, Aimaretti G, Broglio F, Giordano R, Camanni F. Baillieres Clin Endocrinol Metab 1998 Jul; 12 (2), pp. 341–58.

Growth Hormone Deficiency (GHD)
Pyridostigmine and Growth Hormone-Releasing Hormone (GHRH)

Cellular/Molecular Mechanism of Action: Pyridostigmine (acetylcholinesterase inhibitor) potentiates the GH response to GHRH involving inhibition of somatostatin release.

Application: Assess the combination of pyridostigmine (PD) and GHRH as a reliable test probing pituitary somatotropic function.
Background Information: Diagnosis of GHD is currently based on failure to increase plasma GH levels to an arbitrary cutoff point of 7 or 10 μg/l in response to 2 provocative stimuli. False negative responses to these tests, however, frequently occur, thus reducing their diagnostic reliability.
Route of Administration: Pyridostigmine (PO) and GHRH (IV).
Dosage: Pyridostigmine (60 mg oral PD 60 minutes before GHRH) and GHRH (1 μg/kg).
Implementation: Evaluation of the combination PD + GHRH as a test for GHD. Results obtained by PD + GHRH are compared with those obtained by testing with GHRH, clonidine (CLON), and PD alone and by studying spontaneous nocturnal GH secretion over 8 hours.

Human Model: Cases of normal health (n = 27 children and adolescents) and a large group of short children classified as having familial short stature (FSS, n = 24), constitutional growth delay (CGD, n = 34), and GHD (organic, oGHD, n = 6; idiopathic, iGHD, n = 10).
Interpretation and Analysis: Assuming 7 μg/l as minimum normal GH peak, a positive response occurs in only 18/24, 11/12, and 12/13 NS for GHRH, CLON, and PD, respectively. In contrast, even assuming a minimum normal GH peak as high as 20 μg/l, PD + GHRH induces a positive response in 27/27 NS all having a nocturnal GH mean concentration (MC) ≥ 3 μg/l. Therefore PD + GHRH test produces no false negative responses and this is true not only in NS but even in all FSS and CGD having a GH MC ≥ 3 μg/l. On the other hand, PD + GHRH induces a negative GH response in all oGHD and in 8/10 iGHD patients. In the remaining 2 iGHD patients, PD + GHRH demonstrates a normal pituitary GH reserve in spite of a GH MC < 3 μg/l and low insulin-like growth factor-I (IGF-I) level, thus pointing to a hypothalamic pathogenesis for GHD. Considering FSS and CGD children having a GH MC < 3 μg/l, PD + GHRH shows a primary pituitary GH deficiency in 3/12 CGD with low plasma IGF-I levels. Therefore, in slowly growing children PD + GHRH test is the most reliable provocative test for the diagnosis of primary pituitary GHD being capable of discriminating between an unequivocally normal and impaired somatotropic function.

A new test for the diagnosis of growth hormone deficiency due to primary pituitary impairment: Combined administration of pyridostigmine and growth hormone-releasing hormone. Ghigo E, Imperiale E, Boffano GM, Mazza E, Bellone J, Arvat E, Procopio M, Goffi S, Barreca A, Chiabotto P, et al. J Endocrinol Invest 1990 Apr; 13 (4), pp. 307–16.

Growth Hormone Deficiency (GHD): Lack of Diagnostic Efficacy
Diazepam

Background Information: Diazepam as a provocative stimulus for hGH secretion has been suggested as a simple, reliable test in clinical hGH assessment of pituitary disorders.
Application: Detection and evaluation of pituitary disorders as a function of plasma hGH and human prolactin (hPRL) levels. Preliminary investigations imply that diazepam is not a suitable diagnostic pharmaceutical for this purpose.
Route of Administration: Intramuscular.
Dosage: Diazepam (10 mg).
Implemenation: Determination of the influence of diazepam on plasma hGH and hPRL concentrations.

Human Model: Cases of normal health (n = 5); and acromegalic subjects (n = 7).
Interpretation and Analysis: No significant variations in hGH and hPRL levels can be observed in any of the studied subjects. These findings indicate that the drug is ineffective on hGH and hPRL release and that it cannot be considered a valid provocative test either in normal or acromegalic subjects. Diazepam is not a viable diagnostic pharmaceutical for the purpose of characterizing cases of acromegaly.

Lack of effect of IM diazepam administration on hGH and hPRL secretion in normal and acromegalic subjects. Zaccaria M, Giordano G, Ragazzi E, Sicolo N, Foresta C, Scandellari C. J Endocrinol Invest 1985 Apr; 8 (2), pp. 167–70.

Growth Hormone Deficiency (GHD)/ Infantile Malnutrition
Growth Hormone-Releasing Hormone (GHRH)

Background Information: Growth hormone kinetics in infantile malnutrition are characterized by high basal values and decreased responses to various provocative stimuli. The mechanism of this alteration has not been explained.
Application: Detection and characterization of infantile malnutrition as a function of GH kinetics.
Route of Administration: Growth hormone-releasing hormone (1–44) NH_2 (IV bolus).
Dosage: Growth hormone-releasing hormone (1–44) NH_2 (1 mcg/kg).
Implementation: Determine if altered sensitivity to GHRH plays a role in alterated GH kinetics in infantile malnutrition. One approach to achieving this objective can involve diagnostic testing of infants with GHRH that suffer from primary malnutrition of grades II and III (evaluation before and again 6 weeks after nutritional rehabilitation).
Interpretation and Analysis: In affected infants (n = 19), mean baseline GH maximum elevation above baseline (delta GH), and area under the GH response curve (sigma GH) are measured. The injections stimulate a rapid rise in GH that reaches a maximum at 15 to 30 minutes after GHRH. There are no significant differences in the magnitude or time course of the responses between infants in the malnourished and recovering states (delta GH 29 ± 7 vs 34 ± 5 ng ml: sigma GH 3379 ± 571 vs 3459 ± 397 ng min/ml, respectively), and responses are similar to those of normal prepubertal children. Altered sensitivity of pituitary to GHRH appears not to underlie the alterations of GH dynamics observed in malnutrition.

Growth Hormone Deficiency (GHD)
L-dopa/Propanolol

Cellular/Molecular Mechanism of Action: Promotes the release of GH.
Application: Evaluation of GH secretion in short children.
Considerations: The low cost of L-dopa and propranolol, the simplicity and safety of the test, and the acceptable rate of low-responders make the test an effective screening test for GHD.
Implementation: Evaluation of hormones related to growth and maturation. The pituitary release mechanism for GH can be assessed using the propanolol/L-dopa stimulation test. In addition, blood levels of testosterone, sex hormone binding globulin, estradiol, thyroxine, prolactin, cortisol, follicle-stimulating hormone, and luteinizing hormone can be measured as points of reference.

Human Model: Children with idiopathic scoliosis (n = 95); normal reference controls (n = 60); ages between 7 and 17 years. Girls can be divided into age groups and all results evaluated according to chronological and skeletal age.

Interpretation and Analysis: Given the population evaluated, a large number of children will be deemed to have successfully passed the screening test when a GH concentration of > 15 miu/L is elicited after stimulation (n = 133 children passed). A fairly large number of children may fail the screening test (e.g., n = 52; with n = 33/52 with GHD with inadequate GH response to insulin-induced hypoglycemia). The residual cases may be classified as low-responders (e.g., n = 19) associated with the display of adequate GH response to insulin tolerance test (ITT). The low-responder rate to L-dopa-propranolol provocative test among short children who are not GH deficient is 12.5% approximately.

In the evaluation of children diagnosed with idiopathic scoliosis, girls display a significantly higher response to the GH stimulation test than controls between the ages of 7 and 12 years, whereas no significant difference can be detected in older girls. In girls with a skeletal age between 9 and 12 years, significantly higher mean serum levels of testosterone are present ($p < 0.05$). No significant differences can be demonstrated for the remaining hormones. Growth hormone and testosterone are the most important growth factors in prepubertal and pubertal children. Thus, the present findings suggest a hormonal basis for the increased stature in children with idiopathic scoliosis that has previously been reported.

Growth related hormones in idiopathic scoliosis. An endocrine basis for accelerated growth. Skogland LB, Miller JA. Acta Orthop Scand 1980 Oct; 51 (5), pp. 779–80.

Growth Hormone Deficiency (GHD): Obesity/ Hypopituitary Conditions/Hyposomatotropism vs Hypopituitarism: Hypothalamic/Pituitary Function
Growth Hormone-Releasing Hormone (GHRH) + Growth Hormone-Releasing Peptide (GHRP)-6 Test

Background Information: It has been gradually realized that GH may have important physiological functions in adult humans. The biochemical diagnosis of adult GHD is established by provocative testing of GH secretion. The insulin tolerance test (ITT) is the best validation provocative test. However, the insulin-based ITT has been challenged because of its low degree of reproducibility and lack of normal range, and is contra-indicated in common clinical situations. Furthermore, in severely obese subjects the response to the ITT frequently overlaps with those found in non-obese adult patients with GHD.

Application: Detect and evaluate alterations in GH endocrinology.

Implementation: Evaluate the diagnostic capability of 4 different stimuli of GH secretion: ITT, GHRH, GHRH plus acipimox (GHRH + Ac), and GHRH + GHRP-6, in 2 pathophysiological situations (e.g., hypopituitarism and obesity). In this context, in cases of hyposomatotropism of hypopituitarism, the combined test GHRH + GHRP-6 most accurately distinguishes both conditions, without the side effects of ITT.

Route of Administration: Insulin-tolerance test (intravenous insulin), GHRH (intravenous), GHRH (intravenous) preceded by acipimox (oral/per os) (GHRH + Ac), and GHRH (intravenous) plus GHRP-6 (intravenous) (GHRH + GHRP-6).

Dosage: Insulin-tolerance test (0.1 U/kg, 0.15 U/kg for obese, 0 minutes), GHRH (100 μg, 0 minutes), GHRH (100 μg, 0 minutes) preceded by acipimox (250 mg, at −270 minutes and −60 minutes) (GHRH + Ac); and GHRH (100 μg, 0 minutes) plus GHRP-6 (100 μg, 0 minutes) (GHRH + GHRP-6).

Implementation: Individual cases are evaluated with 4 separate provocative tests including ITT; GHRH preceded by acipimox (GHRH + Ac); and GHRH + GHRP-6. Serum GH levels are then measured by radioimmunoassay. Statistical analyses can be performed using the Wilcoxon rank sum and by Mann-Whitney tests.

Human Model: Cases of adult hypopituitarism (n = 8; n = 4/8 female, n = 4/8 male; age = 41–62 years [48.8 ± 1.4 years]; n = 10 normal obese; n = 5/10 female, n = 5/10 male; age = 38–62 years [48.1 ± 2.5 years], body mass index = 34.2 ± 1.2 kg/m^2; n = 10 normal subjects; n = 5/10 female; age = 33–62 years [48.1 ± 2.8 years]).

Interpretation and Analysis: After the ITT the mean peak GH secretions are 1.5 ± 0.3 μg/l for hypopituitary, 10.1 ± 1.7 μg/l ($p < 0.05$ vs hypopituitary) for obese, and 17.8 ± 2.0 μg/l ($p < 0.05$ vs hypopituitary) for normal. GHRH-induced GH secretions are 2 ± 0.7 μg/l for hypopituitary, 3.9 ± 1.2 μg/l (p = NS vs hypopituitary) for obese and 22.2 ± 3.8 μg/l ($p < 0.05$ vs hypopituitary) for normal. After GHRH + Ac, mean peak GH secretions are 3.3 ± 1.4 μg/l for hypopituitary, 14.2 ± 2.7 μg/l ($p < 0.05$ vs hypopituitary) for obese, and 35.1 ± 5.2 μg/l ($p < 0.05$ vs hypopituitary) for normal. GHRH + GHRP-6 induced mean peak GH secretion of 4.1 ± 0.9 μg/l for hypopituitary, 38.5 ± 6.5 μg/l ($p < 0.05$ vs hypopituitary) for obese, and 68.1 ± 5.5 μg/l ($p < 0.05$ vs hypopituitary) for normal subjects. Individually considered, after ITT, GHRH, or GHRH + Ac, the maximal response in hypopituitary cases is lower than the minimal response in normal but higher than the minimal response in obese subjects. In contrast, after GHRH + GHRP-6 the maximal response in hypopituitary cases is lower than the minimal response in normal and obese subjects. In study groups evaluated to date, although both acipimox and GHRP-6 partially reverse the functional hyposomamotropism of obesity after GHRH but are unable to reverse the organic hyposomatotropism of hypopituitarism, the combined test GHRH + GHRP-6 most accurately distinguishes both situations, without the side effects of ITT.

Comparison between insulin tolerance test, growth hormone (GH)-releasing hormone (GHRH), GHRH plus acipimox and GHRH plus GH-releasing peptide-6 for the diagnosis of adult GH deficiency in normal subjects, obese and hypopituitary patients. Cordido F, Alvarez-Castro P, Isidro ML, Casanueva FF, Dieguez C. Eur J Endocrinol 2003 Aug; 149 (2), pp. 117–22.

Growth Hormone Deficiency (GHD): Hypothalamic/Pituitary
Combined Hormonal-Stimulation Test (CHST)/ Thyrotropin-Releasing Hormone (TRH)/Gonadotropin-Releasing Hormone (GnRH)/Levodopa (L-dopa)/ Insulin

Application: Detection and characterization of alterations in GH production in children with pathological growth patterns.

Implementation: Evaluation of children with conditions of short stature to determine their GH production capacity utilizing a CHST involving the sequential administration of insulin, TRH, GnRH, and L-dopa. Growth hormone, thyrotropin, gonadotropins, cortisol, and prolactin levels are sampled over a period of 2 hours.

Human Model: Children with short stature and/or pathologic growth (n = 51).

Interpretation and Analysis: Children with appropriate predicted adult heights, delayed bone ages, and normal growth velocities of 4.0 cm/year or greater demonstrate normal pituitary responses. A small percentage of cases with predicted heights 2.5 standard deviations lower than target height and normal growth velocity demonstrate isolated GHD (e.g., n = 2/12). Conversely, a relatively large percentage of suspected cases have pathologic GHD or panhypopituitarism (e.g., n = 9/11). Evaluation of pituitary function by

combined sequential hormonal stimulation is fruitful in children with pathologic growth patterns but not in children with normal growth velocities and normal predicted adult height.

Pituitary function assessment in short stature by a combined hormonal-stimulation test. Pugliese M, Lifshitz F, Fort P, Cervantes C, Recker B, Ginsberg L. Am J Dis Child 1987 May; 141 (5), pp. 556–61.

Growth Hormone Insufficiency: Noonan's Syndrome (Childhood)/Evaluation of Posttreatment Response
Glucagon

Application: Characterization of the role of GH insufficiency in the pathogenesis of short stature in Noonan's syndrome.
Implementation: Evaluation of spontaneous 12-hour overnight GH secretion by continuous sampling analyzed using Pulsar, plasma insulin-like growth factor-I (IGF-I) and IGFBP-3 levels, and 24-hour urinary GH excretion measured at the start of GH treatment. Insulin-like growth factor-I and IGFBP-3 are remeasured after 1 year of GH treatment. Biological variables measured include height and height velocity, which are monitored before and after 1 year of GH treatment.

Human Model: Cases of Noonan's syndrome (n = 17; n = 13 boys, 4 aged 4.8–13.3; mean 9.2 years) and short stature prior to the initiation of GH treatment.
Interpretation and Analysis: A considerable percentage of children will have a mean overnight GH concentration below the lower limit of the normal range (e.g., n = 9/17). Close to 1/3 of cases will display overnight GH profiles with high trough GH concentrations (e.g., n = 6/17). Glucagon stimulation tests are normal in the vast majority of affected cases (e.g., n = 16/17).

Mean IGF-I levels are below normal (−0.4 SD). None of the parameters regarding GH secretion obtained from the overnight profile or provocative test are related to height or height velocity, nor to 1st-year response to GH treatment. Insulin-like growth factor-I and IGFBP-3 do not correlate with any of the GH secretion data. Insulin-like growth factor-I and IGFBP-3 are related to height and height velocity at the start of GH treatment ($r = 0.53$ [$p < 0.01$] and $r = 0.61$ [$p < 0.03$], respectively). Rises in IGF-I and IGFBP-3 under GH treatment are related to the increment in height velocity ($r = 0.70$ [$p < 0.01$] and $r = 0.71$ [$p < 0.02$], respectively). Abnormalities in GH secretion are frequent in patients with Noonan's syndrome and short stature. These abnormalities are not related to auxology at start of, or response to, GH treatment. Insufficiency of GH is clinically not important in Noonan's syndrome and monitoring spontaneous GH secretion is not necessary before the start of GH treatment.

Growth hormone (GH) secretion in children with Noonan's syndrome: Frequently abnormal without consequences for growth or response to GH treatment. Noordam C, van der Burgt I, Sweep CG, Delemarre-van de Waal HA, Sengers RC, Otten BJ. Clin Endocrinol (Oxf) 2001 Jan; 54 (1), pp. 53–9.

Growth Hormone (GH) Synthetic Capacity/(Children of Short Stature)
Growth Hormone-Releasing Peptide-2 (GHRP-2)/Aginine/Insulin/L-dopa

Cellular/Molecular Mechanism of Action: Hypothalamic-releasing hormones GHRH regulate the secretion of GH. Growth hormone-releasing peptide-2, an investigational biopharmaceutical, is 1 of the most potent members of the GHRP family.
Background Information: The secretion of GH is primarily regulated by the hypothalamic-releasing hormones GHRH and somatostatin. Additionally, several neurotransmitters act at the hypothalamus and pituitary to modulate GH release. The agents commonly used in clinical practice to diagnose GH deficiency, such as arginine, insulin, and L-dopa, act through the neural GH network. Many children with a poor GH response to conventional agents have a significant serum GH response to GHRH (IV). Growth hormone-releasing peptides are synthetic peptides that like GHRH act directly on pituitary somatotrophs to stimulate GH release.
Application: The investigational drug GHRP-2 is one of the most potent members of the GHRP family and can be used to detect abnormalities such as GH deficiency in children of short stature.
Route of Administration: Hypothalamic-releasing GHRP-2 has been shown to be effective in adults.
Dosage: Hypothalamic-releasing hormones (GHRP-2) can induce a significant response in GH secretion over a single dose range between 5 and 20 μg/kg.
Considerations: The intranasal preparation is well tolerated.
Implementation: Preparations of GHRP-2 can be applied to evaluate GH responses and determine their relative effectiveness in this regard compared to other provocative agents in children of short stature. In this context, GHRP-2 is administered intravenously or intranasally to children with short stature and compared to the administration of a combination of GHRP-2 (IV) and GHRH in the same subjects. Comparisons can also be made with conventional agents (arginine, L-dopa/exercise, insulin) in cases being evaluated for GH deficiency (e.g., n = 24 cases) intravenous GHRH and GHRP-2.
Interpretation and Analysis: The GH responses to GHRH or GHRP-2 are similar in children, and both agents are equally reliable predictors of pituitary reserve. Conventional agents used in GH testing are less likely to predict the capacity of pituitary GH release than are either GHRH or GHRP-2. There is no correlation between maximal GH response to standard tests with GH responses to GHRH or GHRP-2. Subsets of children that have a robust response to GHRP-2 (IV) and are then given GHRH + GHRP-2 simultaneously reveal that in such scenarios, these 2 agents exert a synergistic effect similar to previous observations reported in adults of normal stature. Furthermore, children that have a robust response to GH-releasing factors and subsequently receive intranasal GHRP-2 have a significant GH response over a dose range of 5 to 20 μg/kg per dose. The mean peak GH response to 15 μg/kg is 31.3 μg/L.

Diagnostic studies with intravenous and intranasal growth hormone-releasing peptide-2 in children of short stature. Pihoker C, Middleton R, Reynolds GA, Bowers CY, Badger TM. J Clin Endocrinol Metab 1995 Oct; 80 (10), pp. 2987–92.

Growth Hormone (GH)/Thyroid-Stimulating Hormone (TSH) Response/Influence of Fatty Acids on Plasma Levels
Thyrotropin-Releasing Hormone (TRH)

Background Information: Supplemental dietary fat provides excess fatty acids (FA) that can alter circulating concentrations of several hormones.
Route of Administration: Thyrotropin-releasing hormone (intramuscular).
Implementation: Evaluation of the effects of fatty acid isomer type on systemic hormone concentrations and possible sites of regulation. One approach to achieving this objective involves the abdominal infusion of fat mixtures high in cis-C18:1 FA (iCIS), high in trans-C18:1 FA (iTRS), or no infusion (NI) and perform intravenous arginine (ARG) and intramuscular TRH challenges. Test challenges are conducted on days 10 (ARG) and 12 (TRH) after initiation of fat infusion on each of 3 4-week experimental periods. Assessment of fat regulation of the thyroid axis can be tested directly *in vitro* by

characterizing the ability of fatty acids dissolved with sodium taurocholate to affect Type I 5′-deiodinase (5′D) activity in bovine liver homogenates.

Animal Model: Bovine.

***Ex Vivo* Model:** Bovine liver (homogenates).

Experimental Design: Replicated 3 × 3 Latin square.

Interpretation and Analysis: Plasma concentrations of insulin-like growth factor-I (IGF-I) are lower ($p < 0.01$) when cows received iCIS or iTRS compared with no fat infusion. Plasma insulin concentrations increase with ARG but responses are not affected by FA. Plasma GH remains unchanged after ARG. Peak plasma GH and TSH responses to TRH are blunted ($p < 0.05$ and $p < 0.1$, respectively), whereas thyroxine (T4) and triiodothyronine (T3) responses are augmented post-TRH ($p < 0.01$) when cows receive either FA isomer. Prolactin responses to TRH are not different between infusion treatments, although basal plasma concentrations before TRH are higher in cows infused with iTRS ($p < 0.05$). Homogenate 5′D is not affected by C2:0-C10:0 fatty acids, but decreases linearly ($p < 0.01$) with increasing concentrations of C12:0-C16:0 and C18:1 isomers. Cis C18:1 decreases 5′D more than the trans-isomer ($p < 0.01$), but the difference is only apparent at concentrations > 0.25 mM. The data suggest that various aspects of pituitary hormone regulation are differentially affected by FA composition. Fatty acid infusion may accentuate end organ responses in the thyroid axis and decrease IGF-I in the somatotropic axis. The data also suggest that FA isomer may alter patterns of extrathyroidal generation of thyroid hormones via direct influences on 5′D.

Dietary fatty acids modulate hormone responses in lactating cows: Mechanistic role for 5′-deiodinase activity in tissue. Romo GA, Elsasser TH, Kahl S, Erdman RA, Casper DP. Domest Anim Endocrinol 1997 Nov; 14 (6), pp. 409–20.

HYPOTHALAMIC/PITUITARY FUNCTION: REPRODUCTION

Amenorrhea: Pituitary Disturbances
Luteinizing Hormone-Releasing Hormone (LHRH) Gonadorelin/[Progesterone]/[Clomiphene]

Cellular/Molecular Mechanism of Action: Gonadorelin LHRH is a synthetic polypeptide that promotes the release of gonadotropin.

Application: Detection and evaluation of alterations in gonadotropin endocrinology.

Route of Administration: Intravenous.

Dosage: Gonadorelin LHRH (80 μg for the 1st and 2nd administered doses).

Considerations: The "Gonadorelin-test" diagnostically complements the progesterone and clomiphene-test.

Implementation: Evaluation of gonadotropin levels in women during their menstrual cycle in the middle of the follicle phase.

Human Model: Female cases with a normal menstrual cycle during the middle of the follicular phase (n = 11) in addition to cases of secondary amenorrhea (n = 10).

Interpretation and Analysis: Gonadorelin is effective in provoking a typical gonadotropin release. Conditions of secondary amenorrhea respond to the 1st Gonadorelin administration based on the detection of peak gonadotropins levels, in particular with LH levels, after 30 to 60 minutes (delta 1 = for 1st peak level − 0′ level). The relative ascent of gonadotropins (delta 2 = 2nd peak level − 120′ level) after the 2nd step administration at 120 minutes is not significantly higher than delta 1. The profile of pituitary LHRH production/release does not immediately depend on the basal level of gonadotropin values. It reflects the severity of the hypothalamic-pituitary functional disturbance.

Clinical use of Gonadorelin as a diagnostic agent in one- and two-step tests. Vesper B, Rohde W, Groot-Wassink T. Zentralbl Gynakol 1986; 108 (23), pp. 1442–52.

Endometriosis/Prolactin Response: Lack of Discrimination
Thyrotropin-Releasing Hormone (TRH)/Insulin Challenge

Application: Detection and characterization of endometriosis conditions.

Implementation: Evaluation of prolactin secretion applying a thyrotropin-releasing releasing hormone (TRH) test and an insulin challenge test in conditions of female endometriosis.

Human Model: Female cases of endometriosis (n = 10) treated with danazol at a dose of 200 mg 3 times daily for 6 months (evaluated after the reappearance of 2nd menses). Reference controls include normal females (n = 10 follicular phase (test performed only once).

Interpretation and Analysis: The absence of a significant difference in basal prolactin levels, or responses to the TRH and insulin challenge tests between controls and patients with endometriosis, before and after danazol treatment, supports the view that hyperprolactinemia should not be considered as a cause of infertility in endometriotic women. Furthermore, significant reductions in prolactin response to provocative tests during danazol treatment may be associated with the low estrogen levels caused by the medication. Finally, inadequate prolactin responses to insulin-induced hypoglycemia in cases with endometriosis and in normal women shows that this provocative test is not effective for hypothalamic stimulation of prolactin.

Provocative tests of prolactin before, during and after long-term danazol treatment in patients with endometriosis. Panidis D, Vavilis D, Rousso D, Panidou E, Kalogeropoulos A. Gynecol Endocrinol 1992 Mar; 6 (1), pp. 19–24.

Gonadal-Pituitary-Hypothalamic Function/ Estradiol/Luteinizing Hormone (LH)/Follicle-Stimulating Hormone (FSH)
Naltrexone

Cellular/Molecular Mechanism of Action: Opioid antagonist that stimulates LH and FSH in women during the early follicular phase of the menstrual cycle.

Application: Characterization of anterior pituitary function pertaining to LH, FSH, and prolactin (PRL) synthesis and release.

Route of Administration: Naltrexone (intravenous).

Dosage: Naltrexone (0.25, 0.50, and 1.0 mg/kg).

Considerations: These data are not concordant with clinical studies that report significant naltrexone stimulation of LH in men and in women during the early follicular phase.

Implemenation: Evaluation of the acute effects of naltrexone on anterior pituitary (LH, FSH, PRL) and gonadal steroid (T or E2) hormones. Integrated plasma samples can be collected at 20-minute intervals for 60 minutes before and for 300 minutes after intravenous infusion of naltrexone over 10 minutes.

Animal Model: Primate (rhesus/mucaca mulatta; n = 7 female and n = 4 male).

Interpretation and Analysis: In females studied during the early follicular phase (cycle days 1–3), naltrexone does not stimulate LH and significantly suppresses E2 ($p < 0.0003$–0.0001) and FSH

($p < 0.006$–0.0001). Naltrexone (0.50 and 1.0 mg/kg) also does not stimulate LH release in late follicular-phase females (cycle days 10–12) when estradiol levels are in the peri-ovulatory range. Follicle-stimulating hormone and E2 are significantly suppressed ($p < 0.01$–0.05) after 1.0 mg/kg naltrexone, but not after 0.5 mg/kg naltrexone. However, in males all doses of naltrexone significantly stimulate LH ($p < 0.003$–0.0001) and T ($p < 0.001$–0.0001) but not FSH. Luteinizing hormone increases significantly above baseline within 20 to 40 minutes and T increases significantly within 60 minutes. Such gender differences in naltrexone's effects on pituitary gonadotropins and gonadal steroid hormones are unanticipated.

Naltrexone effects on pituitary and gonadal hormones in male and female rhesus monkeys. Mello NK, Mendelson JH, Bree MP, Skupny A. Pharmacol Biochem Behav 1988 Nov; 31 (3), pp. 683–91.

Gonadotropin Insufficiency/[Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) Response]/[Example: Iron Toxicity Induced]
Gonadotropin-Releasing Hormone (GnRH)

Cellular/Molecular Mechanism of Action: Gonadotropin-releasing-hormone (promotes the synthesis/release of gonadotropins (e.g., LH, FSH).
Application: Detection and characterization of gonadotropin insufficiency conditions.
Route of Administration: Gonadotropin-releasing-hormone (IV bolus).
Dosage: Gonadotropin-releasing-hormone (100 mg bolus).
Implementation: Determine whether iron toxicity in blood transfusion dependent beta-thalassemic patients with pubertal failure is associated with gonadotropin (GTH) insufficiency based on responses to spontaneous/dynamic tests. In this context, GnRH-GTH secretory dynamics can be studied by serial ultradian GTH profiles and a GnRH IV bolus test.

Human Model: Cases of beta-thalassemia major patients with failed puberty ($n = 28$ males in FP group; $n = 5$ healthy, non-thalassemic prepubertal males).
Interpretation and Analysis: According to pulse profiles, FP group cases can be subdivided into apulsatile (no FSH and LH pulses, $n = 16$; AFP group) and pulsatile (defective pulse profile, $n = 12$; PFP group) subsets. Groups of FP cases have lower basal FSH ($p < 0.01$), LH ($p < 0.01$) and GnRH stimulated FSH ($p < 0.001$) and LH levels ($p < 0.001$) than the controls. However, basal and GnRH-stimulated FSH ($p < 0.01$ for basal and $p < 0.001$ for peak) and LH ($p < 0.01$ for both basal and peak) levels are lower in the AFP than PFP case groups. Serum ferritin levels in GnRH-non-responders are higher than those in responders ($9{,}052.63 \pm 579.14$ mg/l vs $5{,}933.33 \pm 1{,}819.65$ mg/l; $p < 0.05$). Similarly, symptomatic organ damage is higher in AFP than in PFP case groups (81% vs 42%; $p < 0.001$).

Iron toxicity/overload appears to be a major cause of GnRH-GTH deficiency in cases of failed puberty that suffer from thalassemic conditions. Comparison between spontaneous and dynamic test levels show that there is concordance between the degree of pulse defect and magnitude of LH response to GBT. However, ultradian GTH profiling is a more reliable method for identifying the degree of GTH insufficiency than GBT. Severity of the defect is heterogeneous, ranging from very severe (apulsatile) to less severe (pulsatile) subsets. Such information may be useful for better understanding the pathophysiology of hypogonadotrophic hypogonadism (HH), thereby promoting therapeutic options for induction of puberty and spermatogenesis.

Hypogonadotropic Hypogonadism (HH)/Delayed Growth: Constitutional Growth Delay (CGD)
GnRH/Nafarelin

Background Information: Previous investigations have observed nocturnal luteinizing hormone (LH) levels equal to 5.5 ± 0.9 IU/I for CGD, and 2.7 ± 0.7 IU/I for HH that represent a statistically significant differences. Mean nocturnal follicle-stimulating hormone (FSH) has been reported to be 5.1 ± 1.0 and 2.5 ± 0.2 IU/I, whereas mean nocturnal T concentrations are statistically different at approximately 4.2 ± 0.8 and 0.7 ± 0.2 nmol/I for CGD compared to HH, respectively. Peak LH responses to nafarelin are approximately 36.9 ± 8.9 IU/I for CGD, and 7.0 ± 2.0 IU/I for HH (statistically significant). Peak FSH released by nafarelin is about 14.2 ± 2.4 IU/I for CGD group and 4.8 ± 2.0 IU/I for HH (statistically significant). Peak T are reached by 24 hours following nafarelin injection and are approximately 5.7 ± 1.7 nmol/I for CGD and 0.3 ± 0.2 nmol/I for HH (statistically significant).
Application: Distinguish between CGD in boys with Tanner stage I of sexual development and patients with HH.
Route of Administration: Subcutaneous.
Dosage: Nafarelin (1 μg/kg up to 100 μg).
Implementation: Evalaution of hypothalamic/pituitary function based on the interpretation of changes in LH, FSH, T and E2 concentrations following nafarelin administration. Blood samples are collected at timed intervals every 15 minutes for 24 hours for the measurement of LH, FSH, T, and E2 (e.g., RIA, ELISA). Pretreatment baseline values are obtained by collecting blood samples every 15 minutes during a 12-hour overnight schedule.

Human Model: Cases of suspected constitutional growth delay from hypogonadotropic hypogonadism.
Interpretation and Analysis: Stimulated release of plasma LH, FSH and T is significantly higher with nafarelin compared to GnRH which also takes a longer period time to reach maximum peak concentrations. Experimental findings indicate that in early stages of puberty (before detectable changes of sexual maturation), the nafarelin test combined with the measurement of LH, FSH, and T in the blood or urine is superior to and more practical than overnight hormonal estimates to clearly distinguish CGD from HH.
Instrumentation: Instrumentation and facilities necessary to perform RIA- or ELISA-based methodologies.

Gonadotropin-releasing hormone agonist analog (nafarelin): A useful diagnostic agent for the distinction of constitutional growth delay from hypogonadotropic hypogonadism. Kletter GB, Rolfes-Curl A, Goodpasture JC, Solish SB, Scott L, Henzl MR, Beitins IZ. J Pediatr Endocrinol Metab 1996 Jan–Feb; 9 (1), pp. 9–19.

Infertility: Female/Prolactin: Latent Hyperprolactinaemia/Luteinizing Hormone (LH)/Follicle-Stimulating Hormone (FSH)/17-beta Estradiol (E2)/Testosterone (T) Total
Metoclopramide

Cellular/Molecular Mechanism of Action: Metoclopramide enhances cholinergic activity at muscarinic synapses and suppresses/antagonizes CNS dopaminergic activity.
Implementation: Evaluation of latent hyperprolactinaemia in the midfollicular phase of the cycle in cases of female infertility. Metoclopramide is given to determine prolactin response profiles. Just before metoclopramide is given, blood samples are collected for measuring levels of LH and FSH, 17-beta estradiol (E2) and total testosterone.

Human Model: Cases of female infertility in the midfollicular phase of the cycle (n = 174 randomly chosen; n = 54/174 latent hyperprolactinaemia defined as having prolactin (PRL) levels of at least 150 ng/ml after metoclopramide). Just before metoclopramide is administered, blood is taken to measure levels of LH and FSH, 17-beta estradiol (E2), and total testosterone.

Interpretation and Analysis: Women with latent hyperprolactinaemia have significantly lower levels of LH ($p < 0.01$) and E2 ($p < 0.001$) and higher levels of T ($p < 0.05$) in the midfollicular phase when compared with women without this condition. FSH levels showed no statistically significant difference.

The influence of latent hyperprolactinaemia on the levels of LH, FSH, E2 and T in the midfollicular phase of the cycle. Kostál M; Tosner J. Arch Gynecol Obstet 1997; 259 (2), pp. 65–8.

Infertility and Fertility: Female/Follicular Phase of the Menstrual Cycle/Polycystic Ovarian Syndrome (PCOS): Luteinizing Hormone (LH) Response/ Conserved Central Opioid Tone

Naltrexone

Cellular/Molecular Mechanism of Action: Naltrexone is an opioid antagonist.

Application: Characterization of the endocrinology of normal adult females (e.g., women) during the early follicular phase of the menstrual cycle (day 1 to day 4 following onset of menstruation).

Route of Administration: Naltrexone (oral).

Dosage: Naltrexone (50 mg); gonadotropin-releasing hormone (GnRH)-stimulated LH release (10 μg).

Considerations: Orally administered naltrexone appears to be a safe and effective compound for assessing function of the hypothalamic-anterior pituitary axis in women.

Implementation: Evaluation of enhanced LH levels in females with PCOS to determine if the condition is a consequence of an absent hypothalamic opioid inhibitory control and/or an increased sensitivity of gonadotroph to GnRH, induced by the sensitizing effects of circulating opioid peptides. Delineation of this objective can achieved through the administration of Naltrexone due to its capacity to induce significant elevations in plasma LH, prolactin, adenocorticotropic hormone (ACTH), and cortisol levels. In this context, pulsatile LH secretion (10-minute sampling for 6 hours) and GnRH-stimulated LH release can be characterized in conditions of PCOS before and after a 5-day administration protocol of either a placebo (n = 7) or the opioid antagonist naltrexone.

Human Model: Female with PCOS (n = 14 PCOS; n = 7/14 placebo controls). Seven age- and weight-matched normal cycling women in follicular phase function as controls.

Interpretation and Analysis: In comparison with normal cycling women, PCOS cases show normal frequency and increased amplitude LH pulses, elevated mean LH levels, and increased LH response to GnRH. In PCOS, placebo administration is not associated with any LH modification, whereas naltrexone enhances the frequency and decreases the amplitude of LH pulses, without modifying mean LH levels and the LH response to GnRH. Therefore, naltrexone-induced increment of LH frequency reveals the presence of conserved central opioid tone in PCOS. Reduced LH pulse amplitude, induced by naltrexone, is not associated with a reduced LH response to GnRH or with a reduction in mean LH levels. Present data do not support a role for endogenous opioid peptides in the pathogenesis of increased LH levels in PCOS.

Prolonged opioid blockade with naltrexone and luteinizing hormone modifications in women with polycystic ovarian syndrome. Cagnacci A, Soldani R, Paoletti AM, Falqui A, Melis GB. Fertil Steril 1994 Aug; 62 (2), pp. 269–72.

Infertility: Male/Prolactin (Induced)/Sperm Morphology Correlation/Luteinizing Hormone (LH) Stimulation/Spermiogenetic Defects/Testosterone (Stimulated) Positive Correlation with Sperm Morphology/Estrogen (Stimulated) Negative Correlation with Sperm Morphology/Follicle-Stimulating Hormone (FSH) Poststimulation Response/Male Fertility

Gonadotropin-Releasing Hormone (GnRH)/ Thyrotropin-Releasing Hormone (TRH)/ Adrenocorticotropin Hormone (ACTH)

Background Information: The recognition that discreet hormonal abnormalities may cause ovulation disorders in women suggests that the male partner of infertile women might also suffer from unrecognized hormonal dysfunction amendable to substitution therapy.

Implementation: Evaluation of the combined stimulation test with GnRH, TRH, and ACTH. Concentrations of the following hormones are then determined: estradiol (E), thyroid-stimulating hormone (TSH), prolactin, testosterone (T), dihydrotestosterone (DHT), androstenedione(A), 17-OH-pregnenolone (17-OH-Preg), 17-OH-progesterone (17-OHP), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), cortisone (F), and 21-desoxycortisone (21DF).

Human Model: Cases (n = 225) characterized as males with childless spouses that have actively attempted to have children for at least 1 year.

Interpretation and Analysis: Basal and stimulated, adrenal-testicular steroids with and without ACTH stimulation fail to demonstrate a relevant relationship to semen parameters. Gonadotropin levels have a significant negative correlation to all important semen parameters (testicular volume, sperm count, motility, morphology, and vitality) and are positively correlated to spermiogenetic defects. Stimulated LH values are more clearly associated with spermiogenetic defects than basal LH. Nonetheless, basal FSH concentrations are more informative than LH. Stimulated prolactin values positively correlate with both gonadotropin and with sperm morphology. Estrogen concentrations have a significant positive correlation with both basal and poststimulation DHEAS values, and show a highly negative correlation with sperm count, morphology, and vitality. In comparison, good sperm parameters are associated with high poststimulation testosterone concentrations. Basal FSH and estrogen concentrations, as well as the stimulated LH, testosterone, and prolactin determinations, should be included in the evaluation of male sterility.

Hormone load tests in infertile male patients. Gerhard I, Lenhard HK, Eggert-Kruse W, Runnebaum B. Arch Androl 1991 Nov–Dec; 27 (3), pp. 129–47.

Oocyte Retrieval Prediction (Mature)/Estradiol (E2) Plasma Profiles/Predictor of Performance in the Flare-up IVF Cycle

Gonadotropin-Releasing Hormone Agonist (GnRH-a)

Application: Prediction of the potential yield of retrievable mature oocytes and pregnancy.

Implementation: Evaluation and comparison of the initial versus the early patterns of estradiol (E2) changes after the administration of a GnRH-a. In this context, the GnRH-a stimulation test is compared to E2 patternsas predictors of ovarian response and pregnancy in *in vitro* fertilized (IVF) patients stimulated with a "flare-up" protocol. Candidates are stimulated with a GnRH-a flare-up protocol beginning on menstrual day 2.

Human Model: Cases without any known health abnormalities (n = 228; n = 0 anticipated to have polycystic ovarian disease; n = 0 single ovary; n = 0 ovarian cyst(s)).
Interpretation and Analysis: The GnRH-a stimulation test but not E2 patterns can function as a predictive indicator of the number of mature oocytes that can be retrieved ($r = 0.53$, $p \leq 1 \times 10^{-5}$) and pregnancy ($chi^2 = 8.5$, $p = 0.04$). The E2 pattern is predictive of the duration and number of ampules of gonadotropin required for stimulation. The GnRH-a stimulation test is a sensitive predictor of performance in the flare-up IVF cycle.

The gonadotropin-releasing hormone agonist stimulation test-a sensitive predictor of performance in the flare-up *in vitro* fertilization cycle. Winslow KL, Toner JP, Brzyski RG, Oehninger SC, Acosta AA, Muasher SJ. Fertil Steril 1991 Oct; 56 (4), pp. 711–7.

Oxytoxin and Vasopression Physiology
Glucagon-Like Peptide-1 (7-36)

Application: Characterization of pituitary endocrine function.
Route of Administration: Glucagon-like peptide-1 (7-36) by intracerebroventricular injection.
Dosage: Glucagon-like peptide-1 (7-36) at a dose of 1 μg.
Implementation: Evaluation of the possible effects of glucagon-like peptide-1 (7-36) amide on vasopressin and oxytocin release induced by acute peripheral or central osmotic stimulation. Study Design #1: Intraperitoneally injected isotonic (0.15 M) or hypertonic (*1.5* M) NaCl solutions and then, intracerebroventricular injection of either glucagon-like peptide-1 (7-36) amide dissolved in 5 microl of isotonic saline or with vehicle only. Study Design #2: Glucagon-like peptide-1 (7-36) amide, dissolved in isotonic or hypertonic (0.6 M) saline, is injected into the cerebroventricular system. Control rats are treated with isotonic or hypertonic saline only. All animals are decapitated 10 minutes after the intracerebroventricular injection.

Animal Model: Rat.
Interpretation and Analysis: Glucagon-like peptide-1 (7-36) amide enhances significantly the basal secretion of vasopressin and oxytocin. Moreover, this peptide increases additionally the release of both neurohypophysial hormones stimulated previously by peripheral osmotic challenge. On the other hand, the peptide increases the oxytocin but not vasopressin secretion brought about by an intracerebroventricular injection of hypertonic saline thus suggesting that the central osmotic stimulation decreases the sensitivity of vasopressin neurons to glucagon-like peptide-1 (7-36) amide. Therefore, glucagon-like peptide-1 (7-36) amide may affect the secretory activity of the hypothalamo-neurohypophysial system under acute osmotic challenge.

Effects of glucagon-like peptide-1 (7-36) amide on neurohypophysial hormone secretion induced by acute hyperosmotic challenge. Bojanowska E, Stempniak B. Neuropeptides 2003 Feb; 37 (1), pp. 45–50.

Prolactin: Functional Hyperprolactemia/[Lack of Diagnostic Efficacy]
Metoclopramide/Thyrotropin-Releasing Hormone (TRH)

Application: Detection and characterization of conditions related to alterations in prolactic secretion.
Route of Administration: Thyrotropin-releasing hormone (intravenous); metoclopramide (intravenous).
Dosage: Thyrotropin-releasing hormone (200 μg); metoclopramide (10 mg).
Implementation: Evaluate the diagnostic validity of prolactin response tests by comparing the stimulating effect of TRH and metoclopramide on prolactin secretion in conditions of hyperprolactinemia. Prolactin response studies with TRH and metaclopramide are performed on the 7th, 8th, or 9th day of the follicular phase under standardized conditions (08.00–09.00, after a 1-hour rest and overnight fasting).

Human Model: Cases of functional hyperprolactinemia (n = 18 defined according to prolactin serum levels ≥ 16 and < 50 ng/ml during the follicular phase without evidence of prolactinoma). Reference controls are of a similar age that have normal serum prolactin levels (n = 18). Tests can be done on the 7th, 8th, or 9th day of the follicular phase under standardized conditions (at 08.00 to 09.00, after a 1-hour rest and overnight fasting) with TRH or metoclopramide IV.
Interpretation and Analysis: Higher maximal prolactin concentrations are achieved in affected cases and reference controls after metoclopramide than after TRH (metoclopramide mean: 243 ± 62 ng/ml vs 181 ± 100 ng/ml, U-test: $p = 0.0019$; TRH mean: 101 ± 23 ng/ml vs 41 ± 20 ng/ml, U-test: $p = 0.0001$). There is no significant difference in the relative increment of prolactin serum levels between both groups, neither after metoclopramide nor after TRH (after metoclopramide, mean: 13.5 ± 5.8 vs 17.2 ± 9.2, U-test: $p = 0.137$; after TRH, mean 3.6 ± 1.1 vs 3.8 ± 1.9, U-test: $p = 0.899$). No correlation exits between prolactin basal and peak levels, either after TRH or after metoclopramide neither in the study (metoclopramide test: $p = 0.738$; TRH test: $p = 0.076$) nor in the control group (metoclopramide test: $p = 0.331$; TRH test: $p = 0.360$). While significantly higher prolactin peaks are recorded after metoclopramide compared to TRH in both groups, no difference in the response to TRH and metoclopramide, regarding absolute and relative increment, can be detected.

Prolactin response to metoclopramide and thyrotropin-releasing hormone in normoprolactinemic and hyperprolactinemic women: A comparison of diagnostic validity. Bussen S, Brosemann N, Steck T. Gynecol Endocrinol 1996 Apr; 10 (2), pp. 83–90.

Prolactin Suppression: Ethanol-Induced/ Hypothalamic/Pituitary Dysfunction
Thyrotropin-Releasing Hormone (TRH)/Sulpiride

Physiological Mechanism of Action: Thyrotropin-releasing hormone is produced in the hypothalamus and promotes the synthesis/release of pituitary thyroid-stimulating hormone (TSH).
Route of Administration: Ethanol (indwelling atrial catheter, implanted on day 3 of lactation).
Dosage: Ethanol (0.0, 1.0 or 2.0 g/kg body weight) daily for 8 days from day 5 to 12 of lactation. Following the administration of the initial alcohol dose, infusion is continued at rates required to maintain blood alcohol levels (BALs) for 4 hours every day.
Implementation: Evaluation of the mechanism of action of alcohol-induced inhibition of suckling-induced prolactin release during lactation. Alcohol is administered from day 5 to 12 of lactation. Prolactin responses to sulpiride and TRH are then tested on day 12.

Animal Model: Rat (lactating females).
Interpretation and Analysis: Alcohol administration for 8 days and maintaining the blood alcohol levels for 4 hours daily does not affect the basal or sulpiride and TRH-stimulated plasma prolactin release. Since the prolactin-releasing capacity of pituitary lactotropes of the lactating rat is not compromised following chronic alcohol exposure, it can be concluded that alcohol does not act at the anterior pituitary level to inhibit the suckling-induced prolactin release but probably acts by other mechanisms: either via the hypothalamic and/or higher central nervous system or by disrupting the neural impulse transmission, engendered at the nipples in response to suckling.

Prolactin secretion in lactating rats following chronic alcohol exposure: Provocative tests with secretagogues. Subramanian MG. Life Sci 1995; 57 (6), pp. 533–9.

Puberty (Central): Female/Central Precocious Puberty (CPP)/Premature Thelarche (PT)
Gonadotropin-Releasing Hormone (GnRH)

Cellular/Molecular Mechanism of Action: Gonadotropin-releasing hormone is produced in the hypothalamus and promotes the release of pituitary hormones including luteinizing hormone (LH) and follicle-stimulating hormone (FSH).
Background Information: Precocious puberty is characterized by breast development in girls prior to 8 years old who may have acne, growth spurt, and menstruation. The GnRH stimulation test is the conventional gold standard for diagnosis of CPP but it is a time-consuming procedure that is not practical on an outpatient basis.
Application: Diagnosis of CPP through the evaluation of basal FSH levels in a manner capable of saving time and financial cost.
Implementation: Evaluation of hormonal profiles in response to GnRH stimulation tests.

Human Model: Female cases with breast development, n = 51 at < 8 years old classified as with either CPP (n = 24) or PT (n = 27). In addition, another test group can include comparisons between PT (n = 13) and thelarche variants (TV; n = 12) that develop puberty approximately 1 year later as confirmed by GnRH stimulation test.
Interpretation and Analysis: Girls with CPP have large bone-to-age and bone-to-chronological-age ratios in addition to advanced stages of breast development. Girls with TV have a greater level of basal LH, peak LH, and 120-minute estradiol levels than girls with PT. Basal LH and FSH ratio > 0.2 can be used to diagnose CPP with 75% sensitivity, 85% specificity, 82% positive predictive value (PPV), and 82% negative predictive value (NPV). Girls with CPP have a basal LH/FSH ratio > 0.2 and this can be used as a cutoff point for the diagnosis of CPP.

Basal luteinizing hormone/follicle stimulating hormone ratio in diagnosis of central precocious puberty. Supornsilchai V, Hiranrat P, Wacharasindhu S, Srivuthana S, Aroonparkmongkol S. J Med Assoc Thai 2003 Jun; 86 Suppl 2, pp. S145–51.

Sclerosis/Scleroderma (Systemic)/Follicle-Stimulating Hormone (FSH)/Luteinizing Hormone (LH)/Prolactin/Skin Sclerosis/Prolactin/Peripheral Vascular Involvement/Prolactin/Pulmonary Involvement
Gonadotropin-Releasing Hormone (GnRH)/Thyroid-Stimulating Hormone (TSH)

Application: Evaluation of systemic scleroderma conditions.
Implementation: Evaluation of basal and dynamic levels of pituitary gonadotropin release in female systemic sclerosis (SSc) patients of childbearing age and in postmenopausal SSc patients. One approach to achieving this objective can involve determining responses to GnRH and TSH stimulation tests during the early follicular phase by measuring serum concentrations of FSH, LH, and prolactin. Blood samples are also collected from postmenopausal women with systemic sclerosis after TSH stimulation; only serum prolactin concentrations are determined, because elevated basal concentrations of FSH and LH can be expected to be observed. Hormone concentrations are estimated by radioimmunoassay. Comparisons are made with healthy control women matched for age and reproductive status.

Human Model: Female cases in their childbearing years with systemic sclerosis (n = 12 SSc with mean age [SEM] 34.8 ± 2.4 years). In addition, postmenopausal female cases (n = 6) with systemic sclerosis (mean age 46.8 ± 2.4 years).
Interpretation and Analysis: In cases with systemic sclerosis of childbearing age, basal FSH, LH, and estradiol (E2) levels are not significantly different from reference controls, whereas basal prolactin concentration are significantly higher than reference controls ($p = 0.0001$). After the stimulation test, the peak concentrations of FSH ($p = 0.0001$) and prolactin ($P < 0.0001$) are significantly higher than controls. The net integrated response curves (net area under the curve [AUC]) for FSH and LH do not differ significantly between systemic sclerosis cases and controls. On the contrary, the net AUC for prolactin in response to TSH stimulation is significantly higher than reference controls ($p = 0.001$). In postmenopausal patients, basal estrogen E2, FSH, LH, and prolactin levels are not significantly different between women with systemic sclerosis and controls. However, after TSH stimulation, peak levels and net AUC for prolactin are not significantly higher in cases than those in reference controls. No significant correlations can be appreciated between basal and stimulated FSH, LH, and prolactin levels and the severity of involvement of various organ systems. Multiple regression analysis reveals that basal and stimulated prolactin concentrations can be associated with skin sclerosis and peripheral vascular and lung involvement. Our results suggest that subclinical primary hypogonadism can occur in SSc patients. They also confirm an alteration in the mechanism for prolactin secretion and release, which may not only contribute to further disturbance of the reproductive axis but may also have an influence on the disease.

Assessment of pituitary gonadotropin release to gonadotropin-releasing hormone/thyroid-stimulating hormone stimulation in women with systemic sclerosis. La Montagna G, Baruffo A, Pasquali D, Bellastella A, Tirri G, Sinisi AA. Rheumatology (Oxford) 2001 Mar; 40 (3), pp. 310–4.

Testicular Function: Cryptorchidism (Unilateral or Bilateral) vs Unilateral or Bilateral Castration/ Aggressive Cryptorchid Behavior
Human Chorionic Gonadotropin (hCG)

Cellular/Molecular Mechanism of Action: Human chorionic gonadotropin induces synthesis and release of testosterone from testicular Leydig cells.
Application: Detection of conditions of cryptorchidism (unilateral or bilateral) when it is uncertain if cases may actually be normal but have previously been subjected to bilateral or unilateral castration.
Background Information: Horses with cryptorchidism tend to be ill-tempered. Sometimes insufficient history is available to know if a horse has or has not been previously castrated (e.g., non-castrated cryptorchid vs unilateral castration).
Considerations: Horses < 3 years of age and all donkeys cannot be evaluated using a single measurement of (resting) blood esterone sulfate for the purpose of detecting the presence of cryptorchidism conditions.
Route of Administration: Human chorionic gonadotropin (intravenous injection).
Dosage: Intravenous injection of hCG at a dosage of 6,000 IU per 450 kg with blood sample collected prior to and 30–120 minutes after injection.
Implementation: Detection of conditions of unilateral or bilateral cryptorchidism by measuring increases in serum testosterone levels induced by hCG administration compared to preinjection concentrations.

Interpretation and Analysis: Cryptorchid patients have basal systemic esterone sulfate concentrations in excess of 400 pg/ml. Alternatively, in situations where the esterone sulfate assay is not an appropriate diagnostic approach, the administration of hCG will induce increases in blood testosterone levels. Prior to hCG administration, cryptorchid cases will have resting testosterone levels in excess of 100 pg/ml, whereas horses not affected by cryptorchidism will have resting testosterone concentrations below 40 pg/ml that will not change following hCG administration.
Laboratory Instrumentation: Assay of growth hormone (GH) concentrations by either RIA or ELISA.

Cryporchidism. Cox JE (pp. 571–3). Current Therapy in Equine Medicine 2, ed Robinson NE. WB Saunders, Philadelphia (Copyright 1987).

Is human chorionic gonadotropin useful for identifying and treating nonpalpable testis? Bukowski TP, Sedberry S, Richardson B. J Urol 2001 Jan; 165 (1), pp. 221–3.

Testicular Function/Gonadotropin: Basal Plasma Concentrations
Gonadotropin-Releasing Hormone (GnRH)

Background Information: Provocative GnRH stimulation testing indirectly assesses testicular function with more sensitivity than determination of basal gonadotropin levels alone. Unfortunately, the drawbacks of multiple blood sampling and high cost have limited the clinical usefulness of this particular test.
Implementation: Evaluation of gonadotropin (luteinizing hormone [LH]/follicle-stimulating hormone [FSH]) production using a simplified, 2-point, 30-minute GnRH stimulation test.

Human Model: Cases without any known health abnormalities (n = 55 men with normal baseline gonadotropin levels).
Interpretation and Analysis: Statistical analysis of data reveal that the simplified test is just as accurate as the traditional test ($p \leq 0.0001$) without the latter's attendant difficulties. In addition, normal basal gonadotropin levels have little correlation to the actual responses obtained from GnRH stimulation testing ($r = 0.20$ and $r = 0.39$ for LH and FSH, respectively).

Simplified gonadotropin-releasing hormone (GnRH) stimulation test. Fisch H, Laor E, Lipshultz LI. Urology 1990 Sep; 36 (3), pp. 260–3.

Testicular Function: Cystinosis/Luteinizing Hormone (LH) Response/Gonadotropin-Releasing Hormone (GnRH)/Follicle-Stimulating Hormone (FSH) Response
Human Chorionic Gonadotropin (hCG)

Application: Detection and characterization of reproductive function in conditions of cystinosis.
Implementation: Tanner staging, serum gonadotropin determinations, and testosterone and testosterone-binding globulin assessments. Selected patients also have an hCG stimulation test, a GnRH stimulation test, and serial samplings collected for measurement of LH.

Human Model: Cases of nephropathic cystinosis (n = 10, age = 15–28 years with nephropathic cystinosis and renal allografts) and reference controls (n = 11 renal transplant recipients with a primary renal disorder other than cystinosis, matched with study cases for age and renal function).
Interpretation and Analysis: Although testosterone levels are within normal limits in approximately 70% of cases with cystinosis (e.g., n = 7/10; mean testosterone level in cystinosis = 11.5 ± 2.0 nmol/L compared to 24.2 ± 3.0 nmol/L in control patients $p < 0.005$). Cases with cystinosis reach Tanner stage 5 (full pubertal development), whereas most controls do not (e.g., n = 9/11).

Elevations in LH or FSH can be appreciated in approximately 70% of cases with cystinosis (e.g., n = 7/10), thereby suggesting the presence of testicular failure. Such cases usually also have normal LH and FSH responses after GnRH stimulation, increases in LH pulse frequency, and reduced testosterone response after hCG stimulation. In contrast, a relatively small number of control cases may have minimally elevated gonadotropin levels (e.g., n = 3/11), but the vast majority of these subjects have normal testosterone levels (e.g., n = 11/11). Microscopic testicular examination in some cases shows cystine crystals, germinal dysplasia, increased fibrosis, and Leydig cell hyperplasia (e.g., n = 1). Abnormalities in the pituitary-testicular axis are common in male patients with cystinosis. These changes appear to be related to the disease cystinosis and not to treated renal failure per se.

Pituitary-testicular function in nephropathic cystinosis. Chik CL, Friedman A, Merriam GR, Gahl WA. Ann Intern Med 1993 Oct 1; 119 (7 Pt 1), pp. 568–75.

Testicular Function: Varicoceles
Gonadotropin-Releasing Hormone (GnRH)

Background Information: Ipsilateral testicular catch-up growth has been reported to occur in approximately 80% of adolescents with varicoceles following unilateral varicocelectomy. Observations have noted not only catch-up growth but hypertrophy (left at least 10% larger than right testicular volume) in some adolescents postoperatively. To our knowledge this phenomenon has not been previously described.
Application: Gonadotropin-releasing hormone response can be applied as 1 of the indicator parameters for justifying surgical intervention for testicular varicocele.
Implementation: Assessment of the incidence of left testicular hypertrophy following ipsilateral varicocele ligation and whether it is related to age at operation and/or procedure performed.

Indications for surgery include ipsilateral (left) testicular hypotrophy and exaggerated response to GnRH stimulation test and/or persistent pain. Testes are measured using the Takihara ring orchidometer with relative volume of the left testis expressed as a percentage of the right testis. Results are compared in different age groups as well as by procedure performed to correct the varicocele using chi^2 analysis. Testicular volume is determined before and after surgery, and all patients are followed for a minimum of 6 months.

Human Model: Cases of asymptomatic varicoceles that are candidates for unilateral left varicocelectomy (n = 42; n = 23/42 ipsilateral left testicular hypotrophy; n = 5 grade 2–3 varicocele with palpably softer ipsilateral left testicle; n = 12 grade 3+ varicoceles; n = 1 abnormal GnRH stimulation test; n = 1 persistent pain). Average patient age at operation is 14.7 years (range 9–22) and average follow-up is 22 months (range 6–84). Patients are stratified according to Palomo versus modified Ivanissevich technique and age at operation.
Interpretation and Analysis: Left testicular hypertrophy develops in approximately 43.8% of cases (e.g., n = 13/32) that undergo Palomo repair and in approximately 30% that undergo a modified Ivanissevich repair (e.g., n = 3/10). When compared by age at operation, 40% (e.g., n = 8/20) 14.7 years old or younger have left testicular hypertrophy compared to 36.4% (e.g., n = 8/22) older than 14.7 years. Differences between groups are not statistically significant. Ipsilateral testicular hypertrophy occurs in a substantial

number of adolescents following varicocele ligation. This phenomenon does not seem to be dependent on age at surgery or type of varicocele repair.

The adolescent varicocele I: Left testicular hypertrophy following varicocelectomy. Gershbein AB, Horowitz M, Glassberg KI. J Urol 1999 Oct; 162 (4), pp. 1447–9.

Testicular Function: Varicoceles
Gonadotropin-Releasing Hormone (GnRH)/[Inhibin-B, Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LS), Testosterone]/Lack of Diagnostic Benefit

Background Information: The impact of varicoceles on gonadal function in adolescents has been evaluated using several parameters, including size of testes, hormonal levels, and provocative endocrine testing. Inhibin B is decreased in men with testicular damage from conditions other than varicocele.
Application: Evaluation of conditions of varicocele in adolescent boys.
Implementation: Evaluation of conditions of left or bilateral varicoceles using a GnRH stimulation test and the measurement of basal inhibin B, FSH, LH, and testosterone levels. One objective of such a study is to determine if inhibin B levels are low in varicocele conditions, and if there is a relationship between inhibin B and an exaggerated response to GnRH stimulation testing, testicular hypertrophy, and/or varicocele bilaterality.

Human Model: Cases at Tanner stages III–V of pubertal development who had either left or bilateral varicoceles (n = 9 adolescent boys).
Interpretation and Analysis: The vast majority of cases have essentially normal inhibin B levels for Tanner stage. Less than half of boys have an exaggerated response to GnRH stimulating testing (e.g., n = 4/9). Inhibin B levels do not vary significantly either with the presence of bilateral or unilateral varicoceles or asymmetric testis. The lack of correlation between inhibin B levels and the aforementioned parameters fails to suggest that inhibin B has a significant role in the clinical assessment of testicular function in adolescents with varicocele. Further studies of larger populations may further elucidate the value of inhibin B levels and varicoceles.

Serum inhibin B levels and the response to gonadotropin stimulation test in pubertal boys with varicocele. Carrillo A, Gershbein A, Glassberg KI, Danon M. J Urol 1999 Sep; 162 (3 Pt 1), pp. 875–7.

HYPOTHALAMUS, PITUITARY, AND THYROID AXIS

Carcinoma (Thyroid Medullary)/(Familial Chromaffinomatosis)
Oral Whisky/Pentagastrin

Physiological Mechanism of Action: Induces or promotes the secretion of calcitonin.
Background Information: Familial medullary carcinoma of thyroid (MCT) can be diagnosed early by measuring stimulated plasma calcitonin following provocation with oral whisky, or pentagastrin. The calcitonin release, however, can be variable following both stimuli.
Diagnostic Application: Detection of cases associated with thyroid medullary carcinoma.
Route of Administration: Whisky (oral ingestion); pentagastrin (intravenous).
Dosage: Whisky (50 ml); pentagastrin (0.5 $\mu g\ kg^{-1}$).
Implementation: Determine whether a humoral mechanism might be involved in mediating calcitonin release assessed by measuring 9 intestinal (gut) peptides, histamine, alcohol, calcium, and calcitonin plasma concentrations.

Human Model: Cases affected by multiple endocrine neoplasia Type II syndrome (n = 8).
Interpretation and Analysis: Pentagastrin produces significant rises in plasma calcitonin, histamine and pancreatic polypeptide. Whisky produces a variable rise in plasma alcohol, but significant rises in plasma calcitonin, histamine and pancreatic polypeptide as well as insulin ($p < 0.05$). Such observations suggest that histamine may be the mediator of calcitonin release following provocation by either pentagastrin or alcohol (ethanol). Histamine infusion requires evaluation as a screening test for MCT in MEN II syndrome.

Multiple endocrine neoplasia type II: The role of gastrointestinal humoral factors in calcitonin release following alcohol and pentagastrin stimulation. Fletcher DR, Gamvros O, Man WK, Ahmed Y, Trayner I, Adrian T. Aust N Z J Surg 1984 Jun; 54 (3), pp. 271–5.

Pentagastrin, calcium and whisky stimulated serum calcitonin in medullary carcinoma of the thyroid. Emmertsen KK, Nielsen HE, Mosekilde L, Hansen HH. Acta Radiol Oncol 1980; 19 (2), pp. 85–9.

Whisky: A new provocative test for calcitonin secretion. Dymling JF, Ljungberg O, Hillyard CJ, Greenberg PB, Evans IM, MacIntyre I. Acta Endocrinol (Copenh) 1976 Jul; 82 (3), pp. 500–9.

Carcinoma: Thyroid Medullary: (Hereditary)/(C-Cell Disease)
Omeprazole/Pentagastrin

Cellular/Molecular Mechanism of Action: Pentagastrin and omeprazole both induce the synthesis/release of calcitonin that becomes excessively increased within the plasma in conditions of medullary thyroid carcinoma.
Application: Diagnosis of medullary thyroid carcinoma through induction of serum calcitonin production/release.
Route of Administration: Omeprazole (oral).
Dosage: Omeprazole (20 mg twice a day for 4 days); pentagastrin (0.5 μg/kg of body weight).
Implementation and Validation: Evaluation of conditions of hereditary medullary thyroid carcinoma.
Interpretation and Analysis: In conditions of hereditary medullary thyroid carcinoma, pentagastrin administration produces a significantly higher increase in serum calcitonin than does omeprazole. Occasional, unpleasant side effects are associated with the administration of pentagastrin injection including substantial "tightness" being appreciated in almost all cases. Alternatively, omeprazole is not associated with creating any significant adverse side effects. A direct positive correlation exists between calcitonin-% (ratio of calcitonin peak to basal value $\times$ 100) and gastrin-% (ratio of gastrin peak to basal value $\times$ 100) during the omeprazole test in medullary thyroid carcinoma patients ($r = 0.73$; $p < 0.001$). In spite of several adverse effects, pentagastrin remains the best provocative test for the diagnosis of medullary thyroid carcinoma. Omeprazole may be useful when pentagastrin is contraindicated or refused because of the unpleasant side effects, but further validation is needed.
Instrumentation: Laboratory facilities and reagents necessary for calcitonin radioimmunoassay or ELISA-based methodologies.

Comparison of two provocative tests for calcitonin in medullary thyroid carcinoma: Omeprazole vs pentagastrin. Vitale G, Ciccarelli A, Caraglia M, Galderisi M, Rossi R, Del Prete S, Abbruzzese A, Lupoli G. Clin Chem 2002 Sep; 48 (9), pp. 1505–10.

Carcinoma (Thyroid Medullary)/(Induced Calcitonin Secretion)
Histamine/Calcium/Pentagastrin Infusion/Glucagon

Cellular/Molecular Mechanism of Action: Histamine promotes a secretagogue effect on calcitonin in conditions of medullary thyroid carcinoma.
Application: Characterization of cases of medullary thyroid carcinoma due to its abilty to induce secretion of calcitonin. Glucagon and calcium are other known stimuli for calcitonin secretion in cases of medullary thyroid carcinoma. Histamine will not replace these agents in provocative test, but can provide better insight into the behavior of this neoplastic cell type.
Implementation: Determination of the relative effectiveness of histamine, calcium/pentagastrin, and glucagon as calcitonin secretagogues in cases of medullary thyroid carcinoma.
Interpretation and Analysis: In cases of medullary thyroid carcinoma with undetectable levels of basal plasma calcitonin (e.g., n = 7/15), calcium and pentagastrin can promote detectable elevations in plasma calcitonin concentrations. Similar responses can not be appreciated for histamine or glucagon administration. Alternatively, in cases of modullary thyroid carcinoma that do express detectable base-line levels of calcitonin (e.g., n = 8/15) significant responses can be seen in response to glucagon, calcium/pentagastrin and histamine but the calcium/pentagastrin combination induces the most potent secretagogue effect. Blockade of H2-receptor complexes with cimetidine has no effect on basal calcitonin concentrations and does not suppress responses to histamine, calcium or pentagastrin. The variable secretagogue effect of histamine may be mediated through H1-receptors, through nonspecific vascular dilation "washing out" of preformed calcitonin, or through its destruction to varying degrees by histaminase, present in most medullary thyroid tumors. Histamine is unlikely to replace calcium/pentagastrin as the most discriminative, provocative diagnostic agent in medullary thyroid carcinoma, but correlation of secretory responses with tissue histaminase concentrations and attempted blockade with differing antihistamines will further our understanding of this tumor type.

Some laboratories have proposed that a definitive diagnosis can be established by measuring calcitonin and CEA levels in sera. Cases with normal levels of calcitonin and CEA, the provocative test (Ca-gluconate plus pentagastrin) can be useful (e.g., (pt. no. 11,K.N.)).

Histamine and calcitonin release from medullary thyroid carcinoma. Farndon JR, Lewis KR, Dale JK, Hinshaw WM, Dilley WG, Wells SA Jr. Cancer 1983 Apr 1; 51 (7), pp. 1221–5.

Cancer: Thyroid
Thyroid-Stimulating Hormone (TSH)

Background Information: Detection of recurrent thyroid cancer tumor requires TSH stimulation for radioiodine scanning and thyroglobulin (Tg) measurement. Temporary thyroid hormone withdrawal has previously been used, but causes hypothyroidism and rarely, tumor progression.
Implementation: Evaluation of recombinant thyrotropin (rTSH) assessed in 2 randomized clinical trials in which cases have [^{131}I] and Tg testing twice; (i) initially after rTSH, and (ii) after thyroid hormone withdrawal. Note: Quality of life comparisons have subsequently been made in preliminary investigations.
Interpretation and Analysis: In trials with positive-scan cases (49% of total), rTSH is equivalent to withdrawal in 66%, superior in 5%, and inferior in 29% ($p < 0.05$), suggesting a lesser sensitivity for rTSH scans. In a 2nd trial employing enhanced techniques for patients with positive scans (49%), there are no difference in the number of superior rTSH and withdrawal scans. Furthermore, among all cases with withdrawal study evidence of residual thyroid tissue; 74% of all patients with residual thyroid tissue; and 100% of cases with tumor metastases have rTSH-stimulated thyroglobulin values above 2 ng/mL. Naturally, cases experience significantly more symptoms and diminished quality of life with hypothyroidism than after rhTSH. Therefore, combined rTSH-stimulated radioiodine and Tg testing is as sensitive as thyroid hormone withdrawal to detect recurrent thyroid cancer, and causes less morbidity.

Recombinant thyrotropin for detection of recurrent thyroid cancer. Ladenson PW. Trans Am Clin Climatol Assoc 2002; 113, pp. 21–30.

Cancer: Multiple Endocrine Neoplasia/Multiple Endocrine Neoplasia Type 2A Syndrome (MEN 2A)/Multiple Endocrine Neoplasia Type 2B (MEN 2B)/Familial Medullary Thyroid Carcinoma (FMTC)
Pentagastrin and Calcium Chloride/[Pentagastrin and Calcium Gluconate]

Cellular/Molecular Mechanism of Action: Combined influence of pentagastrin and calcium chloride ($CaCl_2$) promotes the release of calcitonin from certain types of endocrine neoplasias.
Application: Characterization and detection of multiple endocrine neoplasia Type 2A. The pentagastrin provocative test and biochemical screening can be applied to rule out either hyperparathyroidism or pheochromocytoma.

Medullary thyroid carcinoma is a malignancy of the thyroid C-cells that comprises 5–10% of all thyroid cancers. It occurs in both sporadic and familial forms, the latter making up 25% of all MTCs and being comprised of 3 distinct syndromes—MEN 2A, MEN 2B, and FMTC. To date, screening for MTC has been performed using the pentagastrin stimulation test, which is a provocative test for calcitonin release.
Route of Administration: Pentagastrin (intravenous bolus); calcium chloride (intravenous over 5 minutes).
Dosage: Pentagastrin (0.5 μg/kg); calcium infusions (2 mg/kg).
Considerations: RET proto-oncogene analysis can be carried out by automatic DNA sequencing in concert with adequate digestion of PCR amplified products for exons 10 and 11. Two distinct mutations in the RET proto-oncogene have been identified. A T >A transition at position 1783 (codon 618) in exon 10 has been detected in 1 family, and a G >A replacement at position 1832 (codon 634) in exon 11 in others. Germline mutations in the RET proto-oncogene have been identified in families manifesting these syndromes and genetic screening of individuals at risk of 1 of these syndromes has become integral to their clinical management. Majority of the mutations associated with MEN 2A and FMTC are tightly clustered in a cysteine-rich region of the RET receptor. A single mutation associated with MEN 2B is in the tyrosine kinase domain of the RET receptor.
Implementation: Evaluation of MEN 2A as a function of calcitonin release in response to pentagastrin and calcium chloride administration.
Human Model: Cases (n = 44) related to 3 individuals with proven MEN 2A and negative reference controls.
Interpretation and Analysis: Peak calcitonin (CT) levels are usually reached within 2 to 5 minutes after administration of provocative stimulus. Normal subjects have no significant difference in the mean basal CT levels between males (54.8 ± 21.7 pg/ml) and females (56.5 ± 34.8 pg/ml). Mean peak response values for males

is 146.3 ± 120.6 pg/ml, which is significantly different from the mean value of females, namely 71.6 ± 39.0 pg/ml. No significant correlations exists among the basal CT level, peak CT response, and age.

Calcium infusions (2 mg/kg/5 min) in normal individuals (e.g., n = 18 males; n = 37 females) yield a significantly greater secretory response than does pentagastrin (0.5 μg/kg bolus). In cases with medullary thyroid carcinoma (e.g., n = 12), pentagastrin is a better secretagogue than calcium ($p < 0.001$). Therefore, some have recommended routine measurement of plasma silica-extractable calcitonin and pentagastrin injection as the provocative test of choice for the detection and management of medullary thyroid carcinoma. The short calcium-infusion test is a good alternative.

In cases from families with a history of MEN 2A, approximately 20% display exaggerated calcitonin responses to combined pentagastrin/$CaCl_2$ sufficient to warrant thyroidectomy. Histological examination can confirm the presence of C-cell hyperplasia (CCH) (n = 1/9) or medullary thyroid carcinoma (n = 8/9). A relatively high percentage of cases (33%) will have high basal plasma calcitonin. Postoperatively, basal plasma calcitonin levels decline and response to pentagastrin/$CaCl_2$ produces a flat baseline concentration profile. Screening of cases with histological proof of MTC or CCH for a genetic linkage to the gene for MEN 2A using an MCK2 probe usually shows some correlation.

Several families (n = 10) with familial medullary thyroid carcinoma due to V804M mutation have been reported in the literature. Some reports have also described kindreds with not only heterozygous but also homozygous members with the V804M mutation. In gene carriers with the V804M mutation (e.g., n = 4 homozygous; n = 22 heterozygous) the pentagastrin stimulation test result is within normal range for the vast majority of heterozygous gene carriers (thyroidectomy not indicated). Therefore, in some cases, the V804M mutation in heterozygous cases seems not to be enough to express the full disease.

In non-gene carriers, false-positive results for basal calcitonin (6.6%) and for the pentagastrin provocative test (15.4%) can be observed. Based on these observations, the specificity of basal and pentagastrin-stimulated calcitonin is rather limited and RET proto-oncogene analysis is considered by many investigators and clinicians to be the 1st-line screening procedure of choice for the identification of gene carriers.

Experience with provocative and genetic tests strongly supports the concept of the indolent behavior of V804M RET proto-oncogene mutation. Counseling for preventive total thyroidectomy, the specific mutation of RET proto-oncogene, and also the natural history of the disease within a particular family should be considered.

Instrumentation: Scintillation counter and minor laboratory instrumentation necessary for the utilization of a commercial radioimmunoassay for the measurement of serum calcitonin. Radioimmunoassays for human calcitonin in whole plasma are limited in sensitivity and specificity; basal values of calcitonin are often undetectable in normal plasma, and non-specific increases are occasionally found in seemingly healthy persons. Such problems can be avoided by applying a silica-cartridge extraction-concentration technique for calcitonin assay, and the effectiveness of 2 calcitonin stimulation tests in healthy volunteers and patients with medullary thyroid carcinoma has been compared. The radioimmunoassay can be improved by using a new antiserum and a sequential incubation procedure that reduces the previously used sample-volume requirement and incubation period substantially. The method can be used to measure mean basal plasma levels of calcitonin (± SD) in 45 normal men (8.2 ± 5 pg/ml) and 47 normal women (4.8 ± 4 pg/ml) ($p < 0.001$).

Euthyroidism/Euthyroid State: Serum Total Thyroxine (TT4) Response
Thyroid-Stimulating Hormone (TSH)

Physiological Mechanism of Action: Thyrotropin-releasing hormone is produced in the hypothalamus and promotes synthesis/release of pituitary TSH. In turn, recombinant human thyroid-stimulating hormone (rhTSH) induces enhanced thyroid function and the synthesis/release of thyroid hormones.

Background Information: Until a clinically useful canine TSH assay is available, a valid test of thyroid hypofunction should (i) take into consideration valid (or at least predictive) measurement of the free T4 concentration in order to factor out the binding effects of drugs and non-thyroidal illness, and (ii) should assess thyroid functional reserve. A validated equilibrium dialysis measurement of free T4 and the TSH stimulation test provide the most useful diagnostic combination of tests now available. A therapeutic trial with L-thyroxine, despite being relatively safe, should be reserved once any other diagnoses have been ruled out, and should be reevaluated to assure a clinical response that can be attributable to the medication.

Application: Evaluation of normal and diseased thyroid function.

Route of Administration: Thyroid-stimulating hormone (intravenous, intramuscularly, or subcutaneously).

Dosage: Thyroid-stimulating hormone (25 μg, 50 μg, and 100 μg IV compared to 50 μg IM and SQ).

Implementation: Evaluate the effects of rhTSH on serum total thyroxine (TT4) concentration (e.g., euthyroid dogs). Protocol I: TSH response tests can be performed using rhTSH at 3 different total dose levels (IV). Protocol II and Protocol III: TSH response tests are performed using 50 μg of rhTSH (IM vs SQ, respectively).

Animal Model: Canine (n = 6 healthy beagle dogs used in each of the 3 investigation phases).

Interpretation and Analysis: In each phase and following all administered rhTSH doses, an increase in serum TT4 concentration can be appreciated but may not always be significant. Protocol I produces an obvious increase in serum TT4 concentrations. Such observations have fostered the conclusion that 50 μg is the optimal intravenous dose for rhTSH. Conversely, Protocol II and and Protocol III produce no significant increases in serum TT4 after rhTSH administration. Therefore, speculation suggests that rhTSH could be a good substitute for bovine TSH, when used by the intravenous route, for the TSH stimulation test in dogs. Further studies are required to confirm its clinical usefulness.

Update on diagnosis of canine hypothyroidism. Ferguson DC. Vet Clin North Am Small Anim Pract 1994 May; 24 (3), pp. 515–39.

Use of recombinant human thyroid-stimulating hormone for thyrotropin stimulation test in euthyroid dogs. Sauvé F, Paradis M. Can Vet J 2000 Mar; 41 (3), pp. 215–9.

Hypothyroid Obesity/Euthyroid Obesity
Glucocorticoids/Thyrotropin-Releasing Hormone (TRH)

Cellular/Molecular Mechanism of Action: Hypothalamic TRH promotes the release of pituitary thyroid-stimulating hormone (TSH), while glucocorticoids inhibit TSH secretion at the level of the hypothalamus.

Application: Distinguishing conditions of euthyroid obesity from subclinically hypothyroid obese patients.

Route of Administration: Thyrotropin-releasing hormone tests (intravenous bolus injection); dexamethasone (orally).

Dosage: Thyrotropin-releasing hormone test (200 μg) and dexamethasone (2 mg/d in 4 divided doses for 3 days).

Considerations: The presence of an abnormally high TSH response to TRH makes it difficult to distinguish some euthyroid obese subjects from subclinically hypothyroid obese patients.
Interpretation and Analysis: Total thyroxine and triiodothyronine concentrations can be expected to be in the normal range in a high percentage of patients.

Based on the measurement of basal and TRH-stimulated serum thyrotropin (TSH) concentrations, patients can be divided into 3 possible groups including (i) Group I (n = 10), euthyroid subjects; (ii) Group II, euthyroid subjects with normal basal but abnormally elevated TSH responses to TRH; and (iii) Group III (n = 10), subjects with elevated basal and TRH-induced TSH levels (subclinical hypothyroidism). Basal TSH levels are approximately 1.8 ± 0.4 mU/L in Group I, 1.7 ± 0.3 in Group II, and 6.0 ± 0.7 in Group III. In both Groups II and III, TRH-induced TSH increments are above the normal range (maximal increment > 15 mU/L) and significantly higher than values noted for Group I patients. After the 2nd treatment with TRH, pretreatment with dexamethasone significantly decreases both basal TSH levels and peak TSH responses to TRH in all groups. However, a striking percentage of euthyroid obese Group I and Group II patients demonstrate decreases (> 50%) in TRH-induced peak TSH responses, whereas hypothyroid subjects of Group III show only a slight decrement (< 25%). Therefore, it has been proposed that the sensitivity of the TSH secretory system to glucocorticoid inhibitory action is preserved in obese subjects with abnormally elevated TSH response to TRH, but not in subclinically hypothyroid obese patients. The TRH in combination with dexamethozone might be useful in future studies to understand the mechanisms underlying alterations in TSH secretion in obesity.

Thyroid Cancer: Differentiated/Distant Metastatic Disease
Recombinant Thyrotropin

Cellular/Molecular Mechanism of Action: Recombinant thyrotropin stimulates iodine uptake in thyroid tissue.
Application: Detection of differentiated thyroid cancer (± metastasis).
Dosage: Recombinant thyrotropin.
Considerations: Recombinant thyrotropin is a safe and effective diagnostic agent for cases that require radioiodine scanning for routine follow-up. The use of recombinant thyrotropin has fewer adverse effects than does the alternative, which is withdrawal of thyroid hormone replacement, although nausea and headache have been reported. Recombinant thyrotropin is relatively expensive.
Interpretation and Analysis: A serum thyroglobulin > 2 ng/mL and/or a positive whole body scan after recombinant thyrotropin stimulation suggests residual thyroid tissue or neoplastic disease. The combined application of measured serum thyroglobulin concentrations, and whole-body radioiodine scintigraphy can identify virtually all patients with distant metastatic disease.

The use of recombinant thyrotropin in the follow-up of patients with differentiated thyroid cancer. Basaria M, Graf H, Cooper DS. Am J Med 2002 Jun 15; 112 (9), pp. 721–5.

Thyroid Function/Triiodothyronine (T-3); Thyroxine (T4); Free Thyroxine (fT4)/Assay Validation: Phenylbutazone Interference
Thyroid-Stimulating Hormone (TSH)

Application: The TSH stimulation test can be applied to evaluate thyroid function under various physiological conditions, in response to exogenously administered pharmaceutical agents; or validate various analytical assay systems.
Route of Administration: Thyroid-stimulating hormone (intravenous).
Dosage: Thyroid-stimulating hormone (5 IU).
Implementation: Evaluation of the influence of TSH administration on serum concentrations of triiodothyronine (T-3), thyroxine (T4), and free thyroxine (fT4); in addition to determining their serum concentrations at rest and following phenylbutazone administration in healthy horses (7-day administration period).

Animal Model: Equine (normal healthy horses ± phenylbutazone therapy for 7 days).
Interpretation and Analysis: Baseline values range from 0.21 to 0.80 ng of T-3/ml, 6.2 to 25.1 ng of T4/ml, and 0.07 to 0.47 ng of fT-3/dl. Following TSH administration, serum T-3 values increase to 6 times baseline values in 2 hours. Thyroxine values increase to 3 times baseline values at 4 hours and remain high at 6 hours. Free T4 values increase to 4 times baseline values at 4 hours and remain high at 6 hours. Administration of 4.4 mg of phenylbutazone/kg, every 12 hours for 7 days significantly decreases T4 and fT4 values, but does not significantly affect serum T-3 concentrations. Therefore, the TSH stimulation test should be performed when hypothyroidism is suspected. Measurement of serum fT4 concentrations, by the single-stage radioimmunoassay, does not provide any additional information about thyroid gland function over that gained by measuring T4 concentrations. Phenylbutazone given at a dosage of 4.4 mg/kg every 24 hours, for 7 days does significantly decrease resting T4 and fT4 concentrations, but does not significantly affect T-3 concentrations in horses.

Serum triiodothyronine, total thyroxine, and free thyroxine concentrations in horses. Sojka JE, Johnson MA, Bottoms GD. Am J Vet Res 1993 Jan; 54 (1), pp. 52–5.

Thyroid Function/Stimulation Test Optimization (Feline)
Recombinant Human Thyroid-Stimulating Hormone (rhTSH)

Physiological Mechanism of Action: Pituitary or recombinant human TSH activates thyroid gland function and promote the release/synthesis of thyroid hormones.
Application: Evaluate responses of euthyroid cats to the administration of recombinant human thyroid-stimulating hormone (rhTSH).
Route of Administration: Recombinant human TSH (intravenous).
Dosage: Recombinant human TSH (0, 0.025, 0.050, 0.100, and 0.200 mg).
Implementation: Evaluation of serum concentrations of total thyroxine (TT4) and free thyroxine (fT4) measured immediately before each injection (time 0) and 2, 4, 6, and 8 hours after administration of each dose of rhTSH (e.g., n = 5 test doses). Each rhTSH test dose is evaluated at 1-week intervals.

Animal Model: Feline (n = 7 healthy cats).
Interpretation and Analysis: Overall TT4 response does not differ significantly among cats when administered doses are ≥ 0.025 mg. Serum TT4 concentrations peak 6 to 8 hours after administration for all doses ≥ 0.025 mg. For all doses ≥ 0.025 mg, mean ± SEM TT4 concentration at 0, 6, and 8 hours are approximately 33.9 ± 1.7, 101.8 ± 5.9, and 101.5 ± 5.7 nmol/L, respectively. For all doses ≥ 0.025 mg, mean fT4 concentration at 0, 6, and 8 hours are approximately 38.7 ± 2.9, 104.5 ± 7.6, and 100.4 ± 8.0 pmol/L, respectively. At 8 hours, the fT4 response to 0.025 and 0.050 mg is less

than the response to 0.100 and 0.200 mg. Adverse reactions after rhTSH administration usually are not appreciated. The TSH stimulation test can be performed in cats by IV administration of 0.025 to 0.200 mg of rhTSH and measurement of serum TT4 concentrations at time of injection and 6 or 8 hours later. Clinical validation of the TSH stimulation test would facilitate development of additional tests of thyroid gland function, such as a TSH assay.

Use of recombinant human thyroid-stimulating hormone for thyrotropin-stimulation testing of euthyroid cats. Stegeman JR, Graham PA, Hauptman JG. Am J Vet Res 2003 Feb; 64 (2), pp. 149–52.

Thyroid Function/Influence of Physiological Processes and Nutritional Status/Obesity
Thyroid-Stimulating Hormone (TSH)

Cellular/Molecular Mechanism of Action: Thyroid-stimulating hormone is normally produced in the pituitary and when released promotes enhanced thyroid function and the synthesis/release of thyroid hormones.
Background Information: Obesity and weight loss have been shown to alter thyroid hormone homeostasis in humans. In dogs, obesity is the most common nutritional problem encountered and weight loss is the cornerstone of its treatment. Therefore, it is important to clarify how obesity and weight loss can affect thyroid function test results in that species.
Application: Detection and characterization of alterations in thyroid function.
Route of Administration: Thyroid-stimulating hormone test (intravenous).
Dosage: Thyroid-stimulating hormone test (TSH 75 μg).
Implementation: Evaluate thyroid function in obese dogs compared to lean dogs and to explore the effects of caloric restriction and weight loss on thyroid hormone serum concentrations in obese dogs. One approach to achieving this objective is the assessment of thyroid function through the measurement of serum concentrations of total thyroxine (TT4), free thyroxine (FT4), total triiodothyronine (TT-3), thyrotropin (TSH), and reverse triiodothyronine (rT-3) as well as a TSH stimulation test using recombinant human TSH preparations (rhTSH). Complementary investigations can involve the evaluation of obese conditions (e.g., beagles) fed an energy-restricted diet (average 63% maintenance energy requirement [MER]) until an optimal weight is obtained. Blood samples for determination of TT4, FT4, TT-3, TSH, and rT-3 are taken at the beginning and then weekly during periods of weight loss.

Animal Model: Canine (n = 12 healthy lean beagles; n = 12 obese beagles).
Interpretation and Analysis: Only TT-3 and TT4 serum concentrations are significantly higher in obese dogs as compared to lean dogs. In situations where obese dogs are fed an energy-restricted diet, weight loss is associated with a significant decrease in TT-3 and TSH serum concentrations. Thus obesity and energy restriction significantly alter thyroid homeostasis in dogs, but the observed changes are unlikely to affect interpretation of thyroid function test results in clinics.

Evaluation of thyroid function in obese dogs and in dogs undergoing a weight loss protocol. Daminet S, Jeusette I, Duchateau L, Diez M, Van de Maele I, De Rick A. J Vet Med A Physiol Pathol Clin Med 2003 May; 50 (4), pp. 213–8.

Thyroid Function: Hypothyroidism
Thyrotropin-Releasing Hormone (TRH)/Thyroid-Stimulating Hormone (TSH)

Cellular/Molecular Mechanism of Action: Thyrotropin-releasing hormone is produced in the hypothalamus and promotes release of pituitary TSH, which in turn activates the thyroid gland and promotes the release of thyroid hormone.
Application: Characterization of the value of diagnostic tests for the identification of hypothyroid conditions.
Implementation: Evaluation of conditions of hypothyroidism applying the thyrotropin-releasing hormone (TRH) and TSH stimulation tests.

Animal Model: Canine cases with dermatological signs suggestive of hypothyroidism (n = 22; n = 5 normal healthy dogs).
Interpretation and Analysis: Thyroxine (T4) after TRH stimulation is significantly lower than that obtained by TSH stimulation. It is not possible to identify hypothyroid dogs with the TRH-stimulation test, because some euthyroid dogs have either decreases in T4 or only slight increases in T4 concentrations after TRH stimulation. In addition, dogs with pyoderma have decreased responses in serum T4 after TRH stimulation that usually become normal following treatment with antibiotics. Alternatively, the combination of results from a TSH stimulation test, and response to thyroid hormone replacement can be used as a basis for diagnosing hypothyroidism in dogs (e.g., n = 6). It has been concluded that TRH stimulation is not a useful means of diagnosing hypothyroidism in dogs.

Comparison of thyrotropin-releasing hormone (TRH) to thyrotropin (TSH) stimulation for evaluating thyroid function in dogs. Frank LA. J Am Anim Hosp Assoc 1996 Nov–Dec; 32 (6), pp. 481–7.

Thyroid Function: Hyperthyroidism/Critically Ill vs Critically Ill with Hyperthyroidism
Thyroid-Stimulating Hormone (TSH)

Cellular/Molecular Mechanism of Action: Pituitary or recombinant TSH activates the thyroid and promotes synthesis/release of thyroid hormones.
Implementation: Evaluation of conditions of suspected hyperthyroidism. Basal serum thyroxine (T4) concentration and the thyrotropin-releasing hormone (TRH) stimulation test are performed to assess thyroid function.

Animal Model: Feline (n = 22 critically ill hyperthyroid cats with underlying or complicating disease states). Clinical signs include palpable thyroid nodules, and abnormal thyroid gland histology (study group). Cats classified as critically ill that are not hyperthyroid (n = 14).
Interpretation and Analysis: The TRH stimulation test reveals increases in serum T4 of < 50% of the baseline concentration in a large percentage of affected cats (e.g., n = 18/22 or 82% compared to n = 6/22 or 43% of reference controls). Based on the results of serum T4 determinations and the TRH stimulation tests, it is not possible to differentiate between cats with clinical and histologic evidence of thyroid dysfunction (hyperthyroidism) and cats with severe nonthyroidal illnesses.

Thyrotropin-releasing hormone stimulation test to assess thyroid function in severely sick cats. Tomsa K, Glaus TM, Kacl GM, Pospischil A, Reusch CE. J Vet Intern Med 2001 Mar–Apr; 15 (2), pp. 89–93.

Thyroid Function: Hypothyroidism/Euthyroid/Sick Euthyroid
Thyrotropin-Releasing Hormone (TRH)/(Canine Thyroid-Stimulating Hormone [cTSH] Measurement)

Physiological Mechanism of Action: Thyrotropin-releasing hormone is released from the hypothalamus and promotes release of TSH from the pituitary gland.
Application: Adjunct test for characterizing conditions of hypothyroidism, euthyroidism, and sick euthyroidism.
Implementation: Evaluation of cTSH concentrations measured in a variety of clinical conditions. Cases are classified as euthyroid, sick euthyroid, hypothyroid, or hypothyroid on non-thyroidal therapy on the basis of their history, clinical signs, laboratory results (including total thyroxine concentrations and, where indicated, TRH stimulation tests), and response to appropriate therapy. Additional samples are taken during some of the TRH stimulation tests to measure cTSH responses (concentrations) following TRH administration. A reference range can be calculated from the basal concentrations of cTSH (e.g., 0.0–0.41 ng/ml for n = 41 euthyroid dogs).
Animal Model: Canine (n = 72).
Interpretation and Analysis: Approximately 66% of cases of confirmed hypothyroidism will have basal cTSH concentrations above the reference range (e.g., n = 6/9), whereas the remainder will be within the normal range. Some of the remaining cases that do not show a rise in cTSH concentration following TRH stimulation may be due to the presence of pituitary dwarfism (e.g., n = 1/3 or 33%). In contrast, only approximately 15% hypothyroid dogs that have been on non-thyroidal treatment within the previous 4 weeks will have increases in basal cTSH concentrations (e.g., n = 1/6 or 15%). In addition, approximately 30% of dogs with sick euthyroid syndrome have increased cTSH concentrations (e.g., n = 6/15). Therefore, cTSH measurements are a useful additional diagnostic test in cases of suspected hypothyroidism in dogs, but dynamic testing is still required to confirm the diagnosis of hypothyroidism.

Thyroid-stimulating hormone and total thyroxine concentrations in euthyroid, sick euthyroid and hypothyroid dogs. Ramsey IK, Evans H, Herrtage ME. J Small Anim Pract 1997 Dec; 38 (12), pp. 540–5.

Thyroid Function: Hypothyroidism
Thyroid-Stimulating Hormone (TSH)

Cellular/Molecular Mechanism of Action: Thyroid-stimulating hormone is produced in the anterior pituitary (adenohypophysis) and stimulates the production of thyroid hormone by the thyroid gland.
Diagnostic Application #1: The principal application of TSH is for the diagnosis of primary hypothyroidism (e.g., dogs).
Diagnostic Application #2: Hyperthyroid patients (e.g., feline) that display "borderline" or only slightly increased T_3 and T_4 concentrations can be diagnosed with the aid of a TSH response test. Under these circumstances, there is little or no increase in resting T_4 concentrations ($\leq$ 2-fold) within 4 to 6 hours following exogenous TSH administration.
Route of Administration and Dosage: Intravascular injection administered at a dosage of 1.0 IU/kg in feline patients. In canine hypothyroid patients, TSH is administered either intramuscularly or intravenously. Dogs weighing over 20 kg should receive a total dose of 5 IU.
Implementation: Evaluation of thyroid function. Blood samples should be collected from cats prior to TSH administration for the measurement of serum or plasma T_4 concentrations. In dogs, blood samples should be collected at 6 to 8 hours (intravenous TSH), or 8 to 12 hours (intramuscular TSH).
Interpretation and Analysis: Application #1: Pre-TSH T_4 concentrations in the dog are 1.0–4.0 μg/dl, while post-TSH T_4 levels approach 3.5 μg/dl. In cats, pre-TSH T_4 concentrations are 0.8–4.0 μg/dl, with post-TSH T_4 levels $>$ 3.0 μg/dl. Low pre-TSH and post-TSH concentrations of T_4 (less than a 0.5 μg/dl increase) suggest a diagnosis of primary hypothyroidism.
Laboratory Instrumentation: Assay of either cortisol or T_3/T_4 concentrations by either RIA or ELISA.

Thyroid Function/Levothyroxine Efficacy Evaluation
Thyroid-Stimulating Hormone (TSH) Test/Thyrotropin-Releasing Hormone (TRH) Stimulation Test

Cellular/Molecular Mechanism of Action: The stimulating hormone TSH normally is produced in the pituitary, thereby promoting thyroid gland activation and synthesis/release of thyroid hormone.
Application: Characterization of the efficacy of biopharmaceutical agents utilized in the therapeutic intervention for conditions of hyperthyroidism and hypothyroidism.
Implementation: Prospective evaluation of the clinical interchangeability between 2 brands of levothyroxine, Synthroid (Boots Pharmaceuticals, Inc., Lincolnshire, IL) and Levoxine (Daniels Pharmaceuticals, Inc., St. Petersburg, FL) utilizing clinical scores established for hyperthyroidism and hypothyroidism based on the variables/parameters of free thyroxine index (FTI), sensitive TSH, and TRH stimulation testing. This objective can be achieved by delineating observed differences noted with a change in medication from Synthroid to Levoxine (Case Type SL) or Levoxine to Synthroid (Case Type LS). After switching, dosages are kept constant in combination with the recording of clinical scores for hypothyroidism and hyperthyroidism (Billewicz and Crooks scoring systems, respectively), basal FTI, and TRH stimulation test results before and 4 months after therapeutic switching. Comparison of the variables before and after switching is performed separately in each case type (LS or SL) and in the entire group.
Human Model: Cases of hypothyroidism (n = 23/31 with long-standing primary hypothyroidism; n = 6 male; n = 25 female; age range = 30–71 years, mean 47.2 ± 2.2 SEM).
Interpretation and Analysis: No statistically significant differences in the hypothyroid clinical scores (−40.1 ± 1.2 versus −39.7 ± 1.2), the hyperthyroid clinical scores (−19.6 ± 0.9 vs −19.2 ± 1.0), FTI (9.6 ± 0.3 vs 9.6 ± 0.3), basal TSH levels (1.4 ± 0.2 vs 1.4 ± 0.2 mIU/L), or the magnitude of TSH response to TRH (mean delta TSH 9.4 ± 1.5 vs 9.2 ± 1.4 mIU/L) can be detected if they are receiving either Synthroid or Levoxine. Switching does not result in substantial clinical or laboratory changes in any individual patient and the 2 brands of levothyroxine are clinically interchangeable.

Assessment of interchangeability of two brands of levothyroxine preparations with a third-generation TSH assay. Escalante DA, Arem N, Arem R. Am J Med 1995 Apr; 98 (4), pp. 374–8.

Thyroid Hormone Receptor Complex Expression
3,5,3′-triiodo-L-thyronine Analog (GC-1)

Cellular/Molecular Mechanism of Action: High selective binding affinity and activation of thyroid receptor-beta.
Application: Selective detection of thyroid receptor-beta expression that might be useful as an *in vivo* probe for the study of the physiological roles of different thyroid hormone receptor isoforms.
Considerations: Thyroid hormones regulate many different physiological processes in different tissues in vertebrates. Most of the actions of thyroid hormones are mediated by the thyroid hormone receptor (TR), which is a member of the nuclear receptor superfamily of ligand-activated transcription regulators. There are 2 different genes that encode 2 different TRs, TR alpha and TR beta, and these 2 TRs are often co-expressed at different levels in different tissues. Most thyroid hormones do not discriminate between the 2 TRs and bind both with similar affinities.
Molecular Design and Semi-Synthesis: The compound GC-1 has been designed to solve synthetic problems that limit thyroid hormone analog preparation, and contains several structural changes with respect to the natural T-3 hormone (3,5,3′-triiodo-L-thyronine). These changes include replacement of the 3 iodines with methyl and isopropyl groups, replacement of the biaryl ether linkage with a methylene linkage, and replacement of the amino-acid sidechain with an oxyacetic-acid sidechain.
Interpretation and Analysis: The agent GC-1 is a member of a new class of thyromimetic compounds that are more synthetically accessible than traditional thyromimetics and have potentially useful receptor binding and activation properties. The TR beta selectivity of GC-1 is particularly interesting and suggests that GC-1 might be a useful *in vivo* probe for studying the physiological roles of the different thyroid hormone receptor isoforms.

A high-affinity subtype-selective agonist ligand for the thyroid hormone receptor. Chiellini G, Apriletti JW, Yoshihara HA, Baxter JD, Ribeiro RC, Scanlan TS. Chem Biol 1998 Jun; 5 (6), pp. 299–306.

Thyroid-Stimulating Hormone (TSH) and Growth Hormone (GH) Response/Influence of Fatty Acids on Plasma Levels
Thyrotropin-Releasing Hormone (TRH)

Background Information: Supplemental dietary fat provides excess fatty acids (FA) that can alter circulating concentrations of several hormones.
Route of Administration: Thyrotropin-releasing hormone (intramuscular).
Implementation: Evaluation of the effects of fatty acid isomer type on systemic hormone concentrations and possible sites of regulation. One approach to achieving this objective involves the abdominal infusion of fat mixtures high in *cis*-C18:1 FA (iCIS), high in *trans*-C18:1 FA (iTRS), or no infusion (NI) and performed intravenous arginine (ARG) and intramuscular TRH challenges. Challenges are conducted on days 10 (ARG) and 12 (TRH) after initiation of fat infusion on each of 3 4-week experimental periods. Assessment of fat regulation of the thyroid axis can be tested directly *in vitro* by characterizing the ability of fatty acids dissolved with sodium taurocholate to affect Type-I 5′-deiodinase (5′D) activity in bovine liver homogenates.
Animal Model: Bovine and bovine liver homogenates.
Experimental Design: Replicated 3 × 3 Latin square.
Interpretation and Analysis: Plasma concentrations of insulin-like growth factor-I (IGF-I) are lower ($p < 0.01$) when cows receive iCIS or iTRS compared with no fat infusion. Plasma insulin concentrations increase with ARG but responses are not affected by FA. Plasma GH remains unchanged after ARG. Peak plasma GH and TS) responses to TRH are blunted ($p < 0.05$ and $p < 0.1$, respectively), whereas thyroxine (T4) and triiodothyronine (T-3) responses are augmented post-TRH ($p < 0.01$) when cows receive either FA isomer. Prolactin responses to TRH are not different between infusion treatments, although basal plasma concentrations before TRH are higher in cows infused with iTRS ($p < 0.05$). Homogenate 5′D is not affected by C2:0-C10:0 fatty acids, but decreases linearly ($p < 0.01$) with increasing concentrations of C12:0-C16:0 and C18:1 isomers. *Cis* C18:1 decreases 5′D more than the *trans*-isomer ($p < 0.01$), but the difference is only apparent at concentrations > 0.25 mM. The data suggest that various aspects of pituitary hormone regulation are differentially affected by FA composition. Fatty acid infusion may accentuate end organ responses in the thyroid axis and decrease IGF-I in the somatotropic axis. The data also suggest that FA isomer may alter patterns of extrathyroidal generation of thyroid hormones via direct influences on 5′D.

Dietary fatty acids modulate hormone responses in lactating cows: Mechanistic role for 5′-deiodinase activity in tissue. Romo GA, Elsasser TH, Kahl S, Erdman RA, Casper DP. Domest Anim Endocrinol 1997 Nov; 14 (6), pp. 409–20.

PARATHYROID

Hyperparathyroidism (Primary): Normocalcemia
Calcium Gluconolactate

Route of Administration: Calcium gluconolactate (oral).
Dosage: Calcium gluconolactate (1 g).
Implementation: Evaluation of normocalcemic primary hyperparathyroidims using the oral calcium-loading test (OCLT). Calcium and parathyroid hormone (PTH) levels are measured before and 60, 120, and 180 minutes after calcium gluconolactate administration.
Human Model: Cases with high circulating PTH levels (e.g., n = 102 consecutive females) and reference control subjects (e.g., n =25). Patients can be classified as Group A, patients with a parathyroid adenoma identified by 2 imaging modalities; Subgroup AO, hyperparathyroid patients (n = 13, mean age 59 years [SD = 10]) evaluated prior to parathyroidectomy; Subgroup AH, non-operated hypercalcemic patients (n = 29, age 63 years [SD = 11]); Subgroup AN, normocalcemic non-operated women (n = 14, age 59 years [SD = 8]); Group B, normocalcemic individuals (n = 46, age 58 years [SD = 11]) with negative parathyroid imaging; and Group C, control patients (n = 25, age 56 years [SD = 12]).
Interpretation and Analysis: The concentrations of calcium and PTH may overlap in the normocalcemic groups during the OCLT. Product P, defined as circulating PTH nadir (pg/ml) × peak calcium concentration (mg/dl), better discriminates Subgroup AN from Group B, AUC = 0.98 (95% CI 0.95, 1.00) than does Ratio R, defined as relative PTH decline/relative calcium increment, AUC = 0.86 (95% CI 0.73, 0.99). Assuming a normal threshold of Product P and Ratio R at 260 and 17, respectively, the combined parameters diagnose normocalcemic hyperparathyroid patients with 100% sensitivity and 87% specificity.

Diagnosis of normocalcemic hyperparathyroidism by oral calcium loading test. Hagag P, Revet-Zak I, Hod N, Horne T, Rapoport MJ, Weiss M. J Endocrinol Invest 2003 Apr; 26 (4), pp. 327–32.

Hyperparathyroidism: Secondary
Calcium Gluconate/Sodium Citrate

Background Information: The parathyroid glands play a critical role in the maintenance of calcium homeostasis. It has been suggested that the set-point for calcium-regulated parathyroid hormone

(PTH) release is higher in uremic patients than normal subjects. However, these assessments of parathyroid gland function have been performed using methods that differ from the original 4-parameter model.

Route of Administration: Calcium gluconate and sodium citrate (infusion).

Implementation: Dynamic testing of the parathyroid glands can be performed with standardized infusions of calcium gluconate and sodium citrate for the evaluation of conditions of secondary hyperparathyroidism and the efficacy of calcitriol therapy (intermittent over a 4-month period).

Human Model: Cases on dialysis with secondary hyperparathyroidism and normal healthy control volunteers.

Interpretation and Analysis: Derived values for the set-point are 1.21 ± 0.04 mmol/l and 1.24 ± 0.06 mmol/l, respectively in control and dialyzed patients. Furthermore, the values for set-point are 1.21 ± 0.01 and 1.22 ± 0.01 mmol/l (NS), before and after calcitriol therapy. In addition, when subjects are grouped according to severity of secondary hyperparathyroidism, the set-point calcium-regulated PTH release does not differ between case group types. Calcitriol therapy alters the secretory capacity of the parathyroid glands during hypocalcemia. The degree of parathyroid enlargement and the type of cell proliferation may be more important determinants of the severity of secondary hyperparathyroidism.

Parathyroid gland function in secondary hyperparathyroidism. Salusky IB, Goodman WG. Pediatr Nephrol 1996 Jun; 10 (3), pp. 359–63.

Parathyroid Hormone (PTH) Secretion
Calcium

Physiological Mechanism of Action: Acute hypercalcemia suppresses both the pulsatile and the tonic component of PTH secretion.

Route of Administration: Intravascular (intravenous).

Implementation: Evaluate instantaneous PTH secretion as a function of alterations in ionized calcium concentration. Blood sampling (1 minute) is performed under conditions of normo-, hypo-, and hypercalcemia. The characteristics of PTH secretion are analyzed by a deconvolution technique, accounting for subject-specific plasma PTH disappearance half-life, as measured during the 1st 15 minutes of calcium infusion (range, 2.04–2.93 minutes). The process regularity of pulsatile PTH secretion is evaluated by an approximate entropy statistic.

Human Model: Cases without health abnormalities (n = 7 young healthy volunteers).

Interpretation and Analysis: After a baseline period of 75 minutes, ambient ionized calcium is either increased or decreased by 0.2 mmol/L for 105 minutes by clamped infusion of calcium gluconate or sodium citrate. Under baseline conditions, 32% of total PTH secretion is released in a pulsatile fashion, with a burst frequency of 6.9 ± 0.8 h-1 and a PTH mass per burst of 2.6 ± 0.9 pmol/L. The remaining 68% of total secretion is attributed to tonic hormone release. During the initial 30 minutes of induced hypocalcemia, pulsatile secretion increases by 1,140%, whereas tonic secretion does not change. The preferential increase in pulsatile PTH secretion is mediated by a combined rise in burst frequency and mass released per burst. During subsequent steady state hypocalcemia, the tonic secretion rate increases (255% of baseline), whereas burst frequency and burst mass decreases (to 103% and 189% of the baseline values), restoring the baseline ratio of tonic to pulsatile PTH secretion. The regularity of PTH release increases during steady state hypocalcemia. During hypercalcemia, tonic secretion, burst mass, and burst frequency decreases by 75%, 82%, and 32%, respectively, and remains constant throughout the clamp period. Acute hypocalcemia therefore, elicits an immediate pulsatile and a delay in tonic secretory response of the parathyroid gland with increased regularity of PTH release. Acute hypercalcemia suppresses both the pulsatile and the tonic component of PTH secretion.

Control of pulsatile and tonic parathyroid hormone secretion by ionized calcium. Schmitt CP, Schaefer F, Bruch A, Veldhuis JD, Schmidt-Gayk H, Stein G, Ritz E, Mehls O. J Clin Endocrinol Metab 1996 Dec; 81 (12), pp. 4236.

Pseudohypoparathyroidism/Hypoparathyroidism
Synthetic Parathyroid Hormone Fragment (PTH-1-34)/ [cAMP]

Cellular/Molecular Mechanism of Action: A synthetic amino-terminal fragment of PTH (PTH-1-34) has recently been released for clinical testing of PTH responsiveness.

Application: Clinical testing of PTH responsiveness in conditions including pseudohypoparathyroidism and idiopathic hypoparathyroidism.

Route of Administration: Intravenous over a 10-minute time span.

Dosage: Synthetic amino-terminal fragment of PTH, PTH-1-34 (3U/kg BW [200 U maximum]).

Considerations: Induction of hypercalcemia can fail to restore normal cAMP responses to PTH-1-34 infusion in cases of pseudohypoparathyroidism. The synthetic parathyroid hormone fragment (PTH-1-34) is effective for the study of renal responsiveness to PTH. Protocols have been described that facilitate reliable differentiation between pseudohypoparathyroidism from hypocalcemia due to other causes.

Implementation: Synthetic amino-terminal fragment of PTH (PTH-1-34) can be applied to evaluate conditions of pseudohypoparathyroidism and hypoparathyroidism. Urinary cAMP and phosphaturic responses are measured in urine samples collected at 30-minute intervals in addition to the analysis of 4 blood samples drawn following PTH infusion.

Interpretation and Analysis: Calculation of the urinary excretion of cAMP in response to PTH-1-34 in nanomoles/dL of glomerular filtrate during the 1st 30 minutes after infusion provides better differentiation among groups than other parameters of cAMP metabolism. Calculating the phosphaturic response as the percent fall in tubular maximum for phosphate reabsorption during the 1st hour after infusion gives the best degree of statistical separation among groups.

In cases of pseudohypoparathyroidism (hypocalcemia with increased serum immunoreactive PTH concentrations) it is possible to differentiate between suspected pseudohypoparathyroidism, surgical hypoparathyroidism, and normal patients. Pseudohypoparathyroidism conditions are recognized by blunted cAMP and phosphaturic responses to PTH-1-34 administration compared to those of either normal or hypoparathyroid subjects.

Synthetic human parathyroid hormone-(1-34) for the study of pseudohypoparathyroidism. Mallette LE, Kirkland JL, Gagel RF, Law WM Jr, Heath H 3rd. J Clin Endocrinol Metab 1988 Nov; 67 (5), pp. 964–72.

4 Gastrointestinal System

DENTISTRY, ORAL CAVITY, AND SALIVARY GLANDS

Calcium-Binding Peptides
Enamel Proteins/Sheathlin

Cellular/Molecular Mechanism of Action: Enamel proteins possess binding avidity for Ca^{++} cation.
Background Information: Dental enamel is believed to form by the transfer of ions from solution, primarily calcium, phosphate, hydroxyl, and carbonate, to the surface of solid-state mineral. Such precipitation phenomena can be controlled by regulating the degree of saturation of the solution with respect to the potential solid phases that can form. The concentration of free calcium is the factor that most affects the degree of saturation for calcium hydroxyapatite, and its buffering by calcium-binding proteins has been proposed as the mechanism that determines the mineral structure of enamel.
Application: Detection of calcium cation, physically binding to solid calcium containing matrixes dependent upon or involving mechanisms of competitive inhibition of an enzyme requiring calcium as a biochemical co-factor.
Implementation: Application of staining reagents that can be used to identify and isolate calcium-binding proteins from the enamel matrix and to determine their structures and association constants for calcium.
Animal Model: Porcine.
Interpretation and Analysis: Proteolytic cleavage fragments derived from the C-terminus of sheathlin, with apparent molecular weights of 13-kDa, 15-kDa, 27-kDa, and 29-kDa, can be characterized by amino-terminal protein sequencing, amino acid analysis, and sugar, phosphate, and sulphate determinations. Sheathlin C-terminal cleavage products have been shown to have no N-linked glycosylations or phosphorylated amino acids, but Pro(350) is hydroxylated, and there is 1 sulphated O-linked glycosylation at Thr(386), containing galactose and N-acetylgalactosamine. The calcium-binding association constants for enamel proteins range from a high of 1.2×10^4 M(-1) to a low of 4.4×10^1 M(-1). The relative strengths of binding in order of decreasing affinity are: 13-kDa and 15-kDa calcium-binding domain of sheathlin $>$ 27-kDa and 29-kDa calcium-binding proteins $>$ 32-kDa enamelin $>$ 89-kDa enamelin $>$ 6.5-kDa, 25-kDa, 23-kDa, 20-kDa, 13-kDa, 5.3-kDa amelogenins. If enamel proteins have similar calcium-binding properties *in vivo* as they possess when measured *in vitro*, they would tend to buffer the free calcium ion concentration in enamel fluid.

Calcium binding of enamel proteins and their derivatives with emphasis on the calcium-binding domain of porcine sheathlin. Yamakoshi Y, Tanabe T, Oida S, Hu CC, Simmer JP, Fukae M. Arch Oral Biol 2001 Nov; 46 (11), pp. 1005–14.

Dental Caries
Red Acid Dye

Cellular/Molecular Mechanism of Action: Selectively stains dental caries. Deposits of demineralized dentine are preferentially stained in a manner that can be directly visualized.
Application: Detection of dental caries.
Route of Administration: Topical/oral.
Implementation: Determination of the effectiveness of red acid dye in detecting dental caries.
Interpretation and Analysis: A high percentage of junior dental students fail to remove all demineralized dentine without the use of a caries detector reagent (stage 1 = 96% and stage 2 = 79%). In comparison, all senior dental students may fail to diagnose dental caries without the use of a dental caries detector (stage 1 = 100% and stage 2 = 0%). Unlike junior students, a majority (e.g., all) of the senior students during the 2nd stage of their training program will remove all of the demineralized dentine. Without the use of a caries detector dye, the risks of the dental students leaving demineralized dentine is high. This technique may be particularly helpful for the 4th-year students who are performing their 1st restorative treatment.
Instrumentation: None.

In vivo diagnostic assessment of dentinal caries by junior and senior students using red acid dye. Tassery H, Déjou J, Chafaie A, Camps J. Eur J Dent Educ 2001 Feb; 5 (1), pp. 38–42.

Dental Caries
Red Acid Dye/Povidone Iodine Dye

Cellular/Molecular Mechanism of Action: Selectively stains dental caries but does not stain the surface of intact ("mineralized") enamel.
Application: Identification of carious dentin.
Route of Administration: Topical/oral.
Dosage: Acid red (1% acid red in propylene glycol wt/wt) and povidone-iodine (8% povidone-iodine in water wt/vol).
Implementation: Evaluation of the ability for acid red and povodine iodine to detect dental lesions (n = 221 cavities in a double blind study design; n = $\geq$ 2/case).
Interpretation and Analysis: Acid red and povidone-iodine positively stain defects in the dental enamel. Their application reveals that molars and premolars do not appear to be positively stained more frequently by either 1 of the dye formulations. Approximately one-third (36.7%) of teeth may test postive to 1 of the 2 dye reagents. Application of either acid red or povidone-iodine dyes is of value in clinical decision-making processes regarding cavity size while restoring dentinal lesions. However, povidone-iodine may be preferred in clinical settings where acid red is expensive and/or difficult to obtain. Further research is necessary to establish accurately the mechanism of staining by povidone-iodine.

In vivo diagnostic assessment of dentinal caries utilizing acid red and povidone-iodine dyes. Maupomé G, Hernández-Guerrero JC, García-Luna M, Trejo-Alvarado A, Hernández-Pérez M, Díez-de-Bonilla J. Oper Dent 1995 May–Jun; 20 (3), pp. 119–22.

Oral Cancer Lesions/Differentiation: Premalignant vs Malignant Lesions
5-Aminolevulinic Acid (5-ALA)-Induced Protoporphyrin IX (PPIX) Fluorescence

Cellular/Molecular Mechanism of Action: 5-aminolevulinic acid is capable of promoting the fluorescence from protoporphyrin IX compounds.
Background Information: 5-aminolevulinic acid induces fluorescence of PPIX that provides a 95–100% sensitivity level for detecting oral cancer with a corresponding specificity level of only approximately 50–60%.
Application: Differentiation of premalignant from malignant forms of oral cancer applying PPIX fluorescence endoscopy and fluorescence image quantification.
Route of Administration: Topical.
Implementation: Characterization of oral premalignant and malignant lesions applying PPIX fluorescence endoscopy and fluorescence image quantification. To improve diagnostic specificity, 5-ALA mediated digitized fluorescence endoscopic imaging systems have been designed that enable on-line image acquisition, analysis, and fluorescence quantification for early detection of oral cavity neoplasms.

Human Model: Cases of suspected premalignant and malignant lesions of the oral cavity (e.g., n = 16).
Interpretation and Analysis: Head and neck clinical trials have shown that the red-to-blue intensity ratio of malignant tissue is larger than that of benign tissue. By applying the intensity ratio as a diagnostic algorithm, both high specificity and sensitivity can be achieved for discriminating malignant tissue from benign tissue, and the capability of classifying different histopathological stages of oral lesions has also been demonstrated. Red fluorescence intensity distribution in lesion areas could also be obtained to better understand PPIX tissue accumulations. Our initial results indicate that the digitized endoscopic imaging system combined with the fluorescence image quantification method and the ratio diagnostic algorithm has the potential to significantly improve the non-invasive *in vivo* diagnosis of early oral neoplasms.

Detection of neoplasms in the oral cavity by digitized endoscopic imaging of 5-aminolevulinic acid-induced protoporphyrin IX fluorescence. Zheng W, Soo KC, Sivanandan R, Olivo M. Int J Oncol 2002 Oct; 21 (4), pp. 763–8.

Oral Lesions/Differentiation: Oral Premalignant vs Malignant Squamous Cell Carcinoma
5-Aminolevulinic Acid (5-ALA)-Induced Protoporphyrin IX (PPIX) Fluorescence

Background Information: Studies of 5-ALA-induced PPIX fluorescence have shown a sensitivity of 95 to 100% for oral cancer diagnosis, but the specificity is only about 50 to 60%.
Application: Quantifying PPIX fluorescence imaging for specific diagnostic detection of early oral lesions (differentiation of premalignant from malignant disease states).
Implementation: Fluorescence endoscopy and imaging are performed on cases with suspected premalignant or malignant oral cavity lesions in conjunction with the collection of biopsy samples (e.g., n = 70) from tissue sites that will be evaluated by histological analysis. The red-to-blue and red-to-green intensity ratios are calculated from fluorescent images for correlation with histology analysis.

Human Model: Cases with known or suspected premalignant or malignant neoplasia of the oral cavity (e.g., n = 28).
Interpretation and Analysis: Suspicious lesions display bright reddish fluorescence, while normal mucosas exhibit blue color background in the fluorescence images. The red-to-blue and red-to-green intensity ratios of malignant tissues are larger than those of benign tissues. Combining the 2 ratio diagnostic algorithms yields a sensitivity and specificity of 95% and 97%, respectively, exceeding each diagnostic algorithm alone for discriminating malignant tissue from benign tissue. Quantifying PPIX fluorescence endoscopic images combined with ratio diagnostic algorithms has the potential to significantly improve the *in vivo* non-invasive diagnosis of oral cavity lesions.
Instrumentation: Fluorescence endoscopy.

Detection of squamous cell carcinomas and pre-cancerous lesions in the oral cavity by quantification of 5-aminolevulinic acid induced fluorescence endoscopic images. Zheng W, Soo KC, Sivanandan R, Olivo M. Lasers Surg Med 2002; 31 (3), pp. 151–7.

Salivary Gland Secretory Function: Xerostomia
Muscarinic Agonists/Pilocarpine Hydrochloride (CAS 54-71-7)/Cevimeline (CAS 153504-70-2)

Cellular/Molecular Mechanism of Action: Pilocarpine hydrochloride ((3S,4R)-3-ethyl-dihydro-4-[(1-methyl-1H-imidazole-5-yl)methyl]-2(3H)-furanone monohydrochloride, CAS 54-71-7) and cevimeline [(±)-*cis*-2-methylspiro[1,3-oxathiolane-5,3′-quinuclidine] hydrochloride, hemihydrate, CAS 153504-70-2) bind to and stimulate parasympathetic muscarinic receptors (agonist activity).
Physiological Mechanism of Action: Parasympathomimitic activity.
Application: Assess secretion from the submandibular/sublingual (SM/SL) salivary glands relevant to various disease states including xerostomia.
Route of Administration: Pilocarpine hydrochloride (i.d.) and cevimeline (i.d.).
Dosage: Pilocarpine hydrochloride (0.1–0.8 mg/kg) and cevimeline (3–30 mg/kg) in rats.
Implementation: Evaluate the influence of pilocarpine hydrochloride ((3S,4R)-3-ethyl-dihydro-4-[(1-methyl-1H-imidazole-5-yl)methyl]-2(3H)-furanone monohydrochloride, CAS 54-71-7) and cevimeline ((±)-cis-2-methylspiro[1,3-oxathiolane-5,3′-quinuclidine] hydrochloride, hemihydrate, CAS 153504-70-2), muscarinic receptor agonists, on salivary secretion. To clarify their pharmacological safety profiles, the 2 drugs can be further compared with regard to subtype selectivity for muscarinic receptors (M1, M2, and M3) and central nervous, respiratory, and cardiovascular effects.

Animal Model: Rat submandibular/sublingual salivary gland (normal and x-radiation induced xerostomia [15 Gy dose]).
Interpretation and Analysis: Pilocarpine hydrochloride and cevimeline induce dose-dependent increases in salivary flow rate and total salivary volume over a 120-minute period from SM/SL glands in both normal and irradiated rats, the minimum effective doses for their sialagogic effects being 0.2 and 10 mg/kg, respectively. Both drugs also increase protein output from SM/SL glands to a degree that depends on the increase in salivary volume in normal and irradiated rats. In binding studies using radiolabeled antagonists, neither pilocarpine hydrochloride nor cevimeline displays subtype selectivity for muscarinic receptors, indicating non-selective muscarinic agonist

activity. Effects on the central nervous system (CNS) are assessed by monitoring changes in body temperature in conscious normal rats. Pilocarpine hydrochloride (0.4–4 mg/kg p.o.) has no effect on body temperature, but cevimeline (30 and 100 mg/kg p.o.) causes significant hypothermia. In terms of respiratory and cardiovascular effects in anesthetized normal rats, there is no clear difference in safety margin between pilocarpine hydrochloride and cevimeline because both drugs induce significant changes in respiratory rate, heart rate, and blood pressure at doses close to those inducing sialagogic effects. Pilocarpine hydrochloride could be used as a sialagogic drug for postirradiation-induced xerostomia with fewer adverse effects on the CNS.

Effects of pilocarpine hydrochloride and cevimeline on submandibular/sublingual salivation in rat xerostomia model produced by x-ray irradiation. Omori Y, Asari T, Maruyama K, Kusama H, Kojima M, Shibata N. Arzneimittelforschung 2003; 53 (5), pp. 342–50.

ESOPHOGEAL FUNCTION AND DISORDERS

Achalasia and Idiopathic Diffuse Esophageal Spasm (IDES)
Pentagastrin

Route of Administration: Subcutaneous.
Dosage: Pentagastrin (6 μg/kg).
Considerations: Thoracic pain or dysphagia can occur after pentagastrin administration in cases suffering from IDES (e.g., n = 4/9 cases) or achalasia (e.g., n = 7/12). Pentagastrin is not a suitable provocative test for establishing a diagnosis of IDES conditions.
Implementation: Assessment of esophageal motility in conditions of achalasia in cases with idiopathic diffuse esophageal spasm (IDES). Pentagastrin is not an effective diagnostic pharmaceutical for achieving this objective.
Interpretation and Analysis: Pentagastrin in cases of achalasia and IDES produces increases in mean lower esophageal sphincter pressure, amplitude of contractions, esophageal pressure, and repetitive wave activity.

In cases with clinically suspected esophageal motor disease with non-diagnostic routine radiologic and manometric profiles it is invariably almost impossible to establish IDES as a diagnosis.

Pentagastrin in diffuse esophageal spasm. Wexler RM, Kaye MD. Gut 1981 Mar; 22 (3), pp. 213–6.

Achlasia: Idiopathic Diffuse Esophogeal Spasm (IDES)
Pentagastrin

Physiological Mechanism of Action: Pentagastrin produces an increase in mean lower esophageal sphincter pressure, amplitude of contractions, esophageal pressure, and repetitive wave activity in cases of achalasia and IDES.
Application: Differentiation between cardiac and esophageal-associated chest pain.
Route of Administration: Pentagastrin (subcutaneous injection).
Dosage: Pentagastrin (6 μg/kg).
Considerations: Chest pain or dysphagia can occur after pentagastrin administration (4/9 IDES and 7/12 achalasia). Preliminary findings do not support the use of pentagastrin as a provocative test for IDES.
Implementation: Evaluation of esophageal motility in cases with suspected esophageal motor disease (achalasia/idiopathic diffuse esophageal spasm) that have normal radiologic and manometric profiles.
Interpretation and Analysis: Symptoms or manometric changes cannot be appreciated to assist in establishing a diagnosis of IDES. Preliminary findings therefore suggest that pentagastrin is unsuitable for the specific purpose of differentiating esophageal verses cardiac associated causes for chest pain.

The effects of pentagastrin in achalasia and diffuse esophageal spasm. Orlando RC, Bozymski EM. Gastroenterology 1979 Sep; 77 (3), pp. 472–7.

Esophogeal Function: Diffuse Spasmodic Activity
Edrophonium

Application: Detection of diffuse esophogeal spasms. Because of the intermittent nature of the associated symptoms, edrophonium functions as an effective provocative agent to increase diagnostic yield/accuracy.
Route of Administration: Edrophonium (intravenous).
Dosage: Edrophonium (10 mg bolus).
Implementation: Evaluation of esophageal function in conditions of suspected esophageal spasm. Transnasal monometry can be performed using instrumentation with 6 internal transducers arranged at 5 cm intervals from the catheter tip (e.g., Gaeltec system).

Human Model: Criteria for diffuse esophageal spasm (DOS) candidates include the observation of dysphagia and chest pain. Esophageal manometry shows retention of peristalsis with the presence of synchronous contractions in response to wet swallows.
Interpretation and Analysis: A reproduction of symptoms with typical manometry of diffuse esophageal spasms indicates a positive provocation test response. There are no significant side effects that can be detected. Diffuse esophageal spasms can be diagnosed either by typical manometry or a positive edrophonium provocation test. Approximately 66% of positive patients will have diagnostic baseline manometry measurements, and close to 50% of these cases will display a positive provocation test. About 33% of the cases that have normal baseline manometry measurements have positive provocation tests following edrophonium injection. Therefore, without the application of the edrophonium provocation test, about a 3rd of patients would not have been diagnosed as having diffuse esophageal spasms.

Edrophonium provocation test in the diagnosis of diffuse esophageal spasm. Linsell J, Owen WJ, Mason RC, Anggiansah A. Br J Surg 1987 Aug; 74 (8), pp. 688–9.

Esophogeal Motor Function Disorders/ Esophogeal/Gastroesophogeal Reflux
Edrophonium

Cellular/Molecular Mechanism of Action: Edrophonium binds to and directly inactivates the biochemical activity of acetylcholine esterase. As a consequence of acetylcholine esterase inhibition, acetylecholine neurotransmitter concentrations accumulate within the synaptic cleft thereby increasing the (over) stimulation of cholinergic receptor complexes.
Physiological Mechanism of Action: Increased parasympathetic tone.
Application: Diagnosis of esophageal contraction abnormalities involving either painful spasms or acalasia. In this context, edrophonium has been shown to be the most effective and best tolerated provocative agent for the evaluation of esophageal function.
Implementation: Evaluation of the contractile activity of the esophageal body and of the upper and lower esophageal sphincter (LOS) that can reliably be portrayed by means of low compliance

recording systems, either pneumohydraulic or with strain gauge force transducers, and at least 2 pressure sensors. LOS resting pressure can be assessed by both station and rapid pull-through techniques, or by the sleeve method. Conditions of disordered LOS function, such as achalasia, can be diagnosed dependably only by manometric means. Manometry is of high diagnostic yield for motor disorders of the esophageal body as well, although generally accepted diagnostic criteria are still lacking. In patients with angina-like chest pain, provocation tests can prove that esophageal contraction abnormalities cause the symptoms.

Interpretation and Analysis: Transport of swallowed material through the esophagus can reliably be recorded by radionuclide transit studies. Such studies are valuable in identifying patients with absent or impaired peristalsis and in evaluating treatment effects (e.g., effects of mechanic dilatation in achalasia). Gastroesophageal reflux should be recorded not only qualitatively but also quantitatively, although a definition of what is pathologically normal has not been universally standardized. Recording of esophageal intraluminal pH over longer periods of time, preferably 24 hours may have the best diagnostic yield. The advent of computer-aided analysis techniques will replace the cumbersome handscoring of motor and pH tracings and, hopefully, contribute to a better understanding and classification of esophageal pathophysiology.

Methods for measuring the motor activity of the esophagus and gastroesophageal reflux. Stacher G. Z Gastroenterol 1986 Sep; 24 Suppl 2, pp. 26–34.

Esophogeal Motor Function/Gastroesophogeal Reflux Disease (GERD)
Cisapride/Mosapride

Cellular/Molecular Mechanism of Action: Cisapride is a gastric prokinetic agent that promotes acetylcholine release through an action on presynaptic serotonin (5-HT4) receptors.

Application: To evaluate the efficiency of the 5-HT4 agonist and 5-HT3 antagonist mosapride, as compared with cisapride, on esophageal acid reflux variables and esophageal motor function in patients with chronic GERD.

Dosage: Mosapride (60 mg BID); mosapride (30 mg TID); cisapride (20 mg BID) for 7 days (alternating in a randomized order and separated by a washout period of at least 5 days).

Implementation: Evaluation of GERD conditions applying mosapride, mosapride, and cisapride. Recordings of 24-hour gastric pH and motility obtained in an ambulatory manner within 2 weeks before the start of each pharmaceutical exposure period and on day 7 of each exposure period.

Animal Model: Cases of proven GERD (n = 41). Design: double blind, randomized, double-dummy, 3-way crossover study; n = 24/41 ambulatory cases with 24-hour measurement of pH and motility; n = 18/41 cases with acquisition of ambulatory 24-hour pH recordings only on treatment day 7 of each treatment period.

Interpretation and Analysis: Mosapride has no significant effect on the total number of contractions in the esophagus, or on the effectiveness, or possible effectiveness, of the propagations. Significant but numerically small effects on peristaltic durations and amplitudes can be noted during both mosapride and cisapride treatment as compared with baseline values. The effect on acid reflux for both mosapride and cisapride is most pronounced for the duration of the longest reflux episode. The fraction of time with pH < 4 is reduced by mosapride 30 mg TID in the supine position and by cisapride both totally and in the supine position. The number of reflux episodes is reduced significantly only by cisapride. Esophageal clearance is reduced significantly by cisapride only in the supine position. Mosapride has small but statistically significant effects, comparable to those of cisapride, on acid reflux variables and esophageal motor function in patients with GERD.

The effect of mosapride on esophageal motor function and acid reflux in patients with gastroesophageal reflux disease. Ruth M, Finizia C, Cange L, Lundell L. Eur J Gastroenterol Hepatol 2003 Oct; 15 (10), pp. 1115–21.

Thoracic Pain: Atypical Non-Specific/ (Esophagitis Postive Cases)
Bernstein Test (Intraesophageal Acid Perfusion)

Application: Characterization of atypical non-specific thoracic pain.

Considerations: Treatment with either ranitidine or antacids had equivalent effectiveness.

Implemenation: Detection and characterization of thoracic pain of non-cardiac origin.

Human Model: Cases with signs of thoracic pain without signs of heartburn or other characteristic symptoms of esophagitis (n = 60).

Interpretation and Analysis: A positive Bernstein test result in children with non-specific chest pain indicates that thoracic pain is likely associated with esophageal disease. In cases of atypical chest pain without heartburn or other characteristic symptoms of esophagitis, a relatively high percentage (n = 45) have esophagitis. In this subcategory (18/45 or 40%), pain replicating the usual symptoms develops during esophageal acid perfusion. Conversely, cases without esophagitis (n = 15), chest pain does not develop during esophageal acid perfusion. A small number of cases with esophagitis (n = 3), esophageal manometric abnormalities and chest pain developed as a result of esophageal acid perfusion.

Esophageal sensitivity and symptom perception in gastroesophageal reflux disease. Shi G, Tatum RP, Joehl RJ, Kahrilas PJ. Curr Gastroenterol Rep 1999 Jun; 1 (3), pp. 214–9.

The symptom index: A clinically important parameter of ambulatory 24-hour esophageal pH monitoring. Wiener GJ, Richter JE, Copper JB, Wu WC, Castell DO Am J Gastroenterol 1988 Apr; 83 (4), pp. 358–61.

Utility of upper endoscopy in the evaluation of non-cardiac chest pain. Hsia PC, Maher KA, Lewis JH, Cattau EL Jr, Fleischer DE, Benjamin SB. Gastrointest Endosc 1991 Jan–Feb; 37 (1), pp. 22–6.

Thoracic Pain: Non-Cardiac Associated (Esophagitis)
Hypertonic Glucose/Esophogeal HCl/Saline/ Endrophonium

Application: Detection and characterization of non-cardiac related causes of thoracic pain and discomfort.

Route of Administration: Edrophonium (IV); HCl (esophageal perfusion); hypertonic glucose.

Dosage: Hypertonic glucose, compared to esophageal perfusion with HCl (0.1 N) and normal saline; edrophonium (10 mg).

Implementation: Continuous esophageal manometric recordings are performed at the time of testing. The patients' symptoms are monitored every minute. The effect of these solutions and edrophonium on lower esophageal sphincter (LES) pressure and amplitude of esophageal contractions can also be evaluated.

Interpretation and Analysis: Preliminary findings indicate that hypertonic glucose is not effective as a provocative test for non-cardiac chest pain, nor does it contribute to the chest pain in esophagitis. It also has no significant effect on the amplitude of esophageal contractions or LES pressure.

Esophageal perfusion with hypertonic glucose, saline, or acid has no significant effect on LES pressure or amplitude of esophageal contractions in most patients. Edrophonium, however, creates a significant rise in the amplitude of esophageal contractions and the LES pressure in all case groups studied. Hypertonic glucose results in chest pain in 13.6% of cases with non-cardiac chest pain and 20% of those with esophagitis, whereas edrophonium reproduces pain in 38.7 and 37%, respectively. Edrophonium continues to be a relatively sensitive test for non-cardiac chest pain.

Comparison of hypertonic glucose to other provocative tests in patients with non-cardiac chest pain. Nasrallah SM, Hendrix EA. Am J Gastroenterol 1987 May; 82 (5), pp. 406–9.

Thoracic Pain: Non–Cardiac Associated
Endrophonium

Application: Differentiation of cardiac and non-cardiac associated causes for thoracic pain.
Route of Administration: Edrophonium (intravenous bolus).
Dosage: Edrophonium (80 μg/kg of body weight when asymptomatic).
Implemenation: Esophageal motility disorders may be an important cause of non-cardiac chest pain. To improve our diagnostic yield, edrophonium can be applied as a provocative agent for inducing esophageal chest pain in conditions without coronary artery disease.

Human Model: Cases with a history of thoracic pain (n = 50 without coronary artery disease; n = 25 age-matched controls).
Interpretation and Analysis: Edrophonium induces chest pain in about 30% of cases but not in control subjects. Edrophonium increases esophageal amplitude and repetitive contractions to a similar degree in all subjects, but the change in duration (101 ± 13% [SE]) is significantly greater ($p < 0.02$) in patients with induced chest pain. Assessment of drug specificity during cardiac catheterization (n = 9) reveals no significant change detected in coronary artery diameter, blood pressure, or heart rate. Further clinical testing using a placebo control confirms a positivity rate of 28% in cases with chest pain (n = 125 unselected cases) but false-positive tests are infrequent (5.6%). No important side effects can usually be appreciated. Edrophonium is useful for provoking esophageal chest pain

The clinical utility of provocative radionuclide esophageal transit in the evaluation of non-cardiac chest pain. Elloway RS, Jacobs MP, Nathan MF, Mantil JC. Eur J Nucl Med 1992; 19 (2), pp. 113–8.

Comparison of intravenous edrophonium and esophageal acid perfusion during esophageal manometry in patients with non-cardiac chest pain. De Caestecker JS, Pryde A, Heading RC. Gut 1988 Aug; 29 (8), pp. 1029–34.

GASTRIC FUNCTION AND DISORDERS

Carboxylic Acid Metabolism: Gastrectomy/ Nitrogen Metabolism/Hepatic Failure, Renal Failure, Myotonic Dystrophy
1-[^{14}C]-Alpha-Ketoisovaleric Acid (KIV)

Cellular/Molecular Mechanism of Action: 1-[^{14}C]-alpha-ketoisovaleric acid is a keto analogue of valine that can function as a biochemical substrate for assessing mechanisms of carboxylic acid metabolism.
Background Information: The keto analogues of essential amino acids represent a promising therapeutic modality in hereditary and acquired disorders of nitrogen metabolism. The utilization of these substances in humans has been assayed primarily by nitrogen balance studies.
Application: Endogenous biochemical metabolism of KIV can be measured using the [$^{14}CO_2$] breath excretion test. In this context, the agent can be applied as a tool for evaluation of certain diet formulations.
Route of Administration: Oral.
Implementation: Assessement of the *in vivo* decarboxylation of KIV measurement of [$^{14}CO_2$] using a simple and accurate breath excretion test. Protein diets (5 g) supplemented with essential amino acids and KIV (in place of valine) are then evaluated. Diets of 120 g protein/day are then reevaluated in normal volunteers and gastrectomy cases.

Human Model: Normal volunteers and cases with various disease states (n = 6 diseased; gastrectomy, hepatic failure, renal failure, and myotonic dystrophy).
Interpretation and Analysis: In situations of low protein intake, 13–32% of ingested KIV undergoes rapid decarboxylation, and this proportion appeares to correlate inversely with damage to organ systems containing the branched-chain keto acid dehydrogenase. With high protein intake, the proportion of decarboxylated analog increases from 44% to 53%. Such findings confirm that the decarboxylation of KIV in man varies under different conditions of dietary intake and metabolic disease. The [$^{14}CO_2$] breath excretion test is applicable to other related analyses of carboxylic acid metabolism in human subjects.

Decarboxylation of alpha-ketoisovaleric acid after oral administration in man. Epstein CM, Chawla RK, Wadsworth A, Rudman D. Am J Clin Nutr 1980 Sep; 33 (9), pp. 1968–74.

Gastric Acid Secretory Capacity/Antisecretory Agent Evaluation: Transforming Growth Factor-Alpha (TGF-α), Epidermal Growth Gactor (EGF), and Urogastrone (UG)
S-[3-(N,N-Dimethylamino)Propyl]Isothiourea

Cellular/Molecular Mechanism of Action: S-[3-(N,N-Dimethylamino)propyl]isothiourea (dimaprit) functions as a highly specific histamine H2-receptor agonist. The H2-receptor specificity of dimaprit in causing depressor and vasodilator effects was also demonstrated in the cat, in which it had 18–20% of the H2-receptor activity of histamine.
Application: Stimulation of gastric acid secretion (rat, dog, cat).
Considerations: Induces 19%, 58%, and 400–500% the level of activity provided by histamine, respectively, in rats, dogs, and cats. Parallel line assays showed that *in vitro* at H2-receptors dimaprit had approximately 17.5% the activity of histamine on the rat uterus and 71% on the guinea pig right atrium, with similar maximal responses; it had less than 0.0001% the activity of histamine on H1-receptors. The absence of marked cardiovascular effects at doses maximal for the stimulation of gastric acid secretion, as seen in the cat studies, could lead to this compound being of value as a diagnostic agent in the measurement of maximal acid secretory capacity.
Implementation: Evaluation of EGF, UG, and TGF-α and its derivative for their ability to suppress dimaprit- and pentagastrin-induced gastric acid secretion and on acidified ethanol (AE)-evoked ulcer formation in anesthetized rats. Epidermal growth factor, TGF-α, and UG administered subcutaneously (s.c.) 30 minutes before dimaprit inhibited gastric acid secretion.
Interpretation and Analysis: Dimaprit induces 19%, 58%, and 400–500% the level of biological activity exerted by histamine in rats, dogs, and cats, respectively. In the dog and cat the maximum

secretory response to dimaprit is significantly greater than that obtained to histamine.

Against pentagastrin-stimulated secretion, TGF-α inhibits, while EGF and UG potentiate acid secretion dose-dependently. Intraduodenal administration of TGF-α and UG have no effect, while EGF potentiates secretagogue-induced acid secretion in the same dosage schedule. Administration of either EGF, UG, or TGF-α (bolus IV) in response to continuous infusion of dimaprit results in a significant ($p < 0.05$–$p < 0.001$) inhibition of acid secretion that is transient and returns to normal within 30 to 45 minutes for UG while it slowly returns to normal for EGF and TGF-α. The truncated form of TGF-α (amino acids 34–43) does not show any antisecretory effect when administered parenterally. Acidified ethanol produces gastric hemorrhagic lesions in rats 1 hour after oral administration. The gastric mucosal protective effects of TGF-α, EGF, and UG administered either orally or subcutaneously 30 minutes before the administration of AE are dose-dependent against this model of ulcer induction. Indomethacin (Indo), administered 15 minutes before AE to inhibit prostanoids biosynthesis, significantly ($p < 0.001$) reduces the cytoprotective effects of TGF-α, EGF, and UG and aggravates the ulcer index when administered subcutaneously. Results reveal that PGs may be involved in mediating the protective effects of the 3 growth factors. Administration of NG-nitro-L argininemethylester (L-NAME) 15 minutes prior to TGF-α, EGF, and UG s.c. or orally significantly ($p < 0.001$) decreases the degree of ulcer indices and is able to reduce the protective effects of TGF-α, EGF, and UG, thus including the role of NO in mediating the protective effects of these growth factors. In conclusion, these results have demonstrated that EGF, UG, and TGF-α have a short and reversible inhibitory effect on dimaprit-stimulated gastric acid secretion and each is effective parenterally but not orally. Urogastrone and EGF potentiate, while TGF-α inhibits pentagastrin-stimulated acid secretion. In addition, TGF-α seems to lose its activity when it is truncated from the C terminus. The present study also suggests that EGF, UG, and TGF-α are equally effective against AE-induced gastric ulcer and bring about their cytoprotective action through their reduction of acid secretion and through PG and NO pathways.

Comparison of the antisecretory and antiulcer activity of epidermal growth factor, urogastrone and transforming growth factor alpha and its derivative in rodents *in vivo*. Bastaki SM, Chandranath SI, Singh J. Mol Cell Biochem 2002 Jul; 236 (1–2), pp. 83–94.

Gastric Antrum (Retained) vs Zollinger-Ellison/ [Postop Partial Gastrectomy with Billroth II Reconstruction]
Secretin/Barium Sulfate/[^{99m}Tc]

Cellular/Molecular Mechanism of Action: Secretin promotes pancreatic exocrine secretion.
Background Information: If recurrent peptic ulceration follows partial gastrectomy (Billroth II reconstruction), a retained antrum on the duodenal stump may be the culprit. Moderate hypergastrinemia and a high basal acid output (BAO) to maximal acid output (MAO) ratio on gastric analysis should alert the clinician.
Application: Differentiation between retained gastric antrum and Zollinger-Ellison syndrome in postoperative cases that have received a partial gastrectomy using the Billroth II reconstrucution procedure.
Implementation: Careful filling of the afferent loop for barium image analysis or a [^{99m}technetium] meal-scan may serve to verify the diagnosis. The secretin provocative test may be helpful in distinguishing retained antrum from the Zollinger-Ellison syndrome by eliciting a decrease in serum gastrin levels in patients with retained antrum and an increase in serum gastrin levels in patients with Zollinger-Ellison syndrome.
Interpretation and Analysis: Cases suffering from Zollinger-Ellison syndrome will demonstrate elevations in serum gastrin following secretin administration.

Serum gastrin levels in the differential diagnosis of recurrent peptic ulceration due to retained gastric antrum. Webster MW, Barnes EL, Stremple JF. Am J Surg 1978 Feb; 135 (2), pp. 248–52.

Gastric Emptying
Acetaminophen (Paracetamol)

Cellular/Molecular Mechanism of Action: Acetaminophen (paracetamol) is classified as a non-steroid anti-inflammatory agent (cyclooxygenase inhibitor).
Background Information: The delivery rate of enteral nutrition in critically ill patients given via the nasogastric route is often decreased or stopped because of large gastric residual volumes.
Application: Estimation of approximate gastric-emptying rate.
Route of Administration: Acetaminophen (paracetamol) can be given by oral ingestion or nasogastric tube.
Examples of Clinical Implementation: Evaluation of gastric emptying in cases receiving enteral nutrition via a nasogastric tube that have residual volume (assessed every 8 hours) of > 150 ml, or more than twice the hourly infusion rate using the acetaminophen (paracetamol) absorption test.
Interpretation and Analysis: The acetaminophen (paracetamol) absorption test can provide valuable information in cases receiving enteral nutrition. Prokinetic agents facilitate the continuation of enteral nutrition in most (88%) of cases with evidence of abnormal gastic emptying. The paracetamol absorption test may be normal in patients with relatively high gastric residual volumes. These patients may continue to receive enteral nutrition.

The paracetamol absorption test: A useful addition to the enteral nutrition algorithm? Cohen J, Aharon A, Singer P. Clin Nutr 2000 Aug; 19 (4), pp. 233–6.

Gastric Emptying/Amylin
Acetaminophen (Paracetamol)

Diagnostic Cellular/Molecular Mechanism of Action: Non-steroidal anti-inflammatory agents inhibit the biochemical activity of cyclooxygenase similar to other pharmaceutical agents in this class.
Background Information: Amylin is a novel 37 amino acid peptide secreted together with insulin from the pancreas in response to enteral nutrient intake. As a potent inhibitor of gastric motility it plays an important role in the control of carbohydrate absorption.
Application: Evaluation of gastric-emptying function.
Dosage: Paracetamol 6-hour absorption.
Implementation: Evaluation of gastric emptying.
Interpretation and Analysis: Acetominophen (paracetamol) is highly absorbed from the gastrointestinal tract and reaches peak concentration in the blood at about 30 to 60 minutes postoral administration and is subsequently distributed extensively throughout the intravascular compartment and extravascular tissues. The paracetamol absorption test can be used as a parameter for the detection of delayed gastric emptying ($p \leq 0.01$). Median serum amylin concentrations are significantly higher in human (neonate/children) cases of delayed gastric emptying and feed-intolerance. A positive correlation is observed between serum amylin and insulin ($r = 0.46$, $p = 0.02$) but not between amylin and glucose ($r = 0.25$, $p = 0.23$). High

serum amylin levels are associated with delayed gastric emptying in some conditions of chronic disease. Correlation between serum amylin and insulin levels indicates a degree of preservation of pancreatic hormonal co-release.

Amylin is associated with delayed gastric emptying in critically ill children. Mayer AP, Durward A, Turner C, Skellett S, Dalton N, Tibby SM, Murdoch IA. Intensive Care Med 2002 Mar; 28 (3), pp. 336–40.

Gastric Emptying/Orocecal Transit Time *[^{13}C]-Acetate Breath Test*

Background Information: Caffeine is suspected to affect gastrointestinal function.
Implementation: Evaluation of the influence of caffeine supplementation (150 mg/l) in carbohydrate-electrolyte formulations (sports drinks) on gastrointestinal variables compared with a normal CES and water can be established using a standardized rest-exercise-rest protocol. Esophageal motility, gastroesophageal reflux, and intragastric pH can be measured by use of a transnasal catheter. Orocecal transit time can be measured using breath-H_2 measurements. Evaluation of intestinal permeability and glucose absorption can be established using sugar absorption tests, in contrast to the determination of gastric emptying, which can be measured by the [^{13}C]-acetate breath test.
Interpretation and Analysis: Caffeine significantly lowers mid-esophogeal pressures in postexercise periods compared to caffeine-free reference controls ($p = 0.017$). Gastric pH and reflux during pre-exercise periods, cycling exercise, and postexercise time frames, gastric emptying, orocecal transit time, and intestinal permeability are unaffected by caffeine intake.

Gastrointestinal function during exercise: Comparison of water, sports drink, and sports drink with caffeine. Van Nieuwenhoven MA, Brummer RM, Brouns F. J Appl Physiol 2000 Sep; 89 (3), pp. 1079–85.

Gastric Emptying/Motility *[^{13}C]-Acetate/[Breath Test]*

Cellular/Molecular Mechanism of Action: [^{13}C]-acetate is a radioactive analog of acetic acid that is absorbed from the gastrointestinal tract, enters the portal circulation, is removed from the portal circulation by the liver, and is then metabolized within hepatocytes in a manner that results in the generation of [$^{13}CO_2$] that is subsequently exhaled by the respiratory system.
Background Information: Elderly people frequently have symptoms of fullness and appetite loss due to impaired gastric motor activity. These symptoms may cause malnutrition, immunosuppression, and other complications.
Application: Evaluation of gastric motility by [^{13}C]-acetate breath test in combination with electrogastrography techniques.
Route of Administration: [^{13}C]-acetate (oral ingestion).
Implementation: Electrogastrography evaluation of gastric emptying before and after oral ingestion of [^{13}C]-acetate-mixed liquid meal. Expired air is then sampled every 10 minutes for measurement of [$^{13}CO_2$] concentrations.

Human Model: Cases of advanced age ($n = 7$ active/healthy; $n = 7$ reduced mental/physical capacities with inactive behaviors; $n = 7$ young healthy volunteers).
Interpretation and Analysis: The ratio of the incidence of the 3-cpm wave (gastric intrinsic frequency) during the postprandial period compared to the fasting state is reduced in active and inactive geriatric cases compared to young adults. Such reductions are greater in inactive elderly cases than in active elderly cases. The ratio of the amplitude of the peak frequency during the postprandial period to that in the fasting state (power ratio) also decreases with advanced age. The time of peak [$^{13}CO_2$] expiration is delayed in the active elderly cases, but more so in the elderly that are inactive. Postprandial peristalsis and gastric contractile force are reduced in the elderly, and gastric emptying is delayed, indicating a reduction in gastric motor activity.

Evaluation of gastric motor activity in the elderly by electrogastrography and the (13)C-acetate breath test. Shimamoto C, Hirata I, Hiraike Y, Takeuchi N, Nomura T, Katsu K. Gerontology 2002 Nov–Dec; 48 (6), pp. 381–6.

Gastric Emptying/Motility: Prokinetic Activity *Mosapride*

Physiological Mechanism of Action: Mosapride exerts digestive tract prokinetic properties.
Background Information: Delay of gastric emptying is 1 of the factors responsible for unfavorable glycemic control.
Dosage: Mosapride (15 mg per day for 6 months).
Implementation: Evaluation of the effects of the digestive tract prokinetic properties of mosapride on glycemic control in diabetic patients complicated with gastropathy. Gastric emptying can be evaluated according to a marker method (administration of a capsule containing 20 pieces of radiopaque marker during breakfast, followed by abdominal x-ray imaging 3 and 5 hours later; $n = 17$ cases).

Human Model: Cases of diabetes mellitus complicated by gastropathy (e.g., $n = 36$ type II diabetic patients presenting with mild digestive tract symptoms).
Interpretation and Analysis: In approximately 50% of cases, HbA(1C) is improved by more than 0.3% for 6 months (e.g., $n = 18$ with improvement). The other approximately 50% of cases are classified non-improvement cases (e.g., $n = 18$). In gastric-emptying studies, the basal number of residual markers before administration of mosapride is determined 3 and 5 hours later and reveals 18.3 ± 1.8 and 7.6 ± 5.1, respectively, in "improved" cases, while after administration, values decline to 11.2 ± 5.1 and 1.4 ± 2.5, respectively. In sharp contrast, the basal counterparts in the non-improvement group are approximately 19.1 ± 1.5 and 16.4 ± 3.4, respectively, whereas administration fails to reduce the number of the residual markers and they remain high (up to 19.0 ± 1.4 and 11.1 ± 6.4, respectively). Gastric motility in "improved" cases is much more improved by mosapride administration relative to "non-improved" cases. Mosapride might elicit improvement in glycemic control in the patients with diabetic gastropathy.

Effect of mosapride on glycemic control and gastric emptying in type 2 diabetes mellitus patients with gastropathy. Asakawa H, Hayashi I, Fukui T, Tokunaga K. Diabetes Res Clin Pract 2003 Sep; 61 (3), pp. 175–82.

Gastric Emptying/Motility: Functional Dyspnea *[^{13}C]-Octanoic Acid/[Breath Test]*

Background Information: As a non-invasive modality by which to evaluate the gastric emptying of a solid meal, the [^{13}C]-octanoic acid breath test has recently become more widely used. Previously, ultrasonography has also been reported as another non-invasive and reliable method for assessing gastric motility.
Application: Evaluation of gastric motility.
Route of Administration: [^{13}C]-octanoic acid (oral).
Dosage: [^{13}C]-octanoic acid (100 mg).
Implementation: Evaluation of gastric emptying applying the solid meal test consisting of a scrambled egg labeled with [^{13}C]-octanoic acid (100 mg) served with a bowl of rice and boiled chicken

(total 424 kcal). After ingestion of the test meal, examination is performed in a sitting position. Ultrasonography images are obtained every 15 minutes for 3 hours. Breath sampling follows the same time schedule as for ultrasonographic imaging, with an additional 3 hours of sampling at 30-minute intervals. Results are applied to establish half-emptying times ($T_{1/2}$) and lag phase.

Human Model: Cases of functional dyspnea (n = 17 compared to n = 10 normal healthy volunteers).

Interpretation and Analysis: The $T_{1/2}$ values determined by ultrasonography-based methods and the breath test are positively correlated ($r^2 = 0.638$); however, there is no significant agreement between study groups. Both the $T_{1/2}$ and the lag phase are prolonged in cases of functional dyspepsia compared with healthy volunteers, regardless of the method of measurement. The lag phase significantly correlates ($r^2 = 0.864$) with the $T_{1/2}$ established with breath test methods. Although the [^{13}C]-octanoic acid breath test cannot assess gastric emptying of solids as reliably as ultrasonography, both tests are useful for evaluating functional dyspepsia patients with delayed gastric emptying.

Evaluation of gastric emptying measured with the ^{13}C-octanoic acid breath test in patients with functional dyspepsia: Comparison with ultrasonography. Aoki S, Haruma K, Kusunoki H, Hata J, Hara M, Yoshida S, Tanaka S, Chayama K. Scand J Gastroenterol 2002 Jun; 37 (6), pp. 662–6.

Gastric Motility
[^{13}C]-Octane Acid/[Breath Test]/[^{99m}Tc]-Sulphur Colloid

Cellular/Molecular Mechanism of Action: [^{13}C]-octane acid is a radioactive analog of octane acid that is absorbed from the gastrointestinal tract, enters the portal circulation, is removed from the portal circulation by the liver, and is then metabolized within hepatocytes, resulting in the generation of [$^{13}CO_2$] that is then exhaled by the respiratory system.

Background Information: Disorders of gastric motility may be a significant factor in the pathogenesis of many equine conditions. Although tests for liquid phase emptying rate have been validated in the horse, there are no effective tests for solid phase emptying measurement that can be performed routinely in the field.

Application: Assessment of a stable isotope technique, the [^{13}C]-octane acid breath test (^{13}C-OABT), for the measurement of gastric emptying of solid ingesta, compared to the optimum method of gastric scintigraphy.

Implementation: A test meal is used containing baked egg yolk labeled with both [^{13}C]-octanoic acid and [^{99m}Tc]-sulphur colloid. Simultaneous, serial lateral gastric scintigraphic images and expiratory breath samples are obtained after voluntary test meal ingestion. Analysis of breath [$^{13}CO_2$:$^{12}CO_2$] ratio is then performed by mass spectrometry. Power regression is then used to determine the gastric emptying coefficient, the gastric half-emptying time ($T_{1/2}$), and duration of the lag phase (T_{lag}).

Animal Model: Equine (n = 12 healthy horses).

Interpretation and Analysis: Significant correlations ($p < 0.001$) can be appreciated between the 2 techniques for both $T_{1/2}$ and T_{lag} measurements. In addition, the scintigraphic $T_{1/2}$ is significantly correlates with breath test gastric-emptying coefficients ($p < 0.001$).

In has been proposed that the [^{13}C]-octanoic acid breath test is a reliable diagnostic procedure for measuring gastric-emptying rate of solids in the horse. Being safe, non-invasive, and easy to perform, this test has potential value as both sensitive diagnostic modality and humane research tool for motility studies.

Instrumentation: Continuous flow isotope ratio mass spectrometry.

Validation of the ^{13}C-octanoic acid breath test for measurement of equine gastric emptying rate of solids using radioscintigraphy. Sutton DG, Bahr A, Preston T, Christley RM, Love S, Roussel AJ. Equine Vet J 2003 Jan; 35 (1), pp. 27–33.

Gastric Motility Activity: Detection of Stimulation or Inhibition/Identifying Drug-Evoked Changes in Gastric Emptying
Propantheline/Corticotropin-Releasing Factor (CRF)/Metoclopramide/Bethanechol

Physiological Mechanism of Action: Propantheline and CRF inhibit gastric emptying, in contrast to metoclopramide and bethanechol, which accelerate gastric emptying.

Background Information: The increased availability of transgenic mice prompts a need for the adaptation to mice of whole-animal assays traditionally performed in larger laboratory animals. Gastric-emptying studies are frequently conducted in dogs and rats. Mouse-based gastric-emptying models currently available often use inert, non-nutrient liquid meals containing non-absorbable markers or radionuclides. A gastric-emptying assay has been developed in the mouse that features a favorable throughput and the use of a semi-solid, high-calorie meal.

Application: Detection of either stimulation or inhibition of gastric motor activity. This assay should prove useful for identifying drug-evoked changes in gastric emptying as well as assessment of gastric motility effects of altered gene expression in genetically modified mice.

Route of Administration: Propantheline (sq); CRF (ip); metoclopramide (ip); bethanechol (ip).

Dosage: Propantheline (0.3–10 mg/kg); CRF (3–100 nmol/kg); metoclopramide (1–10 mg/kg); bethanechol (91–30 mg/kg).

Implementation: Evaluation of agents known to either inhibit (propantheline, CRF) or accelerate (metoclopramide, bethanechol) gastric emptying. A single time-point variation of the assay can be used for quickly screening compounds for effects on gastric emptying.

A carbohydrate- and protein-rich semisolid test meal is prepared from common laboratory reagents. Gastric emptying is determined by subtracting the mass of test meal remaining in the stomach from the mass of test meal administered. A time-course study of basal emptying of a semisolid, paste-like test meal high in carbohydrate and protein from the stomachs of overnight-fasted is conducted.

Animal Model: Murine.

Interpretation and Analysis: In time-course studies, the test meal empties from the stomach with a half-emptying time of 30.6 minutes (95% CI: 27.3–34.7). The gastric-emptying data can be successfully modeled by a 2-parameter exponential decay function. No lag phase can be observed, indicating that the meal empties from the stomach as a liquid. The anticholinergic agent propantheline increases gastric half-emptying time ($T_{1/2}$) approximately 3-fold, while metoclopramide decreases gastric half-emptying time approximately 2-fold compared to basal emptying. Single time-point screening studies correctly detect the gastrokinetic activity of bethanechol and the inhibitory effect of CRF. The mouse gastric-emptying assay is simple, inexpensive, and not labor-intensive. It is capable of detecting either stimulation or inhibition of gastric motor activity. This assay should prove useful for identifying drug-evoked changes in gastric emptying as well as for assessing the gastric motility effects of altered gene expression in genetically modified mice.

An improved method of evaluation of drug-evoked changes in gastric emptying in mice. Osinski MA, Seifert TR, Cox BF, Gintant GA. J Pharmacol Toxicol Methods 2002 Mar–Apr; 47 (2), pp. 115–20.

Gastric Motility: Detection/[Recurrent Gastric Ulcers]
Acetaminophen

Cellular/Molecular Mechanism of Action: Acetaminophen is a non-steroidal analgesic agent that has minimal anti-inflammatory properties. However, in the context of being applied for characterizing gastric motility, it simply serves as a marker molecule where its rate of absorption into the systemic circulation directly corresponds to rates of gastric emptying.
Background Information: The existence of abnormal gastric motility in gastric ulcer disease remains controversial.
Application: Delineation of gastric motility as a function of plasma acetaminophen concentrations measured 45 minutes after oral ingestion.
Route of Administration: Acetaminophen (oral).
Dosage: Acetaminophen (20 mg/kg).
Implementation: Evaluation of gastric motility in conditions of recurrent gastric ulcers. Gastric motility is evaluated by cutaneous electrogastrography (EGG) and by gastric semi-liquid meal emptying. The EGG is recorded before and after ingestion of a test meal containing 20 mg/kg of acetaminophen. Patients with a dominant EGG frequency of greater than 0.06 Hz are defined as tachygastria, while those with a frequency of less than 0.04 Hz can be defined as bradygastria.

Human Model: Cases of recurrent gastric ulcer based on gastrointestinal endoscopy (n = 24; compared to n = 10 controls).
Interpretation and Analysis: A transient frequency decrease, called postprandial dip (PD), can be identified visually. The degree of gastric emptying is determined from the serum acetaminophen concentration 45 minutes after the meal. Control subjects show no irregularity in their dominant EGG frequency in either fasting or postprandial states. A PD can be observed in a relatively large percentage of reference controls (e.g., n = 8/10). In patients presenting with active gastric ulcers, abnormal patterns in the dominant EGG frequency (either as tachygastria or bradygastria) can be appreciated in slightly greater that 50% of affected cases (e.g., n = 14/24) during fasting or in postprandial states (e.g., n = 15/24). After successful treatment, the number of cases with abnormal patterns in their dominant EGG frequency remains unchanged, while PD can be observed in slightly < 50% of cases (e.g., n = 11/24). No significant difference can be observed in the EGG power ratio as a result of successful treatment. Gastric emptying is significantly delayed compared with controls in both the active and healed stages. These findings suggest that abnormal gastric motility, including gastric electrical abnormalities and delayed gastric emptying, plays an important role in the pathophysiology of recurrent gastric ulcers.

Gastric motility in patients with recurrent gastric ulcers. Kamiya T, Kobayashi Y, Hirako M, Misu N, Nagao T, Hara M, Matsuhisa E, Ando T, Adachi H, Sakuma N, Kimura G. J Smooth Muscle Res 2002 Apr; 38 (1–2), pp. 1–9.

Gastric Motor and Sensory Function
Acetaminophen (Paracetamol)/[^{13}C]-octanoic Acid/Spirulina Breath Tests/Tensostat/Water/Nutrient Drink Test

Background Information: Sensitive and reproducible tests are essential to investigate the mechanisms of gastric motility and sensation in healthy humans and patients with unexplained upper gastrointestinal symptoms. Electrogastrography, manometry, scintigraphic emptying, and barostat studies with an intragastric balloon have been used to better understand the physiology and pathophysiology of gastric motility. However, manometry and barostat studies are time-consuming, costly, and invasive, thus reducing their widespread clinical application.
Implementation: Evaluation of gastric-emptying characteristics can be measured using the paracetamol absorption test, [^{13}C] breath tests, ultrasound, or MRI methodologies. The paracetamol absorption test can measure the gastric emptying of liquids while [^{13}C] breath test can measure gastric emptying of solids or liquids and can achieve accuracy comparable with gastric scintigraphy.
Interpretation and Analysis: The water/nutrient (satiety) test is a non-invasive test proposed as an alternative to sensory studies performed with an intragastric balloon. The satiety test cannot measure gastric accommodation; interpretation of sensory tests usually has required independent assessment of accommodation or compliance. The tensostat can be used as a gastric sensation test because it measures gastric wall tension, which is related to the perception of gastric distention. Ultrasonography requires special skills, and MRI requires costly equipment. To measure gastric accommodation to a meal, ultrasound, MRI, and SPECT have been proposed as effective modalities. The recently introduced SPECT requires an intravenous injection of [^{99m}Tc]-pertechnetate, which the gastric mucosa specifically takes up, and specialized imaging and analysis, which have potential to be automated. Thus, novel, non-invasive approaches assess different dimensions of gastric motility and sensation testing. With further development of these techniques, refinement of their conduct and analysis, and validation of clinical usefulness, they are likely to be applicable in clinical practice to enhance cost-effective, evidence-based management of upper gastrointestinal symptoms. Such applications may provide an alternative to sequential empirical trials for symptoms.

Novel testing of human gastric motor and sensory functions: Rationale, methods, and potential applications in clinical practice. Kim DY, Myung SJ, Camilleri M. Am J Gastroenterol 2000 Dec; 95 (12), pp. 3365–73.

Gastric Ulceration: Stress Associated/Cerebral Trauma/Hypothalamic Injury
Gonadotropin-Releasing Hormone (GnRH)/Thyrotropin-Releasing Hormone (TRH)

Background Information: In patients with severe head injury, hypothalamohypophyseal impairment with subsequent hormone abnormalities has been well documented. Stress ulcers are another commonly encountered problem in such patients. However, little has been reported in the literature about the alterations of pituitary hormones in acute head-injured patients with stress ulcer.
Application: Detection and characterization of alteration in hypothalamic function secondary to cranial trauma reflected by modified responses to gonadotropin-releasing hormone (GnRH) and thyrotropin releasing hormone (TRH).
Implementation: Evaluation of basal serum levels of pituitary hormones measured before, and in response to thyrotropin-releasing hormone (TRH), and gonadotropin-releasing hormone (GnRH) in order to measure pituitary function in conditions of cranial injury.

Human Model: Cases of cranial injury (n = 40 males). Candidate criteria for eligibility may include (i) Glasgow coma scale 4 to 10; (ii) presentation within 24 hours following head injury; (iii) absence of shock or sepsis; and (iv) no past history of peptic ulcer. Stress ulcers can be confirmed by endoscopic examination.
Interpretation and Analysis: Approximately 2/3 of cases show evidence of stress ulcer by endoscope (e.g., n = 27/40 or 67.5%). In the absence of stress ulcer, basal serum levels of thyroid-stimulating hormone (TSH), prolactin (PRL), growth hormone (GH), luetinizing hormone (LH), and follicle-stimulating hormone (FSH) are within

normal range. However, the basal levels of PRL in conditions of stress ulcers are abnormally elevated and are significantly higher than in the absence of stress ulcers ($p < 0.001$). Basal levels of TSH and GH are significantly lower with stress ulcers than in the absence of stress ulcers ($p < 0.001$). In stress ulcer conditions, significant increases ($p < 0.001$) in serum TSH, PRL, LH and FSH levels after thyrotropin-releasing hormone (TRH) and gonadotropin-releasing hormone (GnRH) provocation can be detected. Hypothalamohypophyseal dysfunction and stress ulceration may occur in cases of severe cranial trauma. In the presence of stress ulcers, the abnormalities of pituitary hormones production/release and provocative response of the pituitary to TRH and GnRH reveal normal pituitary function with hypothalamic insufficiency. Therefore, stress ulcers in acute head-injured patients is closely associated with hypothalamic damage.

Hypothalamic dysfunction in acute head-injured patients with stress ulcer. Hwang SL, Lieu AS, Howng SL, Hsieh JS, Huang TJ. Kaohsiung J Med Sci 1998 Sep; 14 (9), pp. 554–60.

Gastric Ulcers/Peptic Ulcer (Recurrent): Post–Partial Gastrectomy/(Billroth II Reconstruction)
Secretin/[^{99m}Tc]-Barium Meal

Application: Differentiation between peptic ulcer and Zollinger-Ellison syndrome.
Implementation: Evaluation of recurrent peptic ulcers following partial gastrectomy.
Interpretation and Analysis: Careful filling of the afferent loop on barium meal or [^{99m}technetium] scanning may verify the diagnosis. The secretin provocative test may be helpful in distinguishing retained antrum from Zollinger-Ellison syndrome by eliciting a decrease in serum gastrin levels in conditions with retained antrum and an increase in serum gastrin levels in patients with Zollinger-Ellison syndrome. In instances of recurrent peptic ulceration following partial gastrectomy with Billroth II reconstruction, a retained antrum on the duodenal stump may be the cause for the complication. Moderate hypergastrinemia and a high basal acid output (BAO) to maximal acid output (MAO) ratio on gastric analysis should initially function as a diagnostic indicator.

Serum gastrin levels in the differential diagnosis of recurrent peptic ulceration due to retained gastric antrum. Webster MW, Barnes EL, Stremple JF. Am J Surg 1978 Feb; 135 (2), pp. 248–52.

Gastric Urease Activity (Intragastric)/Helicobacter pylori (H. pylori)
[^{13}C]-Urea Breath Test

Application: Detection and characterization of *H. pylori* populations residing within the gastrointestinal tract. The reagent can also be applied to evaluate the efficacy of therapeutic agents.
Implementation: Assessment of intragastric urease activity by the [^{13}C]-urea breath test is performed before and after 1 day of dosing with either De-Noltabs (tripotassium dicitrato bismuthate, 1 tablet 1 q.d.s.), Pepto-Bismol liquid (bismuth salicylate 30 ml q.d.s.), or Roter tablets (bismuth subnitrate, 1 tablet q.d.s.) in 12 H. pylori–positive patient volunteers.
Interpretation and Analysis: There is a significant decrease in [$^{13}CO_2$] after 1 day of dosing with each of the 3 bismuth compounds, but analysis of variance detects no difference between the effects of the 3 compounds. Systemic absorption of bismuth following oral dosing with either Pepto-Bismol or Roter is minimal, yet both compounds have a suppressive effect on *H. pylori* similar to that of De-Noltab. The action of all 3 bismuth compounds is within the gastric lumen, and that systemic absorption of bismuth is not necessary for activity against *H. pylori*.

Comparison of 1-day oral dosing with three bismuth compounds for the suppression of Helicobacter pylori assessed by the ^{13}C-urea breath test. Prewett EJ, Luk YW, Fraser AG, Lam WM, Pounder RE. Aliment Pharmacol Ther 1992 Feb; 6 (1), pp. 97–102.

Gastroesophogeal Reflux Disease (GERD)/ Esophogitis/Non-Cardiac Chest Pain (MIBI Negative NCCP)
High-Dose Omeprazole/[^{99m}Tc-SestaMIBI (MIBI)]

Cellular/Molecular Mechanism of Action: Omeprazole inhibits H^+/K^+-ATPase that exchanges hydrogen ion for potassium ion within parietal cell populations. In this fashion, less hydrochloric acid is available for secretion into the gastric lumen.
Background Information: Non-cardiac chest pain presents as a frequent diagnostic challenge, with patients tending to use a disproportionate level of health care resources. Gastroesophageal reflux disease is the most frequent cause of NCCP.
Application: Detection of non-cardiac chest pain etiologies due to esophogistic and gastric esophageal reflux disease in order to avoid nuclear scintigraphy evaluation of cardiac function/physiology.
Route of Administration: Omeprazole (oral).
Dosage: Omeprazole (40 mg twice daily).
Considerations: Gastroesophageal reflux disease is the most frequent cause of NCCP rest/stress perfusion.
Implementation: Evaluation of recurrent chest pain conditions utilizing a prospective, double blind, placebo-controlled, crossover trial using high-dose omeprazole. Findings are then compared with those of endoscopy, manometry, and ambulatory 24-hour 2-channel esophageal pH monitoring. Initial diagnostic upper endoscopy, esophageal manometry, and 24-hour pH monitoring are also applied as evaluation parameters. Cases are then randomly assigned to either placebo or omeprazole for 14 days, washed out for 21 days, and then crossed over. Patient's symptoms are determined using a Visual Analogue Scale to measure the severity of chest pain before and after each period.

Human Model: Cases ($n = 18$ men; $n = 24$ women; age 22–77 years) of recurrent chest pain complaints of a non-cardiac etiology, as determined by rest/stress perfusion imaging with technetium [^{99m}Tc]-sestaMIBI (MIBI).
Interpretation and Analysis: Improvement in perceived chest pain can be detected in approximately 71% of cases receiving omeprazole compared to only 18% for placebo reference controls. Abnormal results on manometry (20%), 24-hour pH monitoring (42%), or endoscopy with visual evidence of esophagitis (26%) can be appreciated less frequently. Application of combinations of the 3 tests does not significantly increase their usefulness. In NCCP patients with GERD, as defined by positive results on a 24-hour pH test or presence of esophagitis on endoscopy, omeprazole treatment results in a response in 95% of cases, whereas 90% of GERD-positive patients treated with placebo do not respond. Of NCCP patients determined to be GERD negative, approximately 39% will respond to omeprazole. Therefore, omeprazole as a 1st diagnostic tool in the evaluation of MIBI-negative NCCP is sensitive and specific for determining the cause of NCCP. Endoscopy, manometry, and 24-hour pH monitoring are not only less sensitive in diagnosing NCCP, they are significantly more expensive.

Short course of omeprazole: A better first diagnostic approach to non-cardiac chest pain than endoscopy, manometry, or 24-hour esophageal pH monitor-

ing. Pandak WM, Arezo S, Everett S, Jesse R, DeCosta G, Crofts T, Gennings C, Siuta M, Zfass A. J Clin Gastroenterol 2002 Oct; 35 (4), pp. 307–14.

Gastroesophogeal Reflux Disease (GERD): Esophogeal Sphincter
Bethanechol/Metoclopramide

Route of Administration: Oral.
Dosage: Metoclopramide (single 20 mg dose) prior to meal ingestion. Esophogeal sphincter function; metoclopramide (10 and 20 mg); bethanechol (25 mg).
Implementation: Evaluation of symptoms of heartburn and regurgitation recognized following a test meal challenge utilizing metoclopramide as a pharmaceutical probe.

In scenarios for the evaluation of lower esophageal sphincter pressure, metoclopramide and bethanechol can be applied.

Human Model: Cases with symptoms of GERD and basal LESP less than 11 mm Hg (n = 15 males).
Interpretation and Analysis: Metoclopramide can prevent symptoms of heartburn and regurgitation induced by a provocative meal challenge. Adverse reactions are rare to non-existent. Metoclopramide reduces the severity of heartburn from the onset, its effect reaching statistical significance within 2 hours and persisting for at least 5 hours. Eighty percent of cases receiving metoclopramide, compared with only 30% of placebo-treated patients, are completely free of heartburn at the end of clinical trials. A significant reduction in regurgitation during the 1 and one-half–4 hours after the test meal is also noted with metoclopramide.

In conditions with symptoms of GERD and basal LESP, metaclopramide and bethanecol produces a significant increase in LESP compared to placebo. Metoclopramide, 20 mg, produces a greater increase than either metoclopramide, 10 mg, or bethanechol, 25 mg. Serum gastrin concentrations are not altered by any of the drugs. Side effects are usually unremarkable. The LESP increasing effect of metoclopramide might be useful in the evaluation/treatment of GERD.

Comparative effects of metoclopramide and bethanechol on lower esophageal sphincter pressure in reflux patients. McCallum RW, Kline MM, Curry N, Sturdevant RA. Gastroenterology 1975 May; 68 (5 Pt 1), pp. 1114–8.

Gastrointestinal Bacteria: Gastric Helicobacter pylori (H. pylori) Detection
[^{13}C]-Urea Breath Test

Background Information: Infection with *H. pylori* is thought to be an important factor in the pathogenesis of gastritis, peptic ulcer, and gastric cancer.
Application: The [^{13}C]-urea breath test (^{13}C-UBT) can be used as a convenient and non-invasive method for the detection of gastric *H. pylori* populations.
Route of Administration: [^{13}C]-urea.
Dosage: [^{13}C]-urea.
Implementation: Assessment of intragastric urease activity using the [^{13}C]-urea breath test is performed before and after 1 day of dosing with either with our without the presence of De-Noltabs (tripotassium dicitrato bismuthate, 1 tablet 1 q.d.s.), Pepto-Bismol liquid (bismuth salicylate 30 ml q.d.s.), or Roter tablets (bismuth subnitrate, 1 tablet q.d.s.).

Human Model: *H. pylori*–positive patient volunteers (n = 12).
Interpretation and Analysis: There is a significant decrease in the excess of [$^{13}CO_2$] after 1 day of dosing with each of the 3 bismuth compounds, but analysis of variance detects no difference between the effects of 3 different bismuth compounds. Systemic absorption of bismuth following oral dosing with either Pepto-Bismol or Roter is minimal, yet both compounds have a suppressive effect on *H. pylori* similar to that of De-Noltab. The action of all 3 bismuth compounds is within the gastric lumen, and that systemic absorption of bismuth is not necessary for activity against *H. pylori.* Compared to histology evaluations, the [^{13}C]-urea breath test (^{13}C-UBT) is more sensitive, specific, and accurate.
Instrumentation: The [$^{13}CO_2$]/[$^{12}CO_2$] ratio is measured by using infra-red spectroscopy (IR) and gas chromatography/mass spectrometry(GC-MS). The IR analyzer should be particularly useful for the diagnostic test of *H. pylori* infection using [$^{13}CO_2$]-UBT.

Comparison of one-day oral dosing with three bismuth compounds for the suppression of Helicobacter pylori assessed by the [^{13}C]-urea breath test. Prewett EJ, Luk YW, Fraser AG, Lam WM, Pounder RE. Aliment Pharmacol Ther 1992 Feb; 6 (1), pp. 97–102.

[^{13}C]-urea breath test. Oshima H, Kajiwara M. Nippon Rinsho. 2003 Jan; 61 (1), pp. 67–71.

Gastrointestinal Bacteria/Helicobacter pylori (H. pylori)
[^{15}N]Urea/Sodium [^{13}C]-Acetate

Application: Detection of gastrointestinal *H. pylori* populations.
Route of Administration: Oral in 75 ml orange juice as a test drink.
Dosage: 150mg [^{15}N]urea and 75 mg sodium [^{13}C]-acetate in 75 ml orange juice as a test drink.
Considerations: The protocols for [^{13}C] and [^{15}N] *H. pylori* tests stipulate that the diagnostic agent should be taken on an empty stomach. It is presumed that food intake prior to the tests leads to less reliable test results due to a prolongation of the gastric residence time of the diagnostic agent urea. This might allow bacteria to split a higher proportion of urea, resulting in an increased number of false positives.

Food intake prior to the test drink does not have much of an influence on the gastric residence time of urea or on qualitative *H. pylori* test results. Due to identical behaviour of [^{13}C]-urea and [^{15}N]-urea in the stomach, this influence is believed to be independent on the labeling isotope. For survey purposes, no fasting conditions are required for the *H. pylori* tests.
Implementation: Evalaution of [^{15}N]-urea as an agent for the diagnosis of gastric *H. pylori* colonization. The [^{15}N] tests are evaluated using a urine sample of the 2nd hour after test start. [^{13}C]-acetate served as a marker of the gastric emptying of water-soluble food including the urea under the influence of food intake. Breath air samples are taken to calculate the gastric-emptying half-life (EHL) and the apparent resorption time (RT) of the urea. The double tests are carried out 4 times within 4 weeks using identical test protocols but different standardized time periods of pretest fasting: overnight, 2 hours prior to test, 1 hour prior to test, and no fasting at all. The food intake amount is standardized.
Interpretation and Analysis: Five probands testing positive in the overnight fasting test are also found to be positive in the other test variants with more or less empty stomachs. Seven other probands testing negative after overnight fasting tested negative in the other test variants as well.

Helicobacter pylori *in vivo* diagnosis after food intake: Results of simultaneous [^{15}N]-urea urine and [^{13}C]-acetate breath tests. Krumbiegel P, Müller DM. Isotopes Environ Health Stud 2000; 36 (2), pp. 99–110.

PANCREAS

Gastric Neoplasia/Carcinoma/Metastasis
Green Fluorescent Protein/Retrovirus Delivery

Refer to Section IV, Delivery Modalities, and Section II, Genetic Disorders, Infectious Disease, Neoplasia, and Metabolic Conditions.

In vivo tumor delivery of the green fluorescent protein gene to report future occurrence of metastasis. Hasegawa S, Yang M, Chishima T, Miyagi Y, Shimada H, Moossa AR, Hoffman RM. Cancer Gene Ther 2000 Oct; 7 (10), pp. 1336–40.

Gastritis (Symptomatic): Bile Reflux Associated
Sodium Hydroxide (Intragastric Infusion)

Application: Detection of symptomatic bile reflux gastritis.
Route of Administration: Blind sequential intragastric infusion of each test solution utilizing a nasogastric tube.
Dosage: Blind sequential administration of HCl (0.1 N, 20 ml); normal saline (0.9%, 20 ml); 0.1 N NaOH, (0.1 N, 20 ml) and endogenous gastric contents (20 ml). Each formulation is given twice.
Considerations: Alkaline reflex gastritis is a disabling clinical syndrome, occurring most often after gastric surgery. It is characterized by abdominal pain and bilious vomiting, and, presumably, is due to gastric irritation by regurgitated duodenal contents. The only known effective treatment is by surgical diversion to prevent the duodenal reflux. Unfortunately, the clinical diagnosis is difficult to prove, and the results of surgery are too often disappointing because of inaccurate patient selection.

The test is simple and appears to be a sensitive, specific, and accurate means for selecting patients for surgical treatment of alkaline reflux gastritis.
Interpretation and Analysis: A positive test is defined as reproduction of pain by NaOH and/or gastric contents equivalent to that experienced during pretest clinical signs. No discomfort is recognized with acid or saline infusions. Cases with clinical symptoms and endoscopic findings suggestive of bile gastritis have positive alkali infusion tests (15/21), while very few (1/18) normal controls or controls with other causes of abdominal pain (0/17) have positive test responses ($p < 0.001$). Of cases with clinical-endoscopic bile gastritis, a relatively large proportion (15/21) have had surgical treatment (Roux-en-Y gastrojejunostomy). In patients with a positive test result, a large number (9/10) will experience excellent symptomatic relief after surgery. Few patients (e.g., 0/5) with a negative test may be relieved of pain after the operation.

Gastritis Syndrome: Alkaline Reflux/Bile Gastritis
Sodium Hydroxide (Intragastric)

Background Information: Accurate diagnosis of the alkaline reflux gastritis syndrome is often difficult to establish and contributes to the relatively high failure rate associated with surgical treatment schemes especially pertaining to pain relief.
Application: Accurate diagnosis of the alkaline reflux gastritis syndrome.
Route of Administration: Intragastric infusion with each test solution administered in a random blind fashion.
Dosage: Diagnostic formulations include (i) saline solution; (ii) hydrochloric acid (HCl 0.1 N); (iii) sodium hydroxide solutions (NaCl 0.1 N); and (iv) endogenous gastric aspirates.
Considerations: Evaluation of the *in vivo* test reveals that positive alkali infusion test results predict that symptoms will likely respond best to surgical diversion of duodenal contents, and that negative test results should be a strong deterrent to surgical diversion. The test is safe, simple, and deserves further trial.
Implementation: Detection of alkaline reflux gastritis syndrome.

Human Model: Cases (n = 147) with symptoms and endoscopic findings suggestive of bile gastritis (asymptomatic controls; n = 19).
Interpretation and Analysis: A positive test result is defined as perceived pain with sodium hydroxide but not with saline solution or hydrochloric acid infusion. Essentially all control subjects have negative test results. Monitoring of symptomatic patients (mean 4.3 years, minimum 1 year) reveals that a large percentage (47/128) suffer from Roux-Y gastrojejunal reconstruction to divert duodenal contents from the stomach. Correlation of test results and the subsequent clinical status shows that those with negative test results (87%) improve spontaneously or with other treatment compared to cases with positive test results ($p < 0.001$) and a large percent (78%) with positive test results have lasting relieve of pain following surgical diversion compared to a 30% success rate if the test results are negative ($p < 0.01$).

Diagnosis of symptomatic alkaline reflux gastritis and prediction of response to bile diversion operation by intragastric alkali provocation. Rutledge PL, Warshaw AL. Am J Surg 1988 Jan; 155 (1), pp. 82–7.

INTESTINAL ABSORPTION BREATH TESTS

Intestinal Absorption: Malabsorption and Compromised Integrity/[Renal Failure]
D-Xylose Absorption/Hydrogen Breath Test

Application: Kinetic analysis of D-xylose absorption and D-xylose breath testing can be performed in an effort to establish an effective absorption test in functionally anephric patients.
Background Information: Evaluation of malabsorption in renal failure is difficult because most absorptive testing requires urinary collections.
Route of Administration: D-xylose (oral and intravenously).
Dosage: D-xylose 15 gram (oral) and 10 gram (intravenous) dosage schemes.
Examples of Clinical Implementation: Evaluation of intestinal absorption. Serum samples are collected and measured for D-xylose in order to calculate the kinetic rate constants and extent of absorption. After the oral D-xylose, end expiratory breaths are collected every 15 minutes for 3 hours and are analyzed for H_2 applying gas chromatography.

Human Model: In fasting renal failure patients with no diarrhea or symptoms suggesting malabsorption on 2 separate non-dialysis days after receiving D-xylose on day 1 (oral) and day 2 (IV).
Interpretation and Analysis: The mean absorption rate constant (Ka) and bioavailability (F) are similar to published values for normal subjects using the 15-g dose (0.936 min^{-1}; range, 0.227–1.96; and 74%, range 46–99, respectively). A number of cases will have a normal 1-hour serum D-xylose concentration (> 20 mg/dL). There is no clear inverse correlation between the rate constant for absorption or bioavailability and peak breath hydrogen or the area under the curve for breath H_2 vs time. Using 15 g of oral D-xylose, mean bioavailability and absorption rate constants are normal in functionally anephric patients with no clinical evidence of malab-

sorption. A small number of cases will have elevated breath peak H_2 concentrations, but there is no clear inverse correlation between bioavailability and the breath H_2 values. A 1-hour serum D-xylose concentration > 20 mg/dL may be considered normal in this patient group, similar to patients with normal renal function.

Instrumentation: Gas chromatography (hydrogen gas measurement).

D-xylose kinetics and hydrogen breath tests in functionally anephric patients using the 15-gram dose. Craig RM, Carlson S, Ehrenpreis ED. J Clin Gastroenterol 2000 Jul; 31 (1), pp. 55–9.

Intestinal Absorption: Malabsorption and Compromised Integrity/Intestinal Permeability/ Gastric Emptying
Glucose Absorption/Lactulose/Rhamnose Absorption Ratio/[^{13}C]-Acetate Breath Test

Background Information: Exercise decreases splanchnic bloodflow to the extent that exercise may induce alterations in gastrointestinal (GI) function.

Application: (i) Glucose and 3-O-methyl-D-glucose can be utilized to assess the presence/integrity of active transport mechanisms within the mucosa of the proximal small intestine; (ii) D-xylose is transported by a passive carrier-mediated process; (iii) L-rhamnose traverses the intestinal mucosal membrane by non-mediated absorptive processes and does not normally cross normal intact intsinal mocosal; (iv) the lactulose:rhamnose ratio serves as a measure of increased intestinal permeability (large-pore defect/ small-pore defect, respectively). In this context, lactulose, mannitol, and rhamnose are not metabolized in the small intestine but may be metabolized in the large intestine. Lactulose and rhamnose are poorly absorbed across intact mucosal surfaces while mannitol is normally absorbed across intact small intestinal mucosa. Lactulose/rhamnose ratios can be applied to detect large-pore/small-pore permeability defects, respectively. The [^{13}C]-acetate breath test can be applied to assess gastric emptying.

Route of Administration: Oral.

Dosage: Lactulose/rhamnose regular dose (50 mg/50 mg) or a high dose (250 mg/250 mg) in humans.

Examples of Clinical Implementation: Evaluate the influence of high-intensity physical exercise on gastrointestinal physiology processes including esophageal motility, gastro-esophageal reflux, gastric pH, gastric emptying, orocecal transit time (OCTT), intestinal permeability and glucose absorption simultaneously. Esophageal motility, gastroesophageal reflux, and intragastric pH can be measured using a trans-nasal catheter while the orocecal transit time is measured using a breath H_2 measurement. A sugar absorption test is used to determine intestinal permeability and glucose absorption. Lastly, gastric emptying is measured using the [^{13}C]-acetate breath test.

Interpretation and Analysis: During exercise, peristaltic velocity is greater than values noted at rest 4.92 (2.86) vs. 4.03 (1. 48) cm s-1, p = 0.015). Mid-esophogeal peristaltic contraction pressure and the duration of the peristaltic contractions at the mid- and distal esophagus are lower during intense exercise. Conversely, no differences can be appreciated between pre-exercise, intraexercise and postexercise measurements for gastric pH values. Exercise does not create noticeable differences in either gastric emptying or orocecal transit time. In contrast, lactulose/rhamnose ratios and intestinal glucose absorption are significantly decreased in response to moderate to intense exercise activity. Normal intestinal absorption profiles reveal absorption of glucose and lactulose but relatively minimal systemic absorption of rhamnose. If the integrity of the small intestinal mucosa is compromised, an increased amount of rhamnose will enter into the systemic circulation. Conversely, if there is a compromise of intestinal mucosa absorptive function, then depressions in glucose and lactulose absorption can be appreciated.

The effect of physical exercise on parameters of gastrointestinal function. van Nieuwenhoven MA, Brouns F, Brummer RJ. Neurogastroenterol Motil 1999 Dec; 11 (6), pp. 431–9.

INTESTINAL ABSORPTION AND PERMEABILITY: NON-BREATH TESTS

Enteropathy: Childhood
D-Xylose Absorption Test/Lactose Breath Hydrogen Test/[Jejunal Biopsy Alternative]

Cellular/Molecular Mechanism of Action: Lactose and D-xylose are normally absorbed across the surface of normal/healthy intestinal mucosa.

Background Information: Three widely used screening tests for enteropathy in childhood are the lactose breath hydrogen test, the 1-hour serum D-xylose absorption test, and the 72-hour fecal fat determination.

Application: Lactose and D-xylose can be applied to detect alterations in the absorptive function of the intestinal mucosa.

Route of Administration: Lactose and D-xylose (oral/per os).

Implementation: Correlate findings from the lactose hydrogen breath test, D-xylose absorption test, and 72-hour fecal fat determination with results from jejunal biopsy evaluations in cases of juvenile enteropathy.

Interpretation and Analysis: Results from D-xylose and fecal fat tests each significantly correlate with jejunal biopsy results (e.g., normal result of either test was highly predictive of a normal biopsy finding). Combining the results of 2 or more screening tests does not improve predictive value. The xylose test is preferable on the basis of greater sensitivity and ease of patient compliance.

Screening tests for enteropathy in children. Levine JJ, Seidman E, Walker WA. Am J Dis Child 1987 Apr; 141 (4), pp. 435–8.

Gastrointestinal Permeability/Mucosal Function
Lactulose/Xylose/Methylglucose/Sucrose

Cellular/Molecular Mechanism of Action: Lactulose, rhamnose, xylose, methylglucose, and sucrose are sugar carbohydrates. Lactulose (L) and rhamnose are not significantly absorbed across intact intestinal mucosa. Alternatively, xylose and methylglucose are normally absorbed across healthy intestinal mucosal surfaces of the jejunum by carrier-mediated transport mechanims. Neither lactulose nor mannitol is metabolized to any significant degree in the small intestine but may be metabolized in the large intestine. Injury to intact intestinal mucosa can promote increases in sucrose absorption.

Application: To describe the kinetics of urinary recovery (UR) of 5 sugars used for gastrointestinal permeability and mucosal function testing following orogastric administration of lactose, rhamnose, xylose, methylglucose, and sucrose. The lactulose/rhamnose ratio can be used to detect large-pore/small-pore defects in mucosal permeability.

Route of Administration: Lactulose, rhamnose, xylose, methylglucose, and sucrose (orogastric intubation).

Implementation: Evaluation of the urinary excretion of sugar solution containing lactulose, rhamnose, xylose, methylglucose, and sucrose following administration by orogastric intubation. Urine samples are collected immediately before sugar solution administration (baseline) and at 2-hour intervals thereafter. Percent urinary recovery (%UR) of the total UR up to 12 hours after sugar solution administration is calculated for each sugar type at 2-hour intervals.

Animal Model: Canine (n = 7 healthy male beagles).

Interpretation and Analysis: Mean %UR exceeds 85% for all 5 sugars at 6 hours after orogastric administration of the sugar solution and exceeds 90% after 8 hours. In healthy dogs, a urine collection period of 6 hours is sufficient for gastrointestinal permeability and mucosal function testing following orogastric administration of lactulose, rhamnose, xylose, methylglucose, and sucrose.

Instrumentation: Analysis of sugars applying high-pressure liquid chromatography and pulsed amperometric detection.

Kinetics of urinary recovery of five sugars after orogastric administration in healthy dogs. Steiner JM, Williams DA, Moeller EM. Am J Vet Res 2002 Jun; 63 (6), pp. 845–8.

Gastrointestinal Permeability/Gastrointestinal Absorption/Primary Biliary Cirrhosis/Cirrhosis
Sucrose/Lactulose/Mannitol

Cellular/Molecular Mechanism of Action: Lactulose is not absorbed across the surface of normal, intact intestinal mucosa. Mannitol is normally absorbed across the surface of healthy small intestinal mucosa. Lactulose and mannitol are not metabolized to any significant degree within the small intestine but may be metabolized in the large intestine. Injury or damage to gastroduodenal mucosal promotes enhanced sucrose absorption.

Application: Lactulose can be applied to detect increases in intestinal permeability, while mannitol is useful in recognizing declines in intestinal absorptive capacity. Sucrose can detect alterations in gastro-duodenal permeability, while lactulose can detect large-pore defects in intestinal permeability

Route of Administration: Lactulose, mannitol, and sucrose can be administered orally for assessing gastrointestinal permeability/absorptive parameters.

Implementation: Evaluation of gastrointestinal permeability in conditions of primary biliary cirrhosis (PBC) determined by measuring the sucrose (gastro-duodenal) and lactose-mannitol (intestinal) excretion profiles as a sensitive method for detecting mucosal epithelial damage Results can then be correlated with the Mayo score, histological stage, ascites, spontaneous bacterial peritonitis, endoscopic signs of portal hypertension and *Helicobacter pylori* infection.

Human Model: Cases of primary biliary cirrhosis or PBC (n = 50) and cirrhosis due to other causes (n = 39). Celiac disease is not evident in any of the case groups.

Interpretation and Analysis: Sucrose excretion was above the limit in both PBC and non-PBC patients. In almost a quarter of primary biliary cirrhosis cases (22%), the lactulose-mannitol test is increased compared to 12.8% of non-PBC cirrhotic patients. Cases of primary biliary cirrhosis (PBC) have high sucrose excretion levels, irrespective of the presence of any esophageal varices, that significantly increase gastroduodenal permeability in non-PBC patients only when associated with hypertensive gastropathy. Sucrose urinary excretion is significantly enhanced by hypertensive gastropathy in non-PBC patients ($p < 0.001$) but not in PBC patients. No significant correlation can be appreciated for gastrointestinal permeability and other parameters in either primary biliary cirrhosis or non-primary biliary cirrhosis. Gastrointestinal permeability is increased in PBC. Portal hypertension contributes to altered gastric mucosal permeability in non-PBC cirrhosis, whereas different epithelial dysfunction can be hypothesized in PBC.

Gastroduodenal and intestinal permeability in primary biliary cirrhosis. Di Leo V, Venturi C, Baragiotta A, Martines D, Floreani A. Eur J Gastroenterol Hepatol 2003 Sep; 15 (9), pp. 967–73.

Intestinal Absorption
D-Xylose/D-Glucose

Cellular/Molecular Mechanism of Action: D-xylose and D-glucose are both carbohydrate sugars that are absorbed across intact mucosal surface of the small intestine (jejunum)involving passive (D-xylose) and active (D-glucose) carrier-mediated transport mechanisms.

Application: Evaluation of the absorptive capacity/function of the small intestine.

Implementation: Evaluation of the potential for D-xylose to accumulate *in vitro* in jejunal mucosa from healthy horses and rabbits.

Animal Model: Equine and rabbit jejunal mucosal preparations.

Interpretation and Analysis: Incubation of jejunal mucosal tissue sheets with D-xylose (1 mM) for 60 minutes, reveals that it accumulates against a concentration gradient within *ex vivo* mucosal preparations from both species. There is no accumulation when equine specimens are incubated with D-xylose (5 mM). By comparison, equine jejunum accumulates D-glucose against a concentration gradient when incubated in 5 mM D-glucose. In equine and rabbit jejunum, 13.3 ± 7.0% and 36 ± 11.0%, respectively, of accumulated D-xylose is phosphorylated when sheets are incubated in 1 mM D-xylose. Short-circuit current and potential difference are lower in equine jejunum than in rabbit jejunum, possibly because of differences in tissue thickness. None of the transmucosal electrical measurements increase after addition of D-xylose (1 mM and 5 mM) or D-glucose (5 mM). The active transport system for D-xylose has a low affinity for this sugar and becomes saturated at low intraluminal concentrations. Therefore, abnormal D-xylose absorption test results in horses are more likely caused by abnormalities in mucosal surface area and mucosal permeability than by abnormalities of nutrient carbohydrate absorption.

In vitro concentrative accumulation of D-xylose by jejunum from horses and rabbits. Freeman DE. Am J Vet Res 1993 Jun; 54 (6), pp. 965–9.

Intestinal Absorption: Neonatal/Colostrum Dietary Intact
D-Xylose

Cellular/Molecular Mechanism of Action: D-xylose is a carbohydrate molecule that is absorbed across the intact mucosa of the small intestine by passive carrier-mediated transport mechanisms.

Background Information: Colostrum intake is important for health and postnatal development of neonatal calves.

Application: Detection and characterization of alterations in intestinal absorptive capacity.

Considerations: Note: Neonatal calves are essentially a simple-stomached animal at this stage of the development of their gastrointestinal tract.

Implementation: Evaluate the influence of enhanced 1st colostrum feeding on growth, health status, and metabolic and endocrine traits and intestinal absorption capacity as measured by the xylose absorption test.

Animal Model: Bovine (7-day old calves). Group 1 calves are fed colostrums from milkings 1–6 twice daily during the 1st 72 hours

of life (n = 7) followed by milk replacer supplementation (MR) for up to 7 days. Group 2 calves are fed colostrum of the 1st milking during the 1st 3 days and then colostrum (of the 1st milking) twice daily, which on day 4, 5, 6, and 7 is diluted with 25, 50, 75, and 75 parts of milk-replacer (MR), respectively (n = 7). Pre- and postprandial blood samples are taken on days 1, 2, 3, and 7 for determination of various metabolic and endocrine traits. On day 5, intestinal absorption capacity is measured using the xylose absorption test.

Interpretation and Analysis: Rectal temperatures and fecal scores are higher ($p < 0.05$) in Group 2 than Group 1 calves. Plasma concentrations of total protein and albumin are higher ($p < 0.05$) on day 7, IgG on days 2 and 3, and urea on days 2, 3, and 7 in Group 2 than in Group 1 calves. Plasma concentrations of triglycerides are higher ($p < 0.05$) on day 2 and of phospholipids and cholesterol are higher ($p < 0.01$) on day 7 in Group 2 than in Group 1 calves. Plasma insulin and glucagon concentrations are higher ($p < 0.05$) in Group 2 than in Group 1 calves, whereas prolactin and growth hormone concentrations are higher ($p < 0.05$) in Group 1 than in Group 2 calves. Enhanced colostrum intake has no effects on xylose absorption on day 5 measurements. Higher plasma protein, urea, and lipid concentrations in Group 2 partly mirror higher protein and fat intake but additionally point to higher protein synthesis and lipid turnover.

Influence of feeding different amounts of first colostrum on metabolic, endocrine, and health status and on growth performance in neonatal calves. Rauprich AB, Hammon HM, Blum JW. J Anim Sci 2000 Apr; 78 (4), pp. 896–908.

Intestinal Absorption: Malabsorption and Compromised Mucosal Integrity
D-Xylose

Cellular/Molecular Mechanism of Action: D-xylose is absorbed from the small intestine (jejunum) by passive carrier-mediated transport mechanisms.

Route of Administration: D-xylose (oral).

Clinical Implementation: Colostrum intake is important for health and postnatal development of neonatal calves. Based on the interpretation of xylose absorption tests performed on day-5, and enhancement of colostrum intake has no significant effect on the absorptive capacity within the intestine.

Influence of feeding different amounts of first colostrum on metabolic, endocrine, and health status and on growth performance in neonatal calves. Rauprich AB, Hammon HM, Blum JW. J Anim Sci 2000 Apr; 78 (4), pp. 896–08.

Intestinal manipulation during elective aortic aneurysm surgery leads to portal endotoxaemia and mucosal barrier dysfunction. Lau LL, Halliday MI, Lee B, Hannon RJ, Gardiner KR, Soong CV. Eur J Vasc Endovasc Surg 2000 Jun; 19 (6), pp. 619–4.

Intestinal Absorption/Escherichia Coli (E. Coli) Enterotoxin-Induced Diarrhea
D-Xylose/[^{51}Cr]-Labeled Ethylenediaminetetraacetic Acid (EDTA)

Cellular/Molecular Mechanism of Action: [^{51}Cr]-EDTA is an organic chelate of radioactive [^{51}chromium] and is normally not significantly absorbed across intact, normal intestinal mucosa. Alternatively, D-xylose is absorbed across the surface of the small intestine (jejunum) mucosa involving passive carrier-mediated transport mechanisms.

Application: Detection and characterization of alterations in the intestinal permeability of [^{51}Cr]-EDTA and absorptive capacity including conditions associated with *E. coli* enterotoxin-induced diarrhea.

Route of Administration: D-xylose and [^{51}Cr]-EDTA can be administered orally for the purpose of evaluating intestinal absorption and permeability.

Implementation: Evaluation of alterations in intestinal absorptive capacity in diarrhea induced by the administration of *E. coli* enterotoxins. Intestinal absorption is evaluated using D-xylose and [^{51}Cr]-EDTA absorption tests.

Animal Model: Murine (*E. coli* heat-stable, heat-labile, and both heat-stable and heat-labile enterotoxin-induced diarrhea).

Interpretation and Analysis: The absorption of D-xylose is greatest 45 minutes after ingestion in both control and enterotoxin-treated groups. There is a significant ($p < 0.01$) decrease in xylose absorption at 45 minutes following exposure to all 3 entertoxin formulations compared to reference control groups. Such findings may be due to change in membrane fluidity and hence changes in membrane permeability, whereas [^{51}Cr]-EDTA absorption is at its maximum 15 minutes after ingestion in both controls and following enterotoxin exposure. No significant ($p < 0.05$) increase can be appreciated for [^{51}Cr]-EDTA absorption in enterotoxin-treated groups as compared with the control group. Findings of this nature suggest a change in tight junctional permeability that may be due to changes in the cytoskeletal microfilaments. Both these tests do, however, demonstrate that *E. coli* enterotoxin-induced diarrhea results in absorption and permeability changes.

[^{51}Cr]-labeled ethylenediaminetetraacetic acid and D-xylose absorption test in Escherichia coli enterotoxin-induced diarrhea in mice. Verma M, Ganguly NK, Majumdar S, Walia BN. Scand J Gastroenterol 1995 Sep; 30 (9), pp. 886–91.

Intestinal Absorption: Malabsorption and Compromised Mucosal Integrity
Glucose

Mechanism of Action: Absorbed from the small intestine by membrane-associated active transport mechanisms.

Diagnostic Application: Diagnosis of malabsorption attributed to the small intestinal segment of the gastrointestinal tract.

Route of Administration and Dosage: Following a 12-hour period of fasting, glucose is administered orally, usually by nasogastic intubation at a dose of approximately 1 g/kg (10% solution). Venous blood samples should be collected at 0, 0.5, 1.0, 1.5, 2.5, 3.0, 3.5, and 4.0 hours into tubes containing fluoride anticoagulant. Peak plasma concentrations are observed at approximately 2 hours, at which time at least a doubling of the systemic glucose concentration should be observed.

Interpretation and Analysis: (i) Solutions greater than 10% concentration will promote delayed gastric emptying, which serves as an artifact complicating interpretation of serum glucose concentrations (false negative). (ii) Glucose is subject to metabolism by gastrointestinal flora and can complicate interpretation of serum glucose concentrations. An example includes diarrhea of an infectious etiology. (iii) Horses on a low carbohydrate plane display higher peak xylose concentrations.

Laboratory Instrumentation: Measured directly through the application of glucose oxidase that rather specifically oxidizes this monosaccharide. An additional specific assay of glucose can be achieved with ortho-toluidine reagent, which reacts with aldohexoses. In the peripheral blood, dextrose is the predominant aldohexose. Many commercial instruments apply this methodology for the assay of serum glucose. Non-specific glucose assay methods include Nelson-Somogyi, ferricyanide, and Folin-Wu.

Intestinal Absorption: Malabsorption and Compromised Mucosal Integrity
Galactose/Glucose

Cellular/Molecular Mechanism of Action: Hydrolysis in the luminal bulk fluid by secreted enzymes is the major pathway for the breakdown of polysaccharides to oligosaccharides, and further hydrolysis is accomplished by a battery of carbohydrases in the brush border of the mature enterocytes. The glucose, galactose, and fructose produced are absorbed across the enterocytes of the upper half of the villus. Glucose and galactose (and other glucalogues) are actively transported into the enterocyte by a Na^+-glucose co-transporter via the trans-membrane electrochemical Na^+ gradient, and exit across the basolateral membrane by a glucose transporter. Fructose absorption is by an Na^+-independent transport system that has not been fully characterized. Absorption of monosaccharides from disaccharides appears to have a kinetic advantage (disaccharide-related transport system). Hexose absorption is enhanced by dietary intake of hexoses by increased activity of Na^+-glucose co-transporter and basolateral-membrane associated glucose transporter and by increased enterocyte numbers. Despite many kinetic and other types of studies in animals, and some in humans that suggest multiple Na^+-glucose transporters, only Na^+-glucose co-transporter is expressed in enterocytes.
Application: Evaluation of intestinal absorption capacity.
Route of Administration: Oral.
Dosage: Swine: Experimental meals containing 392 g dry matter of either hydrolyzed or non-hydrolyzed lactose together with a protein-mineral-vitamin mixture (200 g).
Interpretation and Analysis: Cadmium compounds decrease both D-galactose accumulation in the jejunum tissue, and D-galactose mucosal-to-serosal transepithelial fluxes in a dose-dependent manner. In this context, cadmium does not seem to modify sugar diffusion across the intestinal epithelium. The inhibitory mechanism associated with cadmium is non-competitive and it is partly reversed with dithioerythritol (thiol groups protector). The interpretation of these results implies that cadmium decreases carrier-mediated intestinal absorption of sugar (in rabbits).

The rates of glucose and fructose transport are greater in the proximal intestine prior to weaning and but are highest in mid-intestine after weaning. Galactose transport remains highest in the proximal intestine. Postnatal increases in Km values for galactose, but not glucose indicates that there are multiple aldohexose transporters that undergo separate patterns of development. The presence of fructose transport in neonatal pigs implies that this species may have a more advanced state of development than neonatal rats and rabbits, or may reflect the presence of an adaptation necessary to facilitate early weaning. Changes in fructose-to-glucose and galactose-to-glucose transport ratios before weaning suggest transporter development is at least in part genetically regulated and facilitates weaning in piglets. Curiously, fructose-to-glucose transport ratios are lower than those observed in the rat and rabbit, but closely paralleled those of some carnivorous feline species.

In swine receiving oral lactose (hydrolyzed and non-hydrolyzed), measurements of portal and arterial blood concentrations of glucose, galactose and amino nitrogen together with portal blood flow rate are taken during a postprandial period of 8 hours after oral lactose ingestion. Amounts of hexoses appearing in the portal blood after hydrolyzed-lactose intake are 3- and 2-fold larger within 2 hours and 8 hours, respectively, in contrast to the intake of non-hydrolyzed lactose. Enzymatic hydrolysis within the intestine is therefore the limiting factor of lactose digestion in unadapted pigs. Irrespective of the lactose form ingested, glucose chronologically appears earlier and in larger amounts in the portal blood than does galactose. Following hydrolyzed-lactose intake, the amount of glucose entering the portal blood exceeds the amount administered after 5 hours of intake. The significance of this observation is that a fraction of galactose is transformed into glucose during enterocyte transport.

Intestinal Absorption/Short Bowel Syndrome (SBS): Experimental
3-0 Methylglucose (3-0 MG)/Lactulose/Mannitol

Cellular/Molecular Mechanism of Action: The carbohydrate molecule 3-0 methylglucose is absorbed across the mucosal surface of the small intestine usually involving carrier-mediated processes residing within the jejunum. The lactulose:mannitol ratio can serve as a measure of increased intestinal permeability (large-pore-diameter defects). Lactulose is normally not absorbed across intact intestinal mucosa, while rhamnose and mannitol are absorbed across intact intestinal mucosal surfaces. In this context, lactulose and mannitol are not metabolized in the small intestine but may be metabolized in the large intestine.
Background Information: Inert carbohydrate probes are widely used to study intestinal permeability and examine the passive uptake of markers.
Application: Detection and characterization of alterations in intestinal absorptive capacity and permeability similar to those recognized in SBS.
Implementation: Evaluation of quantitative absorption in an SBS model facilitated by the use of 3-0 MG as a marker of intestinal surface area and active nutrient transport capability. Test solutions containing 3-0 MG, lactulose, and mannitol are gavaged (day 7) followed by a 16-hour period of urine collection. Urine recovery of probes is quantified using high-performance liquid chromotography. Intestinal morphology is then evaluated grossly and by light microscopy.

Animal Model: Rat SBS (sham operation [intestinal transection only], 50% resection [R50], 75% resection [R75], and 90% resection [R90; n = 6 to 8 in each group]). Animals can be pair-fed, and over days 5 and 6 postoperation, a balance study done to quantify absorption of dietary fat, protein, and carbohydrate.
Interpretation and Analysis: Increased extent of surgical resection results in reduced absorption of dietary nutrients and 3-0 MG, which reaches significance in the R90 resected animals. 3-0 MG absorption significantly correlates with intestinal surface area and the absorption of dietary protein and fat ($p < 0.01$ for all comparisons). Interestingly, 3-0 MG absorption does not significantly correlate with the absorption of dietary carbohydrate. Absorption of 3-0 MG is a useful marker of functional intestinal surface area and of the absorption of dietary fat and protein in experimental SBS. 3-0 MG may be a useful marker of nutrient absorptive capacity in patients with SBS but further study is indicated.
Instrumentation: High-performance liquid chromotography.

3-0 methylglucose absorption *in vivo* correlates with nutrient absorption and intestinal surface area in experimental short bowel syndrome. Martin GR, Meddings JB, Sigalet DL. JPEN J Parenter Enteral Nutr 2003 Jan–Feb; 27 (1), pp. 65–70.

Intesinal Absorption: Short Bowel Syndrome
Mannitol/Lactulose/Hydrolyzed Protein vs Non-Hydrolyzed Protein

Cellular/Molecular Mechanism of Action: Lactulose is normally not absorbed across intact intestinal mucosa, in contrast to mannitol, which is absorbed across intact small intestinal mucosal surfaces. In this context, lactulose and mannitol are not metabolized to any significant degree in the small intestine but they can be metabolized in the large intestine.

Application: The lactulose/mannitol ratio can be applied to detect increased small intestinal permeability (large-pore defects).
Implementation: Carbohydrate absorption tests employing lactulose and mannitol as probes to evaluate intestinal mucosa function and integrity have been utilized to evaluate the appropriateness of enteral formulations in cases of neonatal short bowel syndrome. In young children (4.08 ± 2.45 months) several parameters have been measured in attempts to determine the relative benefit of hydrolyzed protein compared to standard fomula properations in conditions of short bowel syndrome.
Interpretation and Analysis: Based on the calculations of intestinal permeability measured by a carbohydrate absorption test (lactulose/mannitol excretion ratio), energy, and nitrogen balance, there are no short-term advantages of hydrolyzed protein preparations (lactulose/mannitol ratio reference control 0.85 ± 0.85; hydrolyzed formula 0.59% ± 0.51%; non-hydrolyzed formula 0.69% ± 0.72%).

Hydrolyzed versus non-hydrolyzed protein diet in short bowel syndrome in children. Ksiazyk J, Piena M, Kierkus J, Lyszkowska M. J Pediatr Gastroenterol Nutr 2002 Nov; 35 (5), pp. 615–8.

Intestinal Absorption: Malabsorption and Compromised Integrity/[Intestinal Cryptosporidiosis]
Lactose Intolerance/Glucose Absorption

Application: Intestinal function and integrity evaluation.
Clinical Implementation: Lactose tolerance test performed to evaluate intestinal lactose intolerance. Glucose is administered for the evaluation of intestinal absorption capacity/function.

Animal Model: Caprine (2-week-old Toggenburg kid with persistent diarrhea and poor body condition).

History: The herd had high morbidity and mortality associated with diarrhea in neonatal kids.
Interpretation and Analysis: Lactose intolerance can be diagnosed on the basis of results of a lactose tolerance test and glucose absorption test. Clinically normal herdmates can be used as control animals. In the clinical case described, the kid responded to lactase supplementation. Cryptosporidium organisms had been detected in feces of several affected kids during episodes of acute diarrhea. Lactose intolerance therefore appears to be a clinical feature associated with intestinal cryptosporidiosis.

Secondary lactose intolerance in a neonatal goat. Weese JS, Kenney DG, O'Connor A. J Am Vet Med Assoc 2000 Aug 1; 217 (3), pp. 372–5.

Intestinal Absorption: Malabsorption and Compromised Integrity
Lactulose/Mannitol

Application: (i) Glucose and 3-O-methyl-D-glucose can be utilized to assess the presence/integrity of active-transport mechanisms within the mucosa of the proximal small intestine; (ii) D-xylose is transported by a passive carrier-mediated process; (iii) lactulose is not absorbed across the surface of intact intestinal mucosa; (iv) mannitol is absorbed across the surface of intact small intestinal mucosa; (v) the lactulose:mannitol ratio serves as a measure of increased intestinal permeability (large-pore defects). Neither lactulose nor mannitol is metabolized to any significant degree in the small intestine but may be metabolized in the large intestine. The [^{13}C]-acetate breath test can be applied to assess gastric emtyping.
Examples of Clinical Implementation: D-xylose absorption tests can be used to investigate the effect of intestinal manipulation on intestinal function and permeability such as the changes that occur during conditions of endotoxaemia associated with elective abdominal aortic aneurysm (AAA) surgery.
Interpretation and Analysis: Applying the D-xylose absorption test, significant increases in intestinal permeability can be detected postoperative day 1 in patients that undergo either transperitoneal or extraperitoneal correction strategies (day 1:0.107 ± 0.04, mean ± S.E.M.). In addition, there is a significant positive correlation between portal endotoxaemia and intestinal permeability ($r = 0.955$ $p = 0.001$). A significantly higher concentration of portal endotoxin can be identified intraoperatively in cases corrected by the transperitoneal approach compared to those corrected applying the extraperitoneal methods ($p < 0.05$). No correlation appears to exist between increased intestinal permeability and aortic clamp time, operation time, amount of blood lost or transfused. In addition, there is a significant positive correlation between portal endotoxaemia and intestinal permeability ($r = 0.955$ $p = 0.001$).

Intestinal manipulation during elective aortic aneurysm surgery leads to portal endotoxaemia and mucosal barrier dysfunction. Lau LL, Halliday MI, Lee B, Hannon RJ, Gardiner KR, Soong CV. Eur J Vasc Endovasc Surg 2000 Jun; 19 (6), pp. 619–24.

Intestinal Absorption: Malabsorption and Compromised Integrity
Lactulose/ Mannitol/Sucrose/[Hyperosmolar vs Non-Hyperosmolar]

Background Information: (i) Lactulose is not absorbed across the surface of intact intestinal mucosa; (ii) mannitol is absorbed across the surface of intact intestinal mucosa; (iii) the lactulose:mannitol ratio serves as a measure of increased intestinal permeability whereby lactose detects the presence of large-pore defects; (iv) increased sucrose absorption occurs with damage/injury to intestinal mucosa. Neither lactulose nor mannitol is metabolized to any significant degree in the small intestine but may be metabolized in the large intestine.
Application: Evaluation of intestinal absorption and permeability as parameters of mucosal integrity.
Dosage: After an overnight fast, individuals are administered a hyperosmolar sugar absorption test solution (2 g mannitol, 5 g lactulose, and 40 g sucrose/100 ml [around 1,560 mmol/l]). A 2nd administration strategy involves the administration of a low-osmolar sugar solution (2 g mannitol and 5 g lactulose/100 ml [around 375 mmol/l]).
Considerations: Reliability of differential sugar absorption tests is hampered by a lack of standardization of the content and osmolarity of the test solutions.
Examples of Clinical Implementation: Evaluation of the effect of sugar test solution osmolality on the 5-hour urine excretion of orally administered lactulose and mannitol. All urine from the 5-hour period following ingestion of the test solution is then collected for analysis.
Interpretation and Analysis: Normal mannitol concentrations imply normal intestinal absorptive capacity and low mannitol concentrations suggest diminished gastrointestinal absorptive capacity. Low lactulose absorption levels suggest that the intestinal mucosa is normal, in contrast to situations of increased passive intestinal lactulose abosorption, which implies that the integrity of the intestinal mucosa barrier has been compromised. Hyperosmolar sugar formulations appear to discriminate better between normal and damaged mucosa of the small bowel such as villous atrophy due to a relative increase in permeability for lactulose.

The sensitivity of hyperosmolar SAT solution and low-osmolar solution for the detection of mucosal abnormalities in celiacs is 64% and 43%, respectively.

Instrumentation: Gas chromatography analysis of lactulose and mannitol urine concentrations.

Sensitivity of a hyperosmolar or "low"-osmolar test solution for sugar absorption in recognizing small intestinal mucosal damage in celiac disease. Uil JJ, van Elburg RM, Janssens PM, Mulder CJ, Heymans HS. Dig Liver Dis 2000 Apr; 32 (3), pp. 195–200.

Intestinal Absorption/Accelerated Physical Development/Correction of Coarctation of the Aorta vs Intestinal Absorption
Xylose

Cellular/Molecular Mechanism of Action: Xylose is absorbed by the intestinal mucosa of the small intestine.
Application: Evaluation of intestinal absorption capacity.
Route of Administration: Xylose (oral/per os).
Implementation: Delineation of surgical correction of coarctation of the aorta or improved intestinal absorption contributes to postoperative accelerated physical development in children.

Human Model: Postoperative surgical correction of coarctation of the aorta (n = 9).
Interpretation and Analysis: Lowered values of the oral xylose absorption *test* are found in all cases prior to surgical correction. In all postoperative cases, sphigmo-oscillometry reveals increases in pulsatile character of the blood flow, pulsatile volume in the lower extremities, and a rise of mean arterial blood pressure in this area. On the other hand, surgical repair has no influence on renal blood flow as evidenced by PAH clearance and isotope renography. There is no correlation between diuresis and the xylose excretion test. Increased values of the xylose test after operation for coarctation of the aorta are due to the increased intestinal absorption rather than to improved renal function. Improved intestinal absorption may be a factor responsible for the accelerated physical development of children operated on for coarctation of the aorta.

Xylose absorption test before and after surgical repair of coarctation of the aorta. Markiewicz A, Wojczuk K, Zmudzicanski J. Acta Hepatogastroenterol (Stuttg) 1975 Apr; 22 (2), pp. 112–7.

Intestinal Absorption (Carrier-Mediated Transport)/Intestinal Permeability (Unmediated Diffusion)/Influence of Age and Body Size
Lactulose:L-Rhamnose Ratio/D-Xylose:3-O-Methyl-D-Glucose Ratio

Cellular/Molecular Mechanism of Action: Lactulose, L-rhamnose, D-xylose, and 3-O-methyl-D-glucose are molecular carbohydrate sugars. Lactulose (L) and rhamnose (R) are not significantly metabolized in the intestine. Lactulose and rhamnose are not normally absorbed across intact mucosal surfaces of the small intestine. The lactulose/rhamnose ratio can be used as an indicator of increased intestinal permeability (large-pore/small-pore defects, respectively). Similarly, xylose and methylglucose are normally absorbed across healthy intestinal mucosa, which is dependent upon carrier-mediated mechanisms residing primarily within the jejunum.
Application: Evaluation of intestinal permeability (unmediated diffusion) as measured by the ratio of urinary lactulose to L-rhamnose (L:R ratio) and intestinal absorption (carrier-mediated transport) as measured by the ratio of urinary D-xylose to 3-O-methyl-D-glucose (X:MG ratio).
Route of Administration: Oral.
Implementation: Evaluation of the influence of the variables of age and body size on estimates of intestinal permeability and absorption using lactulose, rhamnose, xylose, and 3-O-methyl-D-glucose as investigative molecular probes. In this context, solutions containing lactulose and rhamnose (L:R); or xylose and 3-O-methyl-D-glucose (X:MG) are administered orally. Urine is then collected 6 hours later, and urinary L:R and X:MG concentration ratios calculated. Fecal moisture and scoring are recorded during the same periods. Measures of intestinal absorption (unmediated diffusion) and intestinal permeability (carrier-mediated transport) are assessed through the measurement/calculation of urinary L:R ratios and urinary X:MG ratios, respectively. These findings are then compared to the properties of fecal samples to identify possible correlating variables.

Animal Model: Canine (age = 12, 22, 36, and 60 weeks old; n = 6 miniature poodles, n = 6 standard schnauzers, n = 6 giant schnauzers, and n = 6 Great Danes).
Interpretation and Analysis: Age and breed do not appear to affect intestinal absorption. A relationship between X:MG and fecal variables also does not appear to exist. Alternatively, significant effects of age and body size on intestinal permeability can be detected. Puppies (12 weeks old) and large dogs have higher intestinal permeability levels than adult (60 weeks old) and small dogs. The increased intestinal permeability in large dogs is associated with lower fecal quality as indicated by the significant positive correlations between L:R ratio and fecal moisture (r, 0.61) and L:R ratio and fecal scores (r, 0.86) in adult dogs. Such observations indicate that age and body size should be considered when assessing intestinal permeability by use of the L:R urinary excretion test in dogs. High intestinal permeability could be a possible cause of poor fecal quality in large dogs.

Influence of age and body size on intestinal permeability and absorption in healthy dogs. Weber MP, Martin LJ, Dumon HJ, Biourge VC, Nguyen PG. Am J Vet Res 2002 Sep; 63 (9), pp. 1323–8.

Intestinal Absorption: Malabsorption and Compromised Mucosal Integrity
Mannitol/Raffinose/Sucrose/Lactulose

Cellular/Molecular Mechanism of Action: Lactulose and raffinose are poorly absorbed from the small intestine. Mannitol is normally absorbed across the surface of intact small intestinal mucosa. In conditions of injury to the small intestine, sucrose and lactulose absorption increases. Ratios of lactulose/mannitol for example can be therefore be applied to detect increases in intestinal permeability. Lactulose, mannitol, and raffinose are not metabolized in the small intestine, but they can be metabolized in the large intestine.
Application: Detection of conditions associated with increased intestinal permeability.
Implementation: Four enzyme (biochemical-based) methods have been developed for the measurement of the urinary concentration of the probe sugars mannitol, raffinose, lactose and sucrose. Mannitol, lactose and sucrose are measured directly through the use of mannitol dehydrogenase, beta-galactosidase and invertase, respectively. Raffinose measurement needs a 3-hour preincubation with alpha-galactosidase, after which biochemically generated sucrose is measured.

The analytical performance parameters such as within- and between-run precision, linearity, lowest detection limit, interference of other sugars and comparison with a gas chromatographic methods have been described.

Assessment of intestinal permeability: Enzymatic determination of urinary mannitol, raffinose, sucrose and lactose on Hitachi analyzer. Hessels J, Snoeyink EJ, Platenkamp AJ, Voortman G, Steggink J, Eidhof HH. Clin Chem Lab Med 2003 Jan; 41 (1), pp. 33–8.

Intestinal Absorption: Malabsorption and Compromised Integrity/Bacterial Overgrowth: Small Intestine
D-Xylose/[^{14}C]D-Xylose

Cellular/Molecular Mechanism of Action: D-xylose is absorbed from the small intestine (jejunum) by glucose active transport mechanisms. D-xylose compared to L-xylose is relatively poorly metabolized.
Diagnostic Application: Diagnosis of malabsorption attributed to the small intestinal segment of the gastrointestinal tract.
Route of Administration and Dosage: D-xylose can be give as an alternative to glucose at a dose rate of 0.5 to 1.0 g/kg orally as a 10% solution following a 12-hour period of fasting (e.g., equine). Similar to glucose, preparations of D-xylose can be administered orally by nasogastric intubation (e.g., equine). In humans, 25 g is 1 of the "standard" D-xylose doses used diagnostically.
Considerations: D-xylose is superior to glucose for the detection of small intestinal malabsorption because it is more resistant to catabolism by gastrointestinal bacterial flora.
Implementation: The standard D-xylose test in human adults is based on analysis of 5-hour urine collection and a 1-hour serum sample.
Interpretation and Analysis: The D-xylose test can help discriminate between normal subjects and conditions of proximal small intestinal malabsorption with greater than 95% specificity and sensitivity. The 1-hour serum level measured after administering this dose is also useful in evaluating malabsorption in cases with intermediate degrees of renal insufficiency and in geriatric cases. The 1-hour serum test after administration of 5 g of D-xylose should be used in human (and possibly equine) pediatrics and is greater than 91% sensitive and close to 100% specific. The [^{14}C]D-xylose breath test with 1 g of D-xylose has been useful in identifying malabsorption caused by bacterial overgrowth in the small intestine in humans.

D-xylose testing: A review. Craig RM, Atkinson AJ Jr. Gastroenterology 1988 Jul; 95 (1), pp. 223–31.

Intestinal Absorption: Malabsorption Due to Acute Graft vs Host/Myeloablative Conditioning
[^{51}Cr]-Labeled Ethylenediaminetetraacetic Acid (EDTA) Absorption

Application: Detection of alterations in intestinal absorption.
Background Information: The efficacy of allogeneic, hemopoietic stem cell transplantation is limited by concomitant toxicity. This has led to the development of less toxic, reduced intensity conditioning (RIC) protocols, whose therapeutic benefit is largely related to an associated, immunity-mediated graft vs malignancy effect rather than by the cytotoxic treatment itself. Murine hemopoietic stem cell transplantation models suggest that acute graft vs host disease increases with the intensification of the conditioning regimen mediated by loss of integrity of the gut mucosa barrier.
Implementation: Evaluation of gastrointestinal permeability during allogeneic hemopoietic stem cell transplantation procedures can be performed utilizing the [^{51}Cr]-EDTA absorption test.
Interpretation and Analysis: In treatment protocols classified as reduced-intensity conditioning, significant increases in intestinal permeability are not appreciated. Alternatively, in myeloablatively conditioned cases there may be a significant increase in intestinal permeability the day before stem cell infusion ($p < 0.005$), on day 4 ($p < 0.005$), on day 7 ($p < 0.01$), and on day 14 ($p < 0.005$) after stem cell infusion, compared with baseline values. Myeloablative conditioning is also associated with increases in intestinal permeability on day 7 compared with reduced-intensity conditioning ($p < 0.05$). The finding of preserved intestinal-barrier function during allogeneic hemopoietic stem cell transplantation with reduced intensity conditioning is believed to be consistent with the hypothesis that GI tract damage may be an important event in the initiation of acute graft vs host reactions.

The gut mucosa barrier is preserved during allogeneic, hemopoietic stem cell transplantation with reduced intensity conditioning. Johansson JE, Brune M, Ekman T. Bone Marrow Transplant 2001 Oct; 28 (8), pp. 737–42.

Intestinal Absorption/Permeability
Lactulose/Mannitol Excretion Ratio [Urinary]

Cellular/Molecular Mechanism of Action: Lactulose and mannitol are both carbohydrate molecules. Lactulose is not normally absorbed in significant amounts across the surface of intact, healthy intestinal mucosa. In contrast, mannitol is absorbed across the surface of normal small intestinal mucosa. Neither lactulose nor mannitol is metabolized to any significant degree in the small intestine but may be metabolized in the large intestine.
Background Information: The dysfunction of the intestinal barrier is a factor that has been related to bacterial translocation from lumen to extraintestinal sites and consequently to the development of spontaneous bacterial peritonitis.
Application: Detection and characterization of alterations in intestinal mucosal absorptive capacity and assessment of the function of this anatomical structure as an effective barrier (e.g., permeability). In this context, lactulose can be used to detect large-pore defects.
Route of Administration: Lactulose (oral/per os) and mannitol (oral/per os).
Dosage: Lactulose (10 grams) and mannitol (5 g).
Implementation: Evaluation of intestinal permeability in conditions of hepatic cirrhosis utilizing measurements of lactulose/mannitol urinary excretion ratios. Concentrations are measured in amounts of urine collected over a 5-hour period following administration.
Human Model: Cases of hepatic cirrhosis ($n = 79$; $n = 25$ negative reference controls).
Interpretation and Analysis: Lactulose/mannitol ratios are significantly higher in cirrhotic patients than in negative reference controls ($p = 0.03$). The pattern becomes more evident in cases of end-stage cirrhosis. Patients with ascites, or encephalopathy show a statistically significant increase in lactulose/mannitol ratio compared to cases without these complications. Increases in intestinal permeability are related to the progression of liver disease and is more relevant with overt clinical complications. This is due to an increased absorption of lactulose. However, as liver disease progresses, mannitol absorption progressively decreases, probably due to a reduced surface of absorption, and these events are more relevant in patients with overt clinical complications.

Intestinal permeability is increased in patients with advanced cirrhosis. Pascual S, Such J, Esteban A, Zapater P, Casellas JA, Aparicio JR, Girona E, Gutiérrez A, Carnices F, Palazón JM, Sola-Vera J, Pérez-Mateo M. Hepatogastroenterology 2003 Sep–Oct; 50 (53), pp. 1482–6.

Intestinal Absorptive Capacity Posttrauma or during Sepsis/Post-Blunt Trauma/Severe Sepsis
D-Xylose

Cellular/Molecular Mechanism of Action: D-xylose is absorbed across the mucosa surface of the small intestine (jejunum) by passive carrier-mediated transport mechanisms.

Background Information: Although clinical studies suggest enteral, as opposed to parenteral, feeding lowers morbidity and mortality rates following severe trauma and after sepsis, it is unknown whether gut absorptive capacity (GAC) is indeed maintained under such conditions.
Application: Detection and characterization of alterations in intestinal absorptive capacity.
Route of Administration: D-xylose (nasogastric tube).
Dosage: D-xylose (25 g in 200 mL water).
Implementation: Evaluation of gut absorptive capacity (GAC) applying a 1-hour D-xylose absorption test in cases of blunt trauma or sepsis. Trauma and intra-abdominal sepsis cases receive an initial D-xylose test within 24 to 48 hours of admission, at 72 to 96 hours, and then weekly until D-xylose absorption had returned to normal.

Human Model: Cases of blunt trauma ($n = 8$); intense sepsis ($n = 11$); or normal reference controls ($n = 8$) excluding those with ileus, nasogastric output of more than 600 mL/24 hours, or residual gastric content of more than 25 mL after the D-xylose test. Trauma patients (ISS 8-14) and patients with intra-abdominal sepsis are acceptable candidates for being analyzed with the D-xylose test. Cases of ileus, nasogastric output of more than 600 mL/24 hours, or residual gastric content of more than 25 mL after the D-xylose test are excluded.
Interpretation and Analysis: Analyses reveal that gut absorptive capacity is depressed at 24 to 96 hours in both groups but returns to normal by 1 to 3 weeks after trauma or resolution of sepsis. Thus (1) gut absorptive capacity is severely depressed early after trauma and after the onset of sepsis; and (2) the 1-hour D-xylose absorption test represents a simple, quantitative assessment of GAC in critically ill patients. Hence, therapeutic agents that restore gut absorptive capacity may be useful for further reducing morbidity and mortality rates following trauma or the onset of sepsis.

Severe depression of gut absorptive capacity in patients following trauma or sepsis. Singh G, Harkema JM, Mayberry AJ, Chaudry IH. J Trauma 1994 Jun; 36 (6), pp. 803–8; discussion 808–9.

Intestinal Absorptive Function/ Intestinal Permeability
Lactulose (L)/Rhamnose (R)/D-Xylulose (X)/ 3-O-Methylglucose

Cellular/Molecular Mechanism of Action: D-xylulose (X) and 3-O-methylglucose are normally absorbed across healthy mucosa of the small intestine involving passive and active carrier-mediated transport mechanisms, respectively, residing within the jejunum. Alternatively, lactulose and rhamnose are not absorbed across intact intestinal mucosal surface. Neither lactulose nor rhamnose is metabolized to any significant degree within the small intestine but metabolism may occur within the large intestine.
Application: Evaluation of intestinal function and integrity. The lactulose/rhamnose ratio can be applied to detect increased intestinal permeability (large-pore/small-pore defects, respectively).
Route of Administration: The sugar analogs D-xylulose (X), 3-O-methylglucose (G), lactulose (L), and rhamnose (R) can be administered orally.
Implementation: Evaluation of intestinal function applying a differential sugar analog challenge. Intestinal absorption can be evaluated using D-xylulose (X) and 3-O-methylglucose (G). In contrast, intestinal permeability can be assessed using lactulose (L) and rhamnose (R) as molecular probes.

Animal Model: Canine ($n = 48$ healthy Irish setter dogs).
Interpretation and Analysis: Urinary L/R ratios in 48 Irish setters from 1 to 12 years old vary from 0.03 to 0.18, with a mean (SEM) of 0.10 ± 0.01; X/G ratios vary from 0.46 to 0.81, with a mean (SEM) of 0.59 ± 0.01. There are no significant differences between L/R or X/G ratios of dogs of different sex ($p > 0.2$) or age ($p > 0.5$), using analysis of covariance. Lactulose/rhamnose ratios of ≥ 0.18 and X/G ratios ≥ 0.43 are considered normal, defined by the respective mean $\pm$ 2SD. Repeatability is established by performing 3 permeability and function tests at monthly intervals in 12 of the dogs. Analysis of repeated L/R and X/G ratios by means of linear models procedure reveals no significant differences between measurements made on successive occasions ($p > 0.15$), confirming the repeatability of the test.

Reference range and repeatability of a combined intestinal permeability and function test in clinically healthy Irish setter dogs. Garden OA, Rutgers HC, Sørensen SH, Daniels S, Walker D, Batt RM. Res Vet Sci 1997 Nov–Dec; 63 (3), pp. 257–61.

Intestinal Malabsorption and Compromised Mucosal Integrity
Lactulose/Mannitol

Cellular/Molecular Mechanism of Action: Mannitol is, but lactulose is not, absorbed across the surface of intact mucosa of the small intestine. Neither mannitol nor lactulose is metabolized within the small intestine but they may be metabolized within the large intestine. Lactulose and mannitol are both excreted into the urine.
Background Information: In conditions of gastrointestinal cancer, the mean permeability index (PI) significantly increases after chemotherapy compared with the prechemotherapeutic values ($p < 0.01$). No significant difference in PI is observed with most neoplastic states affecting organ systems outside the gastrointestinal tract.
Application: Assessment of the extent of mucosal damage as a consequence of infectous disease and chemotherapeutic administration. In this context, the lactulose/mannitol ratio can be used to detect the presence of increased intestinal permeability.
Implementation: The permeability index (PI) for the intestine is measured by establishing the urinary recovery ratio of excreted lactulose and mannitol.
Interpretation and Analysis: Because the oral L/M absorption test is useful for assessing the degree of mucosal damage and measurement of intestinal permeability, this form of analysis should be recommended to determine the optimum timing and the adequate dosage of antineoplastic chemotherapeutic agents.

Assessment of the intestinal permeability following postoperative chemotherapy for human malignant disease. Inutsuka S, Takesue F, Yasuda M, Honda M, Nagahama S, Kusumoto H, Nozoe T, Korenaga D. Eur Surg Res 2003; 35 (1), pp. 22–5.

Intestinal Malabsorption and Compromised Mucosal Integrity/Neonate Intestinal Integrity and Functional Capacity
Lactulose/Rhamnose/D-Xylose/3-O-Methyl-D-Glucose

Cellular/Molecular Mechanism of Action: Lactulose and rhamnose are not absorbed across intact intestinal mucosal surfaces. Neither lactulose nor rhamnose is metabolized within the small intestine but may be metabolized within the large intestine. The lactulose/rhamnose ratio can be used to detect large-pore/small-pore intestinal defects. In conditions of increased intestinal permeability, both lactulose and rhamnose enter the intravascular compartment and are excreted into the urine. D-xylose and 3-O-methyl-D-glucose are absorbed across the intestinal mucosa by passive and carrier-mediated monosaccharide absorptive mechanisms, respectively, residing largely within the jejunum.

Background Information: Immaturity of intestinal epithelial barrier function and absorptive capacity may play a role in the pathophysiology of intestinal complications in preterm neonates during the early postnatal period.
Application: Evaluation of intestinal mucosal integrity and functional capacity.
Implementation: Establishing the urinary concentration ratios of lactulose and rhamnose following their oral administration can be used to identify conditions of increased intestinal permeability. Urinary excretion percentages of D-xylose and 3-O-methyl-D-glucose can be calculated to assess passive and active carrier-mediated monosaccharide mucosal absorption, respectively.
Interpretation and Analysis: In humans, the barrier function of the intestinal epithelium transiently decreases during the 1st week after birth in preterm neonates who are not enterally fed. Diminished barrier function and low monosaccharide absorptive capacity, particularly in neonates < 28 weeks, can increase vulnerability to the development of intestinal complications during the early postnatal period.

Intestinal permeability transiently increases between day 1 and day 7 in human neonates ($p < 0.05$). Carrier-mediated monosaccharide absorption increases between day 1 and day 14 in neonates of 28 to 30 weeks ($p < 0.05$) to the level observed in the neonates of 30 to 32 weeks gestation. In neonates > 28 weeks, intestinal permeability at day 7 is higher ($p < 0.05$) and carrier-mediated monosaccharide absorption at day 14 is lower ($p < 0.01$) compared with neonates $\geq$ 28 weeks of age.

Intestinal permeability and carrier-mediated monosaccharide absorption in preterm neonates during the early postnatal period. Rouwet EV, Heineman E, Buurman WA, ter Riet G, Ramsay G, Blanco CE. Pediatr Res 2002 Jan; 51 (1), pp. 64–70.

Intestinal Malabsorption and Compromised Mucosal Integrity
Maltose Absorption

Background Information: Successful small bowel transplantation requires effective immunosuppression that preserves intestinal function but avoids opportunistic infection.
Application: Evaluation of intestinal function and integrity.
Example of Clinical Implementation: Maltose absorption studies have been applied to evaluate various types of immunosuppressant agents.
Interpretation and Analysis: Control allografts (heterotopic small bowel transplants) without the benefit of immunosuppressive therapy are abnormal on day 7 and reflect the presence of severely damaged intestinal architecture that can be visualized on histopathological examintion. In contrast, subjects receiving immunosuppressive therapy display well-protected intestinal structure and normal maltose absorption on days 7 and 21. Subnormal plasma concentrations of maltose following administration implies the existence of abnormal function of small intestinal tissues.

Recipient FK506 pretreatment regimens in rat small bowel transplantation: Allograft survival, function, and systemic infection. Guo WH, Tian L, Yuen ZW, Chan KL, Wo JY, Nicholls G, Dallman M, Tam PK. J Pediatr Surg 2000 Nov; 35 (11), pp. 1600–5.

Intestinal Permeability: Crohn's Disease/Genetic Carriers
Polyethylene Glycol 400/Lactulose/ Rhamnose/Mannitol

Background Information: Lactulose, mannitol, and rhamnose are not metabolized in the small intestine but may be metabolized in the large intestine. Lactulose and rhamnose are normally not absorbed across intact intestinal mucosa, in contrast to mannitol, which is absorbed across intact small intestinal mucosal surfaces.
Application: Lactulose/mannitol and lactulose/rhamnose ratios can be applied to detect injury to small intestinal mucosa surfaces (e.g., increased permeability). The lactulose/rhamnose ratio can be used to detect large-pore/small-pore defects in intestinal permeability.
Implementation: Evaluation of intestinal mucosa integrity and function in healthy relatives of cases with Crohn's disease previously found to have increased intestinal permeability to polyethylene glycol 400. Alternative probe carbohydrates that can be applied for this purpose include lactulose, rhamnose, and mannitol.
Human Model: Cases of Crohn's disease ($n = 25$); healthy relatives of Crohn's disease cases ($n = 41$); healthy negative reference controls without a history of inflammatory bowel disease ($n = 29$).
Interpretation and Analysis: Patients with Crohn's disease have increased lactulose permeability when compared with relatives or controls. Lactulose absorption by patients with Crohn's disease is $0.41\% \pm 0.07\%$ (mean $\pm$ SE), whereas that of their relatives and unrelated controls is $0.28\% \pm 0.03\%$ and $0.26\% \pm 0.03\%$, respectively. There is no significant difference between the relatives and controls, but both groups differed from the patients ($p < 0.05$ and $p < 0.025$, respectively). The patients' lactulose/rhamnose ratio is approximately $70.5\% \pm 9.2\%$ vs $37.2\% \pm 3.3\%$ in relatives and $40.6\% \pm 5.7\%$ in unrelated controls ($p < 0.0005$ and $p < 0.0025$, respectively). The 2 intermediate-sized probes, rhamnose and mannitol, are not able to detect permeability differences among the 3 groups. The inability of lactulose, rhamnose, or mannitol to detect permeability abnormalities in healthy relatives of patients with Crohn's disease suggests that these probes penetrate the intestinal barrier by routes or mechanisms that are different from those of polyethylene glycol 400. Lactulose, in particular, detects permeability changes in patients with intestinal inflammation, and polyethylene glycol 400 is able to detect permeability changes in the healthy relatives of patients. Such findings indicate that permeability may be abnormal as a secondary result of inflammation or as a result of a primary genetic abnormality.

Intestinal permeability in patients with Crohn's disease and their healthy relatives. Katz KD, Hollander D, Vadheim CM, McElree C, Delahunty T, Daf-dufalza VD, Krugliak P, Rotter JI. Gastroenterology 1989 Oct; 97 (4), pp. 927–31.

ABSORPTION AND TRANSIT TIME: NON-CARBOHYDRATE-BASED AGENTS

Intestinal Absorption: Idiopathic Bile Acid Malabsorption (IBAM)/Diarrhea as a Rare Etiology
[^{75}Se]-Homocholic Acid Taurine (^{75}SeHCAT)

Cellular/Molecular Mechanism of Action: [^{75}Se]-homocholic acid taurine is a radiolabeled analog of homocholic acid taurine, which is an endogenous bile acid.
Background Information: Idiopathic bile acid malabsorption is a rare cause of diarrhea.
Application: Assessment of IBAM utilizing [^{75}Se]HCAT.
Implementation: Measurement of the proportion of [^{75}Se]-HCAT retained 7 days after its ingestion in cases of IBAM.
Human Model: Cases of IBAM ($n = 23$; $n = 3$ lost to follow-up; $n = 3$ died from malignancy; $n = 3$ inflammatory bowel disease). The mean period of follow-up for the remaining 14 patients = 99.2 months (range 48–140).

Cases that had previously responded well to treatment with a bile acid chelator (cholestyramine or aluminium hydroxide).

Questionnaires relating to current clinical symptoms and prescriptions had been sent to cases and their general practitioners.

Interpretation and Analysis: Many cases show an improvement in symptoms and no longer require treatment with cholestyramine (n = 7/23). Approximately one-third of symptomatic cases (e.g., n = 7/23) experience diarrhea that is well controlled with continued treatment with cholestyramine (n = 5) or standard antidiarrheal treatment (n = 2). In symptomatic (e.g., n = 7/7) and asymptomatic cases (e.g., n = 3) that undergo repeat 7-day [^{75}Se]HCAT tests, tests reveal that asymptomatic cases essentially all improve so that the retention of the tracer after 7 days is above 5%; all but a low percentage of symptomatic cases (e.g., n = 2/7) still have values under 5%. (Note: Small numbers of asymptomatic and symptomatic cases can preclude performing statistical analyses.) Idiopathic bile acid malabsorption is a rare cause of diarrhea and should be diagnosed only after malignancy and inflammatory bowel disease have been excluded by rigorous investigations. Cases should be followed up as some develop other serious gastrointestinal diseases. Approximately 50% of cases have remitted spontaneously and no longer require medication with bile acid chelators or antidiarrheal agents.

Idiopathic bile acid malabsorption: Long-term outcome. Luman W, Williams AJ, Merrick MV, Eastwood MA. Eur J Gastroenterol Hepatol 1995 Jul; 7 (7), pp. 641–5.

Intestinal Absorption: Idiopathic Bile Acid Malabsorption (IBAM)
[^{75}Se]-Homocholic Acid Taurine (^{75}SeHCAT)

Cellular/Molecular Mechanism of Action: [^{75}Se]-homocholic acid taurine is a radiolabeled analog of homocholic acid taurine, which is an endogenous bile acid component.

Application: Detection of cases suffering from IBAM.

Implementation: Assessment of chronic diarrhea of unknown etiology using the [^{75}Se]HCAT test as a "late-stage" diagnostic procedures due to the potential for the condition to be associated with IBAM.

Repeated [^{75}Se]HCAT tests applied in combination with clinical follow-up with patient interview and information from medical records. In this manner, it is possible to describe the long-term clinical course of the disease and assess the reliability of the [^{75}Se]HCAT test in diagnosing IBAM.

Human Model: Cases suffereing from chronic diarrhea (n = 150); chronic diarrhea cases affected by IBAM (n = 20; n = 16/20 that have completed both clinical follow-up and a new [^{75}Se]HCAT test; n = 6/30 lost to follow-up; n = 4/30 excluded from reexamination because of old age [> 80 years] or bowel resection). Median duration of symptoms before initial [^{75}Se]HCAT tests = 2.5 years (1- to 30-year range).

Interpretation and Analysis: On basis of low [^{75}Se]HCAT values and response to cholestyramine treatment, cases can initially be classified as having IBAM (e.g., n = 33/150). The diagnosis of having IBAM may have to be revised in some previously evaluated cases (n = 3; n = 2 inflammatory bowel disease; n = 1 *Clostridium difficile*). In a large percentage of cases (n = 13/16) symptoms persist, and [^{75}Se]HCAT values remain low and almost identical to initial values after a median observation time of 88 (51–113) months. Despite initial response to treatment with cholestyramine, in some instances, treatment has to be discontinued because of adverse effects or other compliance problems (e.g., n = 6/16 cases). In a small percentage of cases (e.g., n = 3/16) the [^{75}Se]HCAT value substantially increases, and bowel function correspondingly normalizes. The [^{75}Se]HCAT test appears to be reliable for diagnosing conditions of IBAM. Despite adult onset of symptoms, only a few patients improve after several years of observation. Treatment with cholestyramine is generally effective but not always tolerated.

Prognosis of adult-onset idiopathic bile acid malabsorption. Rössel P, Sortsøe Jensen H, Qvist P, Arveschoug A. Scand J Gastroenterol 1999 Jun; 34 (6), pp. 587–90.

Intestinal Absorption: Malabsorption of Bile Acid/Chronic Diarrhea and Collagenous Colitis
[^{75}Se]-Homocholic Acid Taurine ([^{75}Se]HCAT)

Cellular/Molecular Mechanism of Action: [^{75}Se]-homocholic acid taurine is a radiolabeled analog of homocholic acid taurine, which is an endogenous bile acid component.

Background Information: Bile acid malabsorption may be observed in patients with collagenous colitis.

Application: Characterization of bile acid malabsorption in conditions like collagenous colitis as a cause of chronic diarrhea.

Implementation: Evaluate the occurrence of bile acid malabsorption and the effect of bile acid binders prospectively in conditions of chronic diarrhea and collagenous colitis. Diagnostic intervention strategies can include the application of gastroscopy with duodenal biopsy, colonoscopy with biopsies, and analysis utilizing the [^{75}Se]HCAT test for bile acid malabsorption. Irrespective of diagnostic findings with [^{75}Se]HCAT, cases are treated with a bile acid binder (n = 24 cases with cholestyramine; n = 3 cases with colestipol).

Human Model: Cases of chronic diarrhea.

Interpretation and Analysis: In cases of collagenous colitis (n = 28; n = 6/28 men, n = 22/28 women), a large percentage have persistent symptoms (n = 27/28). A small number of cases (e.g., n = 4) have had a previous cholecystectomy or a distal gastric resection. The [^{75}Se]HCAT test is abnormal in approximately 44% of cases (e.g., n = 12/27) with collagenous colitis patients with [^{75}Se]HCAT values 0.5–9.7%, and normal in about 56% of cases (e.g., n = 15/27). Bile acid binding treatment is followed by a rapid, marked, or complete improvement in about 78% of collagenous colitis cases (e.g., n = 21/27). Rapid improvement occurs in approximately 92% of cases (e.g., n = 11/12) with bile acid malabsorption compared with 67% (e.g., n = 10/15) of cases with normal [^{75}Se]HCAT tests. Bile acid malabsorption is common in cases with collagenous colitis and is probably an important pathophysiological factor. Because of a high response rate without serious side effects, bile acid binding treatment should be considered for collagenous colitis, particularly patients with bile acid malabsorption.

Role of bile acids and bile acid binding agents in patients with collagenous colitis. Ung KA, Gillberg R, Kilander A, Abrahamsson H. Gut 2000 Feb; 46 (2), pp. 170–5.

Intestinal Absorption: Malabsorption and Compromised Integrity
Cobalamin/[Vitamin B_{12}]

Physiological Mechanism of Action: Cobalamin is normally absorbed across the ileal mucosa of the distal small intestine.

Background Information: Malabsorption of food-bound cobalamin is generally presumed to arise from gastritis and/or achlorhydria.

Application: Evaluation of gastrointestinal absorptive function.

Route of Administration: Cobalamin (oral).

Considerations: Decreased serum vitamin B_{12} can be appreciated with bacterial overgrowth of the small intestine as can occur with hypochlorhydria in atrophic gastritis.

Interpretation and Analysis: In conditions of documented severe malabsorption, histiological evidence of pathology can be appreciated along with lower acid and pepsin secretion values compared to normal reference control subjects. Statistically, there can be an overlap of these same parameters between conditions of severe vs mild malabsorption/achlorhydria/gastric atrophy. In humans, food cobalamin malabsorption is not always synonymous with atrophic gastritis and achlorhydria, and hypochlorhydria does not always guarantee food cobalamin malabsorption.

Heterogeneity of gastric histology and function in food cobalamin malabsorption: Absence of atrophic gastritis and achlorhydria in some patients with severe malabsorption. Cohen H, Weinstein WM, Carmel R. Gut 2000 Nov; 47 (5), pp. 638–45.

Intestinal Absorption: Malabsorption and Compromised Integrity/Jejunum Brush Border
Folic Acid/[Pteroylpolyglutamates]

Physiological Mechanism of Action: Dietary folates (pteroylpolyglutamates) are selectively absorbed from primarily the jejunum and ileal segments of the small intestine.
Background Information: Dietary folates exist as pteroylpolyglutamates (PteGlun) that undergo hydrolysis to pteroylmonoglutamate (PteGlu) forms during the process of intestinal absorption.
Diagnostic Application: Diagnosis of intestinal malabsorption conditions affecting the distal small intestine (ileum) based upon the observation of abnormally low folic acid concentrations following oral administration.
Route of Administration: Oral administration either by ingestion or nasogastric tube.
Considerations: Elevations in serum folic acid concentrations occurs with bacterial overgrowth of the small intestine.
Implementation: Evaluation of jejunal folate hydrolysis applying the technique of jejunal perfusion with separately labeled folate preparations. Note: In clinical practice, "cold" and radiolabeled folate can be applied as diagnostic probes.
Interpretation and Analysis: Pteroylpolyglutamates are hydrolyzed on the surface of the jejunum and is a prerequisite for folate absorption. An intestinal brush border pteroylpolyglutamate hydrolase (BB-PPH) has been identified in human and pig jejunum with characteristics that are distinct from those of an intracellular hydrolase (IC-PPH). Functional parallels of BB-PPH with *in vivo* hydrolysis of PteGlun in human and pig intestine and the clinical responsiveness of BB-PPH to different disease states indicate that this enzyme plays the major physiological role in folate absorption. Folate malabsorption is found in diseases that affect the jejunal mucosa and in response to various drugs. In most of these clinical conditions, folate malabsorption results from suppression of both of the processes of hydrolysis of PteGlun and jejunal uptake of PteGlu. Studies in miniature pigs have defined the sequence of development of folate malabsorption in chronic alcoholism.

The intestinal absorption of dietary folates in health and disease. Halsted CH. J Am Coll Nutr 1989 Dec; 8 (6), pp. 650–8.

Intestinal Absorption: Malabsorption and Compromised Integrity
Theophylline

Application: Evaluation of gastrointestinal absorption capacity, pharmaceutical bioavailability, and owner/patient non-compliance.
Route of Administration: Theophylline (regional perfusion example).
Dosage: Theophylline (5 mg/kg).
Implementation: Evaluation of intestinal integrity and function relative to theophylline absorption. One approach to achieving this objective includes simultaneous perfusion of 4 segments of equal length (jejunum, ileum, right colon and left colon) with saline solution containing [^{3}H]-polyethylene glycol 4000 to measure water shifts and theophylline at a total dose of 5 mg/kg, a typical neonatal dose. Absorption *of* theophylline, appearance in serum, and the effects of the drug on water and electrolyte movement in the intestinal segments are then monitored. Serum concentrations of theophylline after an intravenous and oral dose repesent a more practical approach to the clinical application of theophylline absorption studies.
Animal Model: Swine: (n = 13 newborn piglets; ages 9–14 days with surgically exposed segments of the intestine).
Interpretation and Analysis: Theophyllin displays excellent absorption of the drug from all intestinal segments studied (jejunum 0.97 ± 0.16, ileum 0.7 ± 0.13, right colon 0.7 ± 0.13, and left colon 0.88 ± 0.19 μg/cm/min), despite a concomitant secretion of sodium chloride and water. No statistical differences in absorptive capacity can be appreciated between different segments. The results suggest that as little as 100 cm of residual intestine could, theoretically, absorb a 5 mg dose of theophylline if presented slowly. These findings have ramifications for neonates who may receive theophylline orally or rectally.

Absorption of theophylline from the small and large intestine of the neonatal piglet. Murray RD, Breech L, Ailabouni A, Zingerelli J, Nahata MC. Eur J Drug Metab Pharmacokinet 1993 Oct–Dec; 18 (4), pp. 375–9.

Pharmacokinetic analysis of theophylline to assess non-compliance in therapy. Konishi H, Nakatsuka T, Yoshida M, Tamaki S, Minouchi T, Yamaji A, Kinoshita M. Ann Pharmacother 2002 May; 36 (5), pp. 835–8.

Intestinal Absorption: Malabsorption Syndromes/ Compromised Integrity/Cholestasis
Vitamin D

Cellular/Molecular Mechanism of Action: Cholecalciferol is absorbed from the small intestine and is converted to 1-hydroxycholecalciferol in the liver and to 1,25-dihydroxycholecalciferol in the kidney.
Physiological Mechanism of Action: Vitamin D is absorbed from the duodenum and proximal jejunum. Because vitamin D is a fat-soluble vitamin, its absorption is directly linked to fat absorption and bile production/secretion.
Background Information: Vitamin D is essential for the maintenance of calcium and bone metabolism in humans. The recommended daily allowance (RDA) for vitamin D in the United States of 200 IU (5.0 μg) is reasonable for adults who receive some exposure to sunlight; however, in the absence of any exposure to sunlight, this recommendation may be 2 to 3 times lower than that actually required to satisfy the body's needs. Vitamin D was 1st measured by bioassays. However, bioassays became obsolete in light of the revelation that vitamin D must be activated 1st in the liver to 25-hydroxyvitamin D (25-OH-D) and then in the kidney to 1,25-dihydroxyvitamin D [1,25(OH)2D] before becoming biologically functional. Current assays measure circulating concentrations of vitamin D, 25-OH-D, or 1,25(OH)2D.

Rickets and osteopenia, common problems in chronic childhood cholestasis, have been attributed to vitamin D malabsorption leading to reduced serum levels of 25(OH)-vitamin D. D-alpha-Tocopheryl

polyethylene glycol-1000 succinate (TPGS), a water-soluble form of vitamin E, forms micelles at low concentration.
Application: Potential marker for abnormalities in intestinal integrity and function.
Route of Administration: Vitamin D_3 (oral).
Dosage: Vitamin D_3 1,000 IU/kg (maximum dose of 50,000 IU).
Implementation: Evaluation of the potential role of TPGS in enhancing vitamin D absorption in conditions of cholestasis or intestinal dysfunction. One approach to delineating this objective involves the administration of vitamin D_3 with and without TPGS (25 IU/kg). Serial serum vitamin D_3 levels and areas under the curve are then measured.

Human Model: Cases of severe childhood chronic cholestasis (n = 8; age 5 months–19 years; n = 3 extrahepatic biliary atresia; n = 3 non-syndromic intrahepatic cholestasis; n = 2 Alagille syndrome).
Interpretation and Analysis: Serum concentration of 25-OH-D is the most valuable parameter for determining the overall vitamin D status of an individual, because it is an average of dietary and sunlight-induced vitamin D. The measurement of the serum 1,25(OH) 2D concentrations has been most useful in evaluating disorders in calcium and bone metabolism related to acquired and inborn errors in the conversion of 25-OH-D to 1,25(OH)2D.

d-Alpha-tocopheryl polyethylene glycol-1000 succinate enhances the absorption of vitamin D in chronic cholestatic liver disease of infancy and childhood. Argao EA, Heubi JE, Hollis BW, Tsang RC. Pediatr Res 1992 Feb; 31 (2), pp. 146–50.

Intestinal Absorption/Small Bowel Transplant Rejection: Orthotopic/Jejunum vs Ileum vs Colon vs Rectum

Acetaminophen (Paracetamol)/Indomethacin/ [Acetylsalicylic Acids-Acylsalicylic Acids]/ [Butyrylsalicylic Acylsalicylic Acids]/ [Propionyl Acylsalicylic Acids]

Physiological Mechanism of Action: Lipophilic indomethacin has similar absorption profiles within the large and small intestines, while intermediately lipophilic acetaminophen is more rapidly absorbed in the small intestine than in the large intestine.
Application: Evaluation of small intestine integrity (e.g., transplant viability, reference controls for evaluation of candidate pharmaceutical agents).
Route of Administration: Intragastic.
Dosage: Acetaminophen (0.15 g/kg).
Example of Clinical Implementation: Detection of orthotopic small bowel transplantation rejection.
Implementation: Evalution of profiles for absorption vs drug molecular weight and absorption vs drug lipophilicity within both the small and large intestines. In this context, acetaminophen, indomethacin, and acylsalicylic acid analogs can be applied as a reference control for characterizing the absorption-molecular weight profiles for various experimental compounds (e.g., different-sized polyethylene glycols [PEGs]) to determine if differences occur between the small and large intestines).

Animal Model: Rats (intestine situ loop method).
Interpretation and Analysis: In the application of acetaminophen for assessing small intestine integrity/function, declines in absorption parameters correspond with histological evidence of progressive acute graft rejection (e.g., postoperative day 3 vs day 7).

Alternatively, acetaminophen can serve well as a reference control for assessing intestinal integrity/function. In this context, it has been shown that large-intestinal absorption of PEGs with molecular weights larger than 300 is poor, while PEGs with molecular weights up to 600 are relatively well absorbed in the small intestine. Paracellular route for drug penetration in the large intestine is restricted more than in the small intestine. The absorption-lipophilicity profiles can also be examined in various regions (loops of 6 cm) of (rat) intestine using 3 acylsalicylic acids, acetyl-, propionyl-, and butyrylsalicylic acids. The absorption rates of the acylsalicylic acids varies within different regions of the intestine: the jejunum > the ileum > the colon > the rectum. In each region, the absorption rates increase with the drug lipophilicity. However, it has been shown that the absorption rates in the small intestine tend to reach a ceiling at high lipophilicity. To confirm this tendency, the absorption rates of acetaminophen and indomethacin can be compared in the 4 intestinal regions. The absorption rates of highly lipophilic indomethacin are similar in the large and small intestines, while intermediately lipophilic acetaminophen is more rapidly absorbed in the small intestine than in the large intestine. A thicker unstirred water layer adjacent to the small intestinal mucosa would be 1 of the factors that cause such varying absorption-lipophilicity profiles.

Drug absorption from large intestine: Physicochemical factors governing drug absorption. Kimura T, Sudo K, Kanzaki Y, Miki K, Takeichi Y, Kurosaki Y, Nakayama T. Biol Pharm Bull 1994 Feb; 17 (2), pp. 327–33.

Evaluation of the acetaminophen absorption test for early detection of orthotopic small bowel transplant rejection. Miyauchi T, Ishikawa M, Tashiro S. Surg Today 2001; 31 (8), pp. 695–700.

Intestinal Absorption (Calcium): Changes during Menopause

Strontium Absorption

Cellular/Molecular Mechanism of Action: Strontium is absorbed from the intestinal tract.
Application: Evaluation of intestinal calcium absorption.
Route of Administration: Strontium (oral).
Dosage: Strontium (2.5 mmol).
Examples of Clinical Implementation: Evaluation of the influence of estrogen on intestinal strontium absorption in normal postmenopausal states. To determine the variation within individuals, studies can be performed twice with the 2nd diagnostic test performed 10 days after the 1st procedures in order to obtain 2 baseline strontium absorption values. Fractional absorption and the area-under-the-concentration-time-curve (AUC) are calculated 4 hours after an oral strontium administration (2.5 mmol).
Interpretation and Analysis: The within-subject variation of fractional excretion values (240 hours) and AUC (0–240 hours) are 2.3 ± 0.76 and 1.2 ± 0.41, respectively. Estrogen administration does not influence the magnitude of these parameters. Interpretation of these measurements implies that normal postmenopausal women do not experience a modulating effect of short-term (supra) physiological dose estrogen on intestinal calcium absorption as measured by the strontium absorption test.

Effect of estrogen on intestinal strontium absorption in postmenopausal women. Dijkgraaf-ten Bolscher M, Neele SJ, Barto R, van der Vijgh WJ, Netelenbos JC. Maturitas 2000 Oct 31; 36 (3), pp. 195–201.

Intestinal Absorption: Fat Malabsorption

[^{13}C]-Palmitic Acid

Background Information: Classic fat balance studies detect fat malabsorption but do not discriminate between the potential causes of malabsorption, such as impaired intestinal lipolysis or reduced uptake of fatty acids.

Application: Selective and sensitive detection of impaired intestinal absorption of long-chain fatty acids.
Examples of Clinical Implementation: Evaluation of the absorption and appearance in the plasma of [^{13}C]-palmitic acid in conditions of fat malabsorption due either to chronic bile deficiency (permanent bile diversion) or to oral administration of the lipase inhibitor orlistat (200 mg/kg diet). Comparisons can also be made between [^{13}C]-palmitic acid and the percent absorption of ingested dietary fat determined by fat balance.

Animal Model: Rat (fat malabsorption due to bile deficiency).
Interpretation and Analysis: Decreases in [^{13}C]-palmitate acid absorption compared to negative reference control subjects or normal reference values suggest the detection of impaired intestinal absorption of long-chain fatty acids selectively and sensitively. Examples include the identification of fat malabsorption due to bile deficiency in a rat model. Laboratory data strongly support the use of the [^{13}C]-palmitate acid absorption test for the diagnosis of clinical fat malabsorption syndromes.

Detection of impaired intestinal absorption of long-chain fatty acids: Validation studies of a novel test in a rat model of fat malabsorption. Kalivianakis M, Minich DM, Havinga R, Kuipers F, Stellaard F, Vonk RJ, Verkade HJ. Am J Clin Nutr 2000 Jul; 72 (1), pp. 174–80.

Intestinal Absorptive Capacity/Pancreatic Exocrine Function/[Cystic Fibrosis]/[Celiac Sprue] Maldigestion vs Malabsorption
N-Benzoyl-L-Tyrosyl-P-Aminobenzoic Acid (BT-PABA)/[$^{13}C_6$]PABA/Xylose

Cellular/Molecular Mechanism of Action: N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid (BT-PABA or Bentiromide) is still a synthetic substrate for exocrine pancreatic enzyme fractions. Xylose is absorbed from intact small intestinal mucosal surfaces.
Application: Xylose can be applied to assess small intestinal absorptive capacity/integrity. N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid is of diagnostic value in detecting conditions of suboptimal exocrine pancreatic function.
Route of Administration: N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid and xylose (both can be delivered orally per os).
Implementation: Characterize the potential of a single-step non-invasive stable isotope method of assessing digestive function to selectively identify cases of pancreatic insufficiency (maldigestion) or small bowel dysfunction (malabsorption), in addition to delineating if cases of maldigestion can be simultaneously distinguished from conditions of malabsorption. One approach that can be applied in pursuit of this objective entails the evaluation of the diagnostic attributes of ingesting a liquid test meal in concert with the oral intake of bentiromide, [$^{13}C_6$]PABA, and xylose (PABAX test). Serum is collected 1 hour postadministration and analyzed for PABA, [$^{13}C_6$]PABA, and xylose by stable isotope dilution methods.

Human Model: Cases of cystic fibrosis (n = 18); adult celiac sprue (n = 4); normal volunteers (n = 40).
Interpretation and Analysis: Cases of cystic fibrosis almost invariably have abnormal pancreatic function test results, but a large percentage of sprue cases also have abnormal pancreatic function test results (e.g., n = 3/4). Additionally, a large number of sprue cases have abnormally low bowel absorption test results (e.g., n = 4/4), compared to cases of adult cystic fibrosis that apparently have normal intestinal function. The 1-step, 1-hour PABAX test can reliably separate normal subjects from those with either maldigestion or malabsorption and can also separate subjects with maldigestion from those with malabsorption.
Instrumentation: Gas chromatography mass spectrometry.

A non-invasive stable-isotope method to simultaneously assess pancreatic exocrine function and small bowel absorption. Deutsch JC, Santhosh-Kumar CR, Kolli VR. Am J Gastroenterol 1995 Dec; 90 (12), pp. 2182–5.

Intestinal Malabsorption
Cholicalciferol/Vitamin D

Background Information: Vitamin D is essential for the maintenance of calcium and bone metabolism in humans. The recommended daily allowance (RDA) for vitamin D in the United States of 200 IU (5.0 μg) is reasonable for adults who receive some exposure to sunlight; however, in the absence of any exposure to sunlight, this recommendation may be 2 to 3 times lower than that actually required to satisfy the body's needs. Vitamin D was 1st measured by bioassays. However, bioassays became obsolete in light of the revelation that vitamin D must be activated 1st in the liver to 25-hydroxyvitamin D (25-OH-D) and then in the kidney to 1,25-dihydroxyvitamin D [1,25(OH)2D] before becoming biologically functional.
Interpretation and Analysis: Current assays measure circulating concentrations of vitamin D, 25-OH-D or 1,25(OH)2D. The serum vitamin D concentration is of value for determining the role of sunlight in producing vitamin D in skin and as a provocative test to determine the absorption of vitamin D in patients with malabsorption syndromes. The serum concentration of 25-OH-D is most valuable for determining the overall vitamin D status of an individual, because it is an average of dietary and sunlight-induced vitamin D. The measurement of the serum 1,25(OH)2D concentrations has been most useful in evaluating disorders in calcium and bone metabolism related to acquired and inborn errors in the conversion of 25-OH-D to 1,25(OH)2D.

The use and interpretation of assays for vitamin D and its metabolites. Holick MF. J Nutr 1990 Nov; 120 Suppl 11, pp. 1464–9.

PERMEABILITY AND ULCERATION

Note: See related references in Absorption section of this chapter.

Intestinal Injury: Small Intestine/Diamine Oxidase
Heparin

Application: Assess the extent and severity of acute, severe, small intestinal mucosal injury (adult rats).
Dosage: Heparin (400 U/kg).
Considerations: Diamine oxidase (DAO; EC 1.4.3.6) is an enzyme found in high activity in the mature cells of the upper villus of rat small intestinal mucosa, but has much lower activity in all other tissues in the non-pregnant rat.
Implementation: Determine whether a provocative test for increasing the level of plasma DAO activity by heparin administration can be used to monitor the extent and severity of acute, severe, small intestinal mucosal injury. Plasma postheparin diamine oxidase activity (180 minutes after 400 U/kg i.p. heparin) is measured 7 hours after initiation of perfusion.

Animal Model: Rat (acute small intestinal mucosal injury can be induced by perfusion with sodium sulfate solution [2,100 mosM] for 60 minutes, resulting in damage to villus epithelium).
Interpretation and Analysis: Increasing length of intestinal mucosal injury, precipitates a progressive decrease in both basal and plasma PHD activity. The decrease in plasma postheparin-diamine-oxide activity closely reflects the length of intestinal mucosa injured ($n = 128$, $r = 0.86$, $p < 0.001$), and it is much more sensitive (threshold limit of detection = 13% of total length, range = 67 U/ml

for 100% length of injury) than unstimulated basal levels of plasma diamine oxide (threshold = 40%, range = 2.1 U/ml). Some reports have suggested that diamine oxide is unique among intestinal mucosal enzymes in that circulating levels can serve as a marker of mucosal injury. However, related investigations have demonstrated that low-dose heparin administration enhances the use of diamine oxide even further as a sensitive, quantitative, circulating marker for monitoring the extent of small intestinal mucosal injury in the rat.

Plasma postheparin diamine oxidase. Sensitive provocative test for quantitating length of acute intestinal mucosal injury in the rat. Luk GD, Bayless TM, Baylin SB. J Clin Invest 1983 May; 71 (5), pp. 1308–15.

Intestinal Injury: Devitalization of Structures *Fluorescein Dye*

Cellular/Molecular Mechanism of Action: Functions as a detectable dye marker in biological systems. Reacts with ultraviolet light to produce a fluorescent green color.
Diagnostic Application: Detecting devitalized segments of the gastrointestinal tract.
Route of Administration and Dosage: Intravenous infusion prior to surgical intervention for the purpose of detecting devitalized segments of gastrointestinal tract.
Considerations: Fluorescein dyes can on occasion diffuse rather rapidly across intact barriers such as in the urinary bladder. For this reason, the interval between infusion and collection must be of a relatively short duration. Alternatively, fluorescein dye can be conjugated to large molecular weight substances, such as in a polypeptide protein or dextran molecule, that do not readily diffuse across intact anatomical structures such as the bladder or diaphragm.
Interpretation and Analysis: Segments of affected intestinal tract can be appreciated by direct visual observation based upon localization of fluorescein dye in ischemic, devitalized tissues.
Laboratory Instrumentation: In a clinical environment, presence of fluorescein dye localized within devitalized segments of devitalized intestinal tract or within abdominal fluid samples is usually accomplished by visual observation.

Permeability: Intestinal Permeability/Gastric Permeability/Small Intestinal Permeability/Small Intestinal Mucosa Function *Methylglucose/Rhamnose/Xylose/Sucrose/Lactulose*

Cellular/Molecular Mechanism of Action: Lactulose, rhamnose, xylose, methylglucose, and sucrose are sugar carbohydrates. Lactulose (L) and rhamnose (R) are not significantly absorbed across intact intestinal mucosa. Alternatively, xylose and methylglucose are normally absorbed across healthy intestinal mucosal surfaces in the jejunum and are dependent upon passive and active carrier-mediated transport mechanisms, respectively. Neither rhamnose nor lactulose is significantly metabolized within the small intestine but may be metabolized within the large intestine. Sucrose is absorbed across damaged/injured gastroduodenal mucosa.
Application: Methylglucose, rhamnose, xylose, sucrose, and lactulose can potentially be applied for the simultaneous evaluation of gastric permeability, small intestinal permeability, and small intestinal mucosal function in dogs with gastrointestinal disorders. The lactulose/rhamnose ratio can be applied to detect large-pore/small-pore permeability defects.
Route of Administration: Methylglucose, rhamnose, xylose, sucrose, and lactulose (oral).
Implementation: Develop and validate a method for concurrent separation and quantification of methylglucose, rhamnose, xylose, sucrose, and lactulose in canine urine by using high-pressure anion exchange liquid chromatography and pulsed amperometric detection. The method can be validated by evaluating dilutional parallelism, spiking recovery, intra-assay variability, and interassay variability.
Animal Model: Canine.
Interpretation and Analysis: Ratios for 3 urine samples, and all sugars, range from 77.6% to 106.9% for a 1:2 dilution, 85.2% to 121.4% for a 1:4 dilution, and 91.6% to 163.7% for a 1:8 dilution. Expected ratios for spiking recovery of all sugars, and 5 different spiking solutions, range from 85.5% to 116.7% (mean ± SD, 100.5 ± 6.0%; n = 3 urine samples). Intra-assay coefficients of variation are 1.6%, 3.4%, and 4.7% for methylglucose; 1.6%, 2.0%, and 3.6% for rhamnose; 2.7%, 1.4%, and 1.1% for xylose; 9.8%, 3.4%, and 4.0% for sucrose; and 3.2%, 3.3%, and 3.3% for lactulose. Interassay coefficients of variation are approximately 3.2%, 5.7%, and 4.2% for methylglucose; 4.3%, 5.4%, and 6.4% for rhamnose; 3.3%, 5.0%, and 4.2% for xylose; 9.4%, 9.9%, and 9.4% for sucrose; and 6.1%, 4.9%, and 2.7% for lactulose. Therefore, methodologies exist that can facilitate the simultaneous separation and quantification of 5 different sugars in canine urine (established and found to be linear, accurate, precise, and reproducible). The method may prove useful in the simultaneous evaluation of gastric permeability, small intestinal permeability, and small intestinal mucosal function in dogs with gastrointestinal disorders.
Instrumentation: High-pressure anion exchange liquid chromatography.

Development and validation of a method for simultaneous separation and quantification of 5 different sugars in canine urine. Steiner JM, Williams DA, Moeller EM. Can J Vet Res 2000 Jul; 64 (3), pp. 164–70.

Permeability: Enteropathy/Protein-Losing Conditions/Langerhans Cell Histiocytosis (LCH) *[^{57}Cr]-Radiolabeled Albumin*

Cellular/Molecular Mechanism of Action: Functions as a marker for extravascular leakage or vascular perfusion.
Background Information: Symptomatic involvement of the gastrointestinal (GI) tract as a prominent symptom in LCH is uncommon, occurring in < 1–5% of all cases, even when the disease is in its disseminated form. Up to now, there have been reports of 18 cases of LCH with GI manifestations, including the 2 cases from the referenced study, with diarrhea (77.7%), protein-losing enteropathy (33.3%), and bloody stool being the most frequent findings.
Diagnostic Application: Detection of protein-losing enteropathy conditions.
Dosage: Intravenous injection.
Considerations: Non-specific diagnostic modality.
Implementation: Evaluation of cases susptected of suffering from protein-losing enteropathy conditions utilizing [^{51}Cr]-labeled albumin in "leakage"-based studies.
Human Model: Cases with severe diarrhea, refractory hypoalbuminemia, and protein-losing enteropathy.
Interpretation and Analysis: [^{51}Cr]-labeled albumin can be effectively applied as a marker molecule in imaging studies for the detection of protein-losing enteropathy conditions. A review of the literature indicates that the presence of GI symptoms is often associated with systemic disease as well as with poor prognosis, mainly under 2 years of age. Radioisotopes are useful for documenting protein loss in several diseases with high specificity and sensitivity, and their utilization in such cases can function as an accurate diagnostic procedure as well as a tool for improved therapeutic management.

Disseminated Langerhans cell histiocytosis and massive protein-losing enteropathy. Santos-Machado TM, Cristófani LM, Almeida MT, Maluf PT, Costa PA, Pereira MA, Brito JL, Odone-Filho V. Braz J Med Biol Res 1999 Sep; 32 (9), pp. 1095–9.

Permeability: Gastrointestinal Mucosa
Sucralose/Mannitol

Cellular/Molecular Mechanism of Action: Sucralose and mannitol are carbohydrates that are not metabolized (degraded). Mannitol is normally absorbed across the mucosal surface of the small intestine.
Application: Molecular probes for the detection of alterations in intestinal permeability. Sucralose has also been evaluated as a probe for the measurement of whole gut permeability.
Route of Administration: Sucralose and mannitol (per os).
Analytical Method: Comparison of capillary column gas chromatography (CCGC) and packed column gas chromatography (PCGC) for their ability to accurately measure urinary sucralose (S) and 3 other sugar probes including, sucrose, lactulose (L), and mannitol (M). The 4 sugars can be detected precisely, without interference.
Implementation: Measurement of urinary sucralose (S) and 3 other sugar probes including, sucrose, lactulose (L) and mannitol (M) as *in vivo* molecular probes for the detection of alterations in intestinal permeability using 5- and 24-hour urine collections. Metabolism of the sugars can be evaluated by incubating the intestinal bacteria with an iso-osmolar mixture of mannitol, lactulose, and sucralose at 37°C for 19 hours. Sugar concentrations and the pH of the mixture are then monitored.

Human Model: (n = 14 healthy volunteers).
Interpretation and Analysis: The use of CCGC methodologies improves the detection of sucralose compared to PCGC. The average coefficient of variation decreases from 15% to 4%. It also increases the sensitivity of detection by 200- to 2,000-fold. The GC assay is linear between sucralose concentrations of 0.2 and 40 g/l ($r = 1.000$). Intestinal bacteria metabolize lactulose and acidify the media but do not metabolize sucralose or mannitol. The method for measurement of urinary sucralose permits the simultaneous quantitation of sucrose, mannitol, and lactulose, and is rapid, simple, sensitive, accurate, and reproducible. Because neither S nor M is metabolized by intestinal bacteria, and because only a tiny fraction of either sugar is absorbed, this pair of sugar probes appears to be available for absorption throughout the gastrointestinal tract. Thus, the 24-hour urinary concentrations of S and M, or the urinary S/M ratio following an oral dose of a sugar mixture, might be good markers for whole gut permeability.

Gas chromatographic method for detection of urinary sucralose: Application to the assessment of intestinal permeability. Farhadi A, Keshavarzian A, Holmes EW, Fields J, Zhang L, Banan A. J Chromatogr B Analyt Technol Biomed Life Sci 2003 Jan 25; 784 (1), pp. 145–54.

Permeability: Gastorintestinal Mucosa/Bacterial Translocation: Postoperative Hepatic Resection Conditions
Phenolsulfonphthalein (PSP)

Application: Primarily applied in the gastrointestinal system to determine mucosal integrity/permeability. The peroral PSP test appears to be a possible predictor of bacterial translocation after hepatic resection surgery.
Route of Administration: Phenolsulfonphthalein (peroral).
Dosage: Phenolsulfonphthalein (30 mg).
Implementation: Evaluate perioperative changes in intestinal PSP permeability relevant to conditions of hepatic resection. In this context, peroral PSP tests can be scheduled prior to surgery and then on postoperative days 3, 7, and 14, followed by urine collection over a 24-hour period after surgical intervention. Urinary PSP can be measured applying a colorimetric assay. The correlations between the preoperative PSP value and various clinical variables, such as perioperative changes in urinary PSP excretion, and the relationship between the postoperative PSP value and postoperative complications are then determined.

Human Model: Cases of hepatic resection (n = 50; n = 16 postoperative complications; n = 10/16 sepsis).
Interpretation and Analysis: Preoperative urinary PSP excretion increases in a manner proportional to the degree of liver dysfunction. In contrast, urinary PSP excretion does not significantly change during the perioperative period. However, urinary PSP excretion on postoperative day 3 in patients with postoperative infectious complications (27.3%) is significantly greater than in cases without infectious complications (17.4%; $p < 0.05$). Furthermore, PSP excretion on postoperative day 3 in cases with infectious complications is also significantly greater as compared to the preoperative level. Cases with a urinary PSP excretion level on postoperative day 3 of $> 25\%$ exhibit infectious complications more frequently than patients with a level under 25% (60% vs 10.3%, respectively; $p < 0.05$). The peroral PSP test is thus suggested to be a possible predictor of bacterial translocation after hepatic resection.

Significance of the peroral phenolsulfonphthalein test in hepatic resection: A possible predictor of bacterial translocation. Shimada M, Takenaka K, Fujiwara Y, Gion T, Hasegawa H, Shirabe K, Sugimachi K. Hepatogastroenterology 1998 Mar–Apr; 45 (20), pp. 483–7.

Permeability and Absorption: Gastrointestinal Mucosa/[Feline]
Lactulose/Rhamnose/Xylose/Methylglucose/Sucrose

Cellular/Molecular Mechanism of Action: Lactulose, rhamnose, xylose, methylglucose, and sucrose are sugar carbohydrates. Lactulose (L) and rhamnose (R) are not significantly absorbed across intact mucosa of the small intestine, and neither of these saccharides is metabolized in the small intestine. Alternatively, xylose and methylglucose are normally absorbed across healthy small intestinal mucosal surfaces of the jejunum and are dependent upon passive and active carrier-mediated transport mechanisms, respectively. Sucrose is absorbed across damaged/injured gastroduodenal mucosa.
Application: Lactulose, rhamnose, xylose, methylglucose, and sucrose can be utilized to evaluate intestinal permeability and mucosal function. Selection of these sugar types as investigative probes is based on their prior use for intestinal permeability and mucosal function testing in humans and dogs. The lactulose/rhamnose ratio can be applied to detect large-pore/small-pore permeability defects.
Route of Administration: Lactulose, rhamnose, xylose, methylglucose, and sucrose (intravenous).
Dosage: Isotonic sugar solutions containing lactulose, rhamnose, xylose, methylglucose, and/or sucrose.
Implementation: Determine the kinetics of urinary recovery and evaluate the effects of postmucosal factors on urinary recovery of 5 intravenously administered saccharides. The experimental design of such studies involves the intravenous administration of isotonic sugar solutions containing lactulose, rhamnose, xylose, methylglucose, and sucrose. Urethral catheterization with a closed collection system is then used for the collection of cumulative urine

samples prior to and 2, 4, 6, 8, 10, 12, and 24 hours after sugar solution administration.

Animal Model: Feline (n = 10 cats).

Interpretation and Analysis: Twenty-four-hour cumulative urinary recovery for each sugar in cats is lower than expected compared to dogs and humans. All 5 sugars have the highest percentage of urinary recovery during the 1st 2 hours after administration. Mean sugar elimination rate constants and half-lives range between 0.268/hour for methylglucose to 0.415/hour for lactulose and 1.67/hour for lactulose to 2.59/hour for methylglucose, respectively. Metabolism and incomplete urine collection are possible reasons for lower cumulative urinary recoveries of these 5 sugars in cats compared with dogs. Although these 5 sugars are not ideal marker molecules, they may still be useful for intestinal permeability and mucosal function testing in cats.

Instrumentation: High-pressure anion exchange liquid chromatography with pulsed amperometric detection can be used to measure the concentrations of each sugar in the urine and calculate urinary recovery.

Kinetics and postmucosal effects on urinary recovery of 5 intravenously administered sugars in healthy cats. Krecic MR, Steiner JM, Kern MR, Williams DA. Can J Vet Res 2003 May; 67 (2), pp. 88–93.

Permeability Tendency/Integrity: Gastrointestinal Mucosa
Phenolsufonphthalein (PSP)

Cellular/Molecular Mechanism of Action: Phenolsufonphthalein is a passive marker molecule that is absorbed into the systemic intravascular compartment from the gastrointestinal tract much more extensively in conditions of mucosal damage/injury.

Background Information: The oral PSP absorption test can be used as a simple and non-invasive method for the assessment of either the extent of the permeability of or damage to the gastrointestinal mucosa.

Application: Assessment of gastrointestinal tract mucosa permeability.

Route of Administration: Phenolsufonphthalein (per os).

Dosage: Phenolsufonphthalein (30 mg).

Implementation: Evaluation of the permeability of the gastrointestinal mucosa in conditions of liver cirrhosis and postoperative gastrointestinal surgery cases utilizing oral PSP and subsequent measurement of PSP urine concentrations (e.g., urinary recovery rate).

Interpretation and Analysis: The urinary PSP excretion in conditions of liver cirrhosis (n = 8; 28.8 ± 6.0%) is significantly higher than in cases subjected to vascular surgery (n = 8; 10.0 ± 1.7%; $p < 0.01$). Such findings reflect the presence of increased gastrointestinal mucosa permeability in conditions of liver cirrhosis. The urinary PSP excretion rate in patients who undergo a total gastrectomy with Roux-en-Y reconstruction is 17.3 ± 1.7% (n = 5 and significantly higher than values observed in controls; $p < 0.05$), while the same rates in cases subjected to a partial gastrectomy (n = 10) or colectomy (n = 10) is approximately 10.2 ± 1.8% or 10.6 ± 0.7%, respectively. Parameters of this nature suggest that the intestinal mucosa is damaged by a total resection of the stomach. The oral PSP absorption test, which is non-invasive, simple, and inexpensive, is thus considered to be useful for assessing the degree of damage to the permeability of the gastrointestinal mucosa under various conditions.

Assessing the permeability of the gastrointestinal mucosa after oral administration of phenolsulfonphthalein. Toh Y, Korenaga D, Maekawa S, Matsumata T, Muto Y, Ikeda T, Sugimachi K. Hepatogastroenterology 1997 Jul–Aug; 44 (16), pp. 1147–51.

Permeability: Intestinal/Post–Abdominal Surgery
Enteral Nutrition/Post–Abdominal Surgery
Parenteral Nutrition
Lactulose/Mannitol Urine Ratio

Cellular/Molecular Mechanism of Action: Lactulose and mannitol are carbohydrate molecules that are variably absorbed within the intestinal tract. Lactulose is not normally absorbed across the surface of normal, healthy intestinal mucosa. Conversely, mannitol is normally absorbed across mucosal surfaces of the small intestine. Neither lactulose nor mannitol is significantly metabolized within the small intestine but may be metabolized within the large intestine.

Application: To study the intestinal permeability (IP) following stress of abdominal operation and the different effects on IP of enteral nutrition and parenteral nutrition. The lactulose/mannitol ratio can be applied to detect large-pore permeability defects.

Route of Administration: Lactulose (orally), mannitol (orally).

Dosage: Lactulose (10 g); and mannitol (5 g).

Implementation: Evaluation of the influence of abdominal surgery on intestinal permeability. Lactulose and mannitol are administered as probes to evaluate the integrity of the intestinal mucosa followed by collection of urine samples over a period of 6 hours in order to measure urine excretion ratios for lactulose and mannitol (L/M).

Human Model: Cases of abdominal surgery (n = 40 divided into groups managed with enteral nutrition or parenteral nutrition). Each case group receives nutritional support of the same nitrogen and calories from postoperative day (POD) 3 to POD 11. On the day before surgical intervention (POD−1), POD 7, and POD 12 intestinal permeability studies are performed.

Interpretation and Analysis: The lactulose/mannitol ratios for abdominal surgery cases receiving enteral nutrition group on POD−1, POD 7, and POD 12 are approximately 0.026 ± 0.017, 0.059 ± 0.026, and 0.027 ± 0.017, respectively, and those receiving parenteral nutrition group are 0.025 ± 0.013, 0.080 ± 0.032, and 0.047 ± 0.021, respectively. Cases receiving either parenteral or enteral nutrition have elevated L/M ratios on POD 7 compared to POD−1. The L/M ratio returns toward control levels with enteral nutrition by POD 12, compared to parenteral nutrition cases that still have elevated L/M ratios on POD 12. The L/M ratio increases for a period of time after surgical trauma and the loss of gut mucosal integrity can be reversed by substitution of enteral nutrition.

Intestinal permeability in patients after surgical trauma and effect of enteral nutrition versus parenteral nutrition. Jiang XH, Li N, Li JS. World J Gastroenterol 2003 Aug; 9 (8), pp. 1878–80.

Permeability: Intestinal/Chronic Diarrhea
Lactulose/Mannitol

Cellular/Molecular Mechanism of Action: Lactulose and mannitol are carbohydrate molecules that are variably absorbed within the intestinal tract. Lactulose is not normally absorbed across the surface of normal, healthy intestinal mucosa. Conversely, mannitol is normally absorbed across mucosal surfaces of the small intestine. Neither lactulose nor mannitol is metabolized to any significant degree within the small intestine but they may be metabolized within the large intestinal lumen.

Background Information: Diagnosis of chronic diarrhea in the absence of a distinctive clinical pattern is often challenging, as bio-

chemical tests prescribed at the 1st evaluation do not show enough sensitivity and specificity to tailor further investigation.

Application: Characterization of intestinal absorption and permeability that reflect the presence of mucosal damage/injury. The lactulose/mannitol ratio can be applied to detect large-pore permeability defects.

Implementation: Evaluate the diagnostic value of the lactulose/mannitol (L/M) test in cases of chronic diarrhea.

Human Model: Cases of chronic diarrhea (n = 120 [46%] organic cause for chronic diarrhea; n = 141 [54%] cases with functional conditions).

Interpretation and Analysis: Multivariate logistic regression analysis reveals that the L/M test and C-reactive protein are independent predictors for the final diagnosis of organic causes of chronic diarrhea, with odds ratios of 1.5 (95% CI = 1.29–1.78) and 5.2 (95% CI = 1.90–14.12), respectively. The area under the receiver operating characteristic (ROC) curve of the adjusted model is 0.82, with positive predictive value of 80.4% and negative predictive value of 77.7%. The L/M test appears to be a powerful tool for workup in patients with chronic diarrhea. Introducing the L/M test as 1st-level test effectively improves the selection of patients who need further evaluation.

Lactulose/mannitol test has high efficacy for excluding organic causes of chronic diarrhea. Di Leo V, D'Incà R, Diaz-Granado N, Fries W, Venturi C, D'Odorico A, Martines D, Sturniolo GC. Am J Gastroenterol 2003 Oct; 98 (10), pp. 2245–52.

Permeability: Intestinal/Endotoxemia: Bacterial LPS
Lactulose/Mannitol [Urine]

Cellular/Molecular Mechanism of Action: Lactulose is a carbohydate sugar that is not normally absorbed across the surface of healthy intestinal mucosa. Conversely, mannitol is a carbohydrate sugar that is extensively absorbed across the intact, healthy intestinal mucosa. Neither lactulose nor mannitol is metabolized within the small intestine but they may be metabolized within the large intestine.

Application: Evaluation of intestinal permeability (e.g., protective effect and mechanism of glutamine on the intestinal barrier function in total parenteral nutrition [TPN] rats with trauma or endotoxemia). The lactulose/mannitol ratio can be applied to detect the presence of large-pore permeability defects.

Route of Administration: Lactulose (oral gavaged) and mannitol (oral gavaged).

Dosage: Lactulose (66 mg) and mannitol (50 mg) in 2 ml of saline (rats).

Implementation: Evaluation of intestinal permeability using lactulose and mannitol administered by oral gavage. Urine is collected over a 24-hour period in the presence of a preservative and kept at −20°. The intestine, liver, mesenteric lymph nodes, and blood are then harvested for examination.

Animal Model: Rat (n = 34 male, adult Sprague-Dawley strain rats). Animals can be divided into groups (e.g., n = 8 controls; n = 8 surgical trauma; n = 9 TPN; n = 8 trauma and endotoxemia; n = 9 trauma and endotoxemia with glutamine supplementation). All groups except the control group are given TPN solutions in a 7-day experimental period. For the glutamine group, 1,000 mg/kg/d of glutamine is added to TPN solution during day 1–6. Lactulose and mannitol are administered on day 7 by oral gavaged. On day 8, under intraperitoneal anesthesia using 100 mg/kg ketamine, the intestine, liver, mesenteric lymph nodes, and blood can be collected for examination.

Interpretation and Analysis: Body weight decreases the most in subjects receiving LPS fractions. The structure of small intestinal mucosa with TPN group, LPS, and Gln group show impairments of variable degrees, and the damage of small intestinal mucosa following glutamine is remarkably alleviated. The concentrations of interleukins in small intestine mucosa are lower (for IL-4 and IL-6) or the lowest (IL-10) following glutamine administration. Concentrations of IgA in the blood plasma and the mucosa are highest with glutamine as supplementation. Urine lactulose/mannitol test values reveal that intestinal permeability following bacterial LPS is lower than with TPN ($p < 0.001$), but there is no difference with LPS vs glutamine exposure. The rate of bacterial translocation with glutamine is lower than with LPS exposure ($p < 0.02$). Prophylactic treatment with glutamine could minimize the increments of intestinal permeability and bacterial translocation caused by trauma and endotoxemia in rats treated with TPN.

Effects of glutamine on intestinal permeability and bacterial translocation in TPN-rats with endotoxemia. Ding LA, Li JS. World J Gastroenterol 2003 Jun; 9 (6), pp. 1327–32.

Permeability/Absorption of Intestine: Traumatic Injury Influence
Lactulose/Rhamnose/3-O-Methyl-D-Glucose/Xylose

Cellular/Molecular Mechanism of Action: Lactulose, rhamnose, 3-O-methyl-D-glucose, and xylose are carbohydrate sugars. Lactulose and rhamnose are normally not absorbed across intact surfaces of the intestinal mucosa and neither of the saccharides is metabolized within the small intestine, although metabolism may occur within the large intestine. Conversely, methyl-D-glucose and xylose are normally absorbed across the surface of normal small intestinal mucosa of the jejunum involving active and passive carrier-mediated transport mechanisms, respectively.

Application: Evaluation of intestinal absorption and permeability in canine cases of traumatic injury. The lactulose/mannitol ratio can be applied to detect large-pore permeability defects.

Route of Administration: Lactulose, rhamnose, 3-O-methyl-D-glucose, and xylose can be administered via nasoesophageal tube.

Implementation: Assess the feasibility of using urinary recovery of sugars to evaluate intestinal permeability and absorption in dogs with traumatic injury and to determine if intestinal permeability and absorption becomes altered in dogs with traumatic injury.

After a 6-hour fast, a sugar solution containing lactulose, rhamnose, 3-O-methyl-D-glucose, and xylose is administered via nasoesophageal tube. Urine samples are then collected and quantitated over the 6-hour study period via closed-system collection urinary catheters. Urinary sugar recoveries are measured by high-pressure anion exchange liquid chromatography and pulsed amperometric detection. Urinary sugar recoveries in the trauma group at 24, 48, and 72 hours after trauma are compared to normal controls. In addition, severity of trauma is compared to urinary sugar recoveries.

Animal Model: Canine (n = 12 dogs suffering from clinical traumatic injury; n = 6 healthy negative reference controls).

Interpretation and Analysis: Lactulose recovery and the lactulose:rhamnose recovery ratio are significantly higher in trauma cases at 48 hours but are no longer different from controls by 72 hours. Xylose recovery is significantly higher in canine trauma cases when compared to controls at 72 hours, whereas 3-O-methyl-D-glucose recovery is significantly lower in the trauma group at 24 hours. The xylose:3-O-methyl-D-glucose ratio is higher in the canine cases with trauma at all time points. Significant correlation is found between the severity of trauma and xylose and 3-O-methyl-D-glucose

recoveries 24 hours after injury. Clinical studies therefore support the hypothesis that intestinal permeability and absorption are altered in dogs subjected to traumatic injury.

Instrumentation: High-pressure anion exchange liquid chromatography and pulsed amperometric detection.

Intestinal permeability and absorption in dogs with traumatic injury. Streeter EM, Zsombor-Murray E, Moore KE, Rush JE, Steiner JM, Rozanski EA, Michel KE, Williams DA, Freeman LM. J Vet Intern Med 2002 Nov–Dec; 16 (6), pp. 669–73.

Permeability/Absorption: Intestinal/Chronic Diarrhea/Predictive Value: Organic Etiologies
Lactulose/Mannitol/[Urinary Excretion Ratio]

Cellular/Molecular Mechanism of Action: Lactulose is not but mannitol is significantly absorbed across normal mucosal surfaces of the small intestine. Neither lactulose nor mannitol is metabolized within the small intestine.

Background Information: Diagnosis of chronic diarrhea in the absence of a distinctive clinical pattern is often challenging, as biochemical tests prescribed at the 1st evaluation do not show enough sensitivity and specificity to tailor further investigation.

Application: Intestinal permeability to sugars is an accurate test for detecting intestinal damage. The lactulose/mannitol ratio can be applied to detect large-pore permeability defects.

Route of Administration: Lactulose and mannitol can be administered orally/per os for the purpose of evaluating intestinal absorption/permeability parameters.

Implementation: Evaluate the diagnostic value of the lactulose/mannitol (L/M) test in chronic diarrhea. Complementary biochemical tests include complete blood cell count, acute phase reactive proteins, serum albumin and iron, and stool cultures for bacteria, ova, and parasites.

Human Model: Cases of chronic diarrhea (n = 261; n = ≥ 3 bowel movements per day for ≥ 3 weeks).

Interpretation and Analysis: Approximately 46% of cases have an organic etiology as a cause for their chronic diarrhea (e.g., n = 120/261). Conversely, the remaining cases (e.g., n = 141/261) display evidence of suffering from functional conditions. Multivariate logistic regression analysis reveals that the L/M test and C-reactive protein are independent predictors for the final diagnosis of organic causes of chronic diarrhea, with odds ratios of 1.5 (95% CI = 1.29–1.78) and 5.2 (95% CI = 1.90–14.12), respectively. The area under the receiver operating characteristic (ROC) curve of the adjusted model can be approximately 0.82, with positive predictive value of 80.4% and negative predictive value of 77.7%. The L/M test is a powerful tool for workup in patients with chronic diarrhea. Introducing the L/M test as 1st-level test effectively improves the selection of patients who need further evaluation.

Lactulose/mannitol test has high efficacy for excluding organic causes of chronic diarrhea. Di Leo V, D'Incà R, Diaz-Granado N, Fries W, Venturi C, D'Odorico A, Martines D, Sturniolo GC. Am J Gastroenterol 2003 Oct; 98 (10), pp. 2245–52.

Permeability/Absorption: Intestinal/[Feline]
D-Xylose/3-O-Methyl-D-Glucose/L-Rhamnose/Lactulose/[^{51}Cr]-Labeled Ethylenediaminetetraacetic Acid (EDTA)

Cellular/Molecular Mechanism of Action: Lactulose, rhamnose, xylose, and methylglucose are sugar carbohydrates. Lactulose (L) and rhamnose (R) are not significantly absorbed across intact intestinal mucosa. Neither lactulose nor rhamnose is metabolized within the small intestine. Alternatively, xylose and methylglucose are normally absorbed across healthy intestinal mucosal surfaces by passive and active carrier-mediated transport mechanisms. [^{51}Cr]-EDTA is an organic chelate of radioactive [^{51}Cr] that is not normally absorbed across intact, normal, healthy intestinal mucosa.

Application: The sugars D-xylose, 3-O-methyl-D-glucose, L-rhamnose, and lactulose and the chelation product [^{51}Cr]-EDTA can be applied for the evaluation of intestinal permeability and absorption in the presences of variations in intestinal microflora and dietary intake. The lactulose/rhamnose ratio can be applied to detect large-pore/small-pore permeability defects, respectively.

Route of Administration: Intragastric.

Implementation: Evaluation of a sugar solution containing D-xylose, 3-O-methyl-D-glucose, L-rhamnose, lactulose, and [^{51}Cr]-EDTA administered intragastrically for the purpose of evaluating intestinal absorption and permeability. Urine samples are collected for analysis in order to determine the extent the exogenous sugars are excreted 5 hours after initial administration. After the same cats receive metronidazole for 1 month, the study is repeated. A final study is performed while cats are maintained on a new diet differing in composition and processing.

Animal Model: Feline (n = 6 healthy domestic shorthairs).

Interpretation and Analysis: Lactulose-to-rhamnose ratios, reflecting intestinal permeability, are higher in cats, compared with values for humans or dogs, and values obtained before and after metronidazole administration (mean ± SEM; before, 0.40 ± 0.08; after, 0.45 ± 0.09) are not significantly different. Intestinal absorption also is unaltered after antibiotic administration, and the xylose-to-glucose ratio is 0.70 ± 0.03 before and 0.71 ± 0.06 after metronidazole administration. Sugar recovery does not differ significantly while cats are maintained on canned or dry food.

Reference ranges have been established for the percentage urinary recovery of orally administered D-xylose, 3-O-methyl-D-glucose, L-rhamnose, lactulose, and [^{51}Cr]-EDTA obtained after 5 hours in healthy cats. The intestines of cats appear to be more permeable than those of other species, although the normal bacterial microflora do not appear to influence the integrity or function of the feline intestine, because values obtained for the measured variables before or after antibiotic administration are not significantly different. In addition, differences cannot be detected when dietary intake is completely altered.

Use of an orally administered combined sugar solution to evaluate intestinal absorption and permeability in cats. Johnston KL, Ballèvre OP, Batt RM. Am J Vet Res 2001 Jan; 62 (1), pp. 111–8.

Ulceration: Chronic Duodenal Ulcer vs Pancreatic Gastrinoma (Zollinger-Ellison Syndrome)/[Gastrin-Secreting Tumor]
Secretin/Cimetadine

Application: Characterization of chronic duodenal ulcer case compared to Zollinger-Ellison syndrome.

Route of Administration: Cimetadine (oral and IV).

Dosage: Cimetadine (1 g/day–1.4 g/day).

Considerations: Results from preliminary investigations suggest that previous cimetidine treatment does not alter, and may even increase, the diagnostic sensitivity of the secretin provocative test.

Implementation: Evaluation of conditions that may be either duodenal ulcer or Zollinger-Ellison syndrome using secretin and cimetadine response tests.

Human Model: Cases of chronic duodenal ulcer (n = 16) and Zollinger-Ellison syndrome (n = 5).

Interpretation and Analysis: In cases of chronic duodenal ulcer, a 2nd secretin test can be done during acute cimetidine administration (IV). Only slight variations in plasma gastrin can be detected compared with results from the 1st tests (n = 4 cases). A 3rd test in the same cases with chronic duodenal ulcers after 1 month of cimetidine treatment (1 g/day per os) reveals a time-zero gastrin concentration that is significantly higher than values observed in the previous 2 tests ($p < 0.01$).

Integrated gastrin response after secretin is usually significantly lower in the 3rd test than in the 1st ($p < 0.05$). In Zollinger-Ellison syndrome cases treated with 1.0 and 1.4 g/day cimetidine for 3 months (n = 2 cases), gastrin at time-zero is not markedly increased, whereas compared with the 1st test gastrin levels are higher at each time interval after secretin administration.

Secretin-induced gastrin response in the Zollinger-Ellison syndrome and chronic duodenal ulcer patients before and after cimetidine treatment. Quatrini M, Basilisco G, Conte D, Bardella MT, Bozzani A, Bianchi PA. Am J Gastroenterol 1984 May; 79 (5), pp. 345–7.

INTESTINAL INFLAMMATION

Note: Refer to Section III, Contrast and Image-Enhancing Agents.

Inflammation/Intestinal Lesions: Human Immunodeficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS)/Intestinal Lesions: Crohn's Ileitis *[^{111}In]-Leukocytes/Tauro 23 [^{75}Se]-Selena-25-Homocholic Acid/[^{58}Co]-Labeled Cyanocobalamin*

Cellular/Molecular Mechanism of Action: The agent tauro 23 [^{75}Se]-selena-25-homocholic acid is a bile acid analog, while [^{58}Co]-labeled cyanocobalamin is an analog of vitamin B_{12}. Radiolabeled [^{111}In]-leukocytes behave *in vivo* like endogenous leukocytes of the same cell type.

Background Information: The referenced study examines small intestinal absorption-permeability, intestinal inflammation, and ileal structure and function in HIV-positive male homosexuals.

Application: The agents tauro 23 [^{75}Se]-selena-25-homocholic acid and [^{58}Co]-labeled cyanocobalamin can be applied to evaluate intestinal absorption. Conversely, [^{111}In]-leukocytes can be used to detect foci of inflammation and sepsis.

Implementation: Evaluation of small intestinal absorption-permeability, intestinal inflammation, and ileal structure and function. ^{111}Indium leukocyte studies can be used to detect and characterize intestinal inflammation (e.g., HIV-positive cases). Complementary studies that can be utilized to characterize AIDS cases include the dual radioisotopic ileal function test (whole body retention of tauro 23-[^{75}Se]-selena 25-homocholic acid and ^{58}cobalt-labeled cyanocobalamin). Clinical cases that are HIV positive can also be evaluated using ileocolonoscopy in concert with terminal ileal biopsy.

Human Model #1: Cases of HIV that are seropositive (n = 30 at various stages of disease).

Human Model #2: Cases of AIDS (n = 26; n = 17/26 evaluated by ileocolonoscopy and terminal ileal biopsy).

Interpretation and Analysis: Normal intestinal absorption-permeability can be appreciated in well, HIV-infected cases, but both functions are impaired upon the development of AIDS. The median fecal excretion of ^{111}indium in well patients (0.66%) does not differ significantly ($p > 0.5$) from controls (0.46%), but subjects with AIDS who are well or who have diarrhea have significant ($p < 0.005$) intestinal inflammation (1.33% and 2.18%, respectively). The median 7-day retention of tauro 23-[^{75}Se]-selena 25-homocholic acid in well patients with AIDS (38.9%) does not differ significantly ($p > 0.2$) from controls (39.3%), whereas the absorption of ^{58}cobalt-labeled cyanocobalamin is significantly ($p < 0.05$) lower than controls (32.1% and 59.4%). Patients with AIDS diarrhea have significant ($p < 0.001$) malabsorption of both the bile acid (7.7%) and vitamin B_{12} (8.9%), which is more severe than in Crohn's ileitis (14.2% and 30.3%, respectively). Morphometric analyses of ileal biopsies is unremarkable in AIDS. Such findings depict the presence of low-grade enteropathy in conditions of AIDS, severe ileal malabsorption in cases with AIDS diarrhea, and relatively minor ileal morphologic changes. Malabsorption of bile acids may play a pathogenic role in patients with AIDS and diarrhea.

Intestinal inflammation, ileal structure and function in HIV. Bjarnason I, Sharpstone DR, Francis N, Marker A, Taylor C, Barrett M, Macpherson A, Baldwin C, Menzies IS, Crane RC, Smith T, Pozniak A, Gazzard BG. AIDS 1996 Oct; 10 (12), pp. 1385–91.

Inflammation/Intestinal Lesions: Non-Steroidal Anti-Inflammatory Drug (NSAID)-Associated/Intestinal Lesions: Crohn's Ileitis *[^{111}In]-Leukocytes/Tauro 23 [^{75}Se]-Selena-25-Homocholic Acid/[^{58}Co]-Labeled Cyanocobalamin*

Cellular/Molecular Mechanism of Action: The agent tauro 23 [^{75}Se]-selena-25-homocholic acid is a bile acid analog, while [^{58}Co]-labeled cyanocobalamin is an analog of vitamin B_{12}. Radiolabeled [^{111}In]-leukocytes behave *in vivo* like endogenous leukocytes of the same cell type.

Application: The agents tauro 23 [^{75}Se]-selena-25-homocholic acid and [^{58}Co]-labeled cyanocobalamin can be applied to evaluate intestinal absorption. Conversely, [^{111}In]-leukocytes can be used to detect foci of inflammation and sepsis.

Route of Administration: Intravenous.

Implementation: Evaluation of residual inflammatory lesions following discontinuation of NSAIDs using [^{111}In]-leukocyte scintigraphy imaging methodologies.

Human Model #1: Cases of rheumatoid arthritis (n = 90) and osteoarthritis (n = 7).

Human Model #2: Cases treated with NSAIDs (n = 19); healthy controls (n = 20); and Crohn's ileitis (n = 13).

Interpretation and Analysis: Using [^{111}In]-leukocyte imaging techniques reveals that cases of rheumatoid arthritis and osteoarthritis administered NSAIDs are associated with gastrointestinal lesions. More specifically, approximately two-thirds of all cases evaluated on long-term treatment display inflammatory lesions that may persist for up to 16 months following discontinuation. The prevalence and magnitude of the intestinal inflammation is unrelated to the type and dose of non-steroidal drugs and previous or concomitant 2nd-line drug treatment. There is a significant inverse correlation ($r = -0.29$, $p < 0.05$) between fecal [^{111}In] excretion and hemoglobin levels in cases treated with NSAIDs. The kinetics of fecal [^{111}In] excretion in cases treated with NSAIDs is almost identical to that of patients with small bowel Crohn's disease. Small bowel radiological examination of cases on NSAIDs reveals that approximately one-sixth have asymptomatic ileal disease with ulceration and strictures (e.g., n = 3/18 cases). Evaluation of cases on anti-inflammatory agents, normal healthy controls, and cases of Crohn's ileitis with tauro 23 [^{75}Se]-selena-25-homocholic acid and [^{58}Co]-labeled cyanocobalamin reveals several abnormalities. On day 4, more than half the cases with rheumatoid arthritis have evidence of bile acid malabsorption, but the ileal dysfunction is much milder than seen in patients with Crohn's ileitis.

Non-steroidal anti-inflammatory drug-induced intestinal inflammation in humans. Bjarnason I, Zanelli G, Smith T, Prouse P, Williams P, Smethurst P, Delacey G, Gumpel MJ, Levi AJ. Gastroenterology 1987 Sep; 93 (3), pp. 480–9.

Mucosal Pathology/Erosions/Histopathological Imaging (*In Vivo*)
Cresyl Violet

Cellular/Molecular Mechanism of Action: Cresyl violet functions as a fluorophore.
Application: Cresyl violet can function as a dye reagent that is compatible in viable biological systems.
Route of Administration: Variable.
Implementation: Confocal laser scanning microscope analysis of *ex vivo* esophageal and gastric mucosa preparations. A series of images is recorded for different ranges of cresyl violet concentrations ranging from 0.1% to 10% with staining performed over a time period of 2 minutes after mucolysis with acetylcysteine.

Animal Model: Swine (esophageal and gastric mucosa).
Interpretation and Analysis: Staining with 2% cresyl violet can facilitate the identification of nuclei and cell membranes. Lower concentrations are associated with a lack of homogeneous dye absorption in particular in the gastric mucosa. Cresyl violet is a clinically usable fluorophore for confocal laser scanning microscopy. Its use in combination with an endoluminally applicable system has the potential to allow *in vivo* histopathology analyses.

Cresyl violet as a fluorophore in confocal laser scanning microscopy for future *in vivo* histopathology. George M, Meining A. Endoscopy 2003 Jul; 35 (7), pp. 585–9.

MOTILITY AND TRANSIT TIME OF INTESTINAL TRACT

Colon Motility: Serotonin 5-HT(4)-Receptor Distribution
Mosapride Citrate/Cisapride

Cellular/Molecular Mechanism of Action: Function as 5-HT(4)-receptor agonists.
Route of Administration: Mosapride and cisapride (intragastric).
Dosage: Mosapride and cisapride 3–30 mg/kg.
Considerations: The 5-HT(4) receptors in the colon of guinea pigs show a distribution similar to that in humans.
Interpretation and Analysis: The enhancing effect of mosapride on the colon motility is antagonized by atropine or 5-HT(4)-receptor antagonist (GR113808), but not by methysergide [5-HT(1)- and 5-HT(2)-receptor antagonist]; ondansetron, [5-HT(3)-receptor antagonist]; or CP-99994 [tachykinin NK(1)-receptor antagonist]. *In vitro* receptor autoradiography has shown that mosapride and cisapride inhibit the specific binding of [^{125}I]-SB207710, a selective radioligand of 5-HT(4) receptors, in the colon of guinea pigs. Mosapride therefore enhances colonic motility through 5-HT(4)-receptor activation in guinea pigs and may be useful for treating constipation in patients with colonic motility dysfunction.

Effects of mosapride citrate, a 5-HT4 receptor agonist, on colonic motility in conscious guinea pigs. Inui A, Yoshikawa T, Nagai R, Yoshida N, Ito T. Jpn J Pharmacol 2002 Dec; 90 (4), pp. 313–20.

Colonic Pseudo-Obstruction: Acute
Neostigmine

Cellular/Molecular Mechanism of Action: Neostigmine inhibits acetylcholine esterase, thereby diminishing the enzymatic degradation of acetylcholine and increased stimulation of acetycholine receptor complexes.
Application: Detection or characterization of postoperative ilius, atropine overdose.
Route of Administration: Neostigmine (intravenous) with electrocardiographic monitoring.
Dosage: Neostigmine (2.0 mg over 3–5 minutes).
Implementation: Evaluation of the efficacy of neostigmine in conditions of acute colonic pseudo-obstruction defined as colonic distention with a cecal diameter of at least 10 cm on plain radiographs and no radiographic evidence of mechanical obstruction. Immediately following neostigmine infusion, clinical response is evaluated (passage of flatus or stools associated with decreased abdominal distention within 30 minutes) and sustained response with decreased abdominal girth and reduced colonic dilation on radiographs 3 hours after infusion.

Human Model: Cases failing to respond to conventional management (nothing by mouth, nasogastric suction, postural changes, IV fluids, electrolyte replacement, and discontinuation of any drugs that affect colonic motility) for 24 hours.

Study Design: N = 20 cases in a prospective, randomized, double blind, placebo-controlled study.

Cases with bradycardia (heart rate $<$ 60/minutes), hypotension (systolic blood pressure $<$ 90 mm Hg), active bronchospasm, clinical or radiographic evidence of perforation, history of partial colonic resection, active gastrointestinal bleeding, pregnancy, or serum creatinine $>$ 3 mg/dL are not suitable study candidates.
Interpretation and Analysis: Neostigmine produces an immediate clinical response (median time, 4 minutes) compared to none in the placebo group ($p < 0.001$). Some cases receiving neostigmine (e.g., n = 3/10) or placebo (e.g., n = 8/10) fail to show sustained improvement 3 hours after infusion ($p = 0.04$). Some cases that fail to respond (e.g., n = 1/10 neostigmine; and n = 7/8 placebo) can receive open-label treatment with neostigmine. "Open-label" neostigmine administration ultimately results in a high rate of response (e.g., n = 1/7 placebo group lack of response and eventually required colonic resection).

Therefore, in cases treated with neostigmine, 94% (n = 17/18) have an immediate clinical response, and 89% (n = 16) do not have recurrent colonic dilation. The most common side effect is crampy abdominal pain (e.g., n = 13), although usually mild (n = 9/13). Symptomatic bradycardia may occur in some cases and require atropine intervention therapy (e.g., n = 2).

Neostigmine infusion: New standard of care for acute colonic pseudo-obstruction? Amaro R, Rogers AI. Am J Gastroenterol 2000 Jan; 95 (1), pp. 304–5.

Gastrointestinal-Transit-Absorption (GITA) Model
Salicylazosulfanilic Acid (SASA)

Cellular/Molecular Mechanism of Action: Salicylazosulfanilic acid functions as a prodrug that is converted to the "parent" (active) drug, 5-aminosalicylic acid (5-ASA), after dosing.
Application: The GITA model is useful for the analysis and the prediction of the absorption behavior of drugs orally administered as solutions.

Route of Administration: Salicylazosulfanilic acid (oral).
Implementation: Prediction of plasma concentration-time profiles for SASA and 5-ASA is performed based on a GITA model using parameters describing GI-transit kinetics, the absorption in each GI segment, and the regeneration of 5-ASA within the cecum.
Interpretation and Analysis: Plasma concentration-time profiles of both SASA and 5-ASA after oral administration of SASA are predicted very well by introducing a factor for the 1st-pass elimination of 5-ASA into the GITA model. The simulation study using the parameters shows that about 94.7% of SASA reaches the cecum, where 5-ASA is regenerated very rapidly and 76.0% of 5-ASA is subsequently absorbed into the systemic circulation. Furthermore, the bioavailability of 5-ASA is estimated to be 0.330 because of the 1st-pass elimination through both cecum and liver. In conclusion, the absorption behaviors of a prodrug and its regenerated parent drug can be predicted very well and be clarified successfully using the GITA model. Some concerns exist that because the prodrug SASA is metabolized in the cecum, the estimation of gastrointestinal transit/absorption may be influenced by a relatively large number of variables.

Analysis and prediction of absorption behavior of colon-targeted prodrug in rats by GI-transit-absorption model. Yokoe J, Iwasaki N, Haruta S, Kadono K, Ogawara K, Higaki K, Kimura T. J Control Release 2003 Jan 17; 86 (2–3), pp. 305–13.

Ileus/Adenosine Receptor Antagonist/Ligand Ileus: Reversible (Postoperative)
FK352/DPCPX

Cellular/Molecular Mechanism of Action: (R)-1-[(E)-3-(2-phenylpyrazolo[1,5-a]pyridin-3-yl) acryloyl]-piperidin-2-yl acetic acid (FK352) and 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX) function as adenosine A(1) receptor antagonists.
Physiological Mechanism of Action: Reversal of declines in colonic motility.
Application: Diagnostic interpretation of postoperative ileus.
Implementation: Colonic propulsive motility is evaluated according to migration of dye injected within the lumen of the colon following either pentobarbital anesthesia or surgical trauma (partial gastrectomy, cecectomy, or gentle touching of the colon with fingers).
Animal Model: Rat (unrestrained).
Interpretation and Analysis: Propulsive motility significantly decreases after anesthesia or partial gastrectomy. (R)-1-[(E)-3-(2-phenylpyrazolo[1,5-a]pyridin-3-yl) acryloyl]-piperidin-2-yl acetic acid (FK352) and 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX) (intravenous administration) function as adenosine A(1) receptor antagonists and reverse the slowing of colonic propulsion activity in experimental ileus models. Blockade of adenosine A(1) receptors therefore can potentially be utilized for the diagnosis of intestinal ileus (postoperative).

Adenosine A(1) receptor blockade reverses experimental postoperative ileus in rat colon. Kadowaki M, Nagakura Y, Tokita K, Hanaoka K, Tomoi M. Eur J Pharmacol 2003 Jan 1; 458 (1–2), pp. 197–200.

Neuromuscular Disorders (Colonic): Pediatric/Stimulation of High-Amplitude-Propagating Contractions/Colon Monometry
Bisacodyl/[Edrophonium]

Physiological Mechanism of Action: Functions as a stimulant laxative, thereby promoting accumulation of water and electrolytes within the colonic lumen.
Application: Stimulation of high-amplitude-propagating contractions that can be of benefit during the conduction of pediatric colonic manometry procedures.
Implementation: Evaluation of defecation disorders employing water-perfused manometry catheters inserted into the right colon. Colonic motility is measured during a 3-hour test session (e.g., an hour fasting; an hour after a meal; and 30 minutes after administration of a provocative test agent).
Human Model: Cases suspected of suffering from defecation disorders.
Interpretation and Analysis: Bisacodyl is superior to edrophonium as a stimulant for inducing high-amplitude-propagating contractions. Bisacodyl-induced high-amplitude-propagating contractions are similar in amplitude, duration, propagation velocity, and sites of origin and extinction to naturally occurring high-amplitude-propagating contractions. The effect of intrarectal bisacodyl is similar to that of intracecal bisacodyl, except for a delay of 10 minutes in onset. Bisacodyl induces high-amplitude-propagating contractions in essentially all children (e.g., n = 28/28 children; n = 22/28 with spontaneous high-amplitude-propagating contractions) without evidence of neuromuscular disease and in children with colonic neuromuscular disorder and no spontaneous high-amplitude-propagating contractions (e.g., n = 2/9). In review of the findings noted with bisacodyl, the high-amplitude-propagating contractions it induces are quantitatively and qualitatively similar to naturally occurring high-amplitude-propagating contractions. In selected cases, such as in children receiving total parenteral nutrition or restricted fluid intake, it may be possible to shorten diagnostic colonic manometry using bisacodyl rather than waiting for spontaneous high-amplitude-propagating contractions.

Bisacodyl and high-amplitude-propagating colonic contractions in children. Hamid SA, Di Lorenzo C, Reddy SN, Flores AF, Hyman PE. J Pediatr Gastroenterol Nutr. 1998 Oct; 27 (4), pp. 398–402.

Transit Time (Orocecal): Infants (< 8 months)
Lactose-[^{13}C]-Ureide Breath Test (LUBT)

Background Information: The lactose-[^{13}C]ureide breath test is a novel, non-invasive test to determine orocecal transit time. Lactose ureide resists the action of brush border enzymes and is metabolized by colonic bacteria.
Application: Gastrointestinal tract tracer that can be used to determine orocecal transit time in infants < 8 months of age. In this context, studies have evaluated the potential ability to adapt the LUBT for children of various age groups and to determine whether it can be applied in infants, newborns, and preterms to study the development of small intestinal motility.
Dosage: 500 mg unlabeled lactose ureide; 250 mg lactose-[^{13}C]ureide.
Implementation: A breath test is performed after induction of unlabeled lactose ureide (500 mg) 3 times a day over a 24-hour period. A liquid test meal (chocolate milk) with lactose-[^{13}C]ureide (250 mg) is also ingested. Breath samples are collected every 15 minutes for 10 hours. In a 2nd investigation (age range, 0–3 years), only the *in vitro* stool analysis is performed.

Stools are collected from each subject for *in vitro* stool incubation analyses. The procedure involves utilization of a blank stool sample and a sample produced after induction with unlabeled lactose ureide and then incubated with 10 mg lactose-[^{13}C]ureide within a small, sealed bottle. Ten-milliliter CO_2 samples are aspirated from the bottle using a needle and a syringe every 30 minutes for 24 hours.

The LUBT results are then compared with findings from *in vitro* stool sample analyses.

Human Model: Children (n = 20 at 3 to 17 years of age, compared to n = 32 of which n = 6 at 1 to 3 years; n = 6 infants 6–12 months; n = 13 infants 0–6 months; n = 7 preterm infants).

Interpretation and Analysis: The mean orocecal transit time in cases 3–17 years of age is 255 minutes (range, 165–390 minutes). Stool incubations demonstrated a clear [$^{13}CO_2$] peak in all infants more that 8 months of age, indicating that their colonic bacterial enzymic activity hydrolyzes lactose ureide. However, in all infants < 6 months of age, and in preterm infants, the [$^{13}CO_2$] signal is absent, indicating that those subjects are unable to hydrolyze lactose ureide. Infants < 6 months of age do not host the appropriate bacterial enzymic activity for splitting lactose ureide. The authors of the referenced study conclude that the LUBT can be applied in infants more than 8 months of age, after weaning to solid foods, to determine orocecal transit time.

Lactose-[^{13}C]ureide breath test: A new, non-invasive technique to determine orocecal transit time in children. Van Den Driessche M, Van Malderen N, Geypens B, Ghoos Y, Veereman-Wauters G. J Pediatr Gastroenterol Nutr 2000 Oct; 31 (4), pp. 433–8.

PANCREAS FUNCTION AND DISEASE

Pancreas: Chronic Pancreatitis/Pancreatic Insufficiency
Secretin/N-Benzoyl-L-Tyrosyl-P-Aminobenzoic Acid (BT-PABA)

Cellular/Molecular Mechanism of Action: N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid is a substrate for exocrine pancreatic enzyme fractions. Secretin induces exocrine pancreatic function.

Application: Detection and characterization of exocrine pancreatic insufficiency.

Route of Administration: N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid (oral).

Interpretation and Analysis: The secretin test (ST) is necessary to establish a diagnosis of chronic pancreatitis, especially the non-calcified form, and tubeless tests demonstrate insufficient sensitivity for the diagnosis of chronic pancreatitis.

However, the combined application of the tubeless tests is specific enough to diagnose severe exocrine dysfunction. The sensitivity of the ST for the definitive diagnosis of chronic pancreatitis is 87% (approximately). In patients with probable chronic pancreatitis (CP), 60% have mild exocrine insufficiency and 40% have normal function. The false-positive rate of the ST is 5% for non-pancreatic disease states with the exception of diabetes mellitus.

The correlation between morphological changes in endoscopic retrograde pancreatography (ERP) and exocrine function evaluated by secretin (ST) is 74%. In patients with calcified CP, 81% have parallel results between ERP and the ST, but in non-calcified CP, 47% have parallel results. In cases with severe or moderate exocrine insufficiency demonstrated by the ST, abnormally low levels are observed in 63% by the BT-PABA test, 61% by fecal chymotrypsin test (FCT), and 44% by pancreatic amylase (PA). In patients with normal exocrine function demonstrated by the ST, abnormally low levels can be appreciated in 28% of cases with the BT-PABA test, 28% of cases with the FCT, and 10% with the PA test. A combination assay of BT-PABA test, FCT, and PA improved the specificity for diagnosing CP but not the sensitivity.

Evaluating exocrine function tests for diagnosing chronic pancreatitis. Kitagawa M, Naruse S, Ishiguro H, Nakae Y, Kondo T, Hayakawa T. Pancreas 1997 Nov; 15 (4), pp. 402–8.

Pancreas: Exocrine Function/Efficacy Evaluation of Pancreas, Pankreon, and Pankreatin
[^{14}C]-Triolein-[^{3}H]-Oleic Acid

Cellular/Molecular Mechanism of Action: The agent [^{14}C]-triolein-3H-oleic acid can function as a substrate for pancreatic enzyme fractions. Proteolysis of [^{14}C]-triolein-3H-oleic acid therefore directly corresponds to exocrine pancreatic functional capacity.

Application: [^{14}C]-triolein-[^{3}H]-oleic acid can be used to evaluate exocrine pancreatic function or exocrine pancreatic enzyme supplementation efficacy.

Route of Administration: [^{14}C]-triolein-[^{3}H]-oleic acid (oral).

Implementation: Evaluation or estimation of duodenal enzyme activity and fecal fat excretion related to the efficacy of pancreatic enzyme supplements (e.g.,Pancreas, Pankreon, and Pankreatin) in cases of chronic pancreatitis and severe exocrine insufficiency.

Human Model: Cases of chronic pancreatitis and severe exocrine insufficiency (n = 23).

Interpretation and Analysis: The 3 pancreatic enzyme supplements do not produce any significant difference in treating steatorrhoea. Pankreatin increases the meal-stimulated duodenal enzyme activity ($p < 0.01$) and causes reductions in the fecal fat excretion ($p < 0.05$), whereas no change in these variables is observed with Pankreon or Pancreas. The fecal [^{14}C]-triolein-[^{3}H]-oleic acid test reveals significant improvements in [^{14}C]-triolein digestion with all 3 preparations ($p < 0.01$). The fecal [^{14}C]-triolein-[^{3}H]-oleic acid test is most reliable when monitoring outpatients.

Monitoring the effect of substitution therapy in patients with exocrine pancreatic insufficiency. Jørgensen BB, Pedersen NT, Worning H. Scand J Gastroenterol 1991 Mar; 26 (3), pp. 321–6.

Pancreas: Exocrine Function
N-Benzoyl-L-Tyrosyl-P-Aminobenzoic Acid (BT-PABA)/Xylose

Cellular/Molecular Mechanism of Action: The BT-PABA reagent is a substrate for chymotrypsin resulting in the liberation of the PABA moiety. Proteolytic digestion of BT-PABA can be detected by measurement of released PABA at 405 nm.

Diagnostic Application: Utilized for the diagnosis of exocrine pancreatic insufficiency (EPI) based upon the concept that chymotrypsin is found primarily within the proximal segment of the small intestine. Accuracy of the test is greatly improved if combined with the oral administration of xylose in order to verify gastric emptying has occurred.

Route of Administration and Dosage: Following oral administration, blood samples are collected at 30, 60, 90, 120, and 180 minutes. Assay of para-aminobenzoic acid (PABA) concentrations is assessed by measurement of spectrophotometric absorbance at 405 nm.

Interpretation and Analysis: The PABA moiety of BT-PABA is a detectable chemical marker that is compatible with biological (*in vivo*) systems and can be measured in biological fluid samples. Plasma PABA concentrations should rise above approximately 27 μmol/l within 60 minutes of administration. Markedly low PABA concentrations within the serum in the presence of normal xylose absorption imply that gastric emptying has occurred normally and that there is strong evidence supporting the presence of EPI.

Laboratory Instrumentation: Spectrophotometric absorbance is measured at 405 nm.

Pancreas: Exocrine Function
Porcine Secretin

Cellular/Molecular Mechanism of Action: Secretin promotes exocrine pancreas secretory function.
Dosage: Porcine secretin 1 CU/kg compared to synthetic porcine secretin 0.05, 0.2, and 0.4 μg/kg.
Considerations: Biologically derived porcine secretin has been used as a diagnostic agent in clinical gastrointestinal practice for many years. Pure synthetic porcine secretin is now available for investigational clinical use. Adverse reactions are infrequent and include mild and transient flushing after 0.2 and 0.4 μg/kg doses of synthetic porcine secretin and after the 1 CU/kg dose of biologically derived porcine secretin. Both preparations are considered safe and well tolerated. Experimental evidence justifies the substitution of synthetic porcine secretin for biologically derived porcine secretin.
Interpretation and Analysis: Measurement of bicarbonate concentration and total bicarbonate production in duodenal aspirates reveals that 0.2 and 0.4 μg/kg doses of synthetic porcine secretin are not different from the 1 CU/kg dose of biologically derived porcine secretin for volume, bicarbonate concentration, and total output from 0 to 60 minutes. Synthetic porcine secretin has identical pharmacologic effects to biologically derived porcine secretin in normal subjects.

Comparative effects of synthetic and natural secretin on pancreatic secretion and on secretin, insulin, and glucagon levels in man. Beglinger C, Gyr K, Werth B, Keller U, Girard J. Dig Dis Sci 1982 Mar; 27 (3), pp. 231–3.

Pancreas: Exocrine Insufficiency/Autoimmune-Mediated Pancreatitis/Salivary β_2-Microglobulin
N-Benzoyl-L-Tyrosyl-P-Aminobenzoic Acid (BT-PABA)

Cellular/Molecular Mechanism of Action: N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid can function as a synthetic substrate for exocrine pancreatic enzyme fractions.
Background Information: Autoimmune pancreatitis (AIP) is a distinct clinical entity in which an autoimmune mechanism may be involved in the pathogenesis.
Application: To investigate salivary gland function in addition to pancreatic endocrine and exocrine function in patients with AIP, and to determine changes occurring after steroid therapy. Detection and characterization of exocrine pancreatic insufficiency.
Implementation: Evaluation of AIP conditions applying (i) BT-PABA excretion test; (ii) sialochemistry; (iii) parotid gland scintigraphy; (iv) fasting serum glucose levels; (v) oral glucose tolerance tests; or (vi) glycosylated hemoglobin values in conditions of AIP.
Human Model: Cases of AIP (n = 19). N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid excretion test, sialochemistry, and parotid gland scintigraphy (e.g., n = 8 cases). Some cases may demonstrate evidence of diabetes mellitus (e.g., n = 8).
Interpretation and Analysis: Steroid therapy can improve insulin secretion and glycemic control (n = 3/5 cases). Pancreatic exocrine function is reduced in a large percentage of cases (88%). Impaired pancreatic exocrine function improves after steroid therapy in approximately 50% of cases (e.g., n = 3/6) and these same cases usually/often show treatment-related improvement in endocrine function. Concentration of β_2-microglobulin in saliva is significantly elevated in AIP cases compared to negative reference controls ($p < 0.05$). The ratio of cumulative peak count to injected radionuclide and washout ratio in parotid scintigraphy in AIP conditions is significantly lower than controls ($p < 0.01$). Salivary gland dysfunction improves after steroid therapy in most affected cases (e.g., n = 5/5). Pancreatic endocrine and exocrine and salivary gland function are frequently impaired in conditions of AIP, and steroid therapy is occasionally effective for these dysfunctions.

Pancreatic endocrine and exocrine function and salivary gland function in autoimmune pancreatitis before and after steroid therapy. Kamisawa T, Egawa N, Inokuma S, Tsuruta K, Okamoto A, Kamata N, Nakamura T, Matsukawa M. Pancreas 2003 Oct; 27 (3), pp. 235–8.

Pancreas: Exocrine Function/Intestinal Absorptive Capacity/Maldigestion vs Malabsorption/[Cystic Fibrosis]/[Celiac Sprue]
N-Benzoyl-L-Tyrosyl-P-Aminobenzoic Acid (BT-PABA)/[$^{13}C_6$]PABA/Xylose

Cellular/Molecular Mechanism of Action: N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid (BT-PABA or Bentiromide) is a synthetic substrate for exocrine pancreatic enzyme fractions proteolytic activity. Xylose is absorbed from intact small intestinal mucosal surfaces.
Application: Xylose can be applied to assess small intestinal absorptive capacity/integrity. N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid is of diagnostic value for detecting conditions of suboptimal exocrine pancreatic function.
Route of Administration: N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid and xylose (both can be delivered orally per os).
Implementation: Characterize the potential of a single-step non-invasive stable isotope method of assessing digestive function to selectively identify cases of pancreatic insufficiency (maldigestion) or small bowel dysfunction (malabsorption), in addition to delineating if cases of maldigestion can be simultaneously distinguished from conditions of malabsorption. One approach that can be applied in pursuit of this objective entails the evaluation of the diagnostic attributes of ingesting a liquid test meal in concert with the oral intake of bentiromide, [$^{13}C_6$]PABA, and xylose (PABAX test). Serum is collected 1 hour postadministration and analyzed for PABA, [$^{13}C_6$]PABA, and xylose by stable isotope dilution methods.
Human Model: Cases of cystic fibrosis (n = 18); adult celiac sprue (n = 4); normal volunteers (n = 40).
Interpretation and Analysis: A very high percentage of cystic fibrosis cases have abnormal pancreatic function test results (e.g., n = 18/18), while a large percentage of sprue cases have abnormal results from pancreatic function tests (e.g., n = 3/4 or 75%). A large number of sprue cases have abnormal small bowel absorption tests (e.g., n = 4/4)), compared to cases of adult cystic fibrosis that apparently have normal intestinal function. The 1-step, 1-hour PABAX test can reliably separate normal subjects from those with either maldigestion or malabsorption and can also separate subjects with maldigestion from those with malabsorption.
Instrumentation: Gas chromatography mass spectrometry.

A non-invasive stable-isotope method to simultaneously assess pancreatic exocrine function and small bowel absorption. Deutsch JC, Santhosh-Kumar CR, Kolli VR. Am J Gastroenterol 1995 Dec; 90 (12), pp. 2182–5.

Pancreatic Disease
[^{125}I]-L-3 Iodo-α-Methyl Tyrosine

Background Information: The difficulty of a reliable diagnosis of pancreatic diseases by scintiscanning is mainly derived from the lack of adequate radiopharmaceuticals.
Application: The reagent [^{125}I]-L-3 iodo-α-methyl tyrosine (^{125}I-IMT) has been studied for its ability to detect pancreatic disease and has also been used for the diagnosis of different kinds of brain tumors.

Semi-Synthetic Methods: Quick and easy technique for the synthesis and purification of [^{125}I]-L-3 iodo-α-methyl tyrosine (^{125}I-IMT) in order to be used in a nuclear medicine service. The analog L-alpha-methyl tyrosine can be labeled with [^{125}I] using I-/$I0_3$ and afterward purified by an anionic exchange resin. The labeling yield obtained is approximately 96.0 ± 0.5% when a 15-minute incubation period is employed. No significant statistical differences can be appreciated when the incubation time is extended to 1 hour.
Implementation: Biodistribution investigation performed in mice to determine degree of pancreatic incorporation.
Interpretation and Analysis: Biodistribution studies in mice reveal that the percent of injected dose activity within the pancreas is approximately 34.24 ± 14.03% at 15 minutes postinjection and remains constant for 30 minutes. The pancreas/liver ratio 15 minutes after the injection of the labeled product is 12.22 ± 3.59 and remains constant for an additional 45 minutes. These results demonstrate that [^{125}I]-IMT can be used as a diagnostic agent for pancreatic diseases. The reagent can also be radioiodinated with [^{123}I], [^{124}I], and [^{131}I] if desired.

New methodology for pancreatic scintiscanning imaging using ^{125}I-L-3 iodo-alpha-methyl tyrosine. Zubillaga MB, Bocciot JR, Gentile A, Mitta AE, Nicolini JO, Caro RA. Acta Physiol Pharmacol Ther Latinoam 1996; 46 (2), pp. 83–9.

Pancreatic Exocrine Function/Assessment of Pancreatic Enzyme Replacement
N-Benzoyl-L-Tyrosyl-P-Aminobenzoic Acid (BT-PABA)

Cellular/Molecular Mechanism of Action: N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid is a synthetic peptide substrate for pancreatic enzyme fractions (e.g., chymotrypsin).
Background Information: The clinical diagnosis of chronic pancreatitis is usually based on imaging studies, pancreatic function tests, and the presence of characteristic clinical features. However, the secretin test (a duodenal intubation test) and the combination of non-invasive tests, BT-PABA, and fecal chymotrypsin (FCT) can be applied to characterize exocrine pancreatic function in conditions of chronic pancreatitis.
Implementation: Evaluation of the diagnostic value of non-invasive tests compared to the secretin test.
Interpretation and Analysis: Although non-invasive tests are less sensitive and specific for determining exocrine pancreatic dysfunction than the secretin test, greater reliability for diagnosing chronic pancreatitis can be obtained by performing the BT-PABA and FCT simultaneously. Assessment of exocrine pancreatic function is important not only for the diagnosis of chronic pancreatitis but also to decide a treatment method with pancreatic enzyme preparation.

Assessment of exocrine pancreatic dysfunction in chronic pancreatitis. Kataoka K, Hosoda M, Yasuda H, Sakagami J, Kato M, Kashima K. Digestion 1999; 60 Suppl 1, pp. 86–92.

Pancreatic Exocrine Function/Pancreatic Exocrine Dysfunction/Chronic Pancreatitis
N-Benzoyl-L-Tyrosyl-P-Aminobenzoic Acid (BT-PABA)/P-Aminosalicylic Acid

Cellular/Molecular Mechanism of Action: N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid and *p*-aminosalicylic acid can function as proteolytic substrates for exocrine pancreatic enzyme fractions.
Application: Characterization of pancreatic exocrine function and the efficacy of exocrine pancreatic supplementation.
Analytical Method: Evaluation of spectrofluorimetry as an alternative to HPLC for determining (measuring) *p*-aminobenzoic acid (PABA) and *p*-aminosalicylic acid.
Implementation: Measurement of PABA and *p*-aminosalicylic acid in the BT-PABA/*p*-aminosalicylic acid test of pancreatic exocrine function. Urine specimens are hydrolyzed for 30 minutes in 4 M NaOH at 100°C. The fluorescence of PABA is measured in dimethyl sulphoxide solution (DMSO lambda ex = 300 nm, lambda em = 340 nm) and that of *p*-aminosalicylic acid in sodium acetate buffer, pH 4.0 (lambda ex = 297 nm, lambda em = 394 nm). The linear range is between 0.038 to 8 mM for PABA and 0.051 to 12 mM for *p*-aminosalicylic acid. The level of within-batch precision is approximately 2.2% and 5.5%, respectively, and the entire analysis can be completed within 40 minutes.
Interpretation and Analysis: Although not eliminated, drug interference is greatly reduced in comparison with colorimetry. During the course of 23 consecutive pancreatic function tests there is an excellent correlation between the PABA/*p*-aminosalicylic acid excretion index obtained by fluorimetry and the results from HPLC analysis ($y = 0.914x + 0.070$, $r = 0.987$, $p < 0.001$). The method is simple and cost-effective and may be particularly valuable in developing countries with a high incidence of chronic pancreatitis.
Instrumentation: Spectrofluorimetry.

Spectrofluorimetric determination of urinary p-aminobenzoic and p-aminosalicylic acids in the BT-PABA/PAS test of pancreatic function. Pemberton PW, Gagjee P, Chaloner C, Braganza JM, Lobley RW. Clin Chim Acta 1991 Jul 15; 199 (3), pp. 253–62.

Pancreatic Exocrine Function/[Severe Systemic Sepsis Influence]
Secretin-Cholecystokinin

Background Information: Sepsis in critical illness is associated with the progressive failure of multiple organs.
Application: Detection and characterization of exocrine panacreatic function (e.g., insufficiency conditions) in conditions of severe sepsis.
Implementation: Evaluation of pancreatic exocrine function using a secretin-cholecystokinin test.
Human Model: Cases classified as critically ill (n = 21 mechanically ventilated with sepsis according to criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee [ACCP/SCCM]; n = 11 shock cases; n = 10 cases without shock; n = 7 healthy reference controls).
Interpretation and Analysis: Duodenal fluid volume is not statistically different among the 3 case groups. Sepsis patients without shock have significantly reduced content of amylase and chymotrypsin in duodenal juice compared with healthy controls ($p < 0.01$). Secretion of amylase, chymotrypsin, trypsin ($p < 0.01$ each), and bicarbonate in duodenal fluid ($p < 0.05$) is impaired in cases of septic shock compared with healthy controls. The content of trypsin is different between sepsis patients and septic shock patients ($p < 0.05$). Spearman correlation analysis is significant between the amylase secretion and the APACHE III and SOFA scores ($p < 0.01$). The SOFA score is also related to secretion of trypsin ($p < 0.05$). In patients on pressor therapy, use of norepinephrine is associated with a significant decrease in bicarbonate secretion ($p < 0.05$). Sepsis is associated with secretory pancreatic dysfunction that is worse in septic shock than in sepsis without shock. Impaired exocrine function significantly correlates with APACHE III and SOFA scores.

Exocrine pancreatic dysfunction in sepsis. Tribl B, Sibbald WJ, Vogelsang H, Spitzauer S, Gangl A, Madl C. Eur J Clin Invest 2003 Mar; 33 (3), pp. 239–43.

Pancreatic Exocrine Function: Chronic Pancreatitis/[Chronic Abdominal Pain]
Secretin/Cholecystokinin (CCK)

Physiological Mechanism of Action: Secretin or CCK are potent hormones that markedly influence exocrine pancreatic function.
Application: Hormonal stimulation with secretin or CCK is the most sensitive means of assessing pancreatic function. Secretin is not available, and current CCK tests are cumbersome, requiring dual tube intubation and marker perfusion techniques.
Route of Administration: Cholecystokinin (intravenously).
Dosage: Cholecystokinin (40 ng/kg/hr infused at a constant rate).
Implementation: Test the efficacy of a new CCK-stimulated pancreatic function test measuring peak lipase concentrations. A Dreiling gastroduodenal tube is inserted to the ligament of Treitz, and fluid is collected on ice for 80 minutes in 4 (20-minute) aliquots. Cholecystokinin is infused. Gastric aspirations are discarded. Duodenal aspirates are ultimately analyzed for volume and enzyme concentration with a clinical laboratory autoanalyzer.

Human Model: Cases of chronic pancreatitis (n = 18) and healthy volunteers (n = 19).
Interpretation and Analysis: Lipase concentration and secretory volume display a peak response by 40 minutes of stimulation, whereas amylase response is variable. The mean peak lipase concentrations (± SEM) for normal volunteers and mild, moderate, and advanced chronic pancreatitis patients are 16.9 ± 1.9, 7.9 ± 1.7, 3.7 ± 1.2, and 2.1 ± 0.6 × 10 5 IU/L, respectively. Lower peak lipase concentrations are significantly associated with more advanced chronic pancreatitis ($p < 0.001$). The receiver operating characteristic curve area for all chronic pancreatitis patients is approximately 0.944 (95% CI = 0.825–0.985). A peak lipase concentration of 780,000 IU/L provides a sensitivity and specificity of 0.833 and 0.867, respectively. A CCK test of this design is well tolerated and is usually associated with no complications. Lipase concentration in duodenal fluid increases nearly 3-fold from baseline after CCK stimulation in healthy volunteers but is markedly reduced in patients with chronic pancreatic disease. Peak lipase concentration are a significant predictor of chronic pancreatitis and correlate with the severity of pancreatic disease. Aspiration of duodenal drainage fluid with a Dreiling tube and analysis with a laboratory autoanalyzer are less cumbersome than marker perfusion and back titration techniques. Measurement of enzyme concentration instead of output can lead to the development of an endoscopic or through-the-scope screening method for assessing patients with suspected chronic pancreatitis or chronic abdominal pain.

Cholecystokinin-stimulated peak lipase concentration in duodenal drainage fluid: A new pancreatic function test. Conwell DL, Zuccaro G, Morrow JB, Van Lente F, Obuchowski N, Vargo JJ, Dumot JA, Trolli P, Shay SS. Am J Gastroenterol 2002 Jun; 97 (6), pp. 1392–7.

Pancreatic Exocrine Function/Lipase Secretion
Cholecystokinin (CCK)

Physiological Mechanism of Action: Cholecystokinin is a potent stimulant of pancreatic exocrine secretion, particularly the release of lipase fractions.
Background Information: Hormonal stimulatory agents can be used to assess pancreatic function. Biologically derived secretin, the most widely used pancreatic secretagogue, is no longer available in the United States. Existing secretory tests using CCK alone are cumbersome, requiring a unique dual tube (gastric and duodenal) collection system and constant perfusion of a non-absorbable marker to calculate enzyme output (in international units [IU]). A simpler, quantitative CCK stimulation test that measures enzyme concentrations (in international units per liter [IU/L]) instead of total output would obviate need for marker perfusion/collection.
Application: Detection and evaluation of alterations in exocrine pancreatic secretory function in duodenal fluid following CCK stimulation.
Route of Administration: Cholecystokinin (intravenous).
Dosage: Cholecystokinin (40 ng/kg/hr infused at a constant rate).
Implementation: A Dreiling tube is inserted endoscopically into the ligament of Treitz. Gastric and duodenal aspiration ports are connected to low intermittent suction. A 20-minute baseline is obtained to clear the gastric and duodenal lumina of residual fluid. Cholecystokinin is then infused and duodenal fluid samples collected on ice for 80 minutes (4 20-minute aliquots). Aspirated fluid is then analyzed for enzyme concentration with an automated chemistry analyzer in the hospital biochemistry laboratory.

Human Model: Healthy cases (n = 19).
Interpretation and Analysis: The mean volume (± SEM) of duodenal fluid collected is 85 ± 4.4 mL (range, 48–118 mL). Fluid analysis reveals a significant rise in mean lipase concentration (± SEM) from a baseline of 595,680 ± 11,930 IU/L to a peak of 1,778,847 ± 171,204 IU/L (mean difference = 1,183,167 IU/L; 95% CI = 664,459 IU/L to 1,701,875 IU/L; $p < 0.001$, Student test). Increases in amylase concentrations are markedly less pronounced and do not reach statistical significance. Mean peak lipase concentration occurs within 50 minutes of acinar cell stimulation. The vast majority of cases tolerate tube placement, and there are usually no episodes of acute pancreatitis or abdominal pain. Pancreatic lipase concentrations in duodenal fluid increase nearly 3-fold after CCK stimulation in healthy volunteers. This magnitude of enzyme secretory response may be a marker of pancreatic function and could potentially lead to a more clinically useful and simpler pancreatic function test. This physiologic study serves as the basis for further investigation of CCK-stimulated lipase concentrations as a new test in the assessment of pancreatic insufficiency.

Analysis of duodenal drainage fluid after cholecystokinin (CCK) stimulation in healthy volunteers. Conwell DL, Zuccaro G, Morrow JB, Van Lente F, O'Laughlin C, Vargo JJ, Dumot JA. Pancreas 2002 Nov; 25 (4), pp. 350–4.

Pancreatic Exocrine Insufficiency
N-Benzoyl-L-Tyrosyl-P-Aminobenzoic Acid (BT-PABA)

Cellular/Molecular Mechanism of Action: N-benzoyl-L-tyrosyl-*p*-aminobenzoic acid is a synthetic peptide substrate for pancreatic enzyme fractions (e.g., chymotrypsin).
Background Information: Exocrine pancreatic insufficiency in the dog can be assessed by the oral administration of the synthetic peptide BT-PABA functioning as a specific substrate for pancreatic chymotrypsin. The subsequent assay of PABA in either the plasma or the urine clearly differentiates control animals from those with exocrine pancreatic insufficiency (EPI). The results are not affected by simultaneously performing the pancreatic function with a xylose absorption test.

Implementation: Evaluation of the possible interference of the specificity of the peptide test for the diagnosis of EPI in conditions of small intestinal disease. Previous investigations have delineated if absorption tests compromise the specificity of peptide tests for the diagnosis of EPI.

Animal Model: Canine (n = 6 with small intestinal disease).

Interpretation and Analysis: Assay of PABA in either the plasma or the urine can clearly differentiate control animals from those with EPI, the results being unaffected by combining pancreatic function tests with a xylose absorption test. In animals with features resembling chronic tropical sprue in man, the results are comparable to those of the control group (n = 4).

In a small number of cases, results may be indistinguishable from those of EPI cases, the estimation of sodium PABA absorption and the assay of proteolytic activity in the duodenal juice demonstrating that this is due to defective hydrolysis of the peptide. Conditions of diffuse intestinal lymphosarcoma and marked villous atrophy can be associated with an apparent reduction in the absorption of sodium PABA. However, although the plasma PABA concentrations following oral BT-PABA are subnormal, they are distinctly higher than those of EPI cases. Such observations suggest that small intestinal abnormalities do not affect PABA absorption sufficiently to interfere with the specificity of the peptide test for the detection of severe EPI in the dog. Such insufficiency may occasionally be secondary to small intestinal disease.

Specificity of the BT-PABA test for the diagnosis of exocrine pancreatic insufficiency in the dog. Batt RM, Mann LC. Vet Rec 1981 Apr 4; 108 (14), pp. 303–7.

Pancreatic Exocrine Insufficiency/[Renal Failure: Anuria]
Pancreolauryl Test

Cellular/Molecular Mechanism of Action: Pancreolauryl is proteolytically degraded by pancreatic enzymes within the lumen of the intestinal tract. Fluoroscein is subsequently liberated and absorbed across the surface of the intestinal mucosa.

Application: Although patients with chronic renal failure have a high incidence of chronic pancreatic disease, the condition is frequently overlooked. Pancreolauryl can be applied to detect and characterize alterations in exocrine pancreatic function in conditions of anuria.

Route of Administration: Pancreolauryl (oral).

Implementation: Modification of the pancreolauryl test as an indirect pancreatic function test for application in cases of anuria.

Interpretation and Analysis: The test permits good discrimination between patients with chronic pancreatic disease and those with a normal pancreas when serum levels of fluorescein are measured 10 hours following administration with a standard meal. The sensitivity at this time interval is 80% in concert with an 83% specificity level. Pancreolauryl test with serum measurements provides a simple, non-invasive, and reliable diagnostic test for chronic pancreatic disease in conditions of simultaneous anuria and chronic renal failure.

Diagnostic value of indirect pancreatic function test in serum of anuric patients with chronic renal failure. Lerch MM, Nolte I, Riehl J, Gladziwa U, Mann H, Sieberth HG, Matern S. Scand J Clin Lab Invest 1994 May; 54 (3), pp. 247–50.

Pancreatic Exocrine Insufficiency/Efficacy of Pancreatic Proteolytic Enzyme Supplementation
Schilling Test

Background Information: The pancreatic Schilling test (PST) is a non-invasive, sensitive pancreatic function test.

Application: Detection of pancreatic insufficiency.

Implementation: Evaluation of the ability of the PST to detect pancreatic proteolytic enzyme replacement in conditions of pancreatic insufficiency. The test is performed following supplementation with the enzyme preparations (i) Viokase (4 tablets), (ii) Pancreas (3 capsules), and (iii) Pancreas (10 capsules). The effect of cimetidine on the results of the PST with high-dose Pancreas can also be determined.

Human Model: Cases of well-documented pancreatic insufficiency and with abnormal PST parameters (n = 7). Cimetadine can also be administered to additional case groups (cimetadine and high-dose Pancreass in controls administered to n = 2 affected cases; n = normal reference controls).

Interpretation and Analysis: The PST is a sensitive non-invasive test for detecting the presence of orally administered proteolytic enzymes in subjects with pancreatic insufficiency and in normal cases. Administration of enzymes in a form of enteric-coated microspheres does not enhance the delivery of proteolytic enzymes to the small intestine when compared to conventional high-dose enzyme replacement. Cimetidine appears to decrease the inactivation of the proteolytic enzymes in enteric-coated microspheres, suggesting that a low pH in the small intestine and stomach are responsible for the poor delivery of the enzymes into the small intestine.

Use of pancreatic Schilling test to determine efficiency of pancreatic enzyme delivery in pancreatic insufficiency. Brugge WR, Goldberg HJ, Burke CA, Depping BJ. Dig Dis Sci 1988 Oct; 33 (10), pp. 1226–32.

Pancreatic Gastrinoma/Insulinoma/Intestinal Carcinoids/Zollinger-Ellison Syndrome
Calcium Infusion

Cellular/Molecular Mechanism of Action: Calcium induces the release of certain endogenous hormones including gastrin, secretin, and somatostatin.

Diagnostic Application: (i) Gastrinoma—gastrin assay; (ii) Zollinger-Ellison syndrome—secretin assay; (iii) somatistatinoma—somatostatin (calcium/pentagastrin combination); (iv) insulinoma—diagnostically not reliable; and (v) intestinal carcinoid—diagnostically not reliable.

Interpretation and Analysis: Elevations in gastrin, secretin, and somatostatin following calcium administration when measured concentrations are above normal reference control values, thereby implying the existence of a gastrinoma, Zollinger-Ellison syndrome, or somatistatinoma, respectively.

Pancreatic Gastrinoma/Zollinger-Ellison Syndrome
Secretin and [Ca^{++}]

Cellular/Molecular Mechanism of Action: Induces gastrin secretion.

Application: To evaluate criteria of "positivity" for, and usefulness of both the secretin and calcium gastrin-provocative tests in conditions of Zollinger-Ellison syndrome.

Route of Administration: Kabi-secretin (intravenous bolus); calcium gluconate (10% intravenous).

Dosage: Kabi-secretin (2 U/kg bolus); calcium gluconate 10% (54 mg/kg/hr as 5 mg/kg/hr of calcium continuously infused for 3 hours).

Considerations: The distinct advantages (short test period, low patient morbidity, and relatively great potency) of the calcium plus secretin test make it an attractive alternative to other previously described provocative tests for the diagnosis of gastrinoma. There are usually no false-positive or false-negative tests with the calcium plus secretin.

Implementation: Evaluation of suspected cases of gastrinoma as a function of response to Kabi-secretin and calcium gluconate (10%) (54 mg/kg.h [5 mg/kg.h of calcium]) given by continuous intravenous infusion for 3 hours. Serum gastrin is measured at −15 and −1 minutes before and 2, 5, 10, 15, 20, and 30 minutes after secretin, or every 30 minutes for 3 hours during calcium infusion. Serum calcium and serum gastrin are measured simultaneously during calcium infusion. Criterion for diagnosis is either a 50% increase or a 200 pg/ml increase above the basal gastrin level.

Human Model: Cases suspected of having Zollinger-Ellison syndrome (n = 80).

Design: Prospective trial in consecutive patients.

Interpretation and Analysis: There is no significant difference in the responses of cases with different extents or locations of the tumor, presence or absence of multiple endocrine neoplasia, type-I, or with fasting gastrin $<$ or $>$ 1,000 pg/mL. In cases with fasting gastrin of $<$ 1,000 pg/mL, sensitivity of the secretin test using the criterion of an increase in gastrin of at least 110 pg/mL is 93% (CI, 76–99%) and for an increase of 200 pg/mL it is 85% (CI, 66–96%, $p > 0.05$). With the calcium infusion test, the sensitivity using the criterion of an increase of 395 pg/mL is 43% (CI, 23–66%) and for an increase of 50% is 74% (CI, 52–90%, $p < 0.01$). The calcium infusion test is positive in 33% of cases with a negative secretin test. Applying the secretin test, 75% of cases have a positive response by 5 minutes, 95% by 10 minutes, 100% by 15 minutes, and 6% only at 2 minutes. Utilizing calcium infusions, cases have positive responses at 120 to 180 minutes. The secretin test is preferred over the calcium test because of its greater sensitivity and simplicity. The recommended criteria are a 200 pg/mL increase for the secretin test and a 395 pg/mL increase for the calcium test. The calcium test should be reserved for patients having a negative secretin test, gastric acid hypersecretion, and a strong clinical suspicion of the Zollinger-Ellison syndrome.

Instrumentation: Laboratory facilities and instrumentation for immunodetection methodologies (RIA, ELISA).

Secretin and calcium provocative tests in the Zollinger-Ellison syndrome. A prospective study. Frucht H, Howard JM, Slaff JI, Wank SA, McCarthy DM, Maton PN, Vinayek R, Gardner JD, Jensen RT. Ann Intern Med 1989 Nov 1; 111 (9), pp. 713–22.

Pancreatic Gastrinoma/Zollinger-Ellison Syndrome: Gastrin-Secreting Tumors
Secretin

Cellular/Molecular Mechanism of Action: Secretin induces the production/synthesis/release of gastrin.

Considerations: The provocative secretin-stimulation test has an important role in the diagnosis and management of gastrin-secreting neuroendocrine tumors. However, the molecular basis for positive and false-negative secretin-stimulation test results in cases with this tumor type has previously been poorly characterized.

Application: The secretin-stimulation test can be used to differentiate between cases that have (i) histologically proven tumors but have a normal serum gastrin level and negative secretin-stimulation test results; and (ii) more typical patients with this syndrome. Ancillary diagnostic test can include immunohistochemistry, reverse-transcription polymerase chain reaction (PCR), receptor binding, and signaling assays.

Experimental Interpretation: Tumor expression of the class B G protein-coupled secretin receptor mediates a positive result. False-negative results can be explained by messenger RNA missplicing, resulting in a receptor variant missing exon 3 that encodes residues 44–79 in the amino-terminal tail of the mature receptor. This variant with an in-frame deletion has been shown to be synthesized and to traffic to the cell surface normally, where it can neither bind secretin nor mediate a secretin-stimulated adenosine 3′,5′-cyclic monophosphate response. It is able to act as a dominant negative inhibitor of wild-type secretin receptor function. Some of the atypical presentations of this syndrome provide important insights into basic mechanisms of disease.

Dominant negative action of an abnormal secretin receptor arising from mRNA missplicing in a gastrinoma. Ding WQ, Kuntz S, Bïhmig M, Wiedenmann B, Miller LJ. Gastroenterology 2002 Feb; 122 (2), pp. 500–11.

Pancreatic Gastrinoma: Zollinger-Ellison Syndrome/[Achlorhydria Differential Exclusion]
Secretin

Cellular/Molecular Mechanism of Action: Secretin promotes elevated production and release of gastrin into the intravascular compartment.

Background Information: The secretin stimulation test has become the preferred provocative test to characterize suspected cases of Zollinger-Ellison syndrome.

Application: Dectection and characterization of Zollinger-Ellison syndrome cases and the exclusion of conditions like achlorhydria as a possible differential diagnoses.

Implementation: Detection and characterization of Zollinger-Ellison syndrome conditions applying a pure secretin preparation, a gastrin-specific radioimmunoassay, and an appropriate sequential sampling protocol. These aspects of the testing procedure are vital to performing a successful and accurate test. Gastric acid analysis is necessary in the assessment of hypergastrinemia to confirm acid hypersecretion and exclude achlorhydria.

Interpretation and Analysis: When properly performed and interpreted, the secretin provocation test offers a safe, expeditious, and reliable means of evaluating conditions of hypergastrinemia. Gastric acid analysis is necessary in the assessment of hypergastrinemia to confirm acid hypersecretion and exclude the occurrence of achlorhydria.

Instrumentation: Laboratory facilities and instrumentation necessary to perform gastrin-specific radioimmunoassay methodologies.

Secretin provocation test in the diagnosis of Zollinger-Ellison syndrome. Brady CE 3rd. Am J Gastroenterol 1991 Feb; 86 (2), pp. 129–34.

Pancreatic Gastrinoma: Zollinger-Ellison Syndrome/Gastrinoma/Insulinoma/Carcinoids (Intestinal)
Calcium Infusion (Rapid)/Calcium Infusion (Slow)/Secretin/Calcium Infusion (Rapid) with Secretin or Pentagastrin

Application: Detection of gastrinoma conditions.

Background Information: Calcium infusion has been advocated as a provocative test for the diagnosis of certain endocrine tumors of the pancreas and gastrointestinal tract (gastrinoma, insulinoma, intestinal carcinoids). The release of gastrin from gastrinoma tissue is very sensitive to alterations in serum calcium levels, and the calcium infusion test is recommended in Zollinger-Ellison syndrome when the results of secretin stimulation are equivocal.

Dosage: Comparison of 4 separate diagnostic schedules: (i) rapid calcium infusion (2 mg Ca^{++}/kg/min); (ii) secretin (2 clinical units [CU]/kg/bolus); (iii) long calcium infusion (12 mg Ca^{++}/kg/3 hr); and (iv) a combination test consisting of a rapid calcium infusion followed immediately by secretin.

Implementation: Evaluation of elevated serum gastrin secretion in conditions of gastrinoma in response to provocative test reagents.

Blood is drawn for serum gastrin levels before and following infusion of test agents

Human Model: Cases of gastrinoma (n = 13; n = 10 normal reference controls; n = 1 of presumed gastrinoma).

Interpretation and Analysis: The rapid administration of calcium followed by secretin provokes the greatest increases in serum gastrin above basal levels in both normals (29%) and patients (362%). Peak gastrin levels in patients are similar following the long calcium infusion (341%) but are less following the rapid calcium infusion alone (124%) and secretin alone (207%). There are usually no false-positive or false-negative tests with the calcium plus secretin when the criterion for diagnosis is either a 50% increase or a 200 pg/ml increase above the basal gastrin level. The distinct advantages (short test period, low patient morbidity, and relatively great potency) of the calcium plus secretin test make it an attractive alternative to other previously described provocative tests for the diagnosis of gastrinoma.

The calcium provocative test in the detection of insulinoma and carcinoid tumors is less reliable than other safer and simpler procedures. Intravenous injection of calcium followed by pentagastrin stimulates the release of somatostatin in cases with somatostatinoma and offers a reliable means for establishing the diagnosis of this tumor. Calcium administration has not proven to be useful in the diagnosis of other endocrine tumors of the digestive system.

Comparison of four provocative tests for the diagnosis of gastrinoma. Romanus ME, Neal JA, Dilley WG, Leight GS, Linehan WM, Santen RJ, Farndon JR, Jones RS, Wells SA Jr. Ann Surg 1983 May; 197 (5), pp. 608–17.

Use of calcium provocative test in the diagnosis of gastroenteropancreatic endocrine tumors. Vezzadini C, Poggioli R, Casoni I, Vezzadini P. Panminerva Med 1996 Dec; 38 (4), pp. 255–8.

Pancreatic Gastrinoma: Zollinger-Ellison Syndrome/Recurrent Gastrinoma Post-Resections
Secretin/Fasting Serum Gastrin

Application: Detection and characterization of gastrinoma/Zollinger-Ellison syndrome.

Implementation: Characterization of the best method for determining freedom from disease after gastrinoma resection and for predicting long-term disease-free status in patients with Zollinger-Ellison syndrome. Cases can be assessed after gastrinoma resection, before discharge, 3 to 6 months after surgery, and yearly thereafter. Evaluation can include secretin provocative testing and fasting serum gastrin determinations. Follow-up examinations after the initial postoperative evaluations can include a clinical assessment, acid secretion studies, a calcium provocative test, and various imaging studies.

Human Model: Cases of Zollinger-Ellison syndrome (n = 81 consecutive patients that underwent surgical exploration for gastrinoma resection).

Interpretation and Analysis: Most suspect cases (96%) will have gastrinomas. Freedom from disease is defined by (i) improvement in symptoms; (ii) reduced acid output and antisecretory drug requirements; and (iii) normal gastrin level, normal imaging studies, and negative gastrin provocative studies. Fifty-two percent of patients (n = 42) may be disease-free immediately after surgery, 44% at 3 to 6 months, 42% at 1 year, and 35% by 5 years (mean follow-up, 39 months).

The secretin provocative test may be the 1st test to become positive in 45% of patients with a recurrence, the serum gastrin determination may be the 1st test to become positive in 36%, while both tests may become positive at the same time in less that one-fifth of all cases (18%). Conditions of "no recurrence" are 1st detected by imaging studies or by calcium provocative testing. Fasting serum gastrin levels and secretin provocative test results at different postoperative times can be used to predict the probability of a patient remaining disease free at 3 years. Patients with a normal gastrin level and a normal secretin provocative test immediately after surgery have a 3-year disease-free probability of 75%, and normal results on both tests at 6 months, 1 year, and 2 years yielded respective probabilities of 88%, 95%, and 100%.

Both the secretin provocative test and determination (assay) of fasting serum gastrin are necessary for the early diagnosis of cases of recurrent disease after gastrinoma resection. The calcium provocative test and imaging studies do not detect any recurrences 1st. Fasting serum gastrin determinations and secretin provocative testing at different postoperative times can be used to predict the probability of a patient remaining disease free at 3 years.

Assessment and prediction of long-term cure in patients with the Zollinger-Ellison syndrome: The best approach. Fishbeyn VA, Norton JA, Benya RV, Pisegna JR, Venzon DJ, Metz DC, Jensen RT. Ann Intern Med 1993 Aug 1; 119 (3), pp. 199–206.

Pancreatic Gastrinoma: Zollinger-Ellison Syndrome vs Chronic Duodenal Ulcer/ [Gastrin-Secreting Tumor]
Secretin/Cimetadine

Application: Characterization of chronic duodenal ulcer case compared to Zollinger-Ellison syndrome.

Route of Administration: Cimetadine (oral and IV).

Dosage: Cimetadine (1 g/day–1.4 g/day).

Considerations: Results from preliminary investigations suggest that previous cimetidine treatment does not alter, and may even increase the diagnostic sensitivity of the secretin provocative test.

Implementation: Evaluation of conditions that may be either duodenal ulcer or Zollinger-Ellison syndrome using secretin and cimetadine response tests.

Human Model: Cases of chronic duodenal ulcer (n = 16) and Zollinger-Ellison syndrome (n = 5).

Interpretation and Analysis: In cases of chronic duodenal ulcer, a 2nd secretin test can be done during acute cimetidine administration (IV). Only slight variations in plasma gastrin may be detected compared with results from the 1st tests (n = 4 cases). A 3rd test in the same cases with chronic duodenal ulcers after 1 month of cimetidine treatment (1 g/day PO) reveals a time-zero gastrin concentration that is significantly higher than values observed in the previous 2 tests ($p < 0.01$).

Integrated gastrin response after secretin is usually significantly lower in the 3rd test than in the 1st ($p < 0.05$). In Zollinger-Ellison syndrome cases treated with 1.0 and 1.4 g/day cimetidine for 3 months (n = 2 cases), gastrin at time-zero is not markedly increased, whereas compared with the 1st test gastrin levels are usually higher at each time period analyzed after secretin administration.

Secretin-induced gastrin response in the Zollinger-Ellison syndrome and chronic duodenal ulcer patients before and after cimetidine treatment. Quatrini M, Basilisco G, Conte D, Bardella MT, Bozzani A, Bianchi PA. Am J Gastroenterol 1984 May; 79 (5), pp. 345–7.

Pancreatic Insufficiency
[^{13}C]-Egg White Breath Test/[Indirect Pancreatic Function Test]

Cellular/Molecular Mechanism of Action: [^{13}C]-egg white can function as a substrate for pancreatic enzyme fractions. The pep-

tide fragments are then absorbed in the gastrointestinal tract and metabolized to [$^{13}CO_2$] that is in turn exhaled from the respiratory tract.

Background Information: The recent availability of egg white protein highly enriched with [^{13}C] has allowed breath test technology to be adapted for the study of protein digestion and absorption. Pancreatic trypsin is considered to be the key enzyme in the proteolytic cascade.

Application: Evaluation of pancreatic trypsin activity within the small intestine in conditions of pancreatic disease using the [^{13}C]-egg white breath test.

Route of Administration: [^{13}C]-labeled egg protein (oral).

Dosage: [^{13}C]-labeled egg protein (22 g).

Implementation: Evaluation of pancreatic disease states with the ingestion of a test meal consisting of [^{13}C]-labeled egg protein. Breath samples are taken before and after ingestion of the meal and analyzed for [$^{13}CO_2$] concentration. Moreover, pancreatic trypsin output after maximal stimulation is also measured.

Human Model: Cases with pancreatic disease (n = 30; and n = 48 normal volunteers). Pancreatic trypsin output after maximal stimulation measured in n = 13/30 affected cases and n = 9/48 healthy volunteers.

Interpretation and Analysis: The 6-hour cumulative [$^{13}CO_2$] excretion in breath is significantly lower in patients than observed in controls (mean [SEM]: 6.23 + 0.82% vs 19.16 + 0. 58%, $p < 0.0001$). An excellent correlation can be appreciated between the 6-hour cumulative [$^{13}CO_2$] excretion and trypsin activity after maximal pancreatic stimulation. The non-invasive [^{13}C]-egg white breath test is promising as an indirect pancreatic proteolytic function test.

^{13}C-egg white breath test: A non-invasive test of pancreatic trypsin activity in the small intestine. Evenepoel P, Hiele M, Geypens B, Geboes KP, Rutgeerts P, Ghoos Y. Gut 2000 Jan; 46 (1), pp. 52–7.

Pancreatic/Duodenum Gastrinoma/Zollinger-Ellison Syndrome: Persistent or Recurrent
Methylene Blue/Secretin

Application: Identification of gastrinoma (e.g., 6 mm) in the duodenum in order to facilitate surgical excision.

Route of Administration: Selective intra-arterial (methylene blue).

Implementation: Evaluation of Zollinger-Ellison syndrome when localizing modalities fail to detect a tumor (e.g., ultrasonography, computed tomography, magnetic resonance imaging, duodenal endoscopy, endoscopic ultrasonography, intraoperative endoscopic transillumination of the duodenum). In addition, postoperatively, a negative secretin provocative test result may also be appreciated.

In difficult cases presenting with this type of profile, an novel method can be utilized that involves selective arterial injection of secretin with hepatic venous gastrin sampling to identify the vessel feeding a gastrinoma lesion. An angiographic catheter is then positioned in this artery. At laparotomy, methylene blue is injected through the catheter to selectively stain gastrinoma lesions thereby facilitating its identification.

Human Model: Cases of persistent Zollinger-Ellison syndrome (n = 1 female 40 years) despite previous excision of a 4-cm duodenal gastrinoma. Localizing studies including ultrasonography, computed tomography, magnetic resonance imaging, duodenal endoscopy, endoscopic ultrasonography, and intraoperative endoscopic transillumination of the duodenum failed to detect a tumor.

Interpretation and Analysis: Selective intra-arterial methylene blue injection can enhance intraoperative detection of small gastrinomas and may improve the rate of curative resection in the Zollinger-Ellison syndrome. Further evaluation of this novel localizing technique is warranted.

Endocrine tumors of the pancreas. Meko JB, Norton JA. Curr Opin Gen Surg 1994; pp. 186–94.

Selective intra-arterial methylene blue injection: A novel method of localizing gastrinoma. Ko TC, Flisak M, Prinz RA. Gastroenterology 1992 Mar; 102 (3), pp. 1062–4.

Sphincter of Oddi Dysfunction (Abdominal Pain)
Morphine-Prostigmin

Background Information: Little data is available on the response of control populations to morphine-Prostigmin, or the reproducibility of this test on different days.

Application: diagnose disease of the sphincter of Oddi in cases with chronic abdominal pain. Test subjects included 10 healthy volunteers, 5 patients with symptoms of irritable bowel syndrome and 4 patients with chronic abdominal pain.

Considerations: Results noted in previous reports suggest that (i) neither enzyme rise nor reproduction of abdominal pain produced by morphine-Prostigmin is specific for disease of the sphincter of Oddi, and (ii) the morphine-Prostigmin test may give different results on different days in the same patient. Major clinical decisions should not be made on the basis of this test.

Interpretation and Analysis: A 2-fold rise in amylase or lipase levels, reproduction of abdominal pain or both have been considered positive responses. A positive morphine-Prostigmin test has been used as a justification for a sphincteroplasty, a surgical procedure with significant morbidity.

Sixty percent of healthy controls and patients with irritable bowel syndrome have marked elevations in amylase and lipase. Forty percent of those with irritable bowel syndrome have a reoccurance of typical abdominal pain after morphine-Prostigmin. Cases with chronic abdominal pain (2/4) have a different response to morphine-Prostigmin following the 2nd vs the 1st administration dose.

Manometric diagnosis of sphincter of Oddi spasm as a cause of postcholecystectomy pain and the treatment by endoscopic sphincterotomy. Tanaka M, Ikeda S, Matsumoto S, Yoshimoto H, Nakayama F. Ann Surg 1985 Dec; 202 (6), pp. 712–9.

The morphine-prostigmin provocative test—Is it useful for making clinical decisions? Steinberg WM, Salvato RF, Toskes PP. Gastroenterology 1980 Apr; 78 (4), pp. 728–31.

NEOPLASIA OF THE INTESTINE AND COLON

Colon Cancer (Intestinal/Colon) Optical Lesion Imaging
Indotricarbocyanine (ITCC)/ITCC-Transferrin/ITCC-Human Serum Albumin

Cellular/Molecular Mechanism of Action: Indotricarbocyanine is a dye that can serve as a detectable marker molecule in biological systems.

Background Information: Macromolecules accumulate in solid tumors and can thus be used as carriers for the delivery of attached contrast agents to tumors.

Application: Indotricarbocyanine can be applied as a contrast agent for the optical imaging of neoplastic lesions.

Route of Administration: Intravascular.

Semi-Synthetic Methods: Production of serum protein-dye conjugates consisting of transferrin (Tf) or human serum albumin (HSA) covalently linked to ITCC derivative.

Implementation: Evaluation of semi-synthetic ITCC-HSA and ITCC-Tf conjugates pertaining to (i) photophysical properties; (ii) ability to bind *in vitro* to tumor cells; and (iii) *in vivo* potential to detect/characterize experimental tumors.

Model: Human HT29 colon cancer cell types propagated *in vitro* in a tissue culture environment and in HT29 tumor-bearing nude mice.

Interpretation and Analysis: In contrast to ITCC-HSA, ITCC-Tf displays *in vitro* receptor-mediated uptake by HT29 human colon cancer cells. After intravenous injection, ITCC-HAS and ITCC-Tf both induce increased *in vivo* fluorescence contrast of tumors. After 24 hours the contrast between tumor and normal tissue is significantly higher for ITCC-Tf than for HAS-ITCC-HSA. Dye-induced fluorescence can be found to be predominantly located in perinecrotic areas of tumor lesions. Furthermore, ITCC-Tf produces fluorescence of viable tumor cells, whereas ITCC-HAS fluorescence can be detected along connective tissue. Therefore, ITCC-labeled Tf and HSA can serve as macromolecular contrast agents for the optical imaging of tumors, with ITCC-Tf showing higher efficiency.

Macromolecular contrast agents for optical imaging of tumors: Comparison of indotricarbocyanine-labeled human serum albumin and transferrin. Becker A, Riefke B, Ebert B, Sukowski U, Rinneberg H, Semmler W, Licha K. Photochem Photobiol 2000 Aug; 72 (2), pp. 234–41.

Colon Carcinoma Adhesion to Endothelium/ Intravital Observation of Cancer Cell/ Microcirculation Interactions
CalceinAM

Refer to Section II, Genetic Disorders, Infectious Disease, Neoplasia, and Metabolic Conditions.

Instrumentation: Intravital fluorescence microscopy.

An intravital model to monitor steps of metastatic tumor cell adhesion within the hepatic microcirculation. Haier J, Korb T, Hotz B, Spiegel HU, Senninger N. J Gastrointest Surg 2003 May–Jun; 7 (4), pp. 507–14; discussion 514–5.

Colorectal Cancer (Adenocarcinoma): Sentinel Lymph Node (SLN) Mapping
Isosulfan Blue Dye

Cellular/Molecular Mechanism of Action: Isosulfan blue is a pigment-based dye reagent.

Background Information: Sentinel lymph node biopsy is a widely accepted method for staging breast cancer and melanoma, and it has recently been proposed as a means of improving staging in colorectal cancer. However, lymphatic mapping in colorectal cancer has been plagued by studies demonstrating high false-negative rates.

Application: Evaluate possible mechanisms for high false-negative rates after SLN biopsy in colorectal cancer. Poor accuracy may be due to bulky tumor mass or complete replacement of lymph nodes by tumor lesions.

Route of Administration: Isosulfan blue dye (intralesional injection).

Dosage: Isosulfan blue dye (1 ml volumetric).

Implementation: Intraoperative injection of isosulfan blue dye into colorectal adenocarcinoma lesions for the purpose of lymphatic mapping using either an *in vivo* or *ex vivo* technique. Routine pathological evaluations are then performed. Sentinel node can be examined by hematoxylin and eosin stains, and if they are negative they are subsequently evaluated by cytokeratin immunohistochemistry. The patient's age, operation type, tumor stage, tumor diameter, method of SLN detection, presence of palpable nodes, and pathological description of nodes completely replaced by tumor are recorded.

Human Model: Cases undergoing colorectal cancer resection (n = 50; mean age, 62.8, 50% men) subjected to 51 lymphatic mapping procedures.

Interpretation and Analysis: Right- and left-sided colorectal resections are almost equally distributed (48% vs 42%). Sentinel lymph nodes can be successfully identified in a large percentage of cases (e.g., n = 47/51 or 92%). The mean number of SLNs obtained from each specimen is approximately 1.5 (range, 1–5). Routine pathological evaluation demonstrates lymph node metastasis in less than half of cases (e.g., n = 20/47 or 43%) that have SLN identified. The SLN is positive for metastasis in about half of these cases (e.g., n = 10/20) Approximately half of cases with metastasis have a negative SLN resulting in a false-negative rate of 50% (e.g., n = 10/20 cases). The rate of false-negatives is significantly higher in patients undergoing left-sided procedures vs right-sided procedures. Differences among gender, tumor stage, tumor diameter, method of SLN detection, presence of palpable nodes, and pathological description of nodes completely replaced by tumor are not associated with a higher false-negative rate.

Does tumor burden limit the accuracy of lymphatic mapping and sentinel lymph node biopsy in colorectal cancer? Broderick-Villa G, Ko A, O'Connell TX, Guenther JM, Danial T, DiFronzo LA. Cancer J 2002 Nov–Dec; 8 (6), pp. 445–50.

Colorectal Cancer
Fluorescein Isothiocyanate-Conjugated GS I-A4/Lectins DBA and LBL

Refer to Section II, Genetic Disorders, Infectious Disease, Neoplasia, and Metabolic Conditions, and Section III, Contrast and Image-Enhancing Agents.

The lectin Griffonia simplicifolia I-A4 (GS I-A4) specifically recognizes terminal alpha-linked N-acetylgalactosaminyl groups and is cytotoxic to the human colon cancer cell lines LS174t and SW1116. Chen YF, Boland CR, Kraus ER, Goldstein IJ. Int J Cancer 1994 May 15; 57 (4), pp. 561–7.

Gastrointestinal Cancer/Intestinal/Colon: Intestinal Microcarcinomas/Colorectal Cancer/Gastric Cancer
[Indocyanine Green]-Anti-MUC1 Mucin

Refer to Section II, Genetic Disorders, Infectious Disease, Neoplasia, and Metabolic Conditions, and Section III, Contrast and Image-Enhancing Agents.

Instrumentation: Videoendoscopy.

Vital immunostaining of human gastric and colorectal cancers grafted into nude mice: A preclinical assessment of a potential adjunct to videoendoscopy. Kusaka Y, Ito S, Muguruma N, Tadatsu M, Bando T, Ii K, Irimura T, Shibamura S. J Gastroenterol 2000; 35 (10), pp. 748–52.

5 Hematopoietic System

HEMATOPOIESIS, HEMATOLOGY, AND HEMOSTASIS

Adhesion: Endothelium Interactions/Colon Carcinoma/Intravital Observation of Cancer Cell/Microcirculation Interactions
CalceinAM

Background Information: Organ-specific tumor cell adhesion within the microcirculation of host organs is an important step in the metastatic cascade. Circulating tumor cells have to adhere endothelial surfaces within the microcirculatory vessels, quickly stabilize, and then de-stabilize their adhesion in order to leave the circulation to avoid toxic effects of hydrodynamic shear forces of circulating blood.
Application: Intravital observation of colon carcinoma cell adhesion within the hepatic microcirculation.
Implementation: CalceinAM is used to label colon carcinoma cell populations (e.g., HT-29 [human] and CC531 [rat]) that are then injected intra-arterially as single-cell suspensions. Intravital fluorescence microscopy is then applied to detect adhesive interactions between circulating tumor cells and endothelial surfaces within the hepatic microcirculation. These interactions are then analyzed regarding their time course and site localization within the vascular tree.

Animal Model: Rats (Sprague-Dawley).
Interpretation and Analysis: Autofluorescence of liver parenchyma is sufficient for distinction of hepatic sinusoids. Intravital microscopy is capable of differentiating early events in adhesion formation within hepatic sinusoid, adhesion stabilization, and extravasation of the tumor cells into the liver parenchyma. Tumor cell adhesion occurs almost exclusively within sinusoidal capillaries; however, the diameter of these vessels is usually larger than that of the tumor cells leaving remaining perfused lumen of the capillaries. Colon carcinoma cells rapidly migrate into the liver parenchyma after successful adhesion within the sinusoids. In contrast to common end point assays of the metastatic cascade, this *in vivo* model allows investigations of metastatic colon carcinoma cell adhesion within the liver microcirculation at specific steps during the formation of hematogenous metastasis and their underlying mechanisms.
Instrumentation: Intravital fluorescence microscopy.

An intravital model to monitor steps of metastatic tumor cell adhesion within the hepatic microcirculation. Haier J, Korb T, Hotz B, Spiegel HU, Senninger N. J Gastrointest Surg 2003 May–Jun; 7 (4), pp. 507–14; discussion 514–5.

Anemia: Fanconi's Syndrome
Nitrogen Mustard (NTM)/[Diepoxybutane (DEB)]

Cellular/Molecular Mechanism of Action: Nitrogen mustards and diepoxybutane can function as DNA alkylating agents.
Background Information: Fanconi's anemia (FA) is an autosomal recessive inherited disorder that is associated with a variety of congenital anomalies. These include morphometric abnormalities involving mainly the head and face, skeletal malformations particularly of the radial ray, growth retardation, abnormal skin pigmentation, deafness, and renal, ocular, genital, and cardiac defects. The cardinal clinical feature is a severe progressive pancytopenia.
Application: Compare 2 different alkylating agents that would permit rapid and unequivocal detection of FA.
Implementation: Evaluate the potential to detect conditions of FA rapidly and accurately applying alkylating agents as screening agents. In this context, NTM and DEB tests are performed in concert with the determination of baseline chromosomal breakage analyses.

Human Model: Cases suspected of suffering from Fanconi's syndrome anemia (n = 271).
Interpretation and Analysis: Chromosomal breakage analyses can facilitate the detection of cases with FA (e.g., n = 72) in contrast to cases unaffected by the condition (e.g., n = 136). Studies of family members of affected FA cases (e.g., n = 63 individuals) reveal that NTM seems more specific to identify chromosomal breakages in FA parents than is possible with DEB.

Differential diagnosis of Fanconi's anemia by nitrogen mustard and diepoxybutane. Deviren A, Yalman N, Hacihanefioglu S. Ann Hematol 2003 Apr; 82 (4), pp. 223–7.

Anemia: Iron Deficiency/Renal Tumor/Infectious Anemia
[^{57}Co]Cl_2

Cellular/Molecular Mechanism of Action: [^{57}Co] mimics non-radioactive cobalt, which is an enzyme co-factor required for certain biochemical synthetic mechanisms.
Background Information: In 1971 Sorbie et al. described a simple [^{57}Co]-excretion test as an aid in the diagnosis of iron deficiency anemia. Renal excretion of a tracer dose of 0.5 muCi [^{57}Co]Cl_2 is significantly elevated in cases with iron deficiency anemia (31% of the administered dose in 24 hours urine) as compared with normal reference controls (18%).
Application: Detection and characterization of different types of anemia.
Route of Administration: [^{57}Co]Cl_2 (oral).
Dosage: [^{57}Co]Cl_2 tracer dose (0.5 muCi).
Implementation: Application of the [^{57}Co]-excretion test to evaluate different types of anemia. A modified version of the test entails measurement of serum activity 1, 2, 3, 7, 11, and 24 hours after the oral administration of the test formulation.

Human Model: Human cases and controls (n = 29 of different types of anemia; n = 10 normal controls).
Interpretation and Analysis: In all anemias as well as in the control group the maximum serum activity occurs 3 hours after the oral administration of the tracer. The 3 hours serum activity is elevated only in cases with iron deficiency anemia (5.53%/1 serum) as compared with normal reference controls (1.92%/1) and renal tumor and infectious anemia (1.20%/1, $p < 0.001$). The [^{57}Co] excretion is moderately elevated in most cases with iron deficiency anemia (average 31.5% [^{57}Co]-activity in 24 hours found in the urine) in comparison to healthy controls (average 25.30%). Contrary to the results

obtained by Sorbie et al., some investigations have therefore discovered a wide range of fluctuations in [^{57}Co]-excretion test within case-group types with a poor statistical significance of $p > 0.005$.

The [^{57}Co]-excretion and absorption test in the diagnosis of iron deficiency anemia. Bekier A, Holdener E. Nuklearmedizin 1976 Jun; 15 (3), pp. 126–30.

Anemia: Diamond-Blackfan
Metaclopramide

Cellular/Molecular Mechanism of Action: Metaclopramide promotes the release of prolactin.
Implementation: In cases of Diamond-Blackfan (congenital hypoplastic) anemia that are refractory to corticosteroids, approximately 33% of patients will respond to metoclopramide therapy.
Route of Administration: Metaclopramide (orally).
Dosage: Metaclopramide (10 mg, 3 times daily). Note: Conditions of anemia will usually not improve until 12 to 15 weeks of therapy have been completed.
Considerations: Several clinical scenarios have reported marked improvements in severe macrocytic anemia in women during their 2nd and 3rd trimesters of pregnancy and while breast-feeding children.
Interpretation and Analysis: Serum prolactin levels can increase from 7 to 133 ng/mL (normal < 20 ng/mL) simultaneously accompanied by elevations in hematocrit from 17% to 22% to 35%. With continued therapy (10 mg orally daily), hematocrit values may range from 30% to 40% over a period of 6 years, although the macrocytosis may persist (mean corpuscular volume, 100–112 fL). In pilot studies, metoclopramide therapy has been administered in patients with Diamond-Blackfan anemia that are refractory to low doses of corticosteroids. In these trials, approximately 33% respond the metaclopramide in a manner that suggests that this approach to therapeutic intervention may be universally beneficial.

Response of Diamond-Blackfan anemia to metoclopramide: Evidence for a role for prolactin in erythropoiesis. Abkowitz JL, Schaison G, Boulad F, Brown DL, Buchanan GR, Johnson CA, Murray JC, Sabo KM. Blood 2002 Oct 15; 100 (8), pp. 2687–91.

Cell Cycle Phases: Subpopulation Identification/Identification of Populations in G_0 and G_1 Phases of the Cell Cycle/Primitive Hematopoietic Stem Cell Characterization
Rhodamine 123/Hoechst 33342/Pyronin

Application: Supravital staining reagents that can be used to characterize cells that are in specific stages of mitotic division.
Implementation: Utilization of the supravital dyes rhodamine 123 (Rh), Hoechst 33342 (Ho), and pyronin (PY) to identify G_0 and G_1 phase subsets of primative hemopoietic stem cells (PHSC) for isolation. A competitive repopulation assay is used to evaluate their *in vivo* function.

***Ex Vivo* Model:** Primitive hematopoietic stem cells at phases G_0 and G_1 of the cell cycle.
Interpretation and Analysis: $Rh_{lo}Lin^{-}Kit^{+}Sca\text{-}1^{+}$ PHSC are relatively quiescent compared with the more mature $Rh_{hi}Lin^{-}Kit^{+}Sca\text{-}1^{+}$ HSC and $Rh_{hi}Lin^{-}Kit^{+}Sca\text{-}1^{-}$ progenitors. In addition, cells with $Rh_{lo}Lin^{-}Kit^{+}Sca\text{-}1^{+}$, $Rh_{lo}Ho_{lo}Lin^{-}Sca\text{-}1^{+}$, or $Rh_{lo}Ho_{sp}Lin^{-}Sca\text{-}1^{+}$ phenotypes identify the same cell population. Further subfractionation of $Rh_{lo}Ho(_{lo}/_{sp})Lin^{-}Sca\text{-}1^{+}$ PHSC can be achieved using PY (e.g., PY_{lo} and PY_{hi} subsets). Limiting dilution analysis reveals that the frequency of long-term *in vivo* competitive repopulating units (CRU) of $PY_{lo}Rh_{lo}Ho_{lo}/_{sp}$ PHSC is 1 in 10 cells, whereas there is at least a 3-fold lower frequency in those isolated at the G_1 phase (PY_{hi}). A dose-dependent PY-mediated cytotoxicity exists that at moderate concentration affects most murine hematopoietic compartment but spares the early HSC compartment. The HSC compartment is hierarchically ordered on the basis of quiescence and this concept further extends to PY-mediated cytotoxicity. Pyronin supravital dye can be used to reveal functional heterogeneity within the $Rh_{lo}Ho_{lo}/_{sp}$ PHSC population but is of limited use in dissecting the relatively more mature hematopoietic stem/progenitor cell population.

Functional heterogeneity within rhodamine 123($_{lo}$) Hoechst 33342($_{lo}$/sp) primitive hemopoietic stem cells revealed by pyronin Y. Huttmann A, Liu SL, Boyd AW, Li CL. Exp Hematol 2001 Sep; 29 (9), pp. 1109–16.

Fibrinogen Consumption: Intravascular $T_{1/2}$
[^{125}I]-Fibrinogen/Disseminated Intrascular Coagulation (DIC)/[^{123}I, ^{124}I, ^{125}I, ^{131}I]/[^{99m}Tc] DTPA-Fibrinogen

Cellular/Molecular Mechanism of Action: Fibrinogen is biochemically cleaved to fibrin monomer by thrombin. Fibrin monomers polymerized with 1 another and are covalently cross-linked by coagulation factor XIIIa*.
Physiological Mechanism of Action: Fibrinogen is the primary protein component of intravascular "clots" (thromboses) following biochemical conversion to fibrin monomer by thrombin.
Application: Detection of accelerated fibrinogen consumption or declines in production, or it can be utilized as a diagnostic imaging agent for the detection of intravascular (intravenous) thrombus formation.
Route of Administration: Intravascular.
Considerations: Excessively high labeling rations can compromise biological activity.
Semi-Synthetic Methods: Radioiodination reagents for biological proteins include Choramine-T, iodogen, and monochloroiodine.
Implementation: Fibrinogen intravascular kinetics and *in vivo* imaging of intravascular thromboses.
Interpretation and Analysis: Declines in the fibrinogen intravascular $T_{1/2}$ suggest the presence of elevated consumption. If fibrinogen levels are low but the intravascular $T_{1/2}$ is unchanged, then abnormalities in hepatic production may be present.
Instrumentation: Scintilation counter (intravascular kinetics), gamma camera (imaging).

Extraction, radioiodination, and *in vivo* catabolism of equine fibrinogen. Coyne CP, Hornof WJ, Kelly AB, O'Brien TR, DeNardo SJ. Am J Vet Res 1985; 46, pp. 2572–7.

Rapid extraction, radioiodination, and *in vivo* catabolism of ^{125}I-labeled fibrinogen in the horse. Coyne CP, Hornof WJ, Kelly AB, O'Brien TR, DeNardo SJ. Am J Vet Res 1985; 46, pp. 2578–81.

Lipemia
Heparin (Sodium or Lithium Salt)

Cellular/Molecular Mechanism of Action: Stimulation of lipoprotein lipase activity.
Application: (Bovine lung, porcine mucosal).
Diagnostic Application: (i) Detection of systemic lipemia and/or the temporary resolution of hyperlipemia in order to facilitate

serum electrolyte analysis. Heparine has also been applied in the therapeutic management of lipemia. (ii) Detection of fibrinolytic activity (FDP formation) and platelet consumption due to disseminated intravascular coagulation (DIC).

Route of Administration and Dosage: Intravenous or subcutaneous only.

Considerations: In man and some animal species, heparin becomes associated with the external surface membrane of RBC populations resulting in their premature removal from the intravascular compartment by the reticuloendothelial (RE) system. In general, heparin preparations of porcine intestinal mucoal origin tend to promote less severe anemia than extracts acquired from bovine lung. Heparin should never be administered intramuscularly due to the high probability of hematoma formation.

Laboratory Instrumentation: Serum is simply inspected visually when attempting to detect resolution of systemic hyperlipidemia.

Neutrophils/Neutrophil Margination, Rapid Demargination, and Slow Marginated Pool Transfer/Intravascular Kinetics and Biodistribution/Multiple-Indicator-Dilution Technique

[^{111}In]-Oxide Labeled Neutrophils/[^{51}Cr]-Labeled Red Blood Cells/Evans Blue-Labeled Albumin

Cellular/Molecular Mechanism of Action: Labeled neutrophils respond to chemotactic stimuli and phagocytized material perceived to be a foreign substance.

Application: Detection and characterization of alterations in neutrophil consumption or utilization (intravascular life span and local sequestration in conditions like pulmonary or gastrointestinal inflammatory disease).

Implementation: Evaluate the kinetics and biodistribution of neutrophils in various disease states or physiolocal conditions. One potential application is in multiple-indicator-dilution experiments performed in lungs involving the simultaneous bolus injection of [^{111}In]-labeled neutrophils, [^{51}Cr]-labeled red blood cells, and Evans blue-labeled albumin. Outflow profiles of labeled neutrophils were analyzed on the basis of a recirculatory pharmacokinetic model of labeled albumin.

Animal Model: Canine lung (n = 13 anesthetized dogs).

Interpretation and Analysis: Concomitant counts of unlabeled neutrophils are similar in pulmonary artery and aortic blood samples, demonstrating a dynamic balance across the lungs in the physiological state. The outflow profiles of the recovered neutrophils can be composed of a throughput component of circulating neutrophils and a component of reversibly marginated neutrophils. They can be interpreted by a model incorporating neutrophil margination (transfer coefficient = 0.195 ± 0.081 s-1), rapid demargination (0.054 ± 0.027 s-1), and transfer to a slow marginated pool (0.023 ± 0.018 s-1). Such investigative protocols can be applied as a research or diagnostic tool to delineate interactions between the pulmonary endothelium and neutrophils in physiological conditions and various diseased states.

Kinetic analysis of pulmonary neutrophil retention *in vivo* using the multiple-indicator-dilution technique. Schwab AJ, Salamand A, Merhi Y, Simard A, Dupuis J. J Appl Physiol 2003 Jul; 95 (1), pp. 279–91.

Note: Related citations have also been provided in sections on immunity and inflammation, infection and sepsis, and cellular delivery modalities.

Neutrophils/Monocytes/Consumption vs Production Abnormalities

[^{111}I]-Oxine Neutrophils/Chemotherapeutics: Doxorubicin

Physiological Mechanism of Action: Neutrophils and macrophages migrate into specific locations (tissues/organs) within the body in response to chemotactic stimuli (move toward a concentration gradient).

Application: Includes characterization of elevated platelet consumption or declines in production within the bone marrow. They can also function as a modality for the selective delivery of pharmaceutical agents for various diagnostic and therapeutic purposes.

Route of Administration: Intravascular.

Considerations: Must be prepared fresh.

Semi-Synthetic Methods: Radioisotope markers or chemotherapeutic agents can be passively "loaded" into neutrophil populations at relatively high concentrations without affecting their response to chemotactic stimuli.

Implementation: Selective delivery to foci of infectious disease or pretargeted neoplasia.

Interpretation and Analysis: Declines in intravascular $T_{1/2}$ values for labeled neutrophils implies accelerated consumption or removal. In scenarios where neutrophil numbers are low but the $T_{1/2}$ value is normal, then abnormalities in hematopoeitic production should be considered

Antineoplastic activity of chemotherapeutic "loaded" neutrophils against human mammary carcinoma. Coyne CP, Fenwick BW, Ainsworth J. Biotherapy 1997; 10, pp. 145–59.

Platelet Consumption/Intravascular Kinetics/ Platelet Consumption: Disseminated Intravascular Coagulation, Autoimmune Thrombocytopenia

[^{111}In]-Merc Platelets/[^{111}In]-Oxine Platelets

Cellular/Molecular Mechanism of Action: Platelets when exposed to activating agents like adenosine diphosphate and collagen monomer become activated, degranulate (phase I & II), and aggregate (reversible and irreversible phases). Platelets also repair defects in the vascular endothelium.

Physiological Mechanism of Action: Participate as cellular components in intravascular (especially intra-arterial) thrombus formation.

Background Information: Labeled platelets can be utilized to characterize intravascular platelet kinetics or as a diagnostic imaging modality.

Application: Detection of alterations in platelet intravascular kinetics in conditions of increased consumption (e.g., disseminated intravascular coagulation, autoimmune thrombocytopenia), or decreased production. Labeled platelets can also be utilized for diagnostic imaging of intravascular thromboses (e.g., deep-vein thromboses), and for the treatment of autoimmune thrombocytopenia (vincristine-loaded platelets become coated with autoimmune immunoglobulin and are subsequently phagocytized by macrophages, which are then suppressed by the chemotherapeutic agent).

Route of Administration: Intravascular.

Semi-Synthetic Methods: Usually, chelating agents are used to label cell populations like platelets because agents like [^{111}In] have a cationic or anionic charge that substantially decreases their ability to penetrate intact cell membrane structures.

Interpretation and Analysis: Decreases in the intravascular kinetic patterns for labeled platelet preparations suggest accelerated/elevated platelet consumption. If the platelet intravascular $T_{1/2}$ is normal, but platelet numbers are low, then a decrease in bone marrow platelet production should be considered.
Instrumentation: Scintilation counter (intravascular kinetics), gamma camera (imaging).

Radiolabeling of equine platelets in plasma with ^{111}In-(2-mercaptopyridine-N-oxide) and their *in vivo* survival in the horse. Coyne CP, Kelly AB, Hornof WJ, O'Brien TR, Phelps MS, Lamb JF. Am J Vet Res 1987; 48, pp. 385–91.

Platelet Intravascular Survival
Aspirin/[^{51}Cr]-Platelets/[^{111}In]-Platelets

Cellular/Molecular Mechanism of Action: Aspirin permanently inactivates platelet cyclooxygenase by acetylation. Because platelets do not have a nucleus, they cannot synthesize more enzyme. New platelets gradually over time replace the population of permanently inactived platelets within the intravascular compartment.
Application: Characterization of disease states associated with alterations in platelet survival (e.g., DIC, autoimmune disease, biochemical disorders).
Route of Administration: Aspirin (single oral intake).
Dosage: Aspirin (500 mg).
Implementation: A simultaneous investigation of platelet regeneration time (PRT) based upon malondialdehyde (MDA) recovery after aspirin ingestion and of platelet survival time (PST) after labeling with [^{51}chromium] or [^{111}indium] oxine.
Human Model: Neoplastic disease (n = 25; and n = 9 pilot study in healthy volunteers).
Interpretation and Analysis: In normal healthy individuals, the MDA assay has a high degree of reproducibility and specificity for platelet cycloxygenase activity. The pattern of MDA recovery after aspirin ingestion is linear in the healthy volunteers and in conditions of cancer presenting both a normal and an accelerated platelet turnover. Platelet survival times are calculated using the 4 mathematical models recommended by the International Committee for Standardization in Hematology and the best fit established by the multiple hit model (e.g., n = 22 cases) and by the linear regression model (e.g., n = 3 cases). The mean results obtained in cases investigated with the [^{51}Cr] are consistently shorter than those obtained in cases investigated with the [^{111}In]-oxine, while the mean PRT is almost identical in the 2 case group types. An excellent correlation between PRT and PST is observed after [^{111}In] oxine labeling and using the weighted mean method for PST determination. These results suggest that the [^{111}In] oxine technique is a better method for platelet labeling and that the results provided by the weighted mean method reflect more closely the *in vivo* platelet turnover than those provided by the multiple hit model.

Platelet regeneration time after aspirin ingestion and platelet survival time after labeling with ^{51}chromium or ^{111}indium oxine—A comparative study. Boneu A, Siá P, Bugat R, Caranobe C, Eber M, Cazenave JP, Boneu B. Thromb Haemost 1985 Apr 22; 53 (2), pp. 260–3.

Platelet Plasma Half-Life/Megakaryocyte Cyclooxygenase Activity
Aspirin

Background Information: The *in vivo* inhibitory effect of aspirin on platelet cyclooxygenase is irreversible and lasts for the entire platelet lifespan. Reappearance of cyclooxygenase activity in blood after aspirin has been proposed as a measure of the formation of new platelets and as an indirect indicator of platelet survival. A delay of 24 to 72 hours in recovery, however, has been observed and it has been suggested that aspirin might also inhibit megakaryocyte cyclooxygenase.
Route of Administration: Intraperitoneal.
Dosage: Aspirin (100 mg/kg rat).
Implementation: Evaluate the possibility that aspirin influences megakaryocyte cycloooygenase production. One approach to achieving this objective involves the administration of aspirin or saline, followed by the creation of thrombocytopenia 2 hours later (platelet count < 5% of basal value) by a specific antiplatelet antiserum. Malondialdehyde (MDA) and thromboxane B2 (TxB2) production by platelets is measured spectrophotometry and by radioimmunoassay, respectively, during the period of platelet count restoration. By 24 hours after induced thrombocytopenia, platelet counts are about 15% of basal values in control and aspirin-treated rats.
Interpretation and Analysis: In controls, MDA and TxB2 production is restored to about 20% of basal values, in contrast to aspirin-treated rats with less than 5% return of activity detected. A marked difference between the 2 groups can still be found 96 hours after induction of thrombocytopenia, when platelet count restoration is similar. Because aspirin disappears very rapidly from the circulation, the delay in recovery of cyclooxygenase activity supports the hypothesis of a megakaryocyte effect of this drug.

Impaired thromboxane production by newly formed platelets after aspirin administration to thrombocytopenic rats. Dejana E, Barbieri B, Cerletti C, Livio M, de Gaetano G. Br J Haematol 1980 Nov; 46 (3), pp. 465–9.

Platelet Regeneration/Survival/Consumption: Thrombocytopenia
Aspirin/[Thiobarbituric Acid/Malondialdehyde]

Cellular/Molecular Mechanism of Action: Permanent inhibition of cyclooxygenase biochemical activity through acetylation, thereby preventing the conversion of arachidonic acid generated by phospholipase A_2 to prostaglandin intermediates such as PGG and PGH.
Diagnostic Application: A systemic dose of aspirin is administered, which permanently inactivates cyclooxygenase activity in all exposed platelet populations residing within the intravascular compartment. Aspirin permanently (irreversibly) inactivates platelet cyclooxygenase by acetylation of the enzyme. Because platelets are essentially cellular fragments devoid of a functional nucleus, they are incapable of synthesizing additional cyclooxygenase. Therefore, increases in systemic platelet prostaglandin synthesis detected following the initial administration of aspirin directly (proportionally) correlates with the rate at which new platelet populations enter the peripheral intravascular compartment.

In the malondialdehyde assay, the thiobarbituric acid reagent can be applied to detect and evaluate oxidative stress status as a function of lipid membrane peroxidation and prostaglandin synthesis. In this context, it can be applied to evaluate the efficacy of pharmaceutical agents (e.g., non-steroidal anti-inflammatory drugs [NSAIDs]) and detect tissue peroxidation and the entry of new platelet populations into the systemic circulation.
Route of Administration and Dosage: Aspirin can be applied intravenous or oral administration. The assay method employing thiobarbituric acid, however, is performed *ex vivo* with a prepared cell population.

Considerations: Prolonged administration promotes development of lesions such as gastric ulceration and renal papillary necrosis. This is usually associated with vasoconstriction due to decreased synthesis of vasodilatory prostacyclin (PGI_2).

Assay Procedure: The malondialdehyde assay is based on its reactivity at high temperature and low pH toward thiobarbituric acid. This reaction is very sensitive but its specificity, even with improvement of preanalytical (sampling, preservatives) and analytical stages (fluorescence, high-performance liquid chromatography [HPLC]) is still a matter of debate. At present, the concept of "thiobarbituric acid reactive substances" (TBARS) has merged and progressively replaced the initial malondialdehyde assay. Example: After stimulation of the washed human blood platelets by arachidonic acid (AA), the formation of malondialdehyde (MDA) measured using the photometric thiobarbituric acid (TBA) method. Estimation of platelet lipid peroxidation as a function of malondialdehyde (MDA) production, and the formation of thromboxane B2 (TXB_2) measured by gas chromatography, may reveal that the formed MDA exceeds the amount of TXB_2 on a molar base. However, MDA and TXB2 originating from thromboxane synthase activity should be produced in approximately equimolar amounts. By treatment of stimulated platelet samples with stannous chloride it is possible to reduce all peroxidized products of AA that generate MDA otherwise during the TBA reaction and to estimate MDA and TXB_2 in a ratio of nearly 1:1. The stannous chloride treatment does not destroy the MDA and does not influence the TBA reaction with MDA. Therefore the simple and quick TBA method can be used after stannous chloride treatment for estimation of thromboxane synthase activity in AA-stimulated washed human platelets.

Implementation: Evaluation of platelet survival/production as a function of new arrival of intact platelets into the systemic circulation following univeral aspirin-mediated inactivation of cyclooxygenase is systemic platelet populations. Entry of new platelets into the systemic circulation is therefore detected as a rise in platelet-associated malondialdehyde concentrations measured in an *ex vivo* assay system.

The malondialdehyde assay is the most generally used test in the appreciation of the role of oxidative stress in disease. Malondialdehyde is 1 of several products formed during the radical induced decomposition of polyunsaturated fatty acids. Most often, malondialdehyde assay uses its reactivity at high temperature and low pH toward thiobarbituric acid. This reaction is very sensitive but its specificity, even with improvement of preanalytical (sampling, preservatives) and analytical stages (fluorescence, HPLC) is still a matter of debate. At present, the concept of TBARS has merged and progressively replaced the initial malondialdehyde assay. In the future, oxidative stress appreciation will need the precise analytical determination of different molecules triggered by free radicals. The TBARS assay should be considered as a global test, allowing a global approach of lipoperoxidation, whereas specific determination of malondialdehyde can only appreciate 1 of the end product formed during oxidative stress.

Laboratory Instrumentation: Inhibition of platelet PGI_2 synthesis by NSAIDs is detected by direct assay of PGI_2 applying RIA or ELISA methodologies. Alternatively, inhibited platelet PGI_2 synthesis can be detected by measurement of relative malondialdehyde production applying thiobarbituric acid and measuring spectrophotometric absorbance at 625 nm.

Evaluation of lipid peroxidation by measuring thiobarbituric acid reactive substances. Lefèvre G, Beljean-Leymarie M, Beyerle F, Bonnefont-Rousselot D, Cristol JP, Thérond P, Torreilles J. Ann Biol Clin (Paris) 1998 May–Jun; 56 (3), pp. 305–19.

Platelet Vascular Endothelial Repair/Vascular Endothelial Viability/Integrity
Evans Blue Dye Staining

Cellular/Molecular Mechanism of Action: Evans blue dye is a pigment-based reagent that becomes differentially associated with viable vs compromised cell populations (e.g., vascular endothelium).

Physiological Mechanism of Action: Tumor necrosis factor (TNF) soluble receptor (TNFsr) has been shown *in vitro* and *in vivo* to neutralize TNF-mediated biological activity.

Background Information: Tumor necrosis factor-alpha (TNF-α) is expressed locally in arteries at sites of balloon injury. *In vitro* studies have shown that TNF inhibits cell cycle progression and induces apoptosis in endothelial cells.

Application: Evans blue dye staining can be applied to evaluate viable *ex vivo* preparations of vascular structures for integrity of endothelial surfaces.

Implementation: Evaluation of the hypothesis that inhibiting TNF can accelerate endothelial recovery after angioplasty. One approach to achieving such an objective involves the administration of TNFsr vs control IgG by intraperitoneal injection. De-endothelializing balloon injuries are then created, and the resulting induced lesions evaluated 1 week later for evidence of reendothelialization (Evans blue dye staining), and after 2 weeks to evaluate reendothelialization and endothelial function.

Animal Model: Rat (Sprague-Dawley strain).

Interpretation and Analysis: At both time points, blockade of TNF biological activity with TNFsr results in increased reendothelialization, as measured as absolute area and percent area reendothelialized. Tumor necrosis factor soluble receptor also accelerates functional endothelial recovery that manifests as an increase in nitric oxide production. Neointimal thickening can also be appreciated as being inhibited. Blockade of TNF *in vivo* accelerates functional endothelial recovery after barotraumatic de-endothelializing injury. Locally expressed TNF therefore acts to inhibit functional endothelial recovery after angioplasty and transient blockade of TNF may improve the long-term success of angioplasty.

Evans blue staining reagent facilitates detection of a demarcation between viable endothelial cell populations and subendothelial collagen.

In vivo blockade of tumor necrosis factor-alpha accelerates functional endothelial recovery after balloon angioplasty. Krasinski K, Spyridopoulos I, Kearney M, Losordo DW. Circulation 2001 Oct 9; 104 (15), pp. 1754–6.

Platelet/Endothelium Interactions: *In Vivo*
Anti-P-Selectin Monoclonal Antibody/ Carboxyfluorescein Diacetate Succinimidyl Ester-Labeled Platelets

Cellular/Molecular Mechanism of Action: Carboxyfluorescein diacetate succinimidyl ester can function as a signal or indicator molecular. Anti-P-selectin monoclonal antibody recognizes and physically binds to membrane-associated P-selectin complexes.

Background Information: Accumulating evidence suggests that platelets play an important role in ischemia-reperfusion injury. To fulfill that role, platelets flowing in the bloodstream would have to interact with retinal endothelial cells and to accumulate in the postischemic retina.

Application: Carboxyfluorescein diacetate succinimidyl ester can be used to label cell populations (e.g., platelets) for the purpose of quantitatively characterizing platelet/endothelial interactions in postischemic retina after transient retinal ischemia.

Route of Administration: Intravenous.
Implementation: Isolated platelet samples are labeled with carboxyfluorescein diacetate succinimidyl ester and administered intravenously after various reperfusion periods. Platelet/endothelial interactions in postischemic retina are evaluated *in vivo* with a scanning laser ophthalmoscope. Anti-P-selectin monoclonal antibody (mAb) is administered 5 minutes before the injection of labeled platelets. P-selectin gene expression in the postischemic retina is then studied by semiquantitative polymerase chain reaction (PCR) analyses.

Animal Model: Rats (Long-Evans strain). Transient retinal ischemia is induced for 60 minutes by temporal ligation of the optic nerve.
Interpretation and Analysis: Under basal conditions, infused platelets show minimal interactions with retinal endothelial cells. In contrast, postischemic retinas show active platelet/endothelial interactions. Many platelets are observed rolling along and adhering to the major retinal veins. The number of rolling and adhering platelets reach a peak (555 ± 65/mm per minute and 25.8 ± 3.2/mm^2) 12 hours after reperfusion. However, interactions between platelets and postischemic retinal endothelial cells are substantially inhibited by neutralizing P-selectin expressed on endothelial cells. In addition, P-selectin gene expression in postischemic retina corresponds with the time course of platelet/endothelial interactions during the reperfusion period. Platelets actively interact with retinal endothelial cells in the postischemic retina through P-selectin expressed on the retinal endothelial cells.
Instrumentation: Scanning laser ophthalmoscope.

In vivo evaluation of platelet-endothelial interactions after transient retinal ischemia. Nishijima K, Kiryu J, Tsujikawa A, Honjo M, Nonaka A, Yamashiro K, Tanihara H, Tojo SJ, Ogura Y, Honda Y. Invest Ophthalmol Vis Sci 2001 Aug; 42 (9), pp. 2102–9.

Platelet/Endothelium Interactions/[Retinal Microvascular Pathology]
Carboxyfluorescein Diacetate Succinimidyl Ester/Platelets (Fluorescent Labeled)

Cellular/Molecular Mechanism of Action: Carboxyfluorescein diacetate succinimidyl ester interacts with light and functions as a fluorescent marker molecule suitable for "tracing" the migration of different cell types.
Application: Visualization of *in vivo* platelet behavior in the retinal microcirculation. The methodology allows for quantitative evaluation of platelet dynamics and platelet/endothelial interactions.
Route of Administration: Intravascular.
Considerations: Represents the development of a new method for evaluating the *in vivo* dynamics of platelets in the retinal microcirculation and quantitatively investigating platelet/endothelial interactions.
Implementation: Isolated platelet samples are labeled with carboxyfluorescein diacetate succinimidyl ester. After intravenous administration, platelet behavior in the retinal microcirculation can be evaluated with a scanning laser ophthalmoscope. Images are recorded on S-VHS videotape and analyzed with a computer-assisted image analysis system. The platelet/endothelial interactions in the retinal microcirculation can also be investigated employing lipopolysaccharide-stimulated endothelium or platelets activated with thrombin.

Animal Model: Rat retina.
Interpretation and Analysis: Fluorescent platelets are recognized as distinct dots in the retinal microcirculation and can be traced frame by frame. The velocity of platelets in the retinal arteries, capillaries, and veins is approximately 26.1 ± 6.4, 1.6 ± 0.4, and 19.9 ± 8.2 mm/sec, respectively. In control rats, even activated platelets show minimal interaction with retinal endothelial cells. In contrast, stimulated retinal endothelium show active platelet/endothelial interactions, with many platelets observed rolling and adhering along the major retinal veins. The interactions between platelets and stimulated endothelial cells are substantially inhibited with the injection of P-selectin monoclonal antibody.

In vivo evaluation of platelet-endothelial interactions in retinal microcirculation of rats. Tsujikawa A, Kiryu J, Nonaka A, Yamashiro K, Nishiwaki H, Tojo SJ, Ogura Y, Honda Y. Invest Ophthalmol Vis Sci 1999 Nov; 40 (12), pp. 2918–24.

Stem Cell Migration/Hematopoietic/[Parkinson's Disease Research]
Gadolinium Rhodamine Dextran (GRID)

Cellular/Molecular Mechanism of Action: Rhodamine emits light energy while gadolinium functions as an element that can be selectively detected by magnetic resonance imaging.
Background Information: The ability to track stem cell transplants in the brain by *in vivo* neuroimaging will improve current understanding about how these cells mediate functional recovery after neural transplantation. One major challenge for the development and refinement of stem cell transplantation is to map the spatial distribution and rate of *in situ* migration.
Application: Gadolinium rhodamine dextran is a bifunctional contrast agent that can emit light energy and be detected in a magnetic field. These attributes allow it to be applied as a tracer or marker molecule for profiling the migration of transplanted stem cell populations within ischemia-damaged rat hippocampus by magnetic resonance imaging (MRI). The strategies and techniques could also be applied to tracking hematopoietic stem cell migration.
Route of Administration: Intravenous.
Semi-Synthetic Methods: Conjugation of rhodamine to dextran applying cross-linking strategies. Addition of gadolinium to dextran can potentially be achieved by covalent bond formation or through the application of chelation groups.
Implementation: Assessment of stem cell migration applying *in vitro* labeling of populations with the bifunctional contrast agent GRID prior to transplantation. Detection is achieved applying both MRI and fluorescence microscopy, or with PKH26, visible exclusively under fluorescence microscopy. At different time points following engraftment, brains are evaluated by both histology and *ex vivo* MR imaging.
Interpretation and Analysis: Transplanted stem cells can be identified by MRI only if prelabeled with GRID, whereas fluorescence microscopy can detect transplanted cells using either labeling reagent. Distribution of GRID-labeled stem cells established that applying MRI corresponds to results using fluorescence microscopy. Stem cells and their migration can therefore be reliably identified applying GRID-enhanced MRI methodologies.

Tracking transplanted stem cell migration using bifunctional, contrast agent-enhanced, magnetic resonance imaging. Modo M, Cash D, Mellodew K, Williams SC, Fraser SE, Meade TJ, Price J, Hodges H. Neuroimage 2002 Oct; 17 (2), pp. 803–11.

Stem Progenitor Cell Tracking/Hematopoietic
PKH Dyes

Cellular/Molecular Mechanism of Action: PKH dyes serve as marker molecule.

Background Information: The process of hematopoietic stem and progenitor cell (HSPC) seeding in recipient bone marrow (BM) early after transplantation has not been fully characterized
Application: PKH dyes serve as marker molecule that can be utilized to track the *in vivo* migration of cell populations in biological systems.
Implementation: *In vivo* tracking of HSPCs, labeled with PKH dyes, through an optical window surgically implanted on the mouse femur reveals that transplanted cells cluster in the recipient BM.
Interpretation and Analysis: In the 1st 24 hours after intravenous injection, 86 ± 6% the cells seed in clusters ($p < 0.001$ vs scattered cells) in the endosteal surfaces of the epiphyses. Primary clusters are formed by concomitant seeding of 6 to 10 cells over an area of approximately 70 microm, and secondarily injected cells do not join the already existing clusters but form new clusters. Major antigen-disparate HSPCs participate in formation of primary clusters, and T lymphocytes are also incorporated. After 4 to 5 days, some cellular clusters can be observed in the more central regions of the BM, where the brightness of PKH fluorescence decreases, indicating cellular division. These later clusters are classified as secondary, assuming that the mechanisms of migration in the BM may be different from those of primary seeding. Some clusters remain in the periphery of the BM and retain bright fluorescence, indicating cellular quiescence. The number of brightly fluorescent cells in the clusters decreases exponentially to 2 to 3 cells after 24 days ($p < 0.001$). Hematopoietic niche is a functional unit of the BM stromal microenvironment that hosts seeding of a number of transplanted cells, which form a cluster. This may be the site where auxiliary non-HSPC cells, such as T lymphocytes, act in support of HSPC engraftment.

Transplanted hematopoietic cells seed in clusters in recipient bone marrow *in vivo*. Askenasy N, Zorina T, Farkas DL, Shalit I. Stem Cells 2002; 20 (4), pp. 301–10.

Thalassemia (Beta): Transfusion Dependent/Multiple Endocrine Dysfunction
Thyrotropin-Releasing Hormone (TRH) Stimulation Test

Cellular/Molecular Mechanism of Action: Thyroid-stimulating hormone (TSH) release is promoted by TRH.
Application: Assessment of thyroid dysfunction (example: estimation of the prevalence of thyroid dysfunction in homogenous groups of thalassemic patients).
Considerations: Despite improved hematologic care, multiendocrine dysfunction is a common complication in cases of transfusion-dependent beta-thalassemia.
Implementation: Evaluation of thyroid function in response to a TRH stimulation test for the purpose of possibly detecting cases of beta-thalassemia major. Thyroid dysfunction can be defined as (i) overt hypothyroidism (low FT4 and/or FT3 and increased TSH levels); (ii) subclinical hypothyroidism (normal FT4, FT3, increased TSH levels); (iii) exaggerated TSH response: normal FT4, FT3, normal basal TSH, deltaTSH ≥ 21 μIU/mL (TSH levels measured prior to and 30 minutes after intravenous TRH administration).

Human Model: Case subgroup proven to have normal thyroid hormone values; cases are selected randomly and subjected to a standard TRH stimulation test (e.g., n = 26; n = 12 males, n = 14 females; mean age, 23.6 ± 6.8 years; age range, 15–36 years).
Interpretation and Analysis: In populations of patients suffering from beta-thalassemia major (regularly transfused and desferioxamine chelated), the evaluation of thyroid function and iron-load status (assessed through measurements of free thyroxine [FT4], free triiodothyronine [FT3], thyrotropin [TSH], and serum ferritin levels) has aided in determining that normal thyroid hormone values can be expected in approximately 83.5% of affected patients. Of the remaining patients, about 4% will have overt hypothyroidism, while 12.5% will have subclinical hypothyroidism. Exaggerated TSH response to TRH may be seen in about 27% of patients with normal hormone values. Antithyroglobulin and antithyroid peroxidase (TPO) antibody titers are negative in a high percentage of patients (95%). Mean ferritin levels in hypothyroid and euthyroid patients are 2,707.66 + 1,990.5 mg/L and 2,902.9 + 1,997.3 mg/L, respectively ($p = 0.61$), indicating that no correlation exists between ferritin levels and thyroid functional status. Mean ferritin levels in the patients who respond normally to TRH stimulation and in those who overrespond reveal values of 2,586 + 1,791 mg/L and 3,228 + 2,473 mg/L, respectively ($p = 0.46$; ns). Thyroid failure is a rather rare endocrine complication in patients with beta-thalassemia from Greece and has been validated in previous investigations that have failed to identify any cases of central hypothyroidism. Often, no correlations can be found between thyroid functional status and ferritin plasma levels. Approximately 1 of 5 beta-thalassemic patients with normal thyroid hormone values show exaggerated TSH responses to the TRH test.

Assessment of thyroid function in two hundred patients with beta-thalassemia major. Zervas A, Katopodi A, Protonotariou A, Livadas S, Karagiorga M, Politis C, Tolis G. Thyroid 2002 Feb; 12 (2), pp. 151–4.

von Willebrand's Factor Antigen (vW Fag)
Desmopressin (DDAVP)/Stress Test

Cellular/Molecular Mechanism of Action: Desmopressin directly promotes increases in plasma vW Fag.
Application: Differentiation between conditions of vasculitis, systemic lupus erythematosus (SLE), and rheumatoid arthritis compared to normal (negative) controls. Desmopression is also used to not only to diagnose von Willebrand's disease but also to facilitate the classification of this condition (e.g., mild vs moderate).
Route of Administration: Desmopressin (intravenous).
Dosage: Desmopressin (0.3 μg/kg).
Considerations: von Willebrand's factor antigen has been used as a marker for vasculitis and raised levels have been shown to correlate with active disease. However, certain cases of vasculitis have normal vW Fag levels and are difficult to diagnose; this is of importance because of the high mortality associated with these disorders. Venostatic stress can be used as a provocative test for stimulating vW Fag release, thereby improving the speed and sensitivity of diagnosis.

Unfortunately, the venostatic stress with vW Fag measurement does not produce a more sensitive test for vasculitis. Venostasis should be avoided when measuring vW Fag levels.
Interpretation and Analysis: At baseline, although the levels are not outside the laboratory range, patients with active disease have raised vW Fag compared to simultaneously tested controls. Venostatic stress increased vW Fag activity in all disease groups, control levels also increased, and differences between controls and disease groups diminished in significance.

The diagnosis and classification of von Willebrand's factor (vWF) profiles utilizing intravenous desmopressin infusions involves the interpretation of assays for vWF-related antigens contained in plasma samples collected approximately 60 minutes following administration. In patients with Type 1 von Willebrand's disease a number of fractions become elevated including vWF:Ag (3.7-fold increase); propeptide (7.2-fold increase); and vWF:CBA (2.2-fold increase). Unusually large vWF multimers and traces of provWF also appear during this same time period. A more detailed interpretation of desmopressin-induced alterations in the profile for vW FAg in cases with congenital von Willebrand's disease (CvWD) involves the

interpretation of electrophoresis analyses that are able to facilitate the detection of (i) mild to moderate Type I vWD (24 probands); (ii) severe CvWD Type I (6 probands); and (iii) Type 2 CvWD (12 probands). Congenital von Willebrand's disease Type 1 of mild to moderate severity is featured by proportionally decreased levels of vWF antigen (vWF:Ag), vWF ristocetin co-factor activity (vWF:Rcof), and vWF:CBA between 0.20 and 0.60 u/mL and a normal response to DDAVP of factor (F) VIIIc and all vWF parameters. Severe Type 1 CvWD with vWF parameters below 0.10 or 0.20 u/mL is associated with a decreased response to DDAVP of all vWF parameters, indicating a defective synthesis or secretion vWF by endothelial cells, or both. Congenital von Willebrand's disease 2M may present as severe Type 1 CvWD, as Type 1 "platelet-discordant" CvWD, or with the combination of a discrepant vWF:Rcof/Ag ratio and the presence of all vWF multimers. Ristocetin-induced platelet aggregation (RIPA) is normal in Type 1 CvWD. Congenital von Willebrand's disease 2M is typically featured by decreased RIPA, normal or near normal vWF multimers, and no or only a poor response to DDAVP of vWF:Rcof as compared with a fairly good response to DDAVP of vWF:Ag and vWF:CBA. Conditions of CvWD Vicenza are characterized by unusually large vWF multimers and very low levels of FVIIIc, vWF:Ag, and vWF:Rcof. Congenital von Willebrand's disease Vicenza differs from CvWD 2M because the vWF:Rcof/Ag ratios are completely normal before and after DDAVP; the response to DDAVP is equally good for FVIIIc, vWF:Ag, vWF:Rcof, and vWF:CBA and is followed by very short half-life times for FVIIIc and all vWF parameters. Pertinent findings in Type 2A and 2B CvWD include prolonged Ivy bleeding time (BT), low vWF:Rcof/Ag and vWF:CBA ratios, absence of high vWF multimers, and, depending on the severity of the absence of intermediate vWF multimers, pronounced increase of low vWF multimers and vWF degradation products because of increased proteolysis of the high and intermediate vWF multimers. Ristocetin-induced platelet aggregation is normal in CvWD 2A and increased in CvWD 2B. The response to DDAVP in CvWD 2A is normal for FVIIIc and vWF:Ag but is transient with partial correction and short half-life times of vWF:CBA and vWF:Rcof. DDAVP does not correct BT and multimeric patterns in CvWD Type 2B, despite significant increase of vWF parameters. Congenital von Willebrand's disease Types 2C, 2D, and 2E are featured by very low functional vWF parameters, the presence of typically abnormal vWF multimers, a very poor response of vWF:CBA, a decreased response of vWF:Rcof, and a fairly good response of vWF:Ag to DDAVP with no correction of prolonged Ivy BT and no correction of the vWF multimeric pattern as a consequence of a multimerization or dimerization defect of the vWF molecules. Congenital von Willebrand's disease Type 2N usually presents with much lower levels for FVIIIc as compared with vWF, normal Ivy BT, and normal vWF multimeric pattern. The response to DDAVP is normal for all vWF parameters but is decreased for FVIIIc with a shortened half-life within the intravascular compartment.

The von Willebrand's factor-cleaving protease (ADAMTS13) cleaves vWF and regulates its biological/physiologic function. Evaluation of the influence of desmopressin on plasma ADAMS13 reveals that its concentration noticeably decreases compared to the vWF-related parameters of vWF antigen (vWF:Ag), vWF collagen-binding activity (vWF:CBA), vWF-propeptide, provWF, and vWF multimeric composition in healthy volunteers. More specifically, ADAMTS13 activity decreases to about half the initial value but returns to baseline levels at approximately 24 hours. Patients with Type 1 von Willebrand's disease display similar patterns/results. Therefore, the inverse correlation between ADAMTS13 and vWF-related parameters suggests a consumption of ADAMTS13 after the desmopressin-induced release of higher multimers of vWF.

Prevalence, pathophysiology, diagnosis and treatment of von Willebrand syndrome in orthopedic trauma patients. Kurth AA, Ludwig G, Scharrer I. Orthopade 1999 Apr; 28 (4), pp. 366–74.

IMMUNE SYSTEM

Allergen Immune Responses: Nasal/Late Induction/Allergen Identification/Efficacy Evaluation of Anti-Inflammatory Agents and Corticosteroids/Example Allergen: Grass/Pollen Hypersensitivity

Allergen Challenge Test/Fluticasone

Background Information: Allergen-induced late nasal responses are associated with recruitment and activation of T lymphocytes and eosinophils along with preferential mRNA expression for T-helper type 2 (Th2) cytokines.

Implementation: Detection of allergens responsible for inducing allergic reactions and evaluating the efficacy of potential therapeutic agents (e.g., anti-inflammatory agents and corticosteroids). An example in this regard is the application of an allergen challenge to evaluate the efficacy of topical corticosteroids (fluticasone propionate) and their ability to inhibit late responses by suppressing cellular expression of Th2 type cytokine mRNA sequences. Nasal biopsies are taken at baseline and repeated 24 hr after local nasal allergen provocation following 6 weeks treatment (e.g., fluticasone propionate 200 μg or placebo nasal spray twice daily).

Human Model: Cases of grass pollen sensitivity ($n = 48$ adults) analyzed in a randomized double blind placebo-controlled trial.

Interpretation and Analysis: Baseline mRNA expression for interleukin (IL)-4 ($p = 0.01$) and IL-5 ($p = 0.002$) is higher in cases of grass pollen sensitivity. Topical corticosteroid treatment significantly inhibits immediate nasal symptoms, with almost complete inhibition of the late response following allergen challenge. This is associated with a marked decrease in the allergen-induced increases in cells expressing mRNA for IL-4 ($p = 0.002$) but not for IL-5. Inhibition of the late response is also accompanied by decreases in CD25+ cells, presumed to be T lymphocytes and eosinophils. A significant correlation exists between decreases in IL-4 mRNA+ cells and in eosinophils after treatment ($r = 0.46$, $p < 0.05$). Therefore, prolonged treatment with topical corticosteroid inhibits allergen-induced early and late nasal responses and the associated tissue eosinophilia, and that, at least in part, may result from inhibition of cells expressing IL-4 mRNA sequences.

Topical glucocorticosteroid (fluticasone propionate) inhibits cells expressing cytokine mRNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. Masuyama K, Jacobson MR, Rak S, Meng Q, Sudderick RM, Kay AB, Lowhagen O, Hamid Q, Durham SR. Immunology 1994 Jun; 82 (2), pp. 192–9.

Allergic Reactions: Inhaled Environmental Allergens

Single Allergen Provocation Test/Nasal Biopsy/[Interleukin (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, Interferon-Gamma (IFN-γ), RANTES, Tumor Necrosis Factor-Alpha (TNF-α)]

Background Information: The local production and release of a number of cytokines regulates allergic upper airway inflammation.

Medication is usually used at the presentation of the 1st symptoms. There are, however, clues that it is advisable to start taking corticosteroids before the grass pollen season begins.

Application: The single allergen provocation test can be applied to detect natural allergens responsible for inducing hyperactive immune responses *in vivo* in addition to functioning as a tool for evaluating the efficacy of anti-inflammatory and immunosuppressive agents.

Implementation: Evaluation of preventive therapies for inhaled allergic reactions applying the single allergen provocation protocol conducted in autumn (non–hay fever season). Nasal mucosa biopsies are collected twice before provocation (before and after 4 weeks of preventive treatment) and 3 times after allergen provocation (1 hour, 24 hours, and 1 week). Eosinophils and mRNA positive cells (*in situ* hybridization for IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IFN-γ, RANTES, and TNF-α) are then counted in biopsies.

Human Model: Cases receiving either fluticasone propionate aqueous nasal spray (n = 10 administered FPANS) or a placebo (n = 9).

Interpretation and Analysis: Preventive treatment with FPANS out of season resulted in a decrease in eosinophils and mRNA positive cells for IL-5 and IL-6. After allergen provocation, levels of most of the measured cytokines (IL-3, IL-5, IL-6, IL-13, IFN-γ, RANTES, and TNF-α) and eosinophils are reduced using corticosteroids. The number of cells (eosinophils, IL-3, IL-6, and IL-8) correlates with nasal symptoms. Significant correlations in the early and late allergic phase can be appreciated between eosinophils and cytokines (IL-3, IL-10, and IL-13). These results indicate that preventive treatment with FPANS prior to contact with grass pollen is effective in reducing the increase of cytokine mRNA positive cells in reaction to grass pollen contact.

Preventive treatment of intranasal fluticasone propionate reduces cytokine mRNA expressing cells before and during a single nasal allergen provocation. Kleinjan A, Holm AF, Dijkstra MD, Boks SS, Severijnen L, Mulder PG, Fokkens WJ. Clin Exp Allergy 2000 Oct; 30 (10), pp. 1476–85.

Autoimmune: Graves' Ophthamology/Type Th2 Interleukines: IL-4 and IL-10
Methylprednisolone (MP)/Prednisone

Cellular/Molecular Mechanism of Action: Corticosteroids inhibit general protein synthesis, stabilizes cell membrane structures, and inhibits the biochemical activity of phospholipase A2 in a manner that decreases both leukotriene and prostaglandin synthesis.

Physiological Mechanism of Action: Corticosteroids exert anti-inflammatory and immunosuppressive properties.

Application: Elevated expression of serum interleukin (IL)-4 and IL-10 during successful treatment with corticosteroids indicates the possibility of using these cytokines as predictors of the beneficial effect of corticosteroids in Graves' ophthalmopathy.

Route of Administration: Methylprednisolone (intravenous); prednisone (oral).

Dosage: Methylprednisolone (2 series, 3 g each time); prednisone (60 mg per day according to a tapering dose schedule).

Implementation: Evaluation of Graves' ophthalmology conditions related to the estimated influence of corticosteroids on Th1 and Th2 serum cytokine balance. Cytokines of particular interest include interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), IL-4, and IL-10. In addition, complementary analysis involves delineation of the upregulation of Th2 immune response during successful treatment with corticosteroids to determine if there is a correlation with their beneficial effect in Graves' ophthalmopathy. Corticosteroid challenge/exposure consists of intravenous infusions of and subsequent treatment with oral prednisone in a tapering dose schedule. Serum samples are collected 24 hours before MP, 24 hours after MP and 14 days of treatment with prednisone, and at the end of corticosteroid therapy. Levels of IFN-γ, TNF-α, IL-4, and IL-10 in the serum are determined using ELISA. Statistical significance is estimated by the Mann-Whitney U-test.

Human Model: Cases of Graves' disease without ophthalmopathy (n = 20 Gd); cases with clinical symptoms of ophthalmopathy (n = 16 GO with CAS over 3 points, last consultation record for GO less than a year old); and normal healthy volunteers (n = 16).

Interpretation and Analysis: Deviations exist pertaining to systemic Th2 cytokine profile in Graves' disease. In GO cases, a significant increase in serum IL-10 concentration can be detected. In corticosteroid-responsive patients, the balance of serum cytokines IL-4/IFN-γ, IL-4/TNF-α, IL-10/IFN-γ, and IL-10/TNF-α increases and remains upregulated. In non-responders, the balance of serum cytokines studied increases after methylprednisolone but declines markedly during continuation of the therapy with prednisone. Therefore, efficient corticosteroid therapy may be related to its influence on Th2/Th1 profile cytokine balance. The upregulation of serum IL-4 and IL-10 during successful treatment with corticosteroids indicates the possibility of using these cytokines as predictors of the beneficial effect of corticosteroids in Graves' ophthalmopathy.

Serum Th1 and Th2 profile cytokine level changes in patients with Graves' ophthalmopathy treated with corticosteroids. Myśliwiec J, Kretowski A, Topolska J, Siewko K, Jakubczyk D, Szelachowska M, Mikita A, Kinalska I. Horm Metab Res 2001 Dec; 33 (12), pp. 739–43.

Autoimmune: Multiple Sclerosis (MS)/[Central Nervous System (CNS) Autoimmune Disorder]
Thyrotropin-Releasing Hormone (TRH) Stimulation/ Bromocriptine/(Prolactin)

Cellular/Molecular Mechanism of Action: Thyrotropin-releasing hormone promotes prolactin secretion.

Application: Multiple sclerosis cases reportedly display elevations in plasma prolactin concentrations.

Considerations: Before the onset and during experimental allergic encephalomyelitis (EAE) (animal counterpart of MS), the peripheral concentrations of prolactin can be found to be elevated and bromocriptine has been found to attenuate such attacks. Prolactin may play a role in the immunology of MS.

Implementation: Evaluation of prolactin levels in cases of MS as a diagnostic parameter.

Animal Model: Experimental allergic EAE can be applied as a model for human MS.

Interpretation and Analysis: Multiple sclerosis cases have slightly but significantly higher prolactin levels at baseline (10.2 ± 1.6 vs 6.44 ± 0.57 ng/ml, p = 0.042) than do matched reference controls; however, values are within the normal range. Prolactin levels post-TRH are significantly higher in cases with MS with peak prolactin levels being higher than negative reference controls (57.08 ± 6.144 vs 32.94 ± 4.92 ng/ml, p = 0.006). The area under the curve of prolactin is also higher in patients than in controls (3,421.87 ± 394.53 vs 2,317.62 ± 257.22 ng/ml, p = 0.030).

Prolactin secretion is increased in patients with multiple sclerosis. Azar ST, Yamout B. Endocr Res 1999 May; 25 (2), pp. 207–14.

Autoimmune: Multiple Sclerosis (MS)
Metoclopramide/Bromocriptine/Thyrotropin-Releasing Hormone (TRH)

Cellular/Molecular Mechanism of Action: Bromocriptine blunts elevations in prolactin, while TRH promotes the release of prolactin (PRL). Prolactin belongs to the growth and lactogenic hormone family and has potent immunomodulating properties.
Background Information: Mild hyperprolactinemia has been found to enhance several autoimmune diseases and increased PRL plasma levels have been described in natural and experimental MS models. The PRL antagonist bromocriptine suppresses the disease.
Application: Detection of cases suffering from MS.
Considerations: Prolactin concentrations are elevated in MS. Before the onset and during experimental allergic encephalomyelitis (EAE), the animal counterpart of MS, prolactin levels are elevated, while bromocriptine attenuates attacks.
Implementation: Evaluation of the prolactin baseline values as a function of disease course and activity in a large population of MS patients. Furthermore, inhibitory (bromocriptine) and stimulatory (metoclopramide) tests can be performed in a population subsample to gain functional information.

Human Model: Cases suffering from MS (n = 132; n = 39/132 receiving alternating bromocriptine and metoclopramide).
Interpretation and Analysis: No correlation exists between baseline values and disease course or activity. Nevertheless, in the regression analysis of stimulatory test results, 14% of the variance is attributable to disease activity. In conclusion, PRL does not seem to be relevant as an activity marker in the whole MS population.

Prolactin stimulation in multiple sclerosis—An indicator of disease subtypes and activity? Heesen C, Gold SM, Bruhn M, Mönch A, Schulz KH. Endocr Res 2002 Feb–May; 28 (1–2), pp. 9–18.

Autoimmune: Multiple Sclerosis (MS)/Inflammatory Medicator Profile (Tumor Necrosis Factor-Alpha [TNF-α]; Interleukin [IL]-2; IL-4; IL-6; IL-10; IgG)/Clinical Course and Severity
Azathioprine (AZA)/Cyclosporin/ Cyclophosphamide/ Interferon-Beta 1a (IFN-β 1a)/Corticosteroids (CST)

Physiological Mechanism of Action: Azathioprine, cyclosporin, cyclophosphamide, IFN-β 1a, and/or CST each exert immunosuppressive properties through different individual mechanisms.
Implementation: Discovery of the relationship between various therapies and the clinical activity and course of MS with the *in vitro* production of (i) TNF-α; (ii) IL-2; (iii) IL-4; (iv) IL-6; (v) IL-10; and (vi) oligoclonal IgG (IgG OB). Experimental samples used for analysis can include whole blood stimulated with lipopolysaccharide or concanavalin A.

Human Model: Cases of relapsing-remitting, relapsing-progressive, or chronic progressive MS (n = 61) receiving no therapy, AZA, cyclosporin, cyclophosphamide, subcutaneous IFN-β 1a, and/or CST.
Interpretation and Analysis: The cinetics of cytokine production show that (i) in the absence of treatment, TNF-α and IL-6 drop, respectively, after and during the periods surrounding relapse, while IL-4 is increasing before and IL-10 after relapse; (ii) with AZA, TNF-α and IL-6 decreasing before exacerbation, IL-4 has prolonged high levels after and IL-10 before relapse; and (iii) with IFN-β 1a, IL-10 may already be increasing before relapse, while TNF-α may be higher after relapse. When cytokine levels are analyzed independently from MS clinical activity, the use of AZA inhibits IgG OB and TNF-α synthesis ($p = 0.002$) but increases IL-4 ($p = 0.0024$), whereas IFN-β 1a stimulates TNF-α and inhibits IgG OB and IL-4 production. Corticosteroid inhibits TNF-α, IL-6, IL-4, and IgG OB synthesis. Such studies emphasize both the weight of clinical parameters and of methodology used in results obtained in cytokine analysis in MS.

In vitro cytokine profiles as indicators of relapse activity and clinical course in multiple sclerosis. Schluep M, van Melle G, Henry H, Städler C, Roth-Wicky B, Magistretti PJ. Mult Scler 1998 Jun; 4 (3), pp. 198–202.

Autoimmune: Multiple Sclerosis (MS)
Arginine Vasopressin (AVP)

Background Information: In the LEW/N rat model, a decreased hypothalamic-pituitary-adrenal (HPA) axis response to inflammatory and immune mediators confers susceptibility to the development of a variety of inflammatory and immune diseases, including experimental allergic encephalomyelitis. In humans with optic neuritis, early intervention with steroids is associated with a decrease in the number of patients who go on to develop MS.
Application: Multiple sclerosis cases demonstrate a blunted release of adenocorticotropic hormone (ACTH) in response to AVP stimulation test.
Implementation: Evaluate the potential for MS to show evidence of hypoactive HPA axis function before (baseline) and after provocative testing with several agents including (i) ovine corticotropin releasing hormone (CRH); (ii) AVP; and (iii) ACTH stimulation. Results are then compared to negative reference controls.

Human Model: Cases of MS (n = 13).
Interpretation and Analysis: Compared to matched controls, MS cases have significantly higher plasma cortisol levels at baseline. Despite this hypercortisolism and in contrast to cases with depression that had similar elevations in plasma cortisol levels, cases with MS show normal, rather than blunted, plasma ACTH responses to ovine CRH, suggesting that the pathophysiology of hypercortisolism in MS is different than for depression. Patients with MS also show blunted ACTH responses to AVP stimulation and normal cortisol responses to high- and low-dose ACTH stimulation. Taken together, these findings are compatible with data from studies of experimental animals exposed to chronic inflammatory stress, which show mild increased activation of the HPA axis with increased relative activity of AVP in the regulation of the pituitary-adrenal axis. These data do not support a role for hypocortisolism in MS once the disease is established.

Multiple sclerosis is associated with alterations in hypothalamic-pituitary-adrenal axis function. Michelson D, Stone L, Galliven E, Magiakou MA, Chrousos GP, Sternberg EM, Gold PW. J Clin Endocrinol Metab 1994 Sep; 79 (3), pp. 848–53.

Autoimmune: Myasthenia Gravis (MG)
Edrophonium (Tensilon)

Cellular/Molecular Mechanism of Action: Edrophonium exerts a direct inhibitory effect on cholinesterase activity somewhat analogous to organophosphate insecticides. As a result, the neurotransmitter acetylcholine is allowed to accumulate at the motor end plate. In concept, this will theoretically increase the probability of acetylcholine becoming bound to and continually stimulating the postsynaptic membrane receptor sites in MG patients that have to some degree been inactivated by the presence of bound IgG due to an aberrant (autoimmune) humoral response.

Diagnostic Application: Congenital and acquired MG.
Route of Administration and Dosage: Intravenous injection of 0.5 to 5.0 mg. The response is widely variable, which prevents recommendation of a specific dose regimen. If the capacity to perform physical activity is not restored, a higher dosage can be administered in a matter of a few minutes.
Considerations: Because the response is highly variable, signs of weakness in addition to over-stimulation of parasympathetic muscarinic receptor sites may develop such as salivation, miosis, vomiting, and diarrhea (cholinergic crisis).
Interpretation and Analysis: Correction of exercise intolerance conditions that last a duration of 2 to 3 minutes supports the diagnosis of MG.

Approach to generalized weakness and peripheral neuromuscular disease. LoVecchio F, Jacobson S. Emerg Med Clin North Am 1997 Aug; 15 (3), pp. 605–23

Autoimmune: Myasthenia Gravis (MG) *Edrophonium/Neostigmine/Pyridostigmine/ Vecuronium*

Cellular/Molecular Mechanism of Action: Edrophonium (acetylcholinesterase inhibitor that binds selectively to the active site of the enzyme). Alternatively neostigmine (cholinesterase inhibitor) and pyridostigmine (carbamate cholinesterase inhibitor: charged quaternary compound) contain carbamyl ester linkages that are directly hydrolyzed by acetylcholine esterase. Vecuronium (congener of pancuronium) is a bis-quaternary ammonium steroid that is 5 times more potent than d-tubocurarine as a neuromuscular blocking agent but inhibits plasma butyrylcholinesterase to a lesser degree than pancuronium so that less cardiovascular side effects are produced.
Application: Cases with MG are hypersensitive to non-depolarizing neuromuscular blocking drugs. Vecuronium can be applied in an ocular test for the detection of cases suffering from MG. Other tests include (i) Edrophonium chloride: 2 mg IV followed in 45 seconds by another dose of 8 mg if no improvement is observed. Excessive doses should be avoided in order to prevent weakness from generalized depolarization of motor end plates. (ii) Oral test: Neostigmine (7.5 mg); or pyridostigmine (30 mg); or ambenonium (2.5 mg). Signs for improvement in neuromuscular strength are monitored. In an hour, the dose is then increased 1.5-fold and repeated until a desired effect is achieved. Results can be confirmed with the edrophonium test. (iii) Intravenous test: Neostigmine (0.125 mg) or pyridostigmine (0.5 mg) administered at intervals of several minutes. Atropine (0.4–0.6) is given prior to the initiation of the test to prevent muscarinic side effects. The optimal oral dose is equivalent to 30-fold the intravenous dose that achieved the desired level of effect. (iv) Vecuronium and general anesthesia: 10 μg/kg intravenously under general anesthesia with sevoflurane (2.5%) and nitrous oxide (60%) in oxygen. Neuromuscular transmission measured as twitch tension of the adductor pollicis muscle with supramaximal stimulation can serve as a meaningful evaluation parameter. Following recording of baseline measurements, incremental doses of vecuronium (10 μg/kg IV) are administered sequentially until 90% blockade is exceeded. The degree of blockade and onset time after the initial 10 μg/kg dose of vecuronium are assessed, and the dose required to exceed 90% blockade recorded. In addition, effective doses of 50% and 95% for vecuronium are calculated from a single data point.
Considerations: A diagnosis can usually be obtained from history, presenting complaint, and physical examination, but its differentiation from neurasthenic, infectious, endocrine, neoplastic diseases, or degenerative neuromuscular disorders may be difficult. Provocative testing with an anticholinesterase pharmaceutical agent results in a drastic improvement in conditions of MG.

Although antibodies to the acetylcholine receptor (AChR) often are observed in MG patients, 10–30% of patients do not show an anti-AChR antibody.

In childhood cases of MG, the majority of cases (e.g., 92%, n = 25/27) have localized ocular myasthenia with a median onset of symptoms appreciated at 33 months of age. Approximately 24% of cases progress to generalized MG. A few (8%) may present with respiratory failure that requires ventilatory support with an onset of symptoms being appreciated at about 22 months. Thymectomy (n = 10/27 cases) is used as an option for therapeutic intervention where approximately 70% complete or 26% partial remissions rate can be expected when applied in combination with an immunosuppressant (azathioprine) and a cholinesterase inhibitor (pyridostigmine). The development of a myasthenic crisis is relatively infrequent when patients experience proper management and good follow-up monitoring.
Interpretation and Analysis: Agents should produce a relatively immediate (dramatic) improvement in clinical signs. Seronegative and seropostive MG patients are equally sensitive to vecuronium compared to unaffected negative reference control patients.

The effect of use of pyridostigmine and requirement of vecuronium in patients with myasthenia gravis. Tripathi M, Kaushik S, Dubey P. J Postgrad Med 2003 Oct–Dec; 49 (4), pp. 311–4; discussion 314–5.

Autoimmune: Myasthenia Gravis (MG) *Edrophonium/[Esophogeal Function Screening Test]*

Cellular/Molecular Mechanism of Action: Edrophonium is a short-acting acetylcholinesterase inhibitor.
Background Information: Myasthenia gravis is an autoimmune disease in which impairment of neuromuscular transmission results in a pathological fatigability of striated muscles. Dysphagia is a common symptom in MG. It is caused by a weakness in striated muscles of the pharynx and esophagus.
Application: Evaluate the role of esophageal scintigraphy in the assessment of esophogeal function in MG.
Dosage: Edrophonium chloride (EC; 10 mg).
Implementation: Evaluation of esophageal transit in conditions of MG using scintigraphy with a multiple swallow test protocol. Cases are studied twice: under baseline conditions, and immediately after pharmacological stimulation with edrophonium.

Human Model: Cases of clinically proven MG (n = 15; n = 6/15 oculopharyngeal manifestations; n = 9/15 generalized weakness; n = 10/15 history of dysphagia).
Interpretation and Analysis: Under baseline conditions all cases display impaired esophageal function (emptying [%]= 58 % ± 21; normal range > 85%). In 14/15 individuals esophageal transit improves after administration of EC (emptying [%]= 75% ± 18; $p < 0.01$), reaching the normal range in 6 patients. Some cases show no effects attributable to EC (e.g., n = 1/15). Esophageal transit is often compromised in MG. Functional abnormalities may also be present in patients without a history of dysphagia. Inhibition of cholinesterase positively affects striated muscles in the pharynx and upper esophagus, thus improving esophageal transit. Esophageal scintigraphy may be considered as a simple, non-invasive method for diagnosing impairment of esophageal function in MG and for monitoring the changes under pharmacological stimulation.

Assessment of esophageal function in patients with myasthenia gravis. Linke R, Witt TN, Tatsch K. J Neurol 2003 May; 250 (5), pp. 601–6.

Autoimmune: Myasthenia Gravis (MG)
Vecuronium

Cellular/Molecular Mechanism of Action: Vecuronium is an acetylcholinesterase inhibitor.
Background Information: Conditions of MG are hypersensitive to non-depolarizing neuromuscular blocking drugs. Although antibodies to the acetylcholine receptor (AChR) often are observed in MG patients, 10–30% of patients do not show an anti-AChR antibody. Little is known about differences in sensitivity to non-depolarizing neuromuscular blocking drugs between MG patients with and without anti-AChR antibody.
Application: Determination of the relative sensitivity of seronegative and seropositive MG patients to vecuronium.
Route of Administration: Vecuronium (intravenous).
Dosage: Incremental doses of vecuronium (10 μg/kg) during anesthesia with sevoflurane (2.5%) and nitrous oxide (60%) in oxygen.
Implementation: Measurement and monitoring of neuromuscular transmission can be achieved by assessing twitch tension of the adductor pollicis muscle with supramaximal stimulation. After baseline measurements, 10 μg/kg IV dose increments of vecuronium are administered sequentially until blockade exceeds 90%. The degree of blockade and onset time after the initial 10 μg/kg of vecuronium is assessed, and doses required to exceed 90% blockade recorded. In addition, effective doses of 50% and 95% for vecuronium are calculated from a single data point.
Interpretation and Analysis: Both types of MG patients show increased sensitivity to vecuronium compared with non-MG cases. Therefore, seronegative MG patients, seropositive MG patients, and non-MG patients are equally sensitive to vecuronium.

Vecuronium in the myasthenic patient. Hunter JM, Bell CF, Florence AM, Jones RS, Utting JE. Anaesthesia 1985 Sep; 40 (9), pp. 848–53.

Autoimmune: Rheumatoid Arthritis/Matrix Metalloprotease Protein 3 (MMP-3)
Corticosteroids

Cellular/Molecular Mechanism of Action: Corticosteroids inhibit phospholipase A2 biochemical activity.
Physiological Mechanism of Action: Corticosteroids exert anti-inflammatory and immunosuppressive properties presumably due to their ability to generally suppress protein synthesis and stabilize cellular membrane structures.
Application: Delineation of potential relationships between rheumatoid arthritis, expression profiles for interleukins (IL), and metalloproteases (MMP); and corticosteroid production.
Implementation: Evaluate expression profiles for IL-1β, tumor necrosis alpha-alpha (TNF-α), IL-6, IL-4, IL-10, MMP-1, MMP-3, and MMP-13 in freshly isolated peripheral blood (PBMC) and synovial fluid mononuclear cells (SFMC) in early rheumatoid arthritis (never-treated [ENT-RA] vs non-acute, treated rheumatoid arthritis [NAT-RA]). Characterization of expression profiles entails the application of ELISA and quantitative and semi-quantitative RT-PCR/analyses.

Human Model: Cases suffering from early rheumatoid arthritis (n = 14 RA; n = 7/14 early RA < 1 year of evolution never treated with corticosteroids or disease-modifying antirheumatic drugs; and n = 7/14 non-acute RA > 2 years of evolution treated with disease-modifying antirheumatic drugs). Reference controls: A group of 14 healthy subjects matched for sex and age.

Interpretation and Analysis: Statistically significant differences in protein or transcript levels for the cytokines of interest cannot be appreciated between ENT-RA and NAT-RA case groups. The cytokine mRNA expression by freshly isolated PBMC and SFMC in both case types is IL-1β > TNF-α > IL-10 > IL-6, with no mRNA IL-4 expression. In contrast, cytokine serum levels in ENT-RA and NAT-RA patients exist in an inverse order as follows: IL-6 > IL-10, while IL-1β, TNF-α, and IL-4 are undetectable. MMP-3 mRNA expression by the PBMC of NAT-RA patients is statistically different to that in ENT-RA patients. Similar levels of mRNA expression of MMP-1, MMP-3, and MMP-13 by PBMC and SFMC in both RA groups can be appreciated. A close equilibrium between MMP and pro-/anti-inflammatory cytokine production is observed in ENT-RA and NAT-RA patients. This balance is apparently not influenced by the length of the disease. Highly sensitive methods such as quantitative RT-PCR and ELISA, and even studying freshly isolated MC, show sustained cytokine secretion at the local level (synovial fluid/SFMC) and scarce translation at the peripheral level (serum/PBMC). Expression of MMP mRNA needs to be further evaluated in order to know whether their peripheral expression reflects their local activity in RA patients.

Expression of interleukin-1 beta, tumor necrosis factor-alpha, interleukins-6, -10, and -4, and metalloproteases by freshly isolated mononuclear cells from early never-treated and non-acute treated rheumatoid arthritis patients. Vázquez-Del Mercado M, Delgado-Rizo V, Muñoz-Valle JF, Orozco-Alcalá J, Volk HD, Armendáriz-Borunda J. Clin Exp Rheumatol 1999 Sep–Oct; 17 (5), pp. 575–83.

Autoimmune: Rheumatoid Arthritis: Severe vs Mild-Moderate/Prediction of Disease Severity: G Allele (TT vs TG/GG Genotypes)/Tumor Necrosis Factor-Alpha (TNF-α) Receptor Type II (TNFRII): Exon 6 Polymorphism
Anti-TNF-α Response/Methotrexate

Application: Characterizing severe vs mild-moderate conditions of rheumatoid arthritis as a function of exon 6 TNFRII gene polymorphism. The G allele appears to have utility in predicting the degree of responsiveness to anti-TNF-α treatment while the GG genotype for the exon 6 TNFRII polymorphism may be useful in predicting a more aggressive form of rheumatoid arthritis.
Implementation: Characterization of exon 6 TNFRII gene polymorphism in cases of (i) mild-moderate synovitis classified as responders to methotrexate therapy (e.g., n = 97) for comparison with; (ii) "non-responder" cases receiving anti-TNF-α treatment due to severe and aggressive disease (e.g., n = 78); and (iii) cases with severe rheumatoid arthritis monitored for 6 months during anti-TNF-α therapy. Results are evaluated pertaining to response according to genotype and compared to matched age, sex, and ethnic reference controls (e.g., n = 84).
Interpretation and Analysis: A distinct trend exists toward increases in the frequency of the GG genotype in patients with severe rheumatoid arthritis (6.4%) compared to cases of mild-moderate disease (3.1%) and reference controls (1.2%). Evaluation of the response to anti-TNF-α therapy after 12 weeks of treatment reveals that 37.8% of the TT genotype passes from high to medium-low disease activity. Such observations are in contrast to the 10.7% of the TG/GG genotype that passes from high to medium-low disease activity (p = 0.03).

Collective evaluation of laboratory results therefore suggests that in cases of rheumatoid arthritis classified according to response to the conventional therapy and by disease severity, there is a discern-

able trend toward a higher prevalence of the GG genotype for the exon 6 TNFRII polymorphism in less responsive cases displaying a more aggressive form of disease. In addition, the G allele tends to promote a lower degree of response to anti-TNF-α treatments.

Tumor necrosis factor-alpha receptor II polymorphism in patients from southern Europe with mild-moderate and severe rheumatoid arthritis. Fabris M, Tolusso B, Di Poi E, Assaloni R, Sinigaglia L, Ferraccioli G. J Rheumatol 2002 Sep; 29 (9), pp. 1847–50.

Autoimmune: Systemic Lupus Erythematosis (SLE)/Monocyte Interleukin (IL)-10 and IL-12/ Lymphocyte (CD8+) Poststimulation Interferon-Gamma (IFN-γ) and Tumor Necrosis Factor-Alpha (TNF-α)
Monocyte Activation/Lymphocyte Activation/ Corticosteroids

Application: Detection and characterization of systemic lupus erythematosus (SLE).
Route of Administration: Immersion (*in vitro* tissue culture environment).
Implementation: Determination of the profiles for Th1-type and Th2-type cytokines produced by mononuclear cells in conditions of recent-onset SLE, prior to the initiation of treatment with corticosteroids. Such an objective can be achieved through radioimmunoassay for IL-4, IL-10, IL-12 p40, TNF-α, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) released into the culture supernatants of various unstimulated and stimulated blood mononuclear cell populations.

Human Model: Cases of SLE (n = 10) assessed in comparison to matched healthy controls studied in parallel (n = 10).
Interpretation and Analysis: In early SLE, monocyte-enriched cells constitutively produce increased amounts of IL-10 and decreased amounts of IL-12 following stimulation. Lymphocyte-enriched cells in SLE produced decreased amounts of IFN-γ and TNF-α following stimulation. In "rested" cells, these defects are accentuated and a defect in IL-12 production has been suggested as an explanation. Depletion studies suggest that CD8+ cells are a major source of TNF-α and IFN-γ in controls, but not in SLE patients.

Increased IL-4 production or abnormalities in GM-CSF production cannot be appreciated. Therefore, in this context, even early in the course of SLE, monocyte production of IL-10 is increased and IL-12 production is decreased. Decreased production of Th1-type cytokines in SLE may be secondary to this imbalance between IL-10 and IL-12. A contributory role of dysfunctional CD8+ cells is suggested.

Decreased production of interleukin-12 and other Th1-type cytokines in patients with recent-onset systemic lupus erythematosus. Horwitz DA, Gray JD, Behrendsen SC, Kubin M, Rengaraju M, Ohtsuka K, Trinchieri G. Arthritis Rheum 1998 May; 41 (5), pp. 838–44.

Autoimmune Disease/Example: Multiple Sclerosis
Metaclopramide/Bromocriptine

Cellular/Molecular Mechanism of Action: Metaclopramide promotes the release/secretion of prolactin, which belongs to the growth and lactogenic family of hormones. Physiologically it has been found to promote profound immunomodulating properties. Alternatively, bromocriptine inhibits prolactin secretion.
Application: Metaclopramide does not appear to be an effective determinant.
Considerations: Mild hyperprolactinemia has been found to enhance several autoimmune diseases and increased PRL plasma levels have been described in experimental multiple sclerosis (MS) models and the PRL antagonist bromocriptine is able to suppress the disease. Various studies of PRL serum levels in MS have led to conflicting findings.
Interpretation and Analysis: No correlation appears to exist between baseline values and disease course or activity. Nevertheless in the regression analysis of stimulatory test results, 14% of the variance is attributable to disease activity. In conclusion PRL does not seem to be relevant as an activity marker in the whole MS population.

Immune Hypersensitivity
Praunsnitz-Kustner (PK) Test

Cellular/Molecular Mechanism of Action: Immunoglobulin (IgE) when it forms Ag/Ab complexes subsequently promotes mast cell degranulation.
Application: Detection of immune hypersensitivity conditions.
Implementation: Serum from a potentially immunohypersensitive individual is injected intradermally into a normal subject. After an incubation period of 24 to 48 hours, antigen is then injected intradermally at the same identical location.
Clinical Interpretation: Serum from hypersensitive individuals produces a marked wheal and flare response at the site of intradermal injection following intravenous administration of purified antigen. Serum from a non-hypersensitive individual would create the same response.

Immune Hypersensitivity: Allergies (Grass Pollen)
Recombinant Allergens for Kentucky Bluegrass Pollen

Background Information: Electrophoresis analysis (SDS-PAGE) of extracts from different strains of Kentucky bluegrass pollen (e.g., n = 2) reveals that there is considerable variability in extract allergen content. The significance of this finding is that such variability affects the quantitation of specific IgE antibodies by different immunoassay procedures.
Implementation: Assessment of concentrations (titers) of IgE antibodies in human sera specific for Kentucky bluegrass pollen in a more objective and consistent manner using recombinant grass pollen allergen (e.g., rKBG8.3) formulated at standardized concentrations.
Interpretation and Analysis: It is possible to design an enzyme-based immunoassay to quantify IgE antibodies specific for even 1 single allergen that may be used to discriminate sera of allergic individuals with respect to IgE specific for grass pollen in general. In this context, there is a positive correlation (r = .82) that exists between the binding of IgG to recombinant allergen and crude extracts of grass pollens. Therefore, a single recombinant allergen or a combination of a few major recombinant allergens can substitute for the crude extract for *in vitro* as well as *in vivo* diagnostic purposes.

Recombinant allergens and diagnosis of grass pollen allergy. Olsen E, Mohapatra SS. Ann Allergy 1994 Jun; 72 (6), pp. 499–506.

Immune Hypersensitivity: Anaphylaxis
Evans Blue Dye/Passive Cutaneous Anaphylaxis Test

Cellular/Molecular Mechanism of Action: Immunoglobulin (IgE) when it forms Ag/Ab complexes subsequently promotes mast cell degranulation..
Diagnostic Application: Detection of immune hypersensitivity conditions.

Implementation: Gradient dilutions of serum potentially containing IgE immunoglobulin are injected intradermally over a grid-like pattern. Purified antigen is then administered by intravenous injection. Simultaneous injection of Evans blue dye markedly increases the sensitivity of visually detecting a positive response. In this context, Evans blue dye "leaks" across capillary structures into the surrounding intracellular matrix due to the reaction of vascular capillary endothelium to histamine released from activated mast cell populations.
Interpretation and Analysis: A striking blue discoloration at the site of injection supports a diagnosis of a hypersensitivity reaction.

Immune Hypersensitivity: Asthma/Asthmatic Responses: Early vs Late/Chemotactic Properties Mediated by Mast Cell vs Mononuclear Cells/ Neutrophil Chemotactic Activity (NCA)
Lysine Aspirin Bronchoprovocation (L-ASA BPT)

Application: Differentiation between "early" and "dual" aspirin-sensitive asthma response based on the liberation of serum chemotactic properties by either mast cell or monocyte cell populations.
Route of Administration: Lysine aspirin (inhalation).
Considerations: Chemotactic activity of human neutrophils can be evaluated using a Boyden chamber apparatus. Histamine can be assayed using an automated fluorometric analyzer.
Implementation: Evaluation of pathogenic mechanisms involved in the early vs late asthmatic response as a function of serum NCA and histamine production following bronchoprovocation with lysine aspirin. Sera is collected before and 30 minutes and 240 minutes after L-ASA BPT.

Human Model: Cases of aspirin-sensitive asthma (n = 13 ASA; n = 7 early responders; n = 6 dual responders).
Interpretation and Analysis: Serum NCA increases significantly after 30 minutes ($p = 0.02$) and decreases significantly at 240 minutes ($p = 0.02$) in aspirin-sensitive asthma cases/early responders, while serum NCA in aspirin-sensitive asthma/dual responders increases significantly at 30 minutes ($p = 0.04$) and tends to increase further up to 240 minutes (not statistically significant). Neutrophil chemotactic activity at 240 minutes in aspirin-sensitive asthma/dual responders is significantly higher than baseline NCA ($p = 0.02$). Serum NCA values collected before and at 240 minutes are significantly higher in aspirin-sensitive asthma/dual responders than in aspirin-sensitive asthma/early responders ($p < 0.05$). There are no significant changes in serum histamine levels during lysine aspirin bronchoprovocation in either case group type.

NCA derived from mast cell may contribute to the development of early asthmatic responses induced by L-ASA inhalation. There may be possible involvement of NCA derived from mononuclear cells during late asthmatic response.
Instrumentation: Boyden chemotactic chamber and automated fluorometric analyzer (histamine assay).

Enhanced serum neutrophil chemotactic activity was noted in both early and late asthmatic responses during lysine-aspirin bronchoprovocation test in ASA-sensitive asthmatic patients. Kim SS, Park HS, Yoon HJ, Lee YM, Lee SK, Nahm DH. J Korean Med Sci 2003 Feb; 18 (1), pp. 42–7.

Immune Hypersensitivity: Asthma/Aspirin-Induced Bronchoconstriction
Lysine Acetylsalicylate (LASA)/Cromoglycate (Sodium)/ Nedocromil (Sodium)

Background Information: The protective activity of nedocromil sodium and of sodium cromoglycate against aspirin-induced asthma has rarely been investigated in controlled studies. Reports have described aspirin-induced platelet-mediated cytotoxic activity *in vitro* is inhibited after treatment *in vivo* with nedocromil but not with cromoglycate.
Application: Partial validation of bronchoconstriction induced by aspirin administration.
Route of Administration: Nedocromil sodium (inhalation), sodium cromoglycate (inhalation) or placebo.
Dosage: Nedocromil sodium (4 mg), cromoglycate (10 mg), or placebo.
Implementation: Delineation of the potential protective activity of nedocromil and cromoglycate against aspirin-induced bronchoconstriction. Three bronchial challenges with a single dose of LASA can be performed that are capable of causing decreases in FEV_1 of 25% or more in a preliminary dose-response tests 30 minutes after inhalation of nedocromil sodium, sodium cromoglycate, or placebo. FEV_1 and SRaw are recorded at intervals for 195 minutes.

Human Model: Cases of aspirin-induced asthma (n = 10).
Interpretation and Analysis: Following a placebo, LASA causes a maximal decrease in FEV_1 that is 42 ± 4% of baseline. After cromoglycate and nedocromil the maximal decrease in FEV_1 is reduced to 20 ± 3% and 18 ± 4%, respectively ($p < 0.01$ vs placebo for both treatments), without significant differences between the 2 treatments. Similar results can be observed for SRaw. Therefore, at recommended therapeutic doses, sodium cromoglycate and nedocromil sodium are equally effective in attenuating aspirin-induced bronchoconstriction and it is therefore unlikely that platelet activation participates in the pathogenesis of aspirin-induced asthma.

Attenuation of aspirin-induced bronchoconstriction by sodium cromoglycate and nedocromil sodium. Robuschi M, Gambaro G, Sestini P, Pieroni MG, Refini RM, Vaghi A, Bianco S. Am J Respir Crit Care Med 1997 Apr; 155 (4), pp. 1461–4.

Immune Hypersensitivity: Asthma (Allergic vs Non-Allergic)/Aspirin-Sensitive and Aspirin-Insensitive Asthma/Histamine Release (HR)/Sulfidoleukotriene Release (LTR)
Interleukin (IL)-3/Anti-IgE/C5a/Basophil Stimulation Test

Cellular/Molecular Mechanism of Action: The immunoglobulin fraction anti-IgE binds to and neutralizes the biological activity of IgE, which is an antibody isotype that binds to the surface membrane of basophils and is intimately involved in the initiation of allergic and anaphylactic immune hypersensitivity reponses following the formation of Ag/Ab complexes. The complement fraction C5a, among its many biological properties, is classified as an anaphylatoxin.
Physiological Mechanism of Action: The interleukin IL-3 appears to be necessary for the development/maturation of mast cell populations. The complement fraction C5a functions as an anaphylatoxin related to the initiation of anaphylactic immune hypersensitivity reactions.
Background Information: The differentiation between allergic and non-allergic asthma (NAA) is a common and important clinical challenge. Rare reports exist describing the *in vitro* diagnostic characteristics that distinguish between these 2 types of asthma.
Application: Differentiation between allergic and non-allergic conditions of asthma, but aspirin-sensitive asthma (ASA) cannot be distinguished from NAA without aspirin tolerance.
Implementation: Characterize and profile mediator release induced by anti-IgE and C5a exposure (basophil stimulation test) in different conditions of bronchial asthma. The *ex vivo* analysis proce-

dures involve the isolation of peripheral blood leucocytes (PBL) by dextran sedimentation. After priming with IL-3, peripheral blood leukocytes are stimulated with anti-IgE and C5a to facilitate the measurement of released HR and LTR into the supernatant. Additionally, purified leukocyte fractions can be evaluated to selectively determine the cellular source of mediator release.

Human Model: Cases of ASA (n = 10); NAA without aspirin tolerance (n = 12); allergic asthma (AA; n = 7); negative healthy reference controls (n = 9).

Interpretation and Analysis: Upon stimulation with anti-IgE, LTR is slightly, but not significantly, lower in ASA and NAA without aspirin tolerance compared to AA and controls. In contrast, C5a-triggered LTR is significantly higher in ASA (14.4 ± 12.88 pg/105 cells) and NAA (22.9 ± 22.61 pg/105 cells) than in AA (9.6 ± 3.29 pg/105 cells) and controls (7.5 ± 7.19 pg/105 cells) ($p < 0.05$). This difference between ASA and NAA vs AA and controls is even more pronounced when determining the quotient C5a-/anti-IgE-induced LTR ($p < 0.001$). At an optimal cutoff point of 1.0, calculated by relative operating characteristics (ROC) analysis, the positive predictive value for a donor to belong to ASA or NAA is 0.94. No significant differences can be detected in HR between conditions of asthma and healthy controls in either condition. Delineation of anti-IgE- and C5a-induced LTR from basophils allows differentiation between allergic and non-allergic asthmatic patients. The quotient C5a-/anti-IgE-induced LTR is diagnostically more significant than considering a single parameter. Aspirin-sensitive asthma cannot be distinguished from NAA.

Differential mediator release from basophils of allergic and non-allergic asthmatic patients after stimulation with anti-IgE and C5a. Abrahamsen O, Haas H, Schreiber J, Schlaak M. Clin Exp Allergy 2001 Mar; 31 (3), pp. 368–78.

Immune Hypersensitivity: Asthma/House Dust Mite (HDM)-Provoked Asthma/Aspirin-Insensitive Asthma (AIA)
Pranlukast/HDM Inhalation Provocation

Cellular/Molecular Mechanism of Action: Panlukast is a leukotriene (LT) receptor antagonist (LTRA).

Background Information: Leukotrienes are important in asthma, and LT modifiers modulate antigen-induced asthma. Overproduction of LT by suppression of cyclooxygenase activity is a relevant occurance in conditions of AIA.

Application: Possible differentiation between HDM-provoked asthma (LT overproduction and Ag/Ab reactions) and AIA (LT overproduction and 5-lipoxygenase shift in the arachidonic cascade).

Route of Administration: Aspirin oral provocation.

Implementation: Evaluation of HDM-sensitive asthmatic inpatients without AIA applying an HDM inhalation provocation test. In addition an aspirin oral provocation test can be employed to evaluate cases of asthma that are insensitive to aspirin administration. Tests are repeated using the same regimen after 7 days of treatment with an LTRA. The effects of an LTRA are assessed on changes in sputum LTC_4-LTD_4, eosinophil cationic protein (ECP), eosinophil count, urinary LTE_4/creatinine, 11-dehydrothromboxane B_2 (11-$dhTXB_2$/creatinine), serum LTC_4-LTD_4, ECP, and peripheral blood eosinophil count, during immediate asthmatic reaction (IAR) and late asthmatic reaction (LAR) in the HDM group and during IAR in AIA cases for each test, which are then compared.

Human Model: Cases of house dust mite sensitive asthma without AIA (n = 6 HDM group type); and AIA conditions (n = 7 AIA group type).

Interpretation and Analysis: In cases of house dust mite sensitive asthma without aspirin insensitivity, immediate (IAR) and late (LAR) asthmatic reactions can be observed. Sputum LTC_4-LTD_4 and urinary LTE_4/creatinine both increase significantly during IAR and LAR. Sputum eosinophil cationic protein (ECP) increases during IAR and further increases during LAR. Eosinophil count in the sputum does not increase during IAR but significantly increases during LAR. LT receptor antagonism (Pranlukast) suppresses declines in FEV_1 both during IAR and LAR (73.8% and 51.9%, respectively) and inhibits increases in sputum eosinophil count during LAR and sputum ECP during IAR and LAR. In cases of AIA, aspirin-induced IAR is associated with a fall in urinary 11-$dhTXB_2$/creatinine and increases in levels of sputum LTC_4-LTD_4 and ECP and urinary LTE_4/creatinine. LT receptor antagonism (Pranlukast) suppresses IAR and inhibits increases in the level of sputum ECP, but fails to change aspirin-induced LT production in the sputum and urine. The levels of sputum LTC_4-LTD_4 and urinary LTE_4/creatinine in the stable phase in the ASA group are significantly greater than those in the HDM group. Therefore, it appears that HDM-provoked asthma is associated with overproduction of LT with an antigen-antibody reaction, while AIA is associated with overproduction of LT with a shift to the 5-lipoxygenase series of the arachidonate cascade. Leukotriene receptor antagonist may be useful against both types of asthma through inhibition of LT activity and eosinophilic inflammation of the airways.

Effects of pranlukast on chemical mediators in induced sputum on provocation tests in atopic and aspirin-intolerant asthmatic patients. Obase Y, Shimoda T, Tomari SY, Mitsuta K, Kawano T, Matsuse H, Kohno S. Chest 2002 Jan; 121 (1), pp. 143–50.

Immune Hypersensitivity: Asthma (Aspirin-Induced)/Cysteinyl-Leukotriene Production: Peripheral Blood Leukocytes [Diagnostic Value Limited]
Lysine Acetylsalicylic Acid

Background Information: Aspirin-induced asthma (AIA) is a clinical syndrome related to cysteinyl leukotriene overproduction in airways. The confirmation of the diagnosis requires inconvenient provocation tests with acetylsalicylic acid (ASA).

Implementation: Evaluate the *in vitro* influence of ASA stimulation of isolated peripheral blood leukocytes on measurements for the release of cysteinyl leukotrienes (cys-LTs) as a potential diagnostic molecular marker for AIA. Potential validation of this theory can be achieved through the use of a cellular allergen stimulation test (CAST) adapted for the measurement of leukotriene release from isolated leucocytes. Isolated cell preparations are stimulated with (i) Lys-ASA; (ii) formyl-Met-Leu-Phe (*f*MLP); or (iii) both *f*MLP and Lys-ASA, in a buffer containing interleukin (IL)-3 cytokine. Results are then compared with human leukemia cell line (Hl-60) response to Lys-ASA. Cysteinyl leukotrienes can be measured in cell supernatant fluids by ELISA.

Human Model: Cases of aspirin-tolerant (n = 32) and aspirin-intolerant (n = 26) asthmatics.

Interpretation and Analysis: Acetylsalicylic acid has a rather weak stimulatory effect on cys-LTs released in conditions of both aspirin-tolerant and aspirin-intolerant asthma. In contrast to some related reports, some studies have detected no significant difference between cys-LTs released by leucocytes from AIA and ATA or those released by differentiated Hl-60 cells. Measurement of cys-LT release by peripheral blood leucocytes pretreated with aspirin has no value for diagnosis of AIA.

A moderate and unspecific release of cysteinyl leukotrienes by aspirin from peripheral blood leucocytes precludes its value for aspirin sensitivity testing in asthma. Pierzchalska M, Mastalerz L, Sanak M, Zazula M, Szczeklik A. Clin Exp Allergy 2000 Dec; 30 (12), pp. 1785–91.

Immune Hypersensitivity: Asthma (Grain Dust [GD])/Mast Cells: Tryptase Expression/Neutrophils: Elastase Expression/Interleukin (IL)-8 Expression
GD Bronchoprovocation Test/Bronchial Mucosa Biopsy

Background Information: The immunopathological mechanism for occupational asthma induced by GD remains to be clarified. There have been a few reports that have suggested the involvement of neutrophils in the induction of bronchoconstriction after inhalation of GD.
Implementation: Evaluation of the phenotype of leukocytes that infiltrate the bronchial mucosa in conditions of GD-induced asthma. Bronchial biopsy specimens are obtained by fiber-optic bronchoscopy in GD-induced asthmatics. Bronchial biopsy specimens are examined by immunohistochemistry analyses using a panel of monoclonal antibodies to tryptase-containing mast cell (AA1), activated eosinophil (EG2), pan T-lymphocyte (CD3), and neutrophil elastase (NE). Sputum is then collected before and after initiation of the GD-bronchoprovocation test. The IL-8 level in the sputum is also measured using ELISA techniques.

Human Model: Cases of GD-induced asthma (n = 6); and house dust mite sensitivity (n = 6).
Interpretation and Analysis: There is a significant increase in the number of tryptase-containing mast cell (AA1+) and neutrophil elastase positive (NE+) cells in bronchial mucosa of GD-induced asthma, compared with those of allergic asthma ($p = 0.01$, $p = 0.01$, respectively). No significant differences can be observed in the number of EG2+ and CD3+ cells ($p = 0.13$, $p = 0.15$, respectively). IL-8 is abundant in the sputum of all GD-induced asthma patients and significantly increased after the bronchial challenges compared with baseline values ($p = 0.03$). Such findings support the perspective that neutrophil recruitment together with mast cells may contribute to the bronchoconstriction induced by GD antigen. A possible involvement of IL-8 in such processes has been proposed.
Instrumentation: Fiber-optic bronchoscope.

Neutrophil infiltration and release of IL-8 in airway mucosa from subjects with grain dust-induced occupational asthma. Park HS, Jung KS, Hwang SC, Nahm DH, Yim HE. Clin Exp Allergy 1998 Jun; 28 (6), pp. 724–30.

Immune Hypersensitivity: Asthma/Asthma: Predicted Absence
Skin-Prick Test (SPT)/Methacholine

Cellular/Molecular Mechanism of Action: The SPT employs a battery of foreign environmental substances that can function as immunogens. The biological preparations applied in the SPT are recognized by the immune system and if they are perceived as allergens, activate various immune and inflammatory pathways (e.g., antigen recognition by IgE and Ag:IgE complexes binding to Fc receptors expressed on the exterior surface membrane of mast cell populations leading to their activation and subsequent release of inflammatory mediators like histamine).
Background Information: None of the existing tests for the diagnosis of asthma are considered to be definitive. Certain circumstances require prompt diagnosis, and a test able to predict the absence of asthma would be very useful.
Application: The SPT may be valuable in predicting the probability of asthma in young adults.
Dosage: Methacholine administered at an exposure level capable of causing a 20% fall in FEV_1 (PC^{20}) at < 4 mg/mL while utilizing < 8 mg/mL as diagnostic cutoff values for asthma in the methacholine challenge test (MCT).
Implementation: Evaluation of asthmatic conditions utilizing (i) SPT introduction of a battery of common aeroallergens; and (ii) methacholine challenge test (MCT). The sensitivity, specificity, positive predictive value, and negative predictive values (NPV) of the SPT are calculated using provocative concentrations of methacholine. The Bayes' formula can be used to determine posttest probabilities of having asthma, both for positive and negative SPT results.

Human Model: Cases suffering from asthma including 3 groups of subjects aged 18–24 years: Group A, asthmatic patients (n = 175); Group B, control subjects (n = 100); and Group C, subjects with suspected asthma (n = 150) with normal spirometry findings and a negative exercise challenge test result.
Interpretation and Analysis: A positive SPT result to at least 1 allergen can be detected in approximately 95.5%, 54%, and 69% of cases classified as asthmatics, reference control subjects, and suspected asthmatics, respectively. Sensitivity, specificity, and NPV of the SPT are 90.7%, 52.0%, and 84.8%, respectively, with a cutoff value of $PC^{20} < 8$ mg/mL. The lower cutoff, $PC^{20} < 4$ mg/mL, increases the sensitivity and NPV to 98.2% and 97.8%, respectively. A negative SPT result decreases the probability of having asthma by 10-fold to 20-fold in subjects that have a low to moderate pretest probability. Incorporating an SPT into the workup of subjects with suspected asthma can reduce the cost of this process significantly. The SPT may be used as a simple, fast, safe, inexpensive, and reliable method to predict the absence of asthma in young adults.

Value of a negative aeroallergen skin-prick test result in the diagnosis of asthma in young adults: Correlative study with methacholine challenge testing. Graif Y, Yigla M, Tov N, Kramer MR. Chest 2002 Sep; 122 (3), pp. 821–5.

Immune Hypersensitivity: Asthma/Asthma: Mild to Moderate/Hyperresponsive Airway/Cellular Airway Infiltration
Methacholine/Agonists (B_2)/[Corticosteroids]

Cellular/Molecular Mechanism of Action: Methacholine recognize, binds to, and activates parasympathetic/cholinergic receptor sites.
Physiological Mechanism of Action: Methacholine promotes parasympathomimetic-mediated bronchoconstriction.
Background Information: Current guidelines on the management of asthma advocate the use of anti-inflammatory treatment in all but mild disease. They define disease control in terms of clinical criteria such as lung function and symptoms. However, the relationship between the clinical control of the disease and inflammation of the airways is not clear.
Application: The number of infiltrating leukocytes such as mast cells, (activated) eosinophils, CD8+, and CD45RO+ cells in bronchial biopsy specimens in mild to moderate conditions of asthma appears to correspond to the observation of airway hyperresponsiveness to methacholine.
Implementation: Delineate correlations between airway inflammation and measures of clinical control and bronchial hyperresponsiveness (methacholine challenge test) in asthmatic conditions during simultaneous steroid inhalation. Biopsy specimens are taken by fiber-optic bronchoscopy from the carina of the right lower and middle lobes and from the main carina. Immunohistochemical staining is performed on tissue sections with monoclonal antibodies against eosinophil cationic protein (EG1, EG2), mast cell tryptase (AA1), CD45, CD22, CD3, CD4, CD8, CD25, and CD45RO. The number of positively stained cells in the lamina propria is counted twice by using an interactive display system.

Human Model: Cases of mild to moderate asthma (n = 26 atopic adults; baseline $FEV_1 \geq 50\%$ predicted, concentration of histamine causing a 20% fall in FEV_1 [PC20] 0.02–7.6 mg/ml) on regular treatment with inhaled steroids. Histories and bronchoscopic examinations are utilized to determine use of adrenergic agonists (B_2), peak flow variability, and symptom scores.

Investigation Design: Cross-sectional study.

Interpretation and Analysis: No difference exists in cell numbers in biopsy specimens collected from the carina of the right lower and middle lobes and from the main carina. The PC20 for methacholine is inversely related to the average number of total leucocytes, EG1+, and EG2+ cells, mast cells, CD8+, and CD45RO+ cells in the lamina propria. These relationships are usually similar for each of the biopsy sites. Symptom scores, β_2 agonist usage, FEV_1, and peak flow variability do not appear to consistently correlate with tissue biopsy cell counts. Therefore, infiltration of inflammatory cells in the lamina propria of the airways seems to persist in asthmatic outpatients despite regular treatment with inhaled steroids. The number of infiltrating leucocytes such as mast cells, (activated) eosinophils, CD8+, and CD45RO+ cells in bronchial biopsy specimens from these patients appears to be reflected by airway hyperresponsiveness to methacholine, but not by symptoms or lung function. These findings may have implications for the adjustment of anti-inflammatory treatment of patients with asthma.

Instrumentation: Cryostat histology sectioning unit.

Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. Sont JK, Han J, van Krieken JM, Evertse CE, Hooijer R, Willems LN, Sterk PJ. Thorax 1996 May; 51 (5), pp. 496–502.

Immune Hypersensitivity: Asthma/Airway Reactivity
Methacholine/Corticosteroids/Nitric Oxide (Exhaled)

Cellular/Molecular Mechanism of Action: Methacholine is a parasympathomimetic agent that recognizes, binds to and activates cholinergic receptor complexes. Corticosteroids inhibit phospholipase A2 biochemical activity in a manner that prevents or severely inhibits the subsequent production of prostaglandin species.

Physiological Mechanism of Action: Methacholine can promote bronchoconstriction of the major airways. Salbutamol promotes bronchodilation. Corticosteroids can exert an anti-inflammatory and immunosuppressive response that is in part due to their ability to promote inhibition of protein synthesis and to stabilize biological membrane structures.

Background Information: Bronchial reactivity and exhaled nitric oxide (eNO) are not often used to monitor the control and severity of asthma in clinical practice.

Application: Airway reactivity to methacholine corresponds to alterations in exhaled nitric oxide (eNO) which is also related to conditions of atopy.

Implementation: Evaluation of the relationship between asthma and different physiologic measures (pulmonary function, nonspecific bronchial reactivity, and eNO).

Human Model: Cases of asthma in various stages of severity (n = 392; cross-sectional, hospital-based study design).

Interpretation and Analysis: Inhalation of corticosteroids does not influence eNO levels. However, the percentage of predicted FEV_1 and the provocative dose of methacholine causing a 20% decrease in FEV_1 are significantly lower in inhaled corticosteroids (ICS) case groups compared with cases not receiving inhaled corticosteroids (NICS mean, 83.2%, 95% confidence interval [CI] 80.4–86.0%; vs mean 94.1%, 95% CI 91.1–97.1%, p = 0.001; and geometric mean 0.32 mg, 95% CI 0.23–0.45 mg; vs geometric mean 0.58 mg, 95% CI 0.42–0.81 mg, p = .01, respectively). In conditions with severe bronchial hyperresponsiveness, there is a lower percentage predicted FEV_1 value ($p < 0.001$) and levels of eNO significantly increase with increasing bronchial hyperresponsiveness ($p < 0.001$). There is no relationship between the percentage of predicted FEV_1 and eNO. Conditions of atopy have a significantly higher eNO levels than do nonatopic patients (geometric mean 11.21 ppb, 95% CI 10.07–12.49 ppb; vs geometric mean 7.76 ppb, 95% CI 6.11–9.85 ppb, p = 0.006, respectively). Therefore, values for eNO are not related to the degree of airway obstruction but are related to airway reactivity and atopic status independent of inhaled corticosteroid use. Higher values of eNO are seen with increased airway reactivity.

Relationship among pulmonary function, bronchial reactivity, and exhaled nitric oxide in a large group of asthmatic patients. Langley SJ, Goldthorpe S, Custovic A, Woodcock A. Ann Allergy Asthma Immunol 2003 Oct; 91 (4), pp. 398–404.

Immune Hypersensitivity: Allergic Rhinitis (Seasonal)/Pharmaceutical Efficacy Evaluation/IL-4
Allergen Provocation/Corticosteroids

Cellular/Molecular Mechanism of Action: Allergens stimulate the immune system in a manner that creates a hypersensitivity response.

Application: Detection and characterization of seasonal allergic rhinitis type hyperimmune reactions. Measurement or detection of IL-4 can therefore theoretically be applied as an indicator of disease or a molecular marker for validating pharmaceutical efficacy.

Implementation: Evaluation of conditions of seasonal allergic as a function of cytokine expression analyzed by reverse-transcriptase polymerase chain reaction (RT-PCR). The time-course study during winter includes repeated local allergen provocation test challenges with subsequent nasal biopsies as well as biopsies taken during pollen season.

Human Model: Cases of seasonal allergic rhinitis (n = 17; n = 10 healthy controls).

Interpretation and Analysis: The RT-PCR for CD44 yields positive bands in the vast majority of affected cases (e.g., n = 65/71 cases), in which mRNA sequences for IL-2, IL-4, and IL-5 are then applied for detection by PCR analysis (semi-nested). IL-4 mRNA is almost exclusively expressed by allergic patients. During provocation a significant increase in IL-4 can be detected compared to reference controls (p = 0.043). Similarly, during the natural pollen season, IL-4 mRNA expression is significantly higher in cases not using nasal corticosteroids compared with those who do (p = 0.011). No differences in IL-2 or IL-5 can be appreciated in these same case group types. Such findings, together with the observations of T-cell activation (e.g., phenotype switch toward T-helper 2 (Th2) cells; accumulation (homing) of these T cells in the nasal mucosa) indicate that T cells constitute the main source for IL-4 in the nasal mucosa. Therefore, allergic patients have an increased synthesis of IL-4 when provoked with the allergen, and during natural pollen season this synthesis can be downregulated by corticosteroids. Furthermore, previous investigations illustrate the versatility of molecular biology in surgical pathology and that even low-copy-number cytokine mRNA can be examined in routinely snap-frozen surgical specimens.

Nasal messenger RNA expression of interleukins 2, 4, and 5 in patients with allergic rhinitis. Karlsson MG, Davidsson A, Viale G, Graziani D, Hellquist HB. Diagn Mol Pathol 1995 Jun; 4 (2), pp. 85–92.

Immune Hypersensitivity: Allergen Immune Response/Late Induction (Nasal Mucosa)/ Grass/ Pollen Hypersensitivity Immune Responses/Efficacy Evaluation of Anti-Inflammatory/ Immunosuppresive Agents
Allergen Challenge Test/Fluticasone Proprionate

Background Information: Allergen-induced late nasal responses are associated with recruitment and activation of T lymphocytes and eosinophils and preferential mRNA expression for T-helper Type 2 (Th2) cytokines.
Application: Fluticasone proprionate can be applied as a molecular probe for delineating endogenous inflammatory mediator profiles in conditions of immune hyperreactivity responses and inflammatory reactions in addition to evaluation of the efficacy of immunosuppressive and anti-inflammatory reagents.
Implementation: Detection of allergens responsible for inducing allergic reactions and evaluating the efficacy of potential therapeutic agents (e.g., anti-inflammatory agents and corticosteroids). An example of the use of this investigative strategy involves the application of an allergen challenge to evaluate the efficacy of topical corticosteroids (fluticasone propionate) and their ability to inhibit late responses by suppressing the cellular expression of Th2 type cytokine mRNA sequences. Nasal biopsies are taken at baseline and repeated 24 hr after local nasal allergen provocation following 6 weeks of treatment (e.g., fluticasone propionate 200 μg or placebo nasal spray twice daily).

Human Model: Cases of grass pollen sensitivity (n = 48 adults) analyzed in a randomized double blind placebo-controlled trial.
Interpretation and Analysis: Baseline mRNA expression for interleukin (IL)-4 ($p = 0.01$) and IL-5 ($p = 0.002$) is higher in cases of grass pollen sensitivity. Topical corticosteroid treatment significantly inhibits immediate nasal symptoms, with almost complete inhibition of the late response following allergen challenge. This is associated with a marked decrease in the allergen-induced increases in cells expressing mRNA for IL-4 ($p = 0.002$) but not for IL-5. Inhibition of the late response is also accompanied by decreases in CD25+ cells (presumed T lymphocytes and eosinophils). A significant correlation exists between decreases in IL-4 mRNA+ cells, and in eosinophils after treatment ($r = 0.46$, $p < 0.05$). Therefore, prolonged treatment with topical corticosteroid inhibits allergen-induced early and late nasal responses and the associated tissue eosinophilia, and that, at least in part, may result from inhibition of cells expressing IL-4 mRNA sequences.

Topical glucocorticosteroid (fluticasone propionate) inhibits cells expressing cytokine mRNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. Masuyama K, Jacobson MR, Rak S, Meng Q, Sudderick RM, Kay AB, Lowhagen O, Hamid Q, Durham SR. Immunology 1994 Jun; 82 (2), pp. 192–9.

Immune Hypersensitivity: Ctenocephalides Felis
Ctenocephalides Felis Salivary Extract/Protein/S (MW 40kDa and MW 8-12 kDa)

Cellular/Molecular Mechanism of Action: Hypersensitivity immunogen.
Background Information: An 18 kDa protein isolated from saliva of the cat flea, *Ctenocephalides felis*, elicits a positive intradermal skin test (IDST) in 100 and 80% of experimental and clinical flea allergic dogs, respectively. The protein, named Cte f 1, is the 1st novel major allergen described for canine flea allergy. The relative ability of rCte f 1 to bind IgE in the serum of flea allergic animals, produced in these 3 expression systems, matches that of the native allergen. Competition ELISA techniques demonstrate that approximately 90% of the specific IgE binding to native Cte f 1 can be blocked by the different forms of rCte f 1.
Application: Detection of immune hypersensitivity reactions to *Ctenocephalides felis* (saliva).
Considerations: The cat flea, *Ctenocephalides felis*, is the major initiator of flea-bite hypersensitivity in dogs. Previous analyses of whole extracts of the flea and flea salivary secretions have failed to identify the allergens responsible.

These findings suggest that protein with apparent MW 40k and MW 8k-12k are important in flea bite hypersensitivity. This work also supports a previous finding that mice that had been exposed to flea bites had antibodies to proteins with approximately MW 40k that can be detected in salivary secretions of fleas.
Route of Administration: Intradermal.
Dosage: Harvest by desection of approximately > 2,000 salivary glands from adult female fleas. Extracts are produced in buffered saline containing protease inhibitors and fractionation of the extract can be performed using gel permeation HPLC.
Implementation: Evaluation of the allergenicity of components contained within individual salivary gland extracts tested by intradermal injection. Each sample (aliquots) represents a fraction of whole salivary gland extract separated as a column eluate. Histamine (intradermal) can function as a positive reference control. Negative control injections consist of eluate from the column collected prior to fractions containing any protein.

Animal Model: Canine
Interpretation and Analysis: Canine subjects can be classified as hypersensitive to fleas (flea-feeding positive, FF+) or insensitive (flea-feeding negative, FF−) using a provocative test with live fleas. The skin of FF− dogs either does not respond or has a minimal response (a bleb approximately 2 mm larger than the injection blebs at the negative control injection sites) to all fractions and to the whole extract. Histamine control injections consistently produce positive responses (defined as wheals 5 mm greater than the blebs at the negative control injection sites) in all dogs. The skin of some FF+ dogs (e.g., n = 3/9) reacts positively to injection of fractions containing proteins with apparent MW 40k. Other FF+ dogs may react positively to fractions containing proteins with apparent MW 8-12k (e.g., n = 5/9). On a less frequent basis, dogs may respond with very large, red wheals to injection of both the approximately MW 40k and MW8-12k fractions (e.g., n = 1/9).

Identification, cloning, and characterization of a major cat flea salivary allergen (Cte f 1). McDermott MJ, Weber E, Hunter S, Stedman KE, Best E, Frank GR, Wang R, Escudero J, Kuner J, McCall C. Mol Immunol 2000 May; 37 (7), pp. 361–75.

Immune Hypersensitivity: Eczema (Atopic)/Efficacy Prediction of Therapeutic Intervention
Aeroallergen Challenge/Atopy Patch Test

Background Information: Pharmacologic studies in atopic eczema (AE) are difficult to standardize. Patients with AE differ in the stage of their skin disease (acute, subacute, chronic).
Application: Aeroallergen challenge (atopy patch test) can be applied to evaluate the effect of topical anti-inflammatory agents (topical glucocorticoids; tar) on allergic inflammatory reactions in conditions of AE.
Route of Administration: Aeroallergen challenge (atopy patch test: topical/intradermal injection).

Implementation: Evaluation of non-lesional skin regions on dorsal back region of cases suspected of being affected by AE. Segments of skin are treated for approximately 3 weeks at 3 different sites with triamcin-olonacetonide (1%) in cetamacrogol ointment (GCSs), pix liquida (10%) in cetamacrogol ointment (tar), and cetamacrogol ointment (vehicle), respectively. Atopy patch tests (APT) are then performed, and biopsy specimens collected from each test site (time = 0 and 24 hours) for immunohistochemical analysis.

Human Model: Cases atopic eczema (n = 6 AE).

Interpretation and Analysis: Treatment with both GCSs and tar reduces the macroscopic outcome of the atopy patch test (APT) reactions. Furthermore, both treatment modalities have an almost equal inhibiting effect on the influx of T cells, eosinophils, and CD1+, RFD1$^+$, IFN-γ^+, and IL-4$^+$ cells, as well as on the percentage of vessels expressing the adhesion molecules vascular cell adhesion molecule 1 (VCAM-1) and E-selectin in response to epicutaneous aeroallergen challenge. Although both treatments significantly reduce the various cellular constituents of allergic inflammation, all cell types remain present. In this context, aeroallergen challenge (e.g., atopy patch test) can be used to evaluate the effect of topical anti-inflammatory treatments on allergic inflammation in patients with AE.

Modulation of the atopy patch test reaction by topical corticosteroids and tar. Langeveld-Wildschut EG, Riedl H, Thepen T, Bihari IC, Bruijnzeel PL, Bruijnzeel-Koomen CA. J Allergy Clin Immunol 2000 Oct; 106 (4), pp. 737–43.

Immune Hypersensitivity: Fire Ant Venom
Fire Ant Whole-Body Extract (WBE)

Cellular/Molecular Mechanism of Action: Antigens recognized by humoral immunoglobulin produced as a consequence of previous host exposure resulting in complement activation following antigen/antibody complex formation and release of histamine.

Application: Detection of patients that have developed immune hypersensitivity to fire ant venom.

Route of Administration: Intradermal.

Dosage: Fire ant whole-body extract prepared at a concentration of $1{:}5 \times 10^3$, v/v.

Considerations: Evaluation of cases with a history of immediate hypersensitivity to the sting of the imported fire ant based on a comparison between fire ant whole-body extract (WBE) preparations and fire ant venom (IFAV).

Animal Model: Cases with fire ant hypersensitivity (n = 34).

Interpretation and Analysis: Ninety-one percent (31/34) of skin tests in conditions of hypersensitivity using IFAV at a maximal concentration of $1{:}5 \times 10^3$, v/v, demonstrate a wheal response equal to or greater than a histamine control. Fifty-three percent (18/34) of the same case group skin test positive to a WBE preparation. When the criteria for a positive skin test are relaxed, 82% of the hypersensitive group can be identified with the IFAWBE. A comparison of skin test results in sensitive patients reveals variability in the sensitivity of some WBE preparations. Leukocyte histamine release demonstrates a dose-response release of histamine with both IFAV and SIWBEa preparations. Specific venom antisera produced in rabbits can identify a precipitin line of common identity in a gel-diffusion system containing IFAWBE and IFAV. Such findings can be verified by the competitive inhibition of IFAWBE with IFAV in a solid-phase radioimmunoassay system. In summary, fire ant WBEs contain venom constituents and are effective diagnostic agents in up to 82% of patients with hypersensitivity to the sting of the imported fire ant. Marked variability in the responsiveness of sensitive patients to different WBE preparations mandates standardization of these diagnostic preparations.

In vivo and *in vitro* comparison of fire ant venom and fire ant whole-body extract. Strom GB Jr, Boswell RN, Jacobs RL. J Allergy Clin Immunol 1983 Jul; 72 (1), pp. 46–53.

Immune Hypersensitivity: Pharmaceutical/Example: Beta-Lactam Antibiotics
Molecular Determinants Identification

Cellular/Molecular Mechanism of Action: Molecular components of beta-lactam antibiotics that are recognized by (form complexes with) IgE antibodies.

Background Information: Allergies to antibiotics, mainly the beta-lactam antibiotics (penicillins and cephalosporins), are a common, costly, and potentially dangerous clinical problem encountered in everyday practice. Progress has been made in identifying the spectra of allergenic determinants recognized by IgE antibodies in the sera of subjects sensitized to penicillins and cephalosporins, and this is aiding the development of an appropriate battery of drug conjugates for use as diagnostic agents.

Application: Immunochemical identification of allergenic structures in patients that have developed Type I hypersensitivity reactions to penicillins and cephalosporins.

Considerations: Case sensitivity responses to the beta-lactam antibiotics are frequently heterogeneous, and this factor must be taken into account for any diagnostic strategy or future therapy with a penicillin or cephalosporin. Immunoscreening procedures of this nature have been developed most extensively for this class of pharmaceutical agents.

Interpretation and Analysis: Detection of IgE complex formation with molecular components of penicillins and cephalosporins is highly suggestive of the development of a Type I hypersensitivity response to these antibiotic agents.

beta-Lactam allergenic determinants: Fine structural recognition of a cross-reacting determinant on benzylpenicillin and cephalothin. Zhao Z, Baldo BA, Rimmer J. [Clin Exp Allergy] 2002 Nov; 32 (11), pp. 1644–50.

Fine structural recognition specificities of IgE antibodies distinguishing amoxicilloyl and amoxicillanyl determinants in allergic subjects. Zhao Z, Baldo BA, Baumgart KW, Mallon DF. [J Mol Recognit] 2001 Sep–Oct; 14 (5), pp. 300–7.

Structural determinants of antibiotic allergy. Baldo BA, Zhao Z, Pham NH. Current allergy reports [Curr Allergy Rep] 2001 Jan; 1 (1), pp. 23–31.

Studies on the mechanism of multiple drug allergies. Structural basis of drug recognition. Pham NH, Baldo BA, Puy RM. Journal of immunoassay & immunochemistry [J Immunoassay Immunochem] 2001; 22 (1), pp. 47–73.

Synthesis, characterization and immunochemical evaluation of cephalosporin antigenic determinants. Sánchez-Sancho F, Perez-Inestrosa E, Suau R, Montañez MI, Mayorga C, Torres MJ, Romano A, Blanca M. J Mol Recognit 2003 May-Jun; 16 (3), pp. 148–56.

Immune Hypersensitivity: Pharmaceutical (Erythema Multiforme)
Spironolactone/Immune Desensitization

Cellular/Molecular Mechanism of Action: Immune desensitization.

Application: Desensitization to pharmaceuticals precipitating conditions of erythema multiforme.

Dosage: Spirolactone—using an escalating dosage scheme from 1 mg to 400 mg administered over a 14-day period.

Considerations: In situations where salt/water restriction and other diuretics are ineffective, readministration of spironolactone is sometimes considered appropriate.

Implementation: In the absence of *in vitro* or *in vivo* diagnostic tests for drug allergic responses to spironolactone, it has been reported that serial increases in dose (1 mg to 400 mg administered over a 14 day period) are tolerated uneventfully. Occationally, a mild maculopapular rash appears approximately a month later but resolves despite continuation of spironolactone administration for periods of 20 months or more without developing signs of drug hypersensitivity.
Interpretation and Analysis: Although proven drug hypersensitivity attributable to spironolactone is very infrequent, when allergic manifestations do occur in situations that may require readministration of spironolactone, a suggested protocol for test-dosing should be be considered in order to achieve a successful outcome.

Readministration of spironolactone in the spironolactone-intolerant patient. Greenberger PA, Lazar HP. N Engl Reg Allergy Proc 1986 Jul–Aug; 7 (4), pp. 343–52.

Immune Hypersensitivity (Severe): Bee-Sting Venom
Whole Bee Extract

Cellular/Molecular Mechanism of Action: Antigenic preparations perceived by the immune system as allergens/immunogens.
Application: Stimulation of the immune response for the purpose of surveying for hypersensitivity responses in suspect patients displaying both systemic and localized signs.
Route of Administration: Intradermal skin testing and RAST (Hymenoptera, whole-body extracts of sweet bees, honey bees).
Considerations: Hypersensitivity reactions to sweet-bee stings are distinct and not associated with reactions to other stinging insects. Somewhat infrequently RAST reactions will be observed with whole-body extract of sweat bee. Reliable antigen that is commercially available for diagnosing or treating such conditions is sometimes difficult to find, but sweat bee venom or other venom antigens might be beneficial in patient management.
Interpretation and Analysis: Skin testing and RAST with venom of other Hymenoptera and whole-body extract of sweet bees often reveal negative reactions to many other insect antigens, while less than half of patients will show sensitivity to honey bees.

Whole-body extract of sweat bee causes some irritant reaction when skin testing controls are used for a comparison and it is not a reliable diagnostic agent even though patients are more reactive than controls.

Allergy to honeybee body components: Distinction from bee venom sensitivity. Reisman RE, Hale R, Wypych JI. J Allergy Clin Immunol 1983 Jan; 71 (1 Pt 1), pp. 18–20.

Immune Hypersensitivity and Inflammatory Responses/Bronchoalveolar Lavage Fluid Cell Populations/Endogenous Inflammatory Mediators: Interleukin (IL)-2, IL-4, IL-5, IL-6, IL-10, Interferon-Gamma (IFN-γ), iNOS
Ovalbumin/Aminophylline

Cellular/Molecular Mechanism of Action: Ovalbumin is the avian equivalent to mammalian serum albumin. As a foreign protein, it can function as a potent immunogen/allergen. Aminophylline inhibits guanyl cyclase in a manner that directly inhibits cGMP production and indirectly increases cAMP, thereby promoting bronchial smooth muscle relaxation.
Background Information: Evidence has shown that aminophylline has bronchoprotective, anti-inflammatory, and immunomodulatory effects.
Application: Characterization of immune hypersensitivity respones and intense inflammatory reactions.
Route of Administration: Aminophylline (intraperitoneal).
Dosage: Aminophylline (Group 1 = 25 mg/kg; Group 2 = 5 mg/kg).
Implementation: Evaluate the effect of different doses of aminophylline on the late-phase reaction, bronchial hyperresponsiveness (BHR), and T cell-related cytokine mRNA expression induced by ovalbumin (OA) sensitization.

In this manner, aminophylline can be evaluated at different concentrations/doses to determine its influence on late-phase reactions, BHR, T-cell related cytokine mRNA expression (induced by ovalbumin sensitization), and respiratory function. Pulmonary function tests are used to assess respiratory function and are performed at baseline and after the administration of varying doses of acetylcholine. Thereafter, bronchoalveolar lavage is performed along with histiological analysis of the lungs. Total RNA is extracted from lung tissue and reverse transcriptase-polymerase chain reaction performed using primers for IL-2, IL-4, IL-5, IL-6, IL-10, IFN-γ, inducible nitric oxide synthase (iNOS), and beta-actin.

Animal Model: Rat (n = 40 Brown Norway strain divided into 4 groups. Groups I, II, and III sensitized and subsequently provoked with OA). Group I (aminophylline at 25 mg/kg); Group II (aminophylline at 5 mg/kg); Group III (intraperitoneal normal saline); Group IV (enhaled aerosol saline control).
Interpretation and Analysis: Group III have worse pulmonary function tests, more severe BHR, and more severe lung inflammation, higher IL-4 and IL-10 cytokine levels in bronchoalveolar lavage fluid, and higher IL-4, IL-5, IL-6, IL-10, TNF-α, and inducible nitric oxide synthase (iNOS) mRNA expression than the other 3 groups. Expression of IL-2 and IFN-γ is significantly reduced in Group III. Aminophyllin at both low and high doses is effective in preventing late-phase bronchoconstriction, BHR, and an inflammatory response. Aminopylline decreases T helper cell 2-related cytokine mRNA expression but increases T helper cell 1-related cytokines mRNA expression.

Pulmonary function changes and immunomodulation of Th 2 cytokine expression induced by aminophylline after sensitization and allergen challenge in Brown Norway rats. Lin CC, Lin CY, Liaw SF, Chen A. Ann Allergy Asthma Immunol 2002 Feb; 88 (2), pp. 215–22.

Immune Hypersensitivity Reaction: Asthma/Asthma with Severe Bronchial Obstruction/Aspirin-Intolerant Asthma/Rhinitis
Lysine Acetylsalicylic Acid (L-ASA)

Background Information: Nasal provocation tests with lysine-aspirin have been applied for assessment of aspirin-intolerant asthma.
Application: Nasal challenge with L-ASA can be used as a diagnostic test even in asthmatic patients with severe bronchial obstruction.
Route of Administration: Lysine acetylsalicylic acid (intranasal infusion).
Dosage: Lysine acetylsalicylic acid (25 mg).
Implementation: Evaluate the usefulness of acoustic rhinometry as a non-invasive technique for the diagnosis of aspirin-intolerant asthma/rhinitis. Challenge tests are performed with L-ASA or saline (0.9% NaCl) and 25 mg L-ASA instilled into each nostril of the nose on 2 separate days. Clinical response is evaluated based on nasal symptoms (sneezes, itching, secretion, and blockage). Nasal response is measured by acoustic rhinometry. Symptoms and rhinometry curves are recorded at 10-minute intervals for 3 hours, 1 hour before challenge and 2 hours after challenge.

Human Model: Cases of aspirin-intolerant asthma/rhinitis (n = 15; n = 9/15 females age 54.7 + 14 years; n = 8 aspirin-tolerant asthma/rhinitis; n = 3/8 women mean age = 52.6 + 7.8 years; n = 8 normal healthy volunteers; n = 2/8 women, mean age 32.5 + 9.7 years).

Interpretation and Analysis: L-lysine acetylsalicylic acid challenge induces a significant increase in symptoms in conditions of aspirin-intolerant asthma/rhinitis. No difference in the clinical response can be detected in cases with aspirin-tolerant asthma/rhinitis or healthy subjects. L-lysine acetylsalicylic acid challenge induces a significant decrease in nasal volume measured by acoustic rhinometry in cases of aspirin intolerance. No differences can be detected between challenges in aspirin-tolerant cases. If a 25% decrease in nasal volume is taken as the cutoff point, the specificity of the test is approximately 94% (sensitivity = 73%). The nasal challenge is well tolerated by all subjects. Acoustic rhinometry can be used to effectively study the nasal response to L-ASA.

Instrumentation: Acoustic rhinometry.

Intranasal challenge with aspirin in the diagnosis of aspirin-intolerant asthma: Evaluation of nasal response by acoustic rhinometry. Casadevall J, Ventura PJ, Mullol J, Picado C. Thorax 2000 Nov; 55 (11), pp. 921–4.

Immune Hypersensitivity Reaction/Bronchial Hyperresponsiveness (BHR)/Neutrophil Influx: Differential Response/Allergic Airway Eosinophilia Reaction/Interleukin (IL)-10 and Interferon-Gamma (IFN-γ) Induction: Differential Expression/[IL-2, IL-4, and IL-5]/Type Th1 and Th2 Cytokine Expression Profiles

Ovalbumin/Dexamethasone/Cyclosporin-A

Cellular/Molecular Mechanism of Action: Ovalbumin is the avian equivalent of mammalian serum albumin and can be applied as an immunogenic (antigenic) protein fraction. Dexamethasone is a corticosteroid that has an ability to inhibit phospholipase A2 biochemical activity.

Physiological Mechanism of Action: Dexamethasone is a corticosteroid that promotes immunosuppressive properties in part due to stabilization of cellular membrane structures, which in part inhibits leukocyte degranulation phenomenon and general inhibition of protein synthesis.

Background Information: Cyclosporin A and dexamethasone exhibit different effects on allergic airway eosinophilia and BHR.

Application: Characterization of the molecular pathogenesis of normal and hypersensitivity immune responses including alterations in cytokine production, and changes in the expression profiles for Th1- vs Th2-derived cytokines. Such an investigative strategy can also be applied to delineate the efficacy of various therapeutic agents possessing anti-inflammatory and immunosuppressive properties.

Implementation: Evaluation of the influence of cyclosporine or dexamethasone on the development of immune hypersensitivity reactions to ovalbumin aerosol challenge. Bronchial responsiveness is measured 18–24 hours after aerosol exposure. Airway cellular influx can be determined by bronchoalveolar lavage and tissue immunohistochemistry. Expression of Th1 and Th2 cytokine messenger RNA (mRNA) is then analyzed by reverse transcriptase-PCR.

Animal Model: Rat (Brown Norway rats sensitized to ovalbumin).

Interpretation and Analysis: Ovalbumin exposure induces significant BHR reactions, with increases in eosinophils, lymphocytes, and neutrophils in bronchoalveolar lavage fluid and an increase in eosinophils, CD2+, and CD8+, but not CD4+ T cells, in the airway submucosa. Interleukin 2, IFN-γ, IL-4, and IL-5 mRNA expression in ovalbumin-sensitized lungs increases ($p < 0.05$) compared with controls. Cyclosporin A does not have a significant effect on BHR and neutrophil accumulation but does reduce the number of bronchoalveolar lavage eosinophils ($p < 0.002$), airway submucosal eosinophils, and CD4+ and CD8+ T cells ($p < 0.02$). It also suppresses the induction of mRNA expression for IL-2, IL-4, IL-5, and IFN-γ. By contrast, the inflammatory cell influx and mRNA expression of IL-2, IL-4, and IL-5, as well as BHR, is suppressed by dexamethasone. However, increases in IL-10 and IFN-γ mRNA expression can sometimes not be detected depending on the analytical system evaluated. The differential activities of cyclosporin A and dexamethasone on inflammatory cell influx, particularly neutrophils, or cytokine expression such as IL-10 and IFN-γ may underlie their contrasting effects on BHR.

Differential regulation of cytokine expression after allergen exposure of sensitized rats by cyclosporin A and corticosteroids: relationship to bronchial hyperresponsiveness. Huang TJ, Newton R, Haddad EB, Chung KF. J Allergy Clin Immunol 1999 Sep; 104 (3 Pt 1), pp. 644–52.

Immune System: Lymph Node/Selective Antigen Delivery to Nodes and Processing

[Fluorescein Isothiocyanate]-H-2K(b) CTL Epitope (SIINFEKL)/pH-Sensitive Liposomes

Cellular/Molecular Mechanism of Action: Fluorescein isothiocyanate (FITC) is a molecule that emits fluorescent color when excited by certain wavelengths of light energy. Liposomes that are pH-sensitive provide a modality for protecting their internal contents from metabolic degradation or accelerated elimination but also have the property of degrading upon exposure to environments within a specific hydrogen ion concentration range.

Application: Fluorescein isothiocyanate (FITC) can be applied as a fluorescent tracer molecule in biological systems. Fluorescein isothiocyanate (FITC)-conjugated H-2K(b) CTL epitope (SIINFEKL). Liposomes that have pH-sensitive properties have been designed to release their internal contents through lysis when exposed to environments within a specific pH range.

Interpretation and Analysis: Fluorescence can be initially detected in lymph nodes at 3 hours after immunization, and its intensity reaches a peak value in superficial inguinal lymph node at 9 hours. No trace can be detected in the spleen even with prolonged monitoring for up to 24 hours. These results strongly suggest that the presentation of CTL-peptide antigen vehicled by pH-sensitive liposomes exclusively occurs in lymph nodes. In mice immunized with the H-2K(b) CTL epitope encapsulated pH-sensitive liposomes, peptide-specific CTL responses can be detected on day 3. The response is strongly augmented by a 2nd immunization and persists up to at least 45 days. Therefore, liposomes formulated to be pH-sensitive can function as potential adjuvants of peptide antigens and are useful for the *in vivo* induction of antigen specific CTLsv.

Investigation of antigen delivery route *in vivo* and immune-boosting effects mediated by pH-sensitive liposomes encapsulated with K(b)-restricted CTL epitope. Lee KY, Chun E, Seong BL. Biochem Biophys Res Commun 2002 Apr 5; 292 (3), pp. 682–8.

Immune System: Lymph-Node (Sentinel) Mapping/Adenocarcinoma Colorectal Cancer

Isosulfan Blue Dye

Refer to Section II, Genetic Disorders, Infectious Disease, Neoplasia, and Metabolic Conditions.

Does tumor burden limit the accuracy of lymphatic mapping and sentinel lymph node biopsy in colorectal cancer? Broderick-Villa G, Ko A, O'Connell

TX, Guenther JM, Danial T, DiFronzo LA. Cancer J 2002 Nov–Dec; 8 (6), pp. 445–50.

Immune System: Lymphocyte Function/Skin Test/Organochlorine Exposure/[Gender Differences]
Tetanus Toxoid/Diptheria Toxoid/Candida/ Tricophyton/Streptococcus/Proteus/ Phytohemaglutinin/Concanavalin A (Con-A)/Pokeweed Mitogen

Background Information: Previous studies of gender differences in response to the Multitest CMI skin test have produced conflicting results.
Application: Evaluation of immune system function.
Implementation: Evaluation of immune function applying a skin test using the Multitest CMI skin test to determine if gender influences that nature of observed results. Six of 7 antigens are tested: tetanus toxoid, diphtheria toxoid, Candida, Tricophyton, Streptococcus, and Proteus (excluding tuberculin antigen). Lymphocyte function can also be evaluated *in vitro* using standardized methods of mitogen stimulation with phytohemaglutinin (PHA), concanavalin A (Con-A), and pokeweed mitogen.

Human Model: Cases living near a hazardous waste site containing organochlorine pesticides (n = 297 adults, age = 18–64 years).
Interpretation and Analysis: The frequency of positive responses to the skin tests is significantly ($p < 0.001$) higher among males (80.4%) than among females (55.7%). Males are more likely than females to respond to all 6 test antigens ($p < 0.05$). The mean diameter of positive skin test measurements for males can be found to significantly ($p < 0.05$) exceed female responses to tetanus and diphtheria. Although not statistically significant, male response size exceeds that of females for all other antigens except Trycophyton. Controlling for age, race, smoking, income, and plasma DDE levels do not change these results. Skin test positivity is not associated with mitogen stimulation assay results overall or within gender groups. Significant gender differences in response to the Multitest CMI skin test could limit its use as a marker of anergy in general population studies.

Gender differences in response to the multitest CMI skin test in the general population. Vine MF, Stein L, Weigle K. Ann Allergy Asthma Immunol 2000 Apr; 84 (4), pp. 445–50.

Immune System: Neoplasia/Lymphoma/ Leukemia/Nucleoli Targeting/Gene Expression
Fluorescein-Labeled PS-TFO/Antisense Oligonucleotides

Refer to Section II, Genetic Disorders, Infectious Disease, Neoplasia, and Metabolic Conditions.

Antigene and antiproliferative effects of a c-myc-targeting phosphorothioate triple helix-forming oligonucleotide in human leukemia cells. McGuffie EM, Pacheco D, Carbone GM, Catapano CV. Cancer Res 2000 Jul 15; 60 (14), pp. 3790–9.

Immune System: Neoplasia/Lymphoma/Mammary Carcinoma
Fluorescein-Somatostatin Receptor-Avid Peptide/ Fluorescein-Bombesin/Carbocyanine-Somatostatin Receptor-Avid Peptide/Carbocyanine-Bombesin

Refer to Section II, Genetic Disorders, Infectious Disease, Neoplasia, and Metabolic Conditions.

Synthesis, *in vitro* receptor binding, and *in vivo* evaluation of fluorescein and carbocyanine peptide-based optical contrast agents. Achilefu S, Jimenez HN, Dorshow RB, Bugaj JE, Webb EG, Wilhelm RR, Rajagopalan R, Johler J, Erion JL. J Med Chem 2002 May 9; 45 (10), pp. 2003–15.

Immunity (Cellular/Humoral Response): Combined Deficiency/Combined Immunodeficiency (CID)/ Arabian Foals Hereditary Disorder
Phytohemagglutinin (PHA)

Cellular/Molecular Mechanism of Action: Promotes activation of T-lymphocyte populations.
Background Information: Breeding with heterozygous male and female creates an inherited autosomal recessive trait in the offspring leading to a deficiency in T-lymphocyte and B-lymphocyte production. Affected foals have normal complement systems, neutrophils and macrophages, produce secretory immunoglobulin components and still produce large granular lymphocytes suggestive of killer cells. Affected foals usually have normal colostrally-derived immunity. However, when passively transferred maternal immunoglobulins begins to decline (become depleted) at 3 to 6 weeks and they experience an increased susceptibility to infectious diease (usually opportunistic microorganisms).

Affected animals display a persistent lymphopenia ($< 1 \times 10^9$/l) and histopathology evaluation reveals aplasia or severe hypoplasia of lymphocyte germinal centers.
Application: Detection of deficiencies in T-lymphocyte numbers or function (*in vivo*).
Route of Administration: Phytohemaglutinin (intradermal).
Implemenation Example: Detection of combined immunodeficiency in Arabian foals or Arabian cross-bred foal. Phytohemaglutinin is injected intradermally at 1 site and an equivalent volume of sterile physiological saline is injected intradermally at another nearby location as a negative reference control.
Interpretation and Analsysis: Phytohemaglutinin (PHA) fails to produced an intradermal reaction/response when the total number T-lymphocyte cell production is severely deficient or their function is severely compromised (e.g., either selective or combined immunodeficiency). Presence of cutaneous edema and "sweating" at the site of intradermal injection is considered a positive (normal/healthy) response.

Immunity (Humoral Response): Hypogammaglo-bulinemia (*In Vitro*)/ [Non-Pharmaceutical Agent]
Zinc Sulfate ($ZnSO_4$)

Cellular/Molecular Mechanism of Action: Becomes associated with the cationic and anionic groups of polypeptide amino acid residues. This generally in a reduction in the ionic charge density of protein fractions thereby decreasing their relative solubility in an aqueous environment.
Diagnostic Application: Approximation of immunoglobulin concentrations within serum. Primarily applied in neonatal patients to determine if sufficient ingestion of colostrum has occurred within the 1st 24 hours following parturition.
Route of Administration and Dosage: Serum samples (0.1 ml) are combined with double distilled water (1.0 ml) and thoroughly mixed. This formulation is then combined with zinc sulfate reagent (5 ml) and the reaction mixture thoroughly mixed at room temperature (25°C). After 1 hour, the absorbance (optical density) of the reaction mixture is measured spectrophotometrically at 485 nm. Results are interpreted according to values generated from a standard

reference control curve. If a spectrophotometer is not available, the ability or relative degree to which newsprint can be read through the reaction tube gives a reasonably good indication of the immunoglobulin content within a serum sample. Zinc sulfate reagent is prepared by dissolving 250 mg of zinc sulfate in 1 liter of boiled water. Following cooling to room temperature, the formulation is decanted into a capped dark glass bottle for storage.

Considerations: The zinc sulfate turbidity test is 1 of the most practical means of screening for immunoglobulin deficiency in a field environment. Zinc sulfate also precipitates other large molecular weight protein fractions such as fibrinogen. This mandates that serum rather than plasma be applied as a test medium. Additionally, zinc sulfate reagent decays over time and results tend to vary in relation to extremes in environmental temperature.

Interpretation and Analysis: Following addition of test serum to zinc sulfate reagent formulations, the solution can be evaluated either visually, or by spectrophotometric absorption. In situations where a total lack of passive transfer of humoral immunity has occurred, the reaction mixture remains essentially clear. Readings of less than 400 mg/dl implies neonatal failure of passive transfer of humoral immunity.

Zinc sulfate turbidity has largely been replaced by more accurate laboratory procedures for the quantitation of serum immunoglobulin concentrations (IgG quantation, anti-equine IgG coated latex bead agglutination)

Immunity (Humoral Response): Suppression
Anti-Leu-2a, Leu-3a, and Leu-5 MoAb

Cellular/Molecular Mechanism of Action: Suppression of humoral immune response.

Application: Anti-Leu-2a, Leu-3a, and Leu-5 immunoglobulin for the suppression of the development of AMIA against T cell-specific monoclonal antibodies (cynomolgus monkeys).

Route of Administration: Intravenous.

Dosage: Anti-Leu-2a (1 mg), Leu-3a (1 mg), and Leu-5 (1 mg) for 10 consecutive days. Additional test groups receives (Group 2) cyclosporine, 12.5 mg/kg daily on days -7 to $+14$; (Group 3) group (PI) can be passively immunized with 0.4 ml of hyperimmune monkey AMIA serum on days -7, -1, 2, 4, 6, and 8; and (Group 4) (TLI) received 1,700 rad fractionated total lymphoid irradiation ending on day -1.

Considerations: Murine monoclonal antibodies stimulate the production of human antimouse immunoglobulin antibodies (AMIA) when administered to patients. This limits their long-term usefulness as therapeutic and diagnostic agents.

Interpretation and Analysis: Animals treated with TLI are markedly delayed in the onset of AMIA, which is suppressed to less than 1% of the control group. The AMIA specific for the constant region of animals receiving PI is also suppressed to one-third of controls. Majority of the AMIA in all the animals is anti-idiotypic and wholly anti-idiotypic in TLI animals.

Immunity (Humoral Response to Infection): Bacteria and Fungal Organisms/Corynebacterium/ Tuberculosis/Fungal Infections
Intradermal Testing/Infectious Disease and Hyperimmune Conditions

Cellular/Molecular Mechanism of Action: Allergens injected intradermally stimulate immunocompetent cell populations (T-lymphocytes, B-lymphocytes, marcrophages).

Diagnostic Application: Detection of hyperimmune responses to specific environmental allergens.

Route of Administration and Dosage: Intradermal injection. Examples of products that are applied for this purpose include blastomycin, coccidiodin, cryptococcin, and histoplasmin. Accuracy of these tests is complicated by a variety of factors such as cross-reactions between fungal species, anergy in cases of dissemination, and positive reactions only indicate exposure, and not necessarily ongoing infection.

In the intradermal tuberculin test, 0.1 ml of mammalian tuberculin purified protein derivative is administered intradermally in the caudal tail fold or mucocutaneous junction of the vulva. The injection site is inspected at 72 ± 6 hours (negative, suspect, reactor). The comparative cervical test is applied to determine the species of Mycobacterium that cattle may have been exposed to; it utilizes mammalian and avian tuberculin injected into 2 separate sites on the same side of the neck. Injection sites are inspected at 72 hours and measured with calipers. Other tests include the subcutaneous and intravenous thermal test with positive cases producing a temperature spike of $>$ 104°F (40°C) within 4 to 8 hours postadministration. Alternatively, the Stormont test involves performing the intradermal test twice at the same site over a 7-day interval. Following the last administration, the 2nd injection is evaluated at 24 hours. An increase in skin thickness of $\geq$ 5 mm is considered to be a positive reaction. Relative hyposensitization occurs 4 to 6 weeks after calving due to substantial transfer of antibody into the colostrum. Cattle exposed (infected) within the 1st 6 months may fail to react, and patients with pulmonary lesions may display anergy.

The procedure applied to diagnose *Mycobacterium paratuberculosis* (Johne's disease) in cattle is the intravenous and intradermal Johnin test. The intravenous Johnin is performed by the intravenous injection of Johnin (2–5 ml) on a day with a moderate ambient temperature. A systemic body temperature and CBC are collected at time zero. At intervals of 4, 6, and 8 hours, the body temperature is recorded with a positive response reflected by an increase in body temperature of 3°F (1.6°C). A 2nd CBC is performed on blood collected at 6 hours post-Johnin injection and a $>$ 2:1 alteration in the neutrophil:lymphocyte ratio is diagnostic.

Considerations: Critically important is the administration of intradermal saline reference negative controls.

False positives may be recognized when performing the intradermal tuberculin test if cattle have been exposed to human or avian tuberculosis, Johne's disease, saprophytic Mycobacterium, Nocardia, and other species.

False-positive results with the Johnin test can be appreciated when cattle have been previously exposed to or infected with other species of mycobacteria, Nocardia, Corynebacterium, Dermatophilus, or Actinomyces agents.

Interpretation and Analysis: Increased edema, swelling and redness at the site of allergen injection without an appreciable response at the saline negative reference control suggests a hyperimmune response exists against that particular test reagent.

Immunity (Humoral/Cellular Response to Infection): Johnes Disease
(Mycobacterium Paratuberculois)/Johnin Antigen

Cellular/Molecular Mechanism of Action: Bacterial immunogen.

Application: Detection of cattle infected with Mycobacterium paratuberculosis.

Route of Administration: Intradermal or intravenous.

Dosage: Intravenous (2–4 ml of standardized prepared Johnin antigen).
Considerations: The intravenous Johnin test is diagnostically more reliable than is the interadermal test.
Implementation: Evaluation of the intravascular Johnin test is performed by measuring changes in body temperature at 4.5, 6.0, and 7.5 hours postinjection.
Interpretation and Analysis: Elevations in rectal temperature of ≥ 3°F (1.6°C) are considered a positive response, but the highest temperature that has to be reached is 39.4°C. The pretest temperature must be below 39.4°C. Intradermal sensitivity: Clinical (60–78%); non-clinical (37.5%); specificity (50–54%). Intravenous sensitivity: clinical (84–92%); non-clinical (22–57%); specificity (84–88.5%).

Immunity (Humoral/Cellular Response to Infection): Tuberculosis *(Mycobacterium Bovis, M. Avium)/Tuberculin Antigen*

Cellular/Molecular Mechanism of Action: Intradermal injection of the preparation into cattle infected with *Mycobacterium bovis, M. avium* induces the development of a Type IV hypersensitivity reaction.
Application: Detection of cattle infected with *Mycobacterium bovis* (tuberculosis).
Route of Administration: Mammalian tuberculin purified protein is injected intradermally in the caudal tail fold of the mucocutaneous junction of the vulva using a tuberculin syringe and a 25-gauge needle.
Dosage: Mammalian tuberculin purified protein derivative or PPD (0.1 ml; 1 mg/ml; 3 mg/ml Australia).
Considerations: Bacterial immunogen, which is a concentrated sterile culture filtrate of tubercle bacilli grown on glycerated beef broth or synthetic media. Injection sites are digitally evaluated at 72 hours postinjection for signs of swelling and heat.
Implementation: Following intradermal injection, the site is evaluated for edema and heat relative to a reference control.
Interpretation and Analysis: Antigenic preparations (tuberculin) are not entirely specific and reactions can be seen with infections due to other strains or species of Mycobacterium. In areas where the disease has been eradicated, positive responses should be reevaluated applying the comparative-cervical test (c-c test). In the United States, only veterinarians specifically approved by the state or federal regulatory agencies are permitted to conduct the c-c test utilizing PPD tuberculins of equal biological potency (established in guinea pigs) in order to determine the dispostion of suspect cattle. During the course of this procedure, biologically "equilibrated" mammalian and avian tuberculin are injected simultaneously in 2 sites on the same side of the neck 12 cm apart and 1 above the other. Results are evaluated at 72 hours using calipers for accurate measurement. A nomograph is used to interpret the finding. Federal veterinarians may also use 0.2 ml cervical test in known infected herds. Other tests are the subcutaneous and intravenous thermal tests in which a temperature spike to > 104 (40°C) in 4 to 8 hours is observed in positive reacting cases. The Stormont Test involves performing the intradermal test twice in the same area (7-day interval) and results reviewed at 24 hours. An increase in skin thickness of > 5 mm is considered positive. A temporary hyposensitization may last for up to 6 months. The postparturition period is also associated with hyporeaction to tubuerculin in part due to the contribution of large amounts of dam immunoglobulin into the colostrum
Instrumentation: None.

Immunity (Humoral/Cellular Response to Infection)/Systemic Fungal Infections *Intradermal Allergy Testing/Blastomycin/ Coccidiodin/ Cryptococcin/Histoplasmin*

Cellular/Molecular Mechanism of Action: Allergens injected intradermally stimulate immunological cell populations (T-lymphocytes, B-lymphocytes, macrophages).
Diagnostic Application: Detection of hyperimmune responses to specific environmental allergens.
Route of Administration and Dosage: Intradermal injection. Examples of products that are applied for this purpose include blastomycin, coccidiodin, cryptococcin, and histoplasmin. Accuracy of these tests is complicated by a variety of factors such as cross-reactions between fungal species, anergy in cases of dissemination, and positive reactions only indicate exposure and not necessarily ongoing infection.
Considerations: Important to administer intradermal saline reference negative controls.
Interpretation and Analysis: Increased edema, swelling, and redness at the site of allergen injection without an appreciable response at the saline negative reference control suggests that a hyperimmune response has developed against that specific test antigen reagent.

INFLAMMATION

Inflammation: Hepatitis (Alcohol-Induced)/H_2O_2 *Corticosteroids/[Neutrophils]/L-Selectin/Interleukin (IL)-8/Tumor Necrosis Factor-Alpha (TNF-α)/IL-10*

Background Information: Several observations point to an important role of interactions between polymorphonuclear neutrophils and cytokines in severe alcoholic hepatitis. The polymorphonuclear neutrophil activation status and the local and systemic pro- and anti-inflammatory cytokine responses are then quantified. The effect of corticosteroids, widely used in this setting, can be evaluated using these parameters.
Application: Potential differentiation of alchohol-induced hepatitis and alcohol-induced cirrhosis without hepatitis and normal reference (healthy) controls.
Implementation: Evaluation of blood polymorphonuclear neutrophil functions pertaining to L-selectin and β_2-integrin expression, H_2O_2 production, and IL-8 and TNF-α synthesis capacity. Complementary analysis includes measurement of plasma and hepatic IL-8, TNF-α, and IL-10 levels. The impact of a 28-day course of corticosteroids on blood neutrophil activation status and cytokine levels has been evaluated in patients with alcoholic hepatitis.
Human Model: Cases of alcoholic hepatitis (n = 15; compared to n = 15 conditions of alcoholic cirrhosis without alcoholic hepatitis; compared to n = 10 healthy volunteers).
Interpretation and Analysis: Blood polymorphonuclear neutrophils in conditions of alcoholic hepatitis are activated, as shown by increases in H_2O_2 production (48 ± 6 vs 29 ± 6 MFI in healthy controls), and decreases in L-selectin expression (300 ± 61 vs 449 ± 59 in healthy controls). Upon stimulation, polymorphonuclear neutrophils synthesize large amounts of IL-8 (21.7 ± 9.2 ng/ml vs 8.8 ± 10 ng/ml in healthy controls) and TNF-α (524 ± 132 pg/ml vs 79 ± 144 pg/ml in healthy controls). Tumor necrosis factor-alpha and IL-8 plasma and tissue levels markedly increase in contrast to IL-10, which is barely detectable in alcoholic hepatitis patients, compared to cirrhotic patients and healthy controls. During steroid therapy, plasma levels of the proinflammatory cytokine IL-8 fall as early as day 14, while levels of the anti-inflammatory cytokine IL-10

increase on day 21. Finally, polymorphonuclear neutrophil functions return to normal after treatment. Severe alcoholic hepatitis appears to be associated with polymorphonuclear neutrophil activation and an imbalance between pro- and anti-inflammatory cytokines; during steroid therapy a normalization of these parameters can be observed.

Blood neutrophil functions and cytokine release in severe alcoholic hepatitis: Effect of corticosteroids. Taïeb J, Mathurin P, Elbim C, Cluzel P, Arce-Vicioso M, Bernard B, Opolon P, Gougerot-Pocidalo MA, Poynard T, Chollet-Martin S. J Hepatol 2000 Apr; 32 (4), pp. 579–86.

Inflammatory Responses and Disease States
Cytokines (Interleukins)

Cellular/Molecular Mechanism of Action: Endogenous mediators of inflammation and immunity facilitated by the expression of membrane-associated receptor complexes on the exterior membrane surface of various cell types, most notably including leukocytes (hence the name interleukin) and vascular endothelium.
Considerations: Cytokines are key mediators of immunity and inflammation. These proteins or glycoproteins act as communication signals between different populations of leukocytes but neither their effects nor their production are restricted to immune cells. In the last few years many new cytokines and their effects have been discovered and it has been proposed that when the amino acid sequence of a new cytokine is established it would be assigned the name interleukin (IL), with an added number (e.g., IL-1 through IL-20). The detection of cytokines in disease states promises to provide useful information for diagnostic purposes. The therapeutic utility of cytokines has been explored in many clinical and preclinical studies and in a wide variety of infectious diseases, autoimmune conditions and neoplasia disorders. Because the production of cytokines is modulated by several biological agents such as hormones, prostaglandins, and drugs, these may also serve as therapeutic targets for immunomodulation.

Neutrophils/Monocytes/Consumption vs Production Abnormalities
[^{111}I]-Oxine Neutrophils/Chemotherapeutics: Doxorubicin

Physiological Mechanism of Action: Neutrophils and macrophages migrate into specific locations (tissues/organs) within the body in response to chemotactic agents (move towards a concentration gradient).
Application: Includes characterization of elevated platelet consumption or declines in production within the bone marrow. They can also function as a modality for the selective delivery of pharmaceutical agents for various diagnostic and therapeutic purposes.
Route of Administration: Intravascular.
Considerations: Must be prepared fresh.
Semi-Synthetic Methods: Radioisotope markers and certain chemotherapeutic agents can be passively "loaded" into neutrophil populations at relatively high concentrations without affecting their response to chemotactic stimuli.
Implementation: Selective delivery to foci of infectious disease or neoplasia.
Interpretation and Analysis: Declines in intravascular $T_{1/2}$ values for labeled neutrophils implies accelerated consumption or removal. In scenarios where neutrophil numbers are low but the $T_{1/2}$ value is normal, then abnormalities in hematopoeitic production should be considered

Anti-neoplastic activity of chemotherapeutic "loaded" neutrophils against human mammary carcinoma. Coyne CP, Fenwick BW, Ainsworth J. Biotherapy 1997; 10, pp. 145–59.

Note: Please refer to imaging modality sections for detection and characterization of inflammatory conditions.

EX VIVO LIVING SYSTEMS

CARDIOVASCULAR: *EX VIVO*

Cardiovascular: Cardiomyopathy/Hypertension/ Propanolol β-Adrendergic Receptor Occupancy/ Positron Emission Tomography (PET) vs Single Photon Emission Computed Tomography (SPECT) Spatial Resolution/*Ex Vivo* and *In Vivo* Cardiac and Splenic Tissue Analyses
(1R,2S)-4-[^{18}F]-Fluorometaraminol (4-FM)/[^{125}I]-Iodocyanopindolol/Meta-[^{125}I]-Iodobenzylguanidine (MIBG)

Cellular/Molecular Mechanism of Action: [^{125}I]-Iodocyanopindolol is a radioiodinated analog of propanolol, which is known to have binding avidity for and blocks the activity of β-adrenergic receptor complexes. (1R,2S)-4-[^{18}F]-fluorometaraminol appears to have similar binding-avidity for β-adrenergic receptor complexes. Meta-iodobenzylguanidine is an analog of norepinephrine, which functions as an adrenergic receptor agonist.
Application: [^{125}I]-Iodocyanopindolol can be used to detect alterations in β-adrenergic receptor expression and propanolol β-adrenergic receptor occupancy.
Dosage: [^{125}I]- Iodocyanopindolol (1.5 pmol/L *ex vivo* incubation); propranolol (10 μmol/L *ex vivo* incubation).
Implementation: Evaluation of the biodistribution of no-carrier-added 4-[^{18}F]-FM *in vivo* and *in vitro* in retrograde-perfused hearts. Relevant biodistribution parameters include the assessment of relative uptake, metabolism, and beta-receptor affinity of 4-FM for comparison with analogous values observed for the SPECT pharmaceutical MIBG.

Cardiac and extracardiac distribution can be assessed, in concert with the detection of metabolite formation assessed by thin-layer chromatography techniques. Studies performed *in vivo* are then repeated with no-carrier-added [^{123}I]-MIBG. By means of autoradiography, the beta-receptor affinity of 4-FM can be compared with that of MIBG and propranolol (10 μmol/L) through displacement of [^{125}I]-iodocyanopindolol (1.5 pmol/L) in *ex vivo* tissue slices.

Animal Model: Rats (Wistar-Kyoto (WKY) strain and spontaneously hypertensive [SHR] rats).

***Ex Vivo* Model:** Rat (cardiac and spleen slices).
Interpretation and Analysis: Cardiomyopathic hearts show heterogeneous 4-[^{18}F]-FM uptake with gradients of up to 3.6 *in vivo* and *in vitro* between different regions of the heart. Control hearts display such gradients in 4-[^{18}F]-FM uptake only *in vitro*. [^{125}I]-MIBG exhibits a less heterogeneous *in vivo* distribution in SHR hearts. Extracardiac differences between WKY and SHR can be appreciated for uptake of 4-[^{18}F]-FM in the spleen (63.3% ± 4% vs 38.8% ± 5.7% of cardiac activity) and for renal uptake of [^{125}I]-MIBG (373% ± 27% vs 81.4% ± 17% of cardiac activity). Metabolites of 4-[^{18}F]-FM are found only in the liver and those of [^{125}I]-MIBG are found in the liver and kidney with a nearly equal relative fraction in both types of animals at about 20%, 60%, and 30%, respectively. 4-FM suppresses cardiac-specific beta-receptor binding of [^{125}I]-iodocyanopindolol in heart and spleen of both types of animals significantly, whereas MIBG has almost no effect. The more heterogeneous cardiac distribution of 4-[^{18}F]-FM suggests that it reflects alterations in uptake-1 better than [^{125}I]-MIBG in addition to the possibility of quantification and higher spatial resolution by PET compared with

SPECT. Altered biotransformation in cardiomyopathic diseases may also impair the evaluation of [^{125}I]-MIBG-SPECT data. The beta-receptor binding of 4-[^{18}F]-FM should ideally be further elucidated.

Relative uptake, metabolism, and beta-receptor binding of (1R,2S)-4-[^{18}F]-fluorometaraminol and [^{123}]I-MIBG in normotensive and spontaneously hypertensive rats. Pissarek M, Ermert J, Oesterreich G, Bier D, Coenen HH. J Nucl Med 2002 Mar; 43 (3), pp. 366–73.

Vascular Endothelium: Alpha(V)Beta$_3$ Integrin Complexes/Neoplastic Cell Upregulation *[^{99m}Tc]-(HYNIC)-Arg-Gly-Asp/[^{99m}Tc]-(HYNIC-RGD-4C)*

Cellular/Molecular Mechanism of Action: The peptide amino acids residues, Arg-Gly-Asp (RGD), in vitronectin and fibronectin possess binding-affinity for cell membrane-associated alpha(V)beta$_3$ integrin complexes.

Background Information: Three amino acid residues, Arg-Gly-Asp (RGD), in vitronectin and fibronectin show affinity for alpha(V)beta$_3$ integrins expressed in vascular endothelial cells. That tumor growth can upregulate the expression of these integrins on tumor cells for invasion and metastasis and *in* tissue neovasculature suggests the potential of developing radiolabeled RGD peptides as antagonists of alpha(V)beta$_3$ integrins for broad spectrum tumor specific imaging. The polypeptide RGD-4C, which contains 4 cysteine residues for cyclization, has shown preferential localization on integrins at sites of tumor angiogenesis.

Application: Detection and characterization of neoplastic cell types/tumors that express relatively high alpha(V)beta$_3$ integrin membrane densities.

Route of Administration: Polypeptides RGD-4C and RGE (Arg-Gly-Glu)-4C (intravascular).

Semi-Synthetic Methods: The polypeptide RGD-4C and control RGE (Arg-Gly-Glu)-4C are conjugated with 6-hydrazinopyridine-3-carboxylic acid (HYNIC) to facilitate [^{99m}Tc] radiolabeling. Separation of conjugated products from residual unreacted molecular components can be achieved by C18 chromatography (e.g., Sep-Pak cartridge) applied in combination with 20% methanol solvent. Each of the peptides can be radiolabeled using tricine. For cell binding studies, both [^{99m}Tc] peptides can be further purified by SE HPLC. High specific radioactivity for labeled cyclized RGD/E of about 20 Ci/micromol can be achieved. Both [^{99m}Tc] complexes are stable in the labeling solution for over 24 hours at room temperature.

Implementation: Evaluation of the relative binding avidities of RGD-4C and RGE (Arg-Gly-Glu)-4C peptides for endothelial cell membrane preparations. Determination of such properties can be established by exposure of umbilical vein endothelial (HUVE) to RGD-4C or RGE over an incubation period of 1-hour duration (4°C) at concentrations *in* the picomolar to nanomolar range. Specificity can be approximated by determining at what concentration native cyclized RGD blocks 50% of [^{99m}Tc]-labeled RGD binding to cell preparations.

***Ex Vivo* Model:** Human (umbilical vein endothelium or HUVE).

Interpretation and Analysis: In the human umbilical vein endothelial (HUVE) cell binding-avidity analyses, cell accumulation of [^{99m}Tc] in the case of RGD can be as much as 16 times greater than the RGE control peptide. Specificity analyses reveals that 7 nM of native cyclized RGD blocked 50% binding of [^{99m}Tc]-labeled RGD to cells. The binding percentage of [^{99m}Tc]-labeled RGD to purified alpha(V)beta$_3$ integrin protein, as determined by SE HPLC, increases with integrin concentration while [^{99m}Tc]-labeled RGE displays no binding. The association constant for [^{99m}Tc]-RGD is rather modest at 7×10^6 M^{-1}. In both human renal adenocarcinoma (ACHN) and human colon cancer cell line (LS174T) nude mouse tumor models, the accumulation of [^{99m}Tc]-labeled RGD/E exhibits no statistical difference. In conclusion, possibly because of limited numbers of alpha(V)beta$_3$ integrin receptors per tumor cell and low binding affinity, radiolabeled RGD peptides may have limitations as tumor imaging agents.

In vitro and *in vivo* evaluation of a Technetium-99m-labeled cyclic RGD peptide as a specific marker of alpha(V)beta$_3$ integrin for tumor imaging. Su ZF, Liu G, Gupta S, Zhu Z, Rusckowski M, Hnatowich DJ. Bioconjug Chem 2002 May–Jun; 13 (3), pp. 561–70.

HEPATIC: *EX VIVO*

Hepatic Adrenergic Catechol O-Methyltransferase (COMT)/Genetically Regulated Thermal Stability *Adrenergic Neurotransmitters/(Hepatic Biopsy Specimens)*

Cellular/Molecular Mechanism of Action: Catechol O-methyltransferase activity mediates the biochemical inactivation of epinephrine and norepinephrine neurotransmitters.

Application: Detection of COMT genotypes and characterization of COMT genetic polymorphism.

Considerations: The level of COMT activity and COMT thermal stability in human tissue are controlled by a common genetic polymorphism.

Implementation: Individual hepatic biopsies are collected for analysis (e.g., from individuals with high, low or intermediate COMT biochemical activity and thermal stabilities) to determine if the molecular mass (M_r) and/or isoelectric point (pI) of COMT enzymes differs in tissue from subjects with different presumed genotypes for the COMT genetic polymorphism. Laboratory techniques employed entail the partially purification of COMT from individual hepatic tissue samples by (i) sequential ion exchange and gel filtration chromatography; (ii) photoaffinity labeling performed with [^{3}H-methyl]-S-adenosyl-L-methionine (functions as the methyl donor for the COMT enzymatic reactions); and (iii) 2-dimensional sodium dodecylsulfate polyacrylamide gel electrophoresis (2-D SDS-PAGE) analysis.

Interpretation and Analysis: Two-dimensional sodium dodecylsulfate polyacrylamide gel electrophoresis (2-D SDS-PAGE) analysis of individual samples consistently shows the presence of 3 [^{3}H-methyl]-S-adenosyl-L-methionine photoaffinity-labeled proteins with pI values of 5.4, 5.5 and 5.7 (all 3 have M_r values of approximately 27.1 kDa). The same pattern can be observed in all samples irrespective of COMT phenotype. Western blot analysis of 2-D SDS-PAGE gels performed with rabbit polyclonal antibodies to partially purified human kidney COMT reveals a pattern similar to that found with photoaffinity labeling. Once again, the same pattern is found in all samples irrespective of COMT phenotype. Therefore, neither photoaffinity labeling nor Western blot analysis reveals differences in either M_r or pI of cytoplasmic COMT in hepatic tissue from subjects selected on the basis of different phenotypic expression of the COMT genetic polymorphism.

Catechol O-methyltransferase pharmacogenetics: photoaffinity labeling and western blot analysis of human liver samples. Aksoy S, Klener J, Weinshilboum RM. Pharmacogenetics 1993 Apr; 3 (2), pp. 116–22.

Hepatic Cytochrome P450: Microsomal Fractions *Doxifluridine (5′-DFUR)/Tegafur (FT)*

Cellular/Molecular Mechanism of Action: Doxifluridine (5′-DFUR) and tegafur (FT) are 2 fluoropyrimidine prodrugs that are

metabolized to 5-fluorouracil (5-FU) by hepatic microsomal enzyme fractions.

Application: Characterization of differences in the *in vitro* metabolic profiles for chemotherapeutic agents including doxifluridine (5′-DFUR) and tegafur (FT).

Implementation: Microsomal and cytosolic fractions processed from hepatic tissue (e.g., human liver) are incubated with doxifluridine (5′-DFUR) and tegafur (FT) preparations (500 μM final concentration). Conversion of these 2 fluoropyrimidine prodrugs to 5-FU is measured by high-performance liquid chromatography. The degree of correlation between the 5-FU-forming activities is determined using various cytochrome P450-dependent reactions.

Interpretation and Analysis: Liver microsomes catalyze 5-FU formation from 5′-DFUR at rates of 10.0 to 160.1 pmol/min per mg protein. These findings correlate well with CYP2A6-dependent coumarin 7-hydroxylase activity. The rates of microsomal 5-FU formation from FT range between 44.9 to 808.3 pmol/min per mg protein and correlate with coumarin 7-hydroxylase activity. The cytosol fractions catalyze 5-FU formation from 5′-DFUR at rates of 3,164.6 to 6,026.6 pmol/min per mg protein, or almost 2 orders of magnitude higher than the rates of cytosolic 5-FU formation from FT (46.8–219.0 pmol/min per mg protein). The cytosolic enzymes in hepatic tissue appears to be important for 5-FU formation from 5′-DFUR. Both cytosolic and microsomal enzymes are involved almost equally in 5-FU formation from FT. The increased formation of 5-FU from 5′-DFUR might provide an answer to the question of why similar blood 5-FU levels are retained despite lower blood levels of 5′-DFUR compared to FT concentrations.

Cytosolic and microsomal activation of doxifluridine and tegafur to produce 5-fluorouracil in human liver. Ozawa S, Hamada M, Murayama N, Nakajima Y, Kaniwa N, Matsumoto Y, Fukuoka M, Sawada J, Ohno Y. Cancer Chemother Pharmacol 2002 Dec; 50 (6), pp. 454–8.

HEMATOPOIETIC: LYMPHOCYTES (*EX VIVO*)

Autoimmune: Systemic Lupus Erythematosis (SLE)/Monocyte Interleukin (IL)-10 and IL-12/Lymphocyte (CD8+) Poststimulation Interferon-Gamma (IFN-γ) and Tumor Necrosis Factor-Alpha (TNF-α)

Monocyte Activation/Lymphocyte Activation

Application: Detection and characterization of SLE.

Route of Administration: Immersion (*in vitro* tissue culture environment).

Implementation: Determination of the profiles for Th1-type and Th2-type cytokines produced by mononuclear cells in conditions of recent-onset SLE, prior to the initiation of treatment with corticosteroids. One approach to achieving this objective is the application of radioimmunoassay for the measurement of IL-4, IL-10, IL-12 p40, TNF-α, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) released into the culture supernatants of various unstimulated and stimulated blood mononuclear cell populations.

Human Model: Cases of SLE (n = 10) assessed for comparison to matched healthy controls studied in parallel (n = 10).

Interpretation and Analysis: In early SLE, monocyte-enriched cells constitutively produce increased amounts of IL-10 and decreased amounts of IL-12 following stimulation. Lymphocyte-enriched cells in SLE produce decreased amounts of IFN-γ and TNF-α following stimulation. In "rested" cells, these defects are accentuated and a defect in IL-12 production has been suggested. Depletion studies suggest that CD8+ cells are a major source of TNF-α and IFN-α in controls, but not in SLE patients.

Increased IL-4 production or abnormalities in GM-CSF production cannot be appreciated. Therefore, in this context, even early in the course of SLE, monocyte production of IL-10 is increased and IL-12 production is decreased. Decreased production of Th1-type cytokines in SLE may be secondary to this imbalance between IL-10 and IL-12. A contributory role of dysfunctional CD8+ cells has therefore been suggested.

Decreased production of interleukin-12 and other Th1-type cytokines in patients with recent-onset systemic lupus erythematosus. Horwitz DA, Gray JD, Behrendsen SC, Kubin M, Rengaraju M, Ohtsuka K, Trinchieri G. Arthritis Rheum 1998 May; 41 (5), pp. 838–44.

Immune Hypersensitivity: Severe Atopy (Helper T-Lymphocytes)/Aeroallergen-Specific T-Helper IL-4 and IL-5 Synthesis/Peripheral Blood Mononuclear Cell Proliferation

Beclomethazone/Hydrocortisone/Mometasone/Phytohemagglutinin

Cellular/Molecular Mechanism of Action: Corticosteroids inhibit protein synthesis, stabilize cell membranes, and inhibit phospholipase A2 biochemical activity.

Background Information: Glucocorticoids play an important role in the treatment of allergic disease. The atopic process, itself, may reduce the response of peripheral blood mononuclear cells (PBMC) to these drugs.

Application: Characterization of severe atopy conditions as a function of differential alterations in PBMC proliferation rate and aeroallergen-specific T-helper type 2 cell (Th2) synthesis/release of IL-4 and IL-5 induced by exposure to hydrocortisone (HC), beclomethasone (BDP), and mometasone (MF) (profile of submaximal response in cells from severely affected cases).

Implementation: Evaluation of the relative influence of hydrocortisone (HC), beclomethasone (BDP), and mometasone (MF) on IL-4 and IL-5 secretion by aeroallergen-specific T-helper type 2 cells (Th2) and proliferation of peripheral blood mononuclear cells (PBMC) isolated from cases suffering from conditions of atopy. Isolated cell populations are incubated with steroid agents before stimulation with phytohemagglutinin subsequently followed by assessment of PBMC proliferation and cytokine secretion (Type Th2).

Interpretation and Analysis: Glucocorticoids concentrations as expected inhibit proliferation and cytokine secretion, but have less of an effect on the proliferation of cells from severe atopic cases than on cells from those whose symptoms require little treatment. The rank order of potency is MF (average IC50 0.01 nM) > BDP (4.0 nM) > HC (250 nM). Glucocorticoids are however, able to inhibit IL-4 and IL-5 secretion by human Th2-like cells and proliferation of PBMC from severely and mildly allergic donors.

Glucocorticoids inhibit proliferation and interleukin-*4* and interleukin-5 secretion by aeroallergen-specific T-helper type 2 cell lines. Crocker IC, Church MK, Newton S, Townley RG. Ann Allergy Asthma Immunol 1998 Jun; 80 (6), pp. 509–16.

Lymphocyte Function/Lymphocyte Stimulation Tests

Concanavalin-A/Phytohemaglutinin/Pokeweed Mitogen

Cellular/Molecular Mechanism of Action: Concanavalin-A (Con-A) and phytohemaglutinin are plant lectins that recognize and bind to specific membrane-associated receptor complexes expressed by T-lymphocyte populations. In this context, they promote T-lymphocyte stimulation in most mammalian species. Alternatively pokeweed mitogen stimulates both T and B cells to undergo blastogenic responses.

Application: Evaluation of lymphocyte-mediated immune function.
Route of Administration: Direct incubation with *ex vivo* hematological preparations or isolated (purified/enriched) lymphocyte populations.
Considerations: Lymphocyte proliferative responses are enhanced by exposure to certain proteases and indomethocin.

Stains have been developed to detect or identify the enzymes, acid phosphatase, β-glucuronidase and acid α-naphthyl that can be used as biochemical marker systems to recognize T-cell populations.

Lymphocyte Function: Skin Test/Organochlorine Exposure/[Gender Differences]
Tetanus Toxoid/Diptheria Toxoid/Candida/ Tricophyton/Streptococcus/Proteus/ Phytohemaglutinin/Concanavalin A (Con-A)/ Pokeweed Mitogen

Background Information: Previous studies of gender differences in response to the Multitest CMI skin test have produced conflicting results.
Application: To determine whether gender is associated with response to the Multitest CMI skin test.
Implementation: Evaluation of immune function applying the Multitest CMI skin test. Six of 7 antigens are tested: tetanus toxoid, diphtheria toxoid, Candida, Tricophyton, Streptococcus, and Proteus (excluding tuberculin antigen). Lymphocyte function can also be evaluated *in vitro* using standardized methods of mitogen stimulation with phytohemaglutinin (PHA), concanavalin A (Con-A), and pokeweed mitogen.

Human Model: Cases living near a hazardous waste sites containing organochlorine pesticides (n = 297 adults [age = 18 to 64 years]).
Interpretation and Analysis: The frequency of positive responses to the skin tests is significantly ($p < 0.001$) higher among males (80.4%) than among females (55.7%). Males are more likely than females to respond to all 6 test antigens ($p < .05$). The mean diameter of positive skin test measurements for males significantly ($p < 0.05$) exceeds female responses for tetanus and diphtheria. Although not statistically significant, male response size exceeds that of females for all other antigens except Trycophyton. Controlling for age, race, smoking, and income, plasma DDE levels do not change these results. Skin test positivity is not associated with mitogen stimulation assay results overall or within gender groups. Significant gender differences in response to the Multitest CMI skin test could limit its use as a marker of anergy in general population studies.

Gender differences in response to the multitest CMI skin test in the general population. Vine MF, Stein L, Weigle K. Ann Allergy Asthma Immunol 2000 Apr; 84 (4), pp. 445–50.

HEMATOPOIETIC: MACROPHAGES AND MONOCYTES (*EX VIVO*)

Autoimmune: Rheumatoid Arthritis (Macrophages)/Matrix Metalloprotease Protein 3 (MMP-3)
Corticosteroids

Cellular/Molecular Mechanism of Action: Corticosteroids inhibit phospholipase A2 biochemical activity.
Physiological Mechanism of Action: Corticosteroids exert anti-inflammatory and immunosuppressive properties presumably due to their ability to generally suppress protein synthesis and stabilize cellular membrane structures, which reduces the ability of leukocytes to degranulate following activation/stimulation.
Application: Delineation of potential relationships between rheumatoid arthritis (RA), expression profiles for interleukins (IL), metalloproteases (MMP), and corticosteroid production.
Implementation: Evaluate expression profiles for IL-1β, tumor necrosis factor-alpha (TNF-α), IL-6, IL-4, IL-10, MMP-1, MMP-3, and MMP-13 in freshly isolated peripheral blood (PBMC) and synovial fluid mononuclear cells (SFMC) in early RA (never-treated [ENT-RA] vs non-acute, treated rheumatoid arthritis [NAT-RA]). Characterization of expression profiles entails the application of ELISA and quantitative and semi-quantitative RT-PCR/analyses.

Human Model: Cases suffering from early RA (n = 14; n = 7/14 early RA [< 1 year of evolution] never treated with corticosteroids or disease-modifying antirheumatic drugs, and n = 7/14 non-acute RA [> 2 years of evolution] treated with disease-modifying antirheumatic drugs). Reference controls: A group of 14 healthy subjects matched for sex and age.
Interpretation and Analysis: Statistically significant differences in the protein or transcript levels for the cytokines of interest cannot be appreciated between ENT-RA and NAT-RA case groups. The cytokine mRNA expression by freshly isolated PBMC and SFMC in both case types is IL-1β > TNFα > IL-10 > IL-6, with no mRNA IL-4 expression. In contrast, cytokine serum levels in ENT-RA and NAT-RA patients exist in an inverse order of IL-6 > IL-10, while IL-1β, TNF-α, and IL-4 are usually undetectable. MMP-3 mRNA expression by the PBMC of NAT-RA patients is statistically different to that in ENT-RA patients. Similar levels of mRNA expression of MMP-1, MMP-3, and MMP-13 by the PBMC and SFMC in both RA groups can be appreciated. A close equilibrium between MMP and pro-/anti-inflammatory cytokine production is observed in ENT-RA and NAT-RA patients. This balance is apparently not influenced by duration of disease. Highly sensitive methods such as quantitative RT-PCR and ELISA, and even studying freshly isolated MC, show sustained cytokine secretion at the local level (synovial fluid/SFMC) and scarce translation at the peripheral level (serum/PBMC). Expression of MMP mRNA needs to be further evaluated in order to determine whether their peripheral expression reflects their local activity in RA patients.

Expression of interleukin-1 beta, tumor necrosis factor-alpha, interleukins-6, -10 and -4, and metalloproteases by freshly isolated mononuclear cells from early never-treated and non-acute treated rheumatoid arthritis patients. Vázquez-Del Mercado M, Delgado-Rizo V, Muñoz-Valle JF, Orozco-Alcalá J, Volk HD, Armendáriz-Borunda J. Clin Exp Rheumatol 1999 Sep–Oct; 17 (5), pp. 575–83.

Autoimmune: Systemic Lupus Erythematosis (SLE)/Monocyte Interleukin (IL)-10 and IL-12/ Lymphocyte (CD8+) Poststimulation Interferon-Gamma (IFN-γ) and Tumor Necrosis Factor-Alpha (TNF-α)
Monocyte Activation/Lymphocyte Activation

Application: Detection and characterization of SLE.
Route of Administration: Immersion (*in vitro* tissue culture environment).
Implementation: Determination of the profiles for Th1-type and Th2-type cytokines produced by mononuclear cells in conditions of recent-onset SLE, prior to the initiation of treatment with corticosteroids. Such an objective can be achieved through radioimmunoassay of IL-4, IL-10, IL-12 p40, TNF-α, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) released into

the culture supernatants of various unstimulated and stimulated blood mononuclear cell populations

Human Model: Cases of SLE (n = 10) assessed in comparison to matched healthy controls studied in parallel (n = 10).

Interpretation and Analysis: In early SLE, monocyte-enriched cells constitutively produce increased amounts of IL-10 and decreased amounts of IL-12 following stimulation. Lymphocyte-enriched cells in SLE produce decreased amounts of IFN-γ and TNF-α following stimulation. In "rested" cells, these defects are accentuated due to an apparent abnormality in IL-12 production. Depletion studies suggest that CD8+ cells are a major source of TNF-α and IFN-γ in controls, but not in SLE patients.

Increased IL-4 production or abnormalities in GM-CSF production cannot be appreciated. Therefore, in this context, even early in the course of SLE, monocyte production of IL-10 is increased and IL-12 production is decreased. Decreased production of Th1-type cytokines in SLE may be secondary to this imbalance between IL-10 and IL-12. A contributory role of dysfunctional CD8+ cells has been suggested.

Decreased production of interleukin-12 and other Th1-type cytokines in patients with recent-onset systemic lupus erythematosus. Horwitz DA, Gray JD, Behrendsen SC, Kubin M, Rengaraju M, Ohtsuka K, Trinchieri G. Arthritis Rheum 1998 May; 41 (5), pp. 838–44.

Immune Hypersensitivity (Asthma)/Septic Inflammatory Responses Macrophages (Alveolar)/ Molecular Pathogenesis of Inflammatory Responses in Asthma and Sepis
Lipopolysaccharide (LPS)

Cellular/Molecular Mechanism of Action: Lipopolysaccharide is also referred to as bacterial endotoxin and is a structural component of cell-wall structures. Systemically, it can bind to lipopolysaccharide-binding protein and membrane-associated LPS receptor complexes (CD14).

Physiological Mechanism of Action: Bacterial lipopolysaccharide fractions (LPS, endotoxin) promote inflammatory responses in primarily leukocyte and endothelial cell populations.

Background Information: Inflammation in asthma is characterized by a Th2 response. In many experimental systems, this response can be regulated by interleukin (IL)-10 and IL-12. IL-10 deactivates T cells, and IL-12 reorients the response toward a Th1 pattern. Alveolar macrophages (AMs) can secrete both of these cytokines, and thus regulate T-cell behavior in asthma. They can enhance the Th2 response by turning off their secretion of IL-10 and IL-12, or tend to downregulate it by producing these cytokines.

Application: Delineate the molecular-pathogenesis of immune responses and septic inflammatory reactions in addition functioning as a model agent for evaluating the potential efficacy of anti-inflammatory and immunosuppressive agents.

Implementation: Evaluate IL-10 and IL-12 production in AM populations. In this context, alveolar macrophages are recovered by bronchoalveolar lavage (BAL) from the lung. They are isolated and cultured for 24 hours without stimulation or in the presence of lipopolysaccharide (LPS) fractions. Assays are then performed for the measurement of IL-10 and the p40 subunit of IL-12 in BAL fluid samples and in alveolar macrophage (AM) culture supernatants by ELISA.

Human Model: Cases of adult asthma (n = 11; n = 4 normal healthy controls; n = 6 asthmatics treated with inhaled corticosteroids).

Interpretation and Analysis: Spontaneous AM IL-10 production is higher in asthmatics, particularly in treated cases. Alveolar macrophage IL-10 production after LPS stimulation is also elevated in asthmatics, but mainly so in untreated cases. Levels of IL-12 are higher in BAL fluids from untreated cases than healthy reference controls. The production of IL-12 in LPS-stimulated alveolar macrophages from such cases is increased. Alveolar macrophages are at least primed for the production of IL-10 and IL-12 in asthma. Such phenomenon suggests that these cells could be instrumental in the resolution of asthmatic-associated inflammatory processes.

Alveolar macrophage interleukin (IL)-10 and IL-12 production in atopic asthma. Magnan A, van Pee D, Bongrand P, Vervloet D. Allergy 1998 Nov; 53 (11), pp. 1092–5.

Immune Hypersensitivity: Severe Atopy/Peripheral Blood Mononuclear Cell (PBMC) Proliferation/ Helper T-Lymphocytes/Aeroallergen-Specific T-Helper IL-4 and IL-5 Synthesis
Mometasone/Beclomethazone/Hydrocortisone

Cellular/Molecular Mechanism of Action: Corticosteroids inhibit protein synthesis, stabilize cell membranes, and inhibit phospholipase A2 biochemical activity.

Background Information: Glucocorticoids play an important role in the treatment of allergic disease. The atopic process, itself, may reduce the response of PBMCs to this class of pharmaceuticals.

Application: Delineate differential alterations in PBMC proliferation rate and aeroallergen-specific T-helper type 2 cell (Th2) synthesis/release of IL-4 and IL-5 induced by exposure to hydrocortisone (HC), beclomethasone (BDP), and mometasone (MF) that can potentially be applied to identify cases suffering from severe atopy (submaximal response in cells from severely affected cases).

Implementation: Evaluation of the relative influence of hydrocortisone (HC), beclomethasone (BDP), and mometasone (MF) on IL-4 and IL-5 secretion by aeroallergen-specific T-helper type 2 cells (Th2) and proliferation of PBMCs isolated from cases suffering from conditions of atopy. Isolated cell populations are incubated with steroid agents before stimulation with phytohemagglutinin and assessment of proliferation (PBMC) and cytokine secretion (Th2).

Interpretation and Analysis: Glucocorticoids as expected inhibit proliferation and cytokine secretion, but have less of an effect on proliferation of cells from severe atopics than on cells from those whose symptoms require little treatment. The rank order of potency is MF (average IC50 0.01 nM) > BDP (4.0 nM) > HC (250 nM). Glucocorticoids are however, able to inhibit IL-4 and IL-5 secretion by human Th2-like cells and the proliferation of PBMC from severely and mildly allergic donors.

Glucocorticoids inhibit proliferation and interleukin-4 and interleukin-5 secretion by aeroallergen-specific T-helper type 2 cell lines. Crocker IC, Church MK, Newton S, Townley RG. Ann Allergy Asthma Immunol 1998 Jun; 80 (6), pp. 509–16.

Inflammatory Response: Monocyte Function
Pneumolysin/(mRNA/cDNA Microarrays)

Cellular/Molecular Mechanism of Action: Pneumolysin is an important virulence factor of *Streptococcus pneumoniae* that physically interacts with the external membrane of host cells.

Physiological Mechanism of Action: Pneumolysin elicits a multitude of inflammatory responses.

Application: Detection of alterations in monocyte cell function.

Route of Administration: Incubated with (human) monocyte cell populations *in vitro* in a tissue culture environment.

Methodology and Techniques: The THP-1 human monocytic cell line is co-incubated for 3 hours in medium alone, with the

virulent type-2 S. pneumoniae strain D39, or with the isogenic strain PLN, which does not express pneumolysin. Extracts of RNA are isolated from the monocytes and hybridized on cDNA microarray platforms.

Interpretation and Analysis: Approximately 142 gene sequences (total screened = 4,133) have been found to be responsive in a pneumolysin-dependent fashion, whereas 40 have been found to be responsive independent of pneumolysin. Genes that are upregulated in cells exposed to D39 relative to those exposed to PLN include genes encoding proteins such as mannose binding lectin 1, lysozyme, alpha-1 catenin, cadherin 17, caspases 4 and 6, macrophage inflammatory protein 1beta (MIP-1β), interleukin (IL)-8, monocyte chemotactic protein 3 (MCP-3), IL-2 receptor beta (IL-2Rβ), IL-15 receptor alpha (IL-15Rα), interferon receptor 2, and prostaglandin E synthase. Downregulated genes included those encoding complement component receptor 2/CD21, platelet-activating factor acetylhydrolase, and oxidized low-density lipoprotein receptor 1 (OLR1). Pneumolysin-independent responses include down-regulation of the genes encoding CD68, CD53, CD24, transforming growth factor β_2, and signal transducers and activators of transcription 1.

Pneumolysin-dependent and -independent gene expression identified by cDNA microarray analysis of THP-1 human mononuclear cells stimulated by Streptococcus pneumoniae. Rogers PD, Thornton J, Barker KS, McDaniel DO, Sacks GS, Swiatlo E, McDaniel LS. Infect Immun 2003 Apr; 71 (4), pp. 2087–94.

Neoplasia: Acute Myeloid Leukemia (AML; Bone Marrow Samples)
Chemiluminescent Stain

Application: Determine the monocytic component of FAB-M4 and FAB-M5 and distinguish these AML categories.

Background Information: Classically, the monocytic component of acute myelomonocytic (FAB-M4) and acute monocytic/monoblastic (FAB-M5) leukemias is demonstrated by positive nonspecific detection of esterases applying cytochemical staining reagents. Non-specific esterases from normal monocytes can be determined appling chemiluminescent-based methodologies. Similar techniques can be applied to distinguish unique aspects of AML.

Laboratory Methodologies: Bone marrow samples are processed to obtain cell populations that are incubated with a standard reaction mixture and chemiluminescence is measured for 10 min. Two parameters are assessed including peak (PLE) and the integrated light emission (ILE).

Interpretation and Analysis: In bone marrow samples obtained from patients with acute myeloid leukemia (n = 66), both PLE and ILE values are higher in FAB-M4 and FAB-M5 subtypes compared to other acute myeloid leukemia subtypes ($p < 0.001$). In addition, the classification of AML cases into FAB-M4, FAB-M5 and nonmonocytic subtypes based on ILE analysis correlates with alpha-naphthyl acetate esterase (ANAE) activity in cases of acute myeloid leukemia (97%, kappa coefficient 0.94, $p < 0.001$). Based on these findings, the chemiluminescent assay can be applied to determine the monocytic component of FAB-M4 and FAB-M5 cells, and the classification of acute myeloid leukemia subtypes based on chemiluminescent analysis which strongly agrees with cytochemical ANAE-staining. In conclusion, this chemiluminescent assay is a simple, fast and objective method, which may be useful as an alternative tool in establishing an accurate differential diagnosis of acute myeloid leukemia.

Assessment of monocytic component in acute myelomonocytic and monocytic/monoblastic leukemias by a chemiluminescent assay. da Fonseca LM, Brunetti IL, Campa A, Catalani LH, Calado RT, Falcao RP. Hematol J 2003; 4 (1), pp. 26–30.

Oxidative Stress/Peroxidation of Lipid Membranes/ Prostaglandin/Thromboxane Metabolism/ Thiobarbituric Acid Reactive Substances
Thiobarbituric Acid/Malondialdehyde (MDA)/(Aspirin)

Cellular/Molecular Mechanism of Action: Thiobarbituric acid at a low pH and high temperatures reacts with MDA, producing a molecular product that can be detected spectrophotometrically.

Background Information: Malondialdehyde is a metabolic byproduct of lipid membrane peroxidation and the generation of prostaglandin species. The MDA assay is the most generally used test for characterizing the role of oxidative stress in disease. Malondialdehyde is 1 of several products formed during the radical induced decomposition of polyunsaturated fatty acids.

Application: Detection and evaluation of oxidative stress status as a function of lipid membrane peroxidation and prostaglandin synthesis. In this context, it can be applied to evaluate the efficacy of pharmaceutical agents (e.g., non-steroidal anti-inflammatory drugs [NSAIDs]), detect tissue peroxidation, and recognize the entry of new platelet populations into the systemic circulation.

Route of Administration: *Ex vivo* (topical).

Considerations: At present, the concept of "thiobarbituric acid reactive substances" (TBARS) has merged and progressively replaced the initial MDA assay.

Assay Procedure: The MDA assay is based on its reactivity at high temperature and low pH toward thiobarbituric acid. This reaction is very sensitive but its specificity, even with improvement of preanalytical (sampling, preservatives) and analytical stages (fluorescence, high-performance liquid chromatography [HPLC]), is still a matter of debate. Example: After stimulation of the washed human blood platelets by arachidonic acid (AA), the formation of malondialdehyde (MDA) measured using the photometric thiobarbituric acid (TBA) method. Estimation of platelet lipid peroxidation as a function of MDA production, and the formation of thromboxane B2 (TXB_2) measured by gas chromatography, may reveal that the formed MDA exceeds the amount of TXB_2 on a molar base. However, MDA and TXB_2 originating from thromboxane synthase activity should be produced in approximately equimolar amounts. By treatment of stimulated platelet samples with stannous chloride it is possible to reduce all peroxidized products of AA that generate MDA otherwise during the TBA reaction and to estimate MDA and TXB_2 in a ratio of nearly 1:1. The stannous chloride treatment does not destroy the MDA and does not influence the TBA reaction with MDA. Therefore the simple and quick TBA method can be used after stannous chloride treatment for estimation of thromboxane synthase activity in AA-stimulated washed human platelets.

Interpretation and Analysis: In the future, oxidative stress appreciation will need the precise analytical determination of different molecules triggered by free radicals. The TBARS assay should be considered as a global test, allowing a global approach of lipoperoxidation, whereas specific determination of MDA can only appreciate 1 of the end-product formed during oxidative stress.

Laboratory Instrumentation: Inhibition of platelet PGI_2 synthesis by NSAIDS is detected by direct assay of PGI_2 applying RIA or ELISA methodologies. Alternatively, inhibited platelet PGI_2 synthesis can be detected by measurement of relative MDA production

applying thiobarbituric acid and measuring spectrophotometric absorbance at 625 nm or HPLC analysis.

Evaluation of lipid peroxidation by measuring thiobarbituric acid reactive substances. Lefèvre G, Beljean-Leymarie M, Beyerle F, Bonnefont-Rousselot D, Cristol JP, Thérond P, Torreilles J. Ann Biol Clin (Paris) 1998 May–Jun; 56 (3), pp. 305–19.

An improved malondialdehyde assay for estimation of thromboxane synthase activity in washed human blood platelets. Panse M, Block HU, Förster W, Mest HJ. Prostaglandins 1985 Dec; 30 (6), pp. 1031–40.

Phagocytic Activity/Macrophage Function: Reticuloendothelial System
Nanoparticles: Copolymer Non-Ionic Surfactants/ Polyoxypropylene and Polyoxyethylene

Background Information: A frequently observed but ignored phenomenon following intravenous injection of such polymer-modified long-circulating colloidal systems is their eventual recognition and clearance by macrophages of the reticuloendothelial system.
Application: Nanoparticles composed of polyoxypropylene and polyoxyethylene copolymer non-ionic surfactants can be applied to detect and characterize the function of macrophage populations.
Route of Administration: Intravascular.
Implementation: Evaluation of the design, handling, and biological assessment of long-circulating nanoparticles composed of polyoxypropylene and polyoxyethylene copolymer non-ionic surfactants. Nanoparticles produced in this fashion are recognized by macrophage populations.
Interpretation and Analysis: The pharmacokinetics as well as the tissue distribution of such so-called "stealth" nanoparticles are altered after repeated intravenous injection in a time-dependent manner. An understanding of immunological and pathological factors that control the pharmacokinetic and biological behavior of long-circulating particles after single or repeated administration is therefore crucial for the design of a system with an optimal diagnostic and/or therapeutic performance.

Capture of stealth nanoparticles by the body's defenses. Moghimi SM, Hunter AC. Crit Rev Ther Drug Carrier Syst 2001; 18 (6), pp. 527–50.

Phagocytic/Phagocytic Killing Index: Macrophages/Neutrophils/Macrophage and Neutrophil Phagocytic Effectiveness
Candida albicans/Staphylococcus aureus

Application: Detection of abnormalities in the ability of neutrophils and macrophages to either phagocytize or kill phagocytized pathogens.
Methodology: Enriched neutrophil and macrophage populations are obtained 1st as a leukocyte buffy coat layer following the centrifugation of fresh whole blood. Separation of cell types can be achieved with the application of density gradient media (e.g., Percoll) used in combination with centrifugation. Because a given cell type (e.g., neutrophil) has different densities across different species, a literature review and preliminary tests in the laboratory are required in order to identify the optimum specific gravity at which density gradient medias should be formulated. Usually, a mononuclear cell layer is formed at the plasma/density gradient media interface and contains a combination of monocytes and lymphocytes. Monocyte-derived macrophages are separated from lymphocytes by their ability to adhere to tissue culture flasks (3–12 hours at 37°C, CO_2 5%). The 2nd layer formed is an enriched population of neutrophils that is visualized either mid-level within the density gradient media or on the surface of the red blood cell layer, depending on the specific gravity of density gradient preparations.

Purified cell populations are then separately incubated with suspensions of both non-viable and viable *Candida albicans* populations. The phagocytic index is approximated by calculating the number of cells that have phagocytized Candida organisms and the total number of organisms located intracellularly within each cell. In addition, the phagocytic killing index is calculated as a ratio of the number of total viable organisms phagocytized compared to the number of living *Candida albicans* that have been successfully killed intracellularly. Differential staining techniques are applied to detect viable and non-viable phagocitized Candida organism.
Interpretation and Analysis: Decreases in the phagocytic index imply phagocytic function is suboptimal, while declines in the phagocytic killing index indicate that following the internalization of pathogens, there is an inability to kill such organisms due to lack of phagosome/lysosome formation (e.g., phagolysosome) or the contents of the lysosome are unable to directly inactivate phagocytized bacterial/fungal organisms.

Example: Phagocytosis of *Rhodococcus equi* organisms by equine foal neutrophils is less than that observed for adult neutrophils when autologous serum is used as the source of opsonins in the assay. The use of adult serum does not significantly improve the ability of foal neutrophils to attach bacteria.

A comparison of foal and adult horse neutrophil function using flow cytometric techniques. McTaggart C, Yovich JV, Penhale J, Raidal SL. Res Vet Sci 2001 Aug; 71 (1), pp. 73–9.

Respiratory/Oxidative Burst Potential (Macrophage/Neutrophil)
Nitroblue Tetrazolium (NBT)/Sulfonated Tetrazolium Salt (WST-1)

Cellular/Molecular Mechanism of Action: Neutrophil respiratory burst activity can be detected in histiological samples through the application of the tetrazolium salt, nitroblue tetrazolium, which is reduced intracellularly to an insoluble formazan by superoxide generated by NADPH oxidase. The water-soluble sulfonated tetrazolium salt, WST-1 produces a soluble formazan that can be detected by a simple colorimetric assay for measuring respiratory burst activation and for screening anti-inflammatory agents.
Application: Detection and characterization of leukocyte oxidative burst activity as a function of cell viability and capacity to resolve phagacytized pathogens.
Methodology: Nitroblue tetrazolium (NBT) dye reduction assay.
Considerations: Activation of the respiratory burst of granulocytes and macrophages by invading microorganisms is a key 1st-line of cellular defense against infection. Failure to generate this response leads to persistent life-threatening infection unless appropriate antibiotic treatment is given. Respiratory burst activity in neutrophils is often measured spectrophotometrically following ferricytochrome-C reduction. In tissue or cell preparations, it is detected by the intracellular reduction of the tetrazolium salt, nitroblue tetrazolium to an insoluble formazan crystal. In both assay systems, reduction is mediated by superoxide generated via NADPH oxidase. Because ferricytochrome-C has a high molecular mass and high background absorbance at 550 nm, the assay lacks sensitivity and is not ideally suited to microplate measurement.
Implementation: (i) In activated populations of human peripheral blood neutrophils, the reduction of WST-1 closely correlates

with ferricytochrome-C reduction across a range of PMA concentrations and with time of activation by PMA and *f*MLP. Reduction of WST-1 is inhibited 98% by superoxide dismutase (20 μg/ml); and 88% by the NADPH oxidase inhibitor, diphenyleneiodinium (10 μM). In contrast, WST-1 reduction is resistant to catalase, azide and the NADH oxidase inhibitor, resiniferatoxin. In comparison investigations, the relatively ability of WST-1 and ferricytochrome-C reduction have also been compared using xanthine/xanthine oxidase to generate superoxide. Under optimised assay conditions, both WST-1 and ferricytochrome-C reduction are directly proportional to the quantity of added xanthine. Approximately a 2-fold greater increase in absorbance can be appreciated with WST-1 generated absorbance measurements than is usually observed for absorbance values associated with ferricytochrome-C at their respective wavelengths. These findings imply a direct relationship to increased assay sensitivity. Addition of the intermediate electron acceptor, 1-methoxy phenazine methosulfate, increases the background of the neutrophil assay but does not affect the overall magnitude of the response. In this context, WST-1 based assays have been utilized as investigative tools to assess human neutrophil dysfunction and to compare anti-inflammatory activity (e.g., pharmaceutical therapy).

(ii) Neutrophil chemotaxis and oxidative burst activity has been used to evaluate the immune competence of workers occupationally exposed to mainly silica. Both types of neutrophil functions are significantly reduced in silica-exposed foundry and pottery workers ($p < 0.001$) compared to healthy controls subjects suggesting that chronic exposure mainly to silica and other chemicals originated from foundry and pottery settings may diminish neutrophil functions. Chemotatic function is measured in a Boyden chemotactic chamber using Zymosan-activated serum as a chemotactic preparation. Oxidative burst activity can be measured using a conventional nitroblue tetrazolium (NBT) dye reduction test (2-hour incubation period).

Interpretation and Analysis: The limitation of tetrazolium-based assays can be minimized or eliminated by using a cell-impermeable, WST-1, which exhibits very low background absorbance and is efficiently reduced by superoxide to a stable water-soluble formazan with high molar absorptivity. Improvements provided by this particular reagent have permitted the adaption of the WST-1 assay to a microplate format while still retaining optimum levels of sensitivity.

Declines in oxidative burst activity implies that a given leukocyte cell type has been compromised or incapable of creating a lethal effect on phagocytized pathogens.

Example Application: In cows with subclinical mastitis caused by *Staphylococcus aureus*, the oxidative burst assay has been utilized to analyze whole blood and fresh milk samples and determine the effectiveness of certain therapeutic strategies.

Instrumentation: Spectrophotometer (530 nm, 570 nm or 590 nm).

Alterations of neutrophil functions in foundry and pottery workers. Basaran N, Undeger U, Shubair M. Immunopharmacol Immunotoxicol 2002 Nov; 24 (4), pp. 639–50.

Effect of subcutaneous injection of ginseng on cows with subclinical Staphylococcus aureus mastitis. Hu S, Concha C, Johannisson A, Meglia G, Waller KP. J Vet Med B Infect Dis Vet Public Health 2001 Sep; 48 (7), pp. 519–28.

Superoxide produced by activated neutrophils efficiently reduces the tetrazolium salt, WST-1 to produce a soluble formazan: A simple colorimetric assay for measuring respiratory burst activation and for screening anti-inflammatory agents. Tan AS, Berridge MV. J Immunol Methods 2000 Apr 21; 238 (1–2), pp. 59–68.

HEMATOPOIETIC: NEUTROPHILS (*EX VIVO*)

Adherence: Neutrophil Function/Neutrophil Tissue Infiltration
o-Dianisidine Dihydrochloride/(Myeloperoxidase)

Application: Detection of neutrophils using myeloperoxidase production as a biochemical identification marker. Indirectly can possibly be applied as a vitality marker system due to the relatively short intravascular survival time for neutrophils.

Example Application: The antioxidant system is the primary intracellular defense system of the lung against oxygen toxicity (neutrophil sequestration). One of the major biochemical entities associated with neutrophil function is myeloperoxidase found within cytoplasmic granules. Quantitation of the amount of biochemical activity of this enzyme therefore directly correlates with the degree or extent of neutrophil infiltration within extravascular organ systems and serves as an indirect indicator of lung injury.

In a lamb model of an antioxidant deficient system involving the lung, it has been hypothesized that neutrophil sequestration may play a part in the acute lung injury associated with certain disease states (e.g., congenital diaphragmatic hernia). Preparation of tissue samples prior to performing myeloperoxidase assays involves en bloc dissection of the organ system of interest (e.g., lung) followed by "flash" freezing. Actual processing of tissues and their analysis involves homogenation, sonication, freeze-thawing, and separation of cell types utilizing density gradient media combined with centrifugation. Supernatants are analyzed for myeloperoxidase activity by spectrophotometry with *o*-dianisidine dihydrochloride and hydrogen peroxide at 460 nm. Myeloperoxidase activity is normalized to the protein content/concentration within the supernatant (myeloperoxidase activity/mg protein). In the lamb model for congenital diaphragmatic hernia, myeloperoxidase activity can be found to be significantly higher in ventilated-affected animals than in ventilated-negative controls ($3{,}203 \pm 665$ vs $1{,}220 \pm 194$, $p = 0.001$). Laboratory findings suggest that ventilation and hyperoxia leads to neutrophil accumulation in lung tissue, which is most pronounced in congenital diaphragmatic hernia lung tissue. Such discoveries have provided greater understanding about the pathophysiology of iatrogenic lung injury associated with congenital diaphragmatic hernia. In this context, the myeloperoxidase assay can be used to evaluate antenatal or postnatal antioxidant therapies for iatrogenic lung injury.

In equine studies, flow cytometric assays have been used to compare phagocytic and oxidative burst activity of neutrophils from healthy neonatal foals and adult horses. During the course of these investigations oxidative burst activity of foal neutrophils has been found to be equivalent to that of adult cells. However, when serum or plasma is incorporated into the oxidative burst assay, foal neutrophils demonstrate greatly reduced autofluorescence and a suppressed response to phorbol myristate acetate (PMA), relative to that demonstrated by adult cells. These results suggest that peripheral blood neutrophils from foals have a reduced ability to phagocytose bacteria relative to that exhibited by adult horse neutrophils and that the oxidative burst activity of foal neutrophils is downregulated in response to an unidentified serum factor(s). Such changes may contribute to the increased susceptibility of foals to septic disease.

Methodology: Multi-well tissue culture plates (e.g., 96-well microtiter plate) are coated with fibrinectin (fibronectin [5 μg/ml]) in Hank's Buffered Saline Solution (pH 7.4) incubated with tissue plates overnight (4°C). During the isolation of neutrophils, the plate is washed 3 times (1 minute each) with Hank's solution (200 μl) followed by a 2-hour blocking procedure performed with Hank's

solution containing BSA (1% 250 μl). Fibronectin-coated wells are then rinsed 3 times (1 minute) with Hank's solution containing Tween (1%) followed by a single wash with Hank's solution (200 μl).

Isolated cell populations highly enriched with neutrophils (10^5 to 10^6 cells in 100 μl) are stimulated with either *f*MLP (2×10^{-6} M, final concentration 1×10^{-6}) or PMA (30 ng/ml, final concentration 15 ng/ml) and then incubated at 37°C for 15 minutes (CO_2 5%). A solution of Hanks solution containing bovine serum albumin (1%, 150 μl) is then added into each well and it is sealed with acetate tape. The plate is then centrifuged upside down at 1,200 rpm at 4°C for 5 minutes and the tape removed while the plate is still inverted. In order to detect and quantitatively measure the number of adherent neutrophils, a reaction mixture formulation (100 μl/well) containing *o*-dianisidine (*o*-dianisidine dihydrochloride [1 mM]; H_2O_2 [1 mM]; Triton X-100 [0.1%] in potassium phosphate buffer [100 mM, pH 6.0]) is then pipetted into each fibronective well. Following an incubation period of 30 mintues at 25°C and the spectrophotometric absorbance measured at a wavelength of 450 nm to 460 nm, either Hank's solution with BSA (1%) or cell suspension (100 μl) can be used to generate a standard curve.

Interpretation and Analysis: A direct relationship exists between the amount of myeloperoxidase measured in a tissue/cell sample and the number of neutrophils contained with such preparations.

A comparison of foal and adult horse neutrophil function using flow cytometric techniques. McTaggart C, Yovich JV, Penhale J, Raidal SL. Res Vet Sci 2001 Aug; 71 (1), pp. 73–9.

Myeloperoxidase activity as a lung injury marker in the lamb model of congenital diaphragmatic hernia. McCabe AJ, Dowhy M, Holm BA, Glick PL. J Pediatr Surg 2001 Feb; 36 (2), pp. 334–7.

Chemotactic Activity: Neutrophil Function/Boyden Chemotactic Chamber
formyl-Met-Leu-Phe (fMLP)

Application: Evaluation of neutrophil chemotactic function.
Methodology: Freshly purified populations of neutrophils suspended in MEM growth media are transferred by pipette into individual wells of a Boyden chemotactic chamber. The chemotactic agent *f*MLP (10^{-6} M final concentration) is placed in the well on the opposite side of the membrane filter and the Boyden chamber is then incubated at 37°C for 90 minutes. Non-adherent cells are discarded and the remaining neutrophil population is incubated with 350 μl of calcium/magnesium-free phosphate-buffered saline (CMF-PBS) containing EDTA (20 mM). Following the harvest of this supernatant, this procedure is repeated again. Adherent neutrophils are detected by incubation with MTT vitality staining reagent (375 μl) previously formulated in serum free medium (60 ml/300 ml v/v). The preparations are then incubated at 37°C over a 4-hour period. Cell populations stained with MTT reagent are then destained with isopropyl alcohol (200 μl) at 25°C for 25 minutes with the aid of gently horizontal agitation on a horizontal plateform. The resulting blue/purple supernatant is harvested by pipette and transferred to a 96-well microtiter plate for measurement of spectrophometric absorbance (550nm).
Dosage: Chemotactic agent *f*MLP (10^{-6} M final concentration).
Interpretation and Analysis: Deminished chemotactic responses by neutrophils is an indiction of altered cellular function and may indicate a condition of a reduced ability to control septic disease states.
Instrumentation: Boyden chemotactic chamber and spectrophotometer capable of reading absorbance levels at 550 nm wavelength.

Chemotactic Activity: Neutrophil Function/[Respiratory Burst Activity]/[Bacterial Phagocytic Activity]
Interleukin (IL)-8/formyl-Met-Leu-Phe (fMLP)/Antithrombin/C5a/Protein C

Cellular/Molecular Mechanism of Action: The interleukin IL-8 promotes or functions as a neutrophil chemotactic agent.
Background Information: Activation of protein C by thrombin bound to thrombomodulin is enhanced by endothelial protein C receptor. This pathway may inhibit inflammation.
Implementation: Evaluate the influence of protein C and activated protein C on neutrophils, as well as determine the potential for endothelial protein C receptor to mediate protein C–mediated effects. Achievement of this objective can be determined by isolating neutrophils from venous whole blood. Cell migration, respiratory burst, phagocytic activity, and apoptosis are determined by micropore filter assay and fluorometry. Receptor expression can be delineated by reverse transcriptase-polymerase chain reaction (PCR) for mRNA, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), in combination with autoradiography of immunoprecipitated receptor protein, and fluorescence-activated cell-sorter scanner (FACS) analysis using the antiendothelial protein C receptor antibody RCR-252.

Human Model: Cases without any evidence of disease or physiological abnormalities.
Interpretation and Analysis: Neither protein C nor activated protein C induce migration, yet both agents inhibit neutrophil chemotaxis triggered by IL-8, *f*MLP, antithrombin, or C5a. A protein C activation-blocking antibody against endothelial protein C receptor diminishes inhibitory effects of protein C or activated protein C on migration. No effect of either protein C preparation can be appreciated in neutrophil's respiratory burst, bacterial phagocytosis, or apoptosis assays. Endothelial protein C receptor immunoreactivity can be confirmed on neutrophils by FACS. Reverse transcriptase PCR and immunoprecipitation SDS-PAGE analyses suggest the occurrence of de-novo synthesis of endothelial protein C receptor mRNA expression. Data suggest that an endothelial protein C receptor is expressed by human neutrophils whose active site ligation with either protein C or activated protein C results in arrested directional cell migration. Inhibitory effects of these components of the protein C pathway on neutrophil function may play a role in the protein C–based treatment of severe sepsis.

Expression and function of the endothelial protein C receptor in human neutrophils. Sturn DH, Kaneider NC, Feistritzer C, Djanani A, Fukudome K, Wiedermann CJ. Blood 2003 Aug 15; 102 (4), pp. 1499–505.

Chemotactic Activity: Neutrophil Function/Phagocytic Capacity/Super-Oxide Production/Adherence Characteristics
Interleukin (IL)-8/Phorbol Myristate Acetate

Background Information: Polymorphonuclear granulocyte (PMN) phagocytosis may be affected by many pathological changes.
Dosage (*Ex Vivo*): Interleukin-8 (25 ng/mL); phorbol myristate acetate (10^{-6} mol/L).
Implementation: Evaluation of neutrophil function applying a panel of tests requiring relatively small volumes of blood in order to

establish normal values and to standardize techniques. Utilizing discontinuous Percoll gradient media, PMNs are isolated from whole blood samples. Chemotaxis capacity is determined in a modified Boyden chamber using the leading front method. Phagocytic activity is evaluated by determining the rate of fluorescinated yeast uptake (slide format). Superoxide (SO) production and adherence is established within the wells of a microtitre plate.

Animal Model: Canine (n = 16 healthy dogs).

Interpretation and Analysis: Different aspects of phagocytosis show no correlation with 1 another. Better results can be obtained using a 60-minute incubation period and the application of IL-8 (25 ng/mL) as an activator for chemotaxis, and incubating plates for 30 minutes with phorbol myristate acetate (10^{-6} mol/L) to assess SO production.

Evaluation *in vitro* of canine neutrophil function. Comazzi S, Paltrinieri S, Spagnolo V, Sartorelli P. Vet J 2001 Nov; 162 (3), pp. 219–25.

Degradulation: Neutrophil Function
Complement (C5a)/Calium Ionophor (A23187)/formyl-Met-Leu-Phe (fMLP)/Phorbol Myristate Acetate (PMA)

Mechanisms of Action: (i) Selective induction of specific granule secretion can be achieved with concanavalin-A, phorbol myristate acetate, *f*MLP, and complement (C5a). (ii) Induced secretion of specific and azurophilic granules can be achieved with immune complexes and calcium ionophors (e.g., A23187), and lithium also is capable of promoting similar responses. (iii) Inhibition of degranulation events (possible microtubule disassembly) occurs in the presence of adenylate cyclase stimulation (e.g., β-adrenergic agonists and PGE_1). (iv) Promotion of degranulation events, by microtubule assembly, induction of cGMP synthesis/activation. (v) Lysosomal enzyme release induced by complement (C3a and C5a).

Application: Detection of conditions associated with neutrophil dysfunction.

Glycogen Storage Disease Type Ib (GSD-Ib)/ Neutrophil Respiratory Burst Activity/[Glucose-6-Phosphate Levels]
Phorbol Myristate Acetate (PMA)/Glucose

Background Information: Neutrophils from patients suffering from glycogen storage disease type Ib (GSD-Ib) show several defects, 1 of which is a decreased rate of glucose utilization.

Implementation: Evaluation of the stimulation of the neutrophil respiratory burst by extracellular glucose using phorbol-myristate-acetate as a stimulating agent.

Interpretation and Analysis: The activity of NADPH oxidase in GSD-Ib neutrophils hardly increases upon addition of glucose. In control and GSD-type Ia neutrophils, a clear increase can be observed. The lack of response to extracellular glucose in GSD-Ib neutrophils is correlated with an inability to raise intracellular glucose-6-P levels on glucose addition, thereby limiting the activity of the generation of NADPH in the hexose-monophosphate shunt. The test protocol can be applied for the diagnosis of neutrophil function abnormalities in GSD-Ib conditions.

A convenient diagnostic function test of peripheral blood neutrophils in glycogen storage disease type Ib. Verhoeven AJ, Visser G, van Zwieten R, Gruszczynska B, Tien Poll-The DW, Smit GP. Pediatr Res 1999 Jun; 45 (6), pp. 881–5.

Mononuclear/Neutrophil Phagocytosis (Opsonin Independent)
Fluorescent Polymethacrylic Nanoparticles

Cellular/Molecular Mechanism of Action: Fluorescent polymethacrylic nanoparticles are recognized by and intracellularly assimilated by mononuclear cells and neutrophils.

Application: Quantitative evaluation of phagocytosis by human mononuclear and polymorphonuclear cells. One possible application of the fluorescent nanoparticles is the simultaneous analysis of cell surface antigens and cell phagocytic activity.

Route of Administration: Intravascular or in the use of *ex vivo* cellular preparations.

Semi-Synthetic Methods: Preparation of the nanoparticles, by emulsion copolymerization of methacrylic monomers, and assessment of their physicochemical properties.

Implementation: Evaluation of fluorescent polymethacrylic nanoparticles (0.3 micron) as a flow cytometric reagent in the quantitative evaluation of phagocytosis by human mononuclear and polymorphonuclear cells. In this context, nanoparticles coupled with a fluorescent agent (ethidium bromide) can be used in flow cytometric assays to study opsonin-independent phagocytosis by human polymorphonuclear cells and by human monocytes. Phagocytosis of nanospheres by monocytes is determined by flow cytometry from the fluorescence distribution, while ingestion is visualized by scanning and transmission electron microscopy.

Instrumentation: Flow cytometry and scanning and transmission electron microscopy.

Flow cytometric quantitative evaluation of phagocytosis by human mononuclear and polymorphonuclear cells using fluorescent nanoparticles. Rolland A, Merdrignac G, Gouranton J, Bourel D, Le Verge R, Genetet B. J Immunol Methods 1987 Feb 11; 96 (2), pp. 185–93.

Phagocytic Activity: Neutrophil Function/ Superoxide (O_2^-) Synthetic Capacity/[Neutrophil Bendiazepine Receptors: Peripheral Type]
Diazepam-Binding Inhibitor-Derived Peptides/ Triakontatetraneuropeptide/Eiksoneuropeptide

Cellular/Molecular Mechanism of Action: Triakontatetraneuropeptide (DBI 17-50, TTN) and eiksoneuropeptide (DBI 51-70, ENP) are peptides that form a complex with and block diazepam binding sites.

Application: Triakontatetraneuropeptide (DBI 17-50, TTN) and eiksoneuropeptide (DBI 51-70, ENP) could potentially be applied to detect and characterize neutrophil chemotactic and phagocytic properties.

Implementation: Evaluation of neutrophil function based on changes in free [Ca^{2+}] cation concentrations [Ca^{2+}]; chemotaxis activity; superoxide anion (O_2^-) generation; and phagocytic activity. The 2 diazepam-binding inhibitor (DBI)-derived peptides, triakontatetraneuropeptide (DBI 17-50, TTN) and eiksoneuropeptide (DBI 51-70, ENP) can be applied as molecular probes for evaluating such neutrophil parameters.

Interpretation and Analysis: Both TTN and ENP induce a rapid and transient rise in [Ca^{2+}]i. The effect of TTN depends on the presence of extracellular [Ca^{2+}], whereas the effect of ENP also persists after extracellular [Ca^{2+}] chelation. TTN induces neutrophil chemotaxis, stimulated O_2^- generation, and enhanced phagocytosis. ENP does not affect cell migration or oxidative metabolism but does enhance phagocytosis. Both peptides modulate formyl-Met-Leu-Phe

(*f*MLP) and phorbol myristate acetate-induced O_2^- generation. Because neutrophils express benzodiazepine receptors of the peripheral type (pBRs) and DBI-derived peptides may interact with such receptors, investigations have characterized the possible role of pBRs in TTN- or ENP-induced effects. The synthetic pBR ligand RO 5-4864 increases $[Ca^{2+}]i$ through extracellular Ca^{2+} influx and this effect is prevented by the pBR antagonist PK-11195. RO 5-4864, however, has no effect on neutrophil migration or O_2^- generation and only slightly affects phagocytosis. Moreover, PK-11195 delays $[Ca^{2+}]i$ rises induced by TTN but does not significantly affect its extent, and has no effect on the $[Ca^{2+}]i$ rise induced by ENP. Therefore, it appears that DBI-derived peptides induce $[Ca^{2+}]i$ changes and modulate neutrophil function mainly through pBR-independent pathways. In view of the wide cell and tissue distribution of DBI in the brain and in peripheral organs, modulation of neutrophil function by DBI-derived peptides may be relevant for both the neuroimmune network and the development and regulation of the inflammatory processes.

Diazepam-binding inhibitor-derived peptides induce intracellular calcium changes and modulate human neutrophil function. Cosentino M, Marino F, Cattaneo S, Di Grazia L, Francioli C, Fietta AM, Lecchini S, Frigo G. J Leukoc Biol 2000 May; 67 (5), pp. 637–43.

Phagocytic/Phagocytic Killing Index: Neutrophils/Macrophages Neutrophil and Macrophage Phagocytic Effectiveness
Candida Albicans/Staphylococcus Aureus

Application: Detection of abnormalities in the ability of neutrophils and macrophages to either phagocytize or kill phagocytized pathogens.

Methodology: Enriched neutrophil and macrophage populations are obtained 1st as a leukocyte buffy coat layer following the centrifugation of fresh whole blood. Separation of cell types can be achieved with the application of density gradient media (e.g., Percoll) used in combination with centrifugation. Since a given cell type (e.g., neutrophil) has different densities across different species, a literature review and preliminary tests in the laboratory are required in order to identify the optimum specific gravity of the density gradient media required. Usually, a mononuclear cell layer is formed at the plasma/density gradient media interface and contains a combination of monocytes and lymphocytes. Monocyte-derived macrophages are separated from lymphocytes by their ability to adhere to tissue culture flasks (3–12 hours at 37°C, CO_2 5%). The 2nd layer formed is an enriched population of neutrophils that is visualized either mid-level within the density gradient media or on the surface of the red blood cell layer depending on the specific gravity of the preparation

Purified cell populations are then separately incubated with suspensions of both non-viable and viable Candida albicans preparations. The phagocytic index is approximated by calculating the number of cells that have phagocytized Candida organisms and the total number of organisms located intracellularly within each cell. In addition, the phagocytic killing index is calculated as a ratio of the number of total viable organisms phagocytized compared to the number of living Candidate albicans that have been successfully killed intracellularly.

Differential staining techniques are applied to detect viable and non-viable phagocitized Candida organism.

Interpretation and Analysis: Decreases in the phagocytic index imply phagocytic function is suboptimal, while declines in the phagocytic killing index indicate that following the internalization of pathogens, there is an inability to kill such organisms due to lack of phagosome/lysosome formation (e.g., phagolysosome) or the contents of the lysosome are unable to directly inactivate phagocytized bacterial/fungal organisms. Example: Phagocytosis of *Rhodococcus equi* organisms by foal neutrophils is less than that observed for adult neutrophils when autologous serum is used as the source of opsonins in the assay. The use of adult serum does not significantly improve the ability of foal neutrophils to attach bacteria.

A comparison of foal and adult horse neutrophil function using flow cytometric techniques. McTaggart C, Yovich JV, Penhale J, Raidal SL. Res Vet Sci 2001 Aug; 71 (1), pp. 73–9.

Respiratory/Oxidative Burst (Neutrophils/ Macrophages)
Nitroblue Tetrazolium (Tetrazolium Salt)

Cellular/Molecular Mechanism of Action: Respiratory burst activity can be monitored spectrophotometrically with enzyme-specific substrates that detect ferricytochrome-c reduction reactions, or histiologically through the application of nitroblue tetrazolium (reduced intracellularly to insoluable formazan crystals). Both substrates are reduced by superoxide generated by NADPH oxidase. Sulfonated tetazolium salts are reduced to a water-soluble formazan that has a high molar absorptivity.

Application: Detection of permanent deficiencies or transient dysfunctions in granulocyte and macrophage respiratory burst activity. Evaluation of such properties is important due to their intimate role in cellular defense mechanisms against invading microorganisms that can potentially cause overwhelming (life-threatening) infectious disease in situations where antibiotic therapy has not been initiated in a timely manner. The water-soluble characteristics of formazan (metabolized sulfonated tetrazolium salts or WST-1) and its very low background absorbance levels facilitate its adaptation to microtiter plate formats without loss of assay sensitivity.

Methodology: Biologically viable cell populations are incubated with the cell-impermeable, sulfonated *tetrazolium* salt (WST-1). This reagent exhibits very low background absorbance in a manner that allows adaptation of the WST-1 assay to microplate format with no sacrifice of sensitivity.

Reduction of WST-1 is inhibited (98%) by superoxide dismutase (20 μg/ml) and by the NADPH oxidase inhibitor, diphenyleneiodinium (88% for 10 μM). In contrast, the enzyme substrate is resistant to reduction activity in the presense of catalase, azide and the NADH oxidase inhibitor, resiniferatoxin. The sulfonated tetrazolium salt (WST-1) and ferricytochrome-c reductions have also been compared using xanthine/xanthine oxidase to generate superoxide. Under optimized assay conditions, both WST-1 and ferricytochrome-c reduction occurs in a manner directly proportional to the amount of added xanthine. A 2-fold greater level of absorbance is observed with WST-1 in contrast to ferricytochrome-c at their respective wavelengths. The implications of this result is that it reflects an increased level of assay sensitivity when applying this substrate reagent. Addition of the intermediate electron acceptor, 1-methoxy phenazine methosulfate, increases the background of the neutrophil assay but does not affect the overall magnitude of the response.

Considerations: Because ferricytochrome-c has a high molecular mass and high background absorbance at 550 nm, this assay lacks sensitivity and is not ideally suited to microplate measurements.

Interpretation and Analysis: Reduction of WST-1 by activated peripheral blood neutrophils (human) closely correlates with

ferricytochrome-c reduction across a range of activating agent concentrations (e.g., PMA) and time-initiated activation (e.g., PMA/*f*MLP).

Superoxide produced by activated neutrophils efficiently reduces the tetrazolium salt, WST-1 to produce a soluble formazan: A simple colorimetric assay for measuring respiratory burst activation and for screening anti-inflammatory agents. Tan AS, Berridge MV. J Immunol Methods 2000 Apr 21; 238 (1–2), pp. 59–68.

Respiratory/Oxidative Burst Potential (Neutrophil/Macrophage) *Nitroblue Tetrazolium (NBT)/Sulfonated Tetrazolium Salt (WST-1)*

Cellular/Molecular Mechanism of Action: Neutrophil respiratory burst activity can be detected in histiological samples through the application of the tetrazolium salt, nitroblue tetrazolium, which is reduced intracellularly to an insoluble formazan by superoxide generated by NADPH oxidase. The water-soluble sulfonated tetrazolium salt, WST-1 produces a soluble formazan that can be detected by a simple colorimetric assay for measuring respiratory burst activation and for screening anti-inflammatory agents.
Application: Detection and characterization of leukocyte oxidative burst activity as a function of cell viability and capacity to resolve phagacytized pathogens.
Example Application: In cows with subclinical mastitis caused by *Staphylococcus aureus*, the oxidative burst assay has been utilized to analyze whole blood and fresh milk samples and determine the effectiveness of certain therapeutic strategies.
Methodology: Nitroblue tetrazolium (NBT) dye reduction assay.
Considerations: Activation of the respiratory burst of granulocytes and macrophages by invading microorganisms is a key 1st-line of cellular defense against infection. Failure to generate this response leads to persistent life-threatening infection unless appropriate antibiotic treatment is given. Respiratory burst activity in neutrophils is often measured spectrophotometrically following ferricytochrome-C reduction. In tissue or cell population preparations, it is detected by the intracellular reduction of the tetrazolium salt, nitroblue tetrazolium to an insoluble formazan crystal. In both assay systems, reduction is mediated by superoxide generated via NADPH oxidase. Because ferricytochrome-C has a high molecular mass and high background absorbance at 550 nm, the assay lacks sensitivity and is not ideally suited to microplate measurement.
Implementation: (i) In activated populations of human peripheral blood neutrophils, the reduction of WST-1 closely correlates with ferricytochrome-C reduction across a range of PMA concentrations and with time of activation by PMA and *f*MLP. Reduction of WST-1 is inhibited 98% by superoxide dismutase (20 μg/ml); and 88% by the NADPH oxidase inhibitor, diphenyleneiodinium (10 μM). In contrast, WST-1 reduction is resistant to catalase, azide and the NADH oxidase inhibitor, resiniferatoxin. In comparison investigations, the relatively ability of WST-1 and ferricytochrome-C reduction have also been assessed using xanthine/xanthine oxidase to generate superoxide. Under optimised assay conditions, both WST-1 and ferricytochrome-C reduction are directly proportional to the quantity of added xanthine. Approximately a 2-fold greater increase in absorbance can be appreciated with WST-1 generated absorbance measurements than is usually observed for absorbance values associated with ferricytochrome-C at their respective wavelengths. These findings imply a direct relationship to increased assay sensitivity. Addition of the intermediate electron acceptor, 1-methoxy phenazine methosulfate, increases the background of the neutrophil assay but does not affect the overall magnitude of the response. In this context, WST-1 based assays have been utilized as investigative tools to assess human neutrophil dysfunction and to compare anti-inflammatory activity (e.g., pharmaceutical therapy).

(ii) Neutrophil chemotaxis and oxidative burst activity has frequently been used to evaluate immune competence in workers occupationally exposed to silica. Both types of neutrophil functions are significantly reduced in silica-exposed foundry and pottery workers ($p < 0.001$) compared to healthy controls subjects suggesting that chronic exposure mainly to silica and other chemicals originating from foundry and pottery settings may diminish neutrophil functions. Chemotatic function can be measured in a Boyden chemotactic chamber using Zymosan-activated serum as a chemotactic preparation. Oxidative burst activity is measured using a conventional nitroblue tetrazolium (NBT) dye reduction test (2-hour incubation period).
Interpretation and Analysis: The limitation of tetrazolium-based assays can be minimized or eliminated by using a cell-impermeable, WST-1, which exhibits very low background absorbance and is efficiently reduced by superoxide to a stable water-soluble formazan with high molar absorptivity. Improvements provided by this particular reagent have permitted the adaption of the WST-1 assay to a microplate format while still remaining optimum levels of sensitivity.

Declines in oxidative burst activity implies that a given leukocyte cell type has been compromised or incapable of creating a lethal effect on phagocytized pathogens.
Instrumentation: Spectrophotometer (530 nm, 570 nm or 590 nm).

Alterations of neutrophil functions in foundry and pottery workers. Basaran N, Undeger U, Shubair M. Immunopharmacol Immunotoxicol 2002 Nov; 24 (4), pp. 639–50.

Effect of subcutaneous injection of ginseng on cows with subclinical Staphylococcus aureus mastitis. Hu S, Concha C, Johannisson A, Meglia G, Waller KP. J Vet Med B Infect Dis Vet Public Health 2001 Sep; 48 (7), pp. 519–28.

Superoxide produced by activated neutrophils efficiently reduces the tetrazolium salt, WST-1 to produce a soluble formazan: A simple colorimetric assay for measuring respiratory burst activation and for screening anti-inflammatory agents. Tan AS, Berridge MV. J Immunol Methods 2000 Apr 21; 238 (1–2), pp. 59–68.

HEMATOPOIETIC: PLATELETS (*EX VIVO*)

Activation of Platelets: General Function/P-Selectin Expression/GPIIb/IIIa Receptor: Activated *Collagen-Epinephrine/Collagen-Adenosine Diphosphate (ADP)*

Background Information: Flavanols modulate platelet function *in vitro*, but less is known of their *in vivo* effects and how they compare to pharmacological platelet inhibitors.
Application: Detection and characterization of platelet function.
Route of Administration: Collagen, epinephrine, and ADP are applied in an *ex vivo* manner where they are incubated with highly enriched platelet suspensions.
Dosage: Aspirin (81 mg).
Implementation: Evaluation of separate and collective influences of flavanol-rich cocoa (897 mg/ml) and aspirin on platelet function and activation. Platelet activation can be measured *in vitro* by detection of P-selectin surface expression and PAC-1 binding to the activated GPIIb/IIIa receptor (GPIIb/IIIa-act). Platelet function can be measured on an analyzer (the PFA-100) that measures shear stress-induced platelet plug formation in response to collagen-epinephrine or collagen-ADP.
Human Model: Cases of normal health (n = 16).

Interpretation and Analysis: Plasma epicatechin concentrations peak approximately 2 hours after subjects are given either cocoa or aspirin plus cocoa. After 6 hours, cocoa inhibits epinephrine-induced platelet function. Epinephrine-induced platelet function is inhibited 2 and 6 hours after aspirin, and after aspirin plus cocoa. Epinephrine-stimulated P-selectin expression is inhibited by aspirin at 6 hours, and after 2 and 6 hours by aspirin plus cocoa. Adenosine diphosphate–stimulated P-selectin expression is not affected by the treatments. Cocoa and aspirin, given separately, reduce epinephrine-stimulated GPIIb/IIIa-act expression at 2 and 6 hours, respectively, and at 2 and 6 hours when given together, suggesting an additive effective. Acetylsalicylic acid plus cocoa inhibits ADP-stimulated GPIIb/IIIa-act expression at 6 hours. In summary, flavanol-rich cocoa inhibit epinephrine-stimulated platelet activation and function. These effects are qualitatively similar to aspirin, but less profound. These results emphasize the need to further examine the effects of flavonol-containing foods for platelet modulating effects.

Instrumentation: An analyzer (the PFA-100) capable of measuring shear stress-induced platelet plug formation.

The effects of flavanol-rich cocoa and aspirin on *ex vivo* platelet function. Pearson DA, Paglieroni TG, Rein D, Wun T, Schramm DD, Wang JF, Holt RR, Gosselin R, Schmitz HH, Keen CL. Thromb Res 2002 May 15; 106 (4–5), pp. 191–7.

Adhesion Function: Endothelial Repair Potential/ von Willebrand's Disease (vWD)/Thrombasthenic Platelets

Bead Column

Cellular/Molecular Mechanism of Action: The 2 primary functions of platelets include their participation in hemostatic processes (platelet aggregation), in addition to their role in the repair of the vascular endothelial surface (platelet adhesion function). In part, this process inovolves the recognition of defects in the vascular endothelium and physical attraction to subendothelial (negatively-charged) collagen that is recognized as a foreign surface by circulating platelets. This concept has been utilized in the design of platelet adhesion assays that employ glass bead retention columns because their anionic properties are recognized as a foreign surface.

In the 1st stage, platelets adhere to the glass beads; this requires fibrinogen but not von Willebrand's factor (vWF). The platelet-platelet adhesion in the 2nd stage is dependent on release of ADP, and fails to occur if thrombasthenic platelets are tested.

Application: The platelet retention test provides a measure of the number of platelets retained in a column of glass beads and can be used to detect the presence of acquired, congenital or hereditary abnormalities in platelet adhesion properties. It is also 1 of the few *in vitro* platelet function tests that is abnormal in vWD. Retention is usually normal when blood from a patient with afibrinogenemia are used as Sample-B blood.

Example Application #1: Monoclonal antibodies have been used to elucidate the mechanism of platelet retention. Antibodies directed at 5 different vWF epitopes essentially abolishes platelet retention in the 1-stage test and 2nd stage of 2-stage tests, but has no effect on the 1st stage. Thus, the entire vWF molecule must be free of antibody to function in the platelet-platelet adhesion of the 2nd stage of this test. Binding of the antigen-antibody complex to the Fc receptor of platelets appears to have an insignificant impact in this regard because Fab and $F_{ab'}$[2] immunoglobulin fragments are as effective as intact antibody, and neither heat-aggregated IgG nor a polyclonal antibody to plasma factor IX inhibits retention. Immunoglobulin $F_{ab'}$[2] fragments with binding avidity for platelet GP Ib prevents binding of vWF to platelets and inhibits the 2nd phase of platelet retention. Antibody that inhibits binding of fibrinogen and vWF to GP IIb/IIIa (LJ-CP8) inhibits both the 1st and 2nd stages of platelet retention, whereas LJ-P5 antibody that inhibits only the binding of vWF to GP IIb/IIIa causes only slight inhibition of platelet retention when normal or afibrinogenemic blood is used as sample-B blood. The same antibody also reportedly causes only partial inhibition of ADP-induced platelet aggregation in instances of afibrinogenemia. Interpretation of these *ex vivo* investigations suggests that vWF is altered during rapid passage of blood through the glass-bead column to the extent that it attaches to GP Ib, exposing GP IIb/IIIa, which then binds the altered vWF or fibrinogen, either of which can induce platelet aggregation (platelet-platelet adhesion) and thus retention within the column.

Example Application #2: Platelet glass-bead retection columns have been applied in delineating bleeding tendencies in cases of aortic valvular stenosis. High and low shear platelet function tests performed *in vitro* on normal controls have been compared with that observed in cases with severe aortic valve stenosis with a mean Doppler systolic gradient of 75 (SD = ± 18) mm Hg before and at least 4 months after aortic valve replacement. Affected cases show reduced retention in high shear platelet function tests. More specifically, platelet retention in the filter test is approximately 53.6 (12.6)% in cases of aortic valve stenosis compared to 84.8 (9.6)% in negative reference controls ($p < 0.001$). In addition, retention in the glass bead column test is approximately 49.8 (± 19.2) in affected cases, and 87.4 (± 8.7) in negative reference controls ($p < 0.001$). (c) The standard bleeding time is also longer in cases with aoritic valvular stenosis ($p < 0.06$). Results observed with high shear tests after aortic valve replacement are usually within the normal range. Platelet counts are low but also within normal limits range before surgery and increased postoperatively ($p < 0.01$). Standard clotting tests, plasma, intraplatelet von Willebrand's factor, or platelet aggregation tests using 5 agonists are often no different than negative reference controls.

Laboratory findings from these investigations imply that the high shear hemodynamics of aortic valve stenosis modifies platelet function *in vivo* predisposing to a bleeding tendency. A platelet function abnormality of this type is detectable only *in vitro* using high shear tests. The abnormal function is reversed by aortic valve replacement. High shear forces *in vitro* activate and then inactivate platelets. By this same mechanism aortic valve stenosis seems to lead to high shear damage *in vivo*, resulting in clinically relevant bleeding tendency in certain patients.

Methodology: Enriched preparations of platelets are initially harvested from fresh whole blood collected into sodium citrate or acid-citrate-dextrose anticoagulant (9:1 to 5:1 v/v) in the form of platelet rich plasma following a brief period of low-speed centrifugation. The platelet rich plasma is then harvested after the formation of a leukocyte buffy coat layer on top of the red blood cell layer. Either the platelet rich plasma itself can be used directly in the evaluation of aggregation potential or if necessary, preparations can be recentrifuged at low speed to obtain a platelet pellet that can be suspended in a smaller volume of plasma in order to obtain a more concentrated platelet preparation. Alliquots of fresh whole-blood or platelet rich plasma are then pipetted onto a glass-bead column following performing of a platelet count. The ratio of platelets that become adheared to the glass beads compared to the number that pass through the column is a reflection of the degree of platelet adhesion potential.

In a 2-stage test, 1 mL of blood (Sample A: 5.0 ml) is passed through a glass bead column, followed by isotonic saline (5.0 ml). A

2nd aliquote of blood (Sample-B: 5.0 ml) is applied to the column and the degree of platelet retention measured. With normal blood as A and B, retention is very high in all 5 mL of Sample-B blood.

Interpretation and Analysis: Platelet retention in a standard glass bead column is measured and then compared to values for normal reference controls.

Decreased platelet function in aortic valve stenosis: High shear platelet activation then inactivation. O'Brien JR, Etherington MD, Brant J, Watkins J. Br Heart J 1995 Dec; 74 (6), pp. 641–4.

Effect of monoclonal antibodies against von Willebrand factor and platelet glycoproteins IIb/IIIa on the platelet retention test. McPherson J, Brownlea S, Zucker MB. Blood 1987 Aug; 70 (2), pp. 546–50.

Aggregation of Platelets: (*Ex Vivo*)/Bleeding Disorders (Primary)/von Willebrand's Disease (vWD)/Acetylsalicylic Acid (ASA) Intake: Differential Effect Induced by Adenosine Diphosphate (ADP) and Epinephrine (EPI)
ADP/EPI

Cellular/Molecular Mechanism of Action: Adenosine diphosphate is produced/released by platelets during the process of platelet activation and promotes aggregation phenomenon in addition to the activation of other platelets that may be in close proximity. Epinephrine is another endogenous agent (adrenergic neurotransmitter) that activates platelet populations.

Application: Detection and characterization of primary bleeding disorders (e.g., vWD) and ASA intake that are associated with alterations in platelet function (e.g., adhesion/aggregation), which can be detected applying comparative ADP vs EPI-induced aggregation tests.

Considerations: Evaluation of platelet function applying aggregation methodologies is more sensitive than the evaluation of bleeding times. In this context, EPI is a more sensitive diagnostic reagent than ADP.

Implementation: Evaluation of bleeding disorders. The procedure involves the collection of citrated venous whole blood that is subsequently aspirated through a capillary toward an aperture of a collagen-coated membrane containing either ADP or EPI. The time needed for occluding this aperture by plug formation is called the closure time (CT). A specific type of citrated whole blood has to be prepared for analysis within 0.5 to 4.0 hours after collection. In addition, duplicate measurements are necessary, and results differ between blood samples collected in the morning compared to those obtained in the afternoon.

Human Model: Cases with suspected mild bleeding disorders ($n = 43$; and $n = 70$ normal healthy reference controls).

Interpretation and Analysis: The reference range for the PFA-100 is approximately 82–159 seconds for EPI/closure time (EPI-CT) and 62.5–120.5 seconds for ADP/closure time (ADP-CT). Duplicate analyses reveal a mean coefficient of variations of 7.1% (EPI-CT) and 5.7% (ADP-CT). The EPI- and ADP-CT for blood samples collected during evening hours are significantly longer ($p = 0.002$ and $p = 0.004$, respectively) than the CT for blood samples collected in the morning. Acetylsalicylic acid (100 mg, 300 mg, or 500 mg) administered as a single dose or daily on 10 consecutive days results in prolongation of the EPI-CT, whereas the ADP-CT is not affected. EPI-CT is more sensitive in detecting ASA ingestion than is bleeding time (BT) measurements. Sensitivity and specificity of the PFA-100 for detecting vWD are comparable to the results obtained with BT measurements. The PFA-100 is a simple and easy to use test for the investigation of primary hemostasis. The test procedures are sensitive to the extent that the test can detect ASA intake and vWD. Its use is preferable to BT determination because it is less invasive and more sensitive to abnormalities of primary hemostasis.

Evaluation of a Platelet Function Analyser (PFA-100) in patients with a bleeding tendency. Wuillemin WA, Gasser KM, Zeerleder SS, Lämmle B. Swiss Med Wkly 2002 Aug 10; 132 (31–32), pp. 443–8.

Aggregation of Platelets
Adenosine Diphosphate (ADP)/Collagen Monomer/Epinephrine/Thromboxane (TXA_2)

Cellular/Molecular Mechanism of Action: During the process of platelet activation, ADP is released from intracellular granules (platelet degranulation) and increased amounts of thromboxane (TXA_2) are produced. Exposure of non-activated platelets to ADP promotes platelet activation and platelet aggregation.

Application: Detection of acquired, congenital, or hereditary forms of platelet dysfunction. Examples in this regard include nonsteroidal anti-inflammatory agents (e.g., NSAIDs) such as aspirin due to its ability to irreversibly inactivate cyclooxygnease through direct enzyme acetylation.

Methodology: Enriched preparations of platelets are initially harvested from fresh whole blood collected into sodium citrate or acid-citrate-dextrose anticoagulant (9:1 to 5:1 v/v) in the form of platelet rich plasma following a brief period of low-speed centrifugation. The platelet rich plasma is then harvested after the formation of a leukocyte buffy coat layer on top of the red blood cell layer. Either the platelet rich plasma itself can be used directly in the evaluation of aggregation potential or they can be recentrifuged at low speed to obtain a platelet pellet that can in turn be suspended in a smaller volume of plasma in order to obtain a more concentrated platelet preparation. Alliquots of the platelet rich plasma are pipetted into the wells of a pletelet aggregometer and an activating agent such as ADP are added to promote platelet aggregation. Based on a simple light detection sensor, the amount of transmitted light through the activated platelet rich plasma increases as more and more platelet aggregates are forms. The slope of the reaction curve (light-transmitteance as a function of time) is a reflection of the rate of platelet aggregation and the plateau of the reaction curve represents the degree of total platelet aggregation.

Considerations: Various therapeutic pharmaceuticals can interfer with attempts to diagnose acquired, congenital or hereditary forms of dysfunctional platelet aggregation .

Interpretation and Analysis: Decreases in the steepness of the slope or plateau of the platelet aggregation curve indicate the presence of diminished rate of platelet aggregation (aggretatory dysfunction).

Instrumentation: Platelet aggregometer integrated to either a paper tracing system or computer display.

Aggregation of Platelets: General Function/Device Implantation Evaluation/Pharmaceutical Efficacy/Sequelae Evaluation
Adenosine Diphosphate (ADP)

Cellular/Molecular Mechanism of Action: Adenosine diphosphate promotes the activation of platelets (degranulation, release of ADP, calcium, reversible aggregation, irreversible aggregation, alterations in the expression of suface membrane receptor complexes).

Application: Evaluation of platelet and coagulation function following device implantation or characterization of pharmaceutical efficacy or unexpected sequelae.

Route of Administration: Usually applied in *ex vivo* cell preparations where it is pipetted directly into a suspension and the degree of platelet aggregation measured using various types of instrumentation (e.g., change in light-impedence in a platelet aggregometer well).

Implementation: Evaluation of platelet and coagulation function in 4 different animal species utilizing a clot signature analyzer (CSA). Clot signature analyzer is unique in that it simultaneously measures global platelet and coagulation function under flow using whole blood.

No anticoagulant, chemical, or immunologic agent is required. Three CSA parameters are measurable: platelet mediated hemostasis time (PHT), collagen-induced thrombus formation time (CITF), and clotting time (CT). Bovine, ovine, and canine animal models are appropriate since these species are most frequently used in *in vivo* testing of cardiovascular implants. These parameters, as well as data from whole blood platelet aggregometry (commonly used for platelet function studies because of the response to exogenous agonists), and platelet counts from these animals, are then measured and compared with values observed in humans.

Interpretation and Analysis: In all 3 parameters, the canine species shows distinctively shorter time values than other species, including humans, suggesting that the dog is not an ideal animal model for the evaluation of blood-surface interactions. Ovine and human blood show similar PHT and CT values, but CITF time values are significantly shorter in sheep than in humans. With bovine blood, PHT is most prolonged among the 4 species compared. CT and CITF times in calves are shorter than those in humans, although the difference in CITF time is not statistically significant. Adenosine diphosphate–induced platelet aggregation shows the same order of responsiveness in 4 species as does CITF. It has been observed that the intermeasurement variation is rather high for CSA parameters, especially in PHT, warranting caution when this parameter is used to study time-dependent changes after device implantation.

Evaluation of platelet and coagulation function in different animal species using the xylum clot signature analyzer. Sato M, Harasaki H. ASAIO J 2002 Jul–Aug; 48 (4), pp. 360–4.

Arterial Disease: Platelet Function/Peripheral Arterial Disease (PAD) of the Lower Limb/Carotic Artery Disease/Abdominal Aortic Aneurysm (AAA)
Adenosine Diphosphate (ADP)/Collagen

Cellular/Molecular Mechanism of Action: Adenosine diphosphate is released by platelets during activation phenomenon and promotes activation/aggregation of nearby platelets. Soluble collagen binds membrane-associated receptors expressed by platelets and promotes aggregatory responses.

Background Information: Conditions of PAD are associated with a 3-fold increase in cardiovascular mortality. Standard antiplatelet treatments may not confer uniform benefit in different patient groups.

Application: Detection and chacterization of arterial disease related to alterations in platelet function.

Implementation: Compare platelet function in disease states of lower limb PAD carotid disease and AAA. One viable investigative approach in this regard involves the evaluation of PAD as a function of spontaneous platelet aggregatory (SPA) properties assessed in whole-blood samples applying ADP and collagen to induce aggregation responses. Flow cytometry analyses can also be used to detect platelet P-selectin and the PAC-1 antigen as markers of platelet activation and aggregation.

Human Model: Cases of PAD of the lower limbs ($n = 20$); carotid artery disease ($n = 40$); AAA ($n = 13$); and age-/sex-matched healthy controls ($n = 20$).

Interpretation and Analysis: Conditions of lower limb PAD or AAA have higher baseline SPA compared to normal controls ($p < 0.01$). There is significantly higher collagen-induced aggregation in IC patients compared to normal controls ($p < 0.01$). However, there is no difference in ADP-induced aggregation between lower limb PAD and control patients. There is no difference in PAC-1 binding between control patients and the patients with lower limb PAD, carotid disease, or AAA. Conditions of carotid artery disease express higher levels of P-selectin compared to normal controls ($p < 0.05$).

Evidence therefore exists that suggests platelet hyperactivity is present in conditions of PAD despite the use of antiplatelet therapy. Further antiplatelet strategies may be indicated for the therapeutic management of such disease states.

Instrumentation: Flow cytometry instrumentation.

Increased platelet aggregation and activation in peripheral arterial disease. Robless PA, Okonko D, Lintott P, Mansfield AO, Mikhailidis DP, Stansby GP. Eur J Vasc Endovasc Surg 2003 Jan; 25 (1), pp. 16–22.

Cardiovascular Disease Risk Indicator/Aggregation of Platelets
Garlic Extract (Aged)/(Adenosine Diphosphate [ADP]/Collagen/Epinephrine)/Platelet Adhesion (Collagen/Fibrinogen/von Willebrand's Factor)

Physiological Mechanism of Action: Aged garlic extract (AGE) has previously been shown to have moderate cholesterol-lowering and blood pressure–reducing effects.

Route of Administration: Aged garlic extract administered orally (per os).

Dosage: Aged garlic extract between 2.4 and 7.2 grams/day.

Laboratory Analysis: Measurement of platelet aggregation and adhesion performed at 2-week intervals reveals moderated elevations in the threshold concentrations for epinephrine and collagen compared to preadministration baseline values and individuals receiving a placebo. Only at the highest supplementation level does AGE show a slight increase in the threshold level of ADP-induced aggregation. Platelet adhesion to collagen, fibrinogen, and von Willebrand's factor can also serve as a diagnostic aid in detecting AGE-induced changes in platelet function. Adhesion properties can be determined by perfusing whole blood through a laminar flow chamber under controlled flow conditions. Adherence of platelets is inhibited by AGE in a dose-dependent manner when collagen is applied as an adhesive surface and perfused at low shear rates (approximately 30 s-1). At high shear rates (1,200 s-1), AGE also inhibits platelet adhesion to collagen but only at higher intake levels of the extract. Adhesion to von Willebrand's factor is reduced only at 7.2 g/d AGE, but adherence to fibrinogen is potently inhibited at all levels of supplementation.

Interpretation: Preparations of AGE selectively inhibit platelet aggregation and adhesion. Each of these is a platelet function that may be important for the development of cardiovascular events such as myocardial infarction and ischemic stroke.

Aged garlic extract, a modulator of cardiovascular risk factors: A dose-finding study on the effects of AGE on platelet functions. Steiner M, Li W. J Nutr 2001 Mar; 131 (3s), pp. 980S–4S.

HEMATOPOIETIC: RED BLOOD CELLS (*EX VIVO*)

Anemia: Fanconi's Syndrome
Nitrogen Mustard (NTM)/[Diepoxybutane (DEB)]

Cellular/Molecular Mechanism of Action: Nitrogen mustards and DEB can function as DNA alkylating agents.
Background Information: Fanconi's anemia (FA) is an autosomal recessive inherited disorder that is associated with a variety of congenital anomalies. These include morphometric abnormalities involving mainly the head and face, skeletal malformations particularly of the radial ray, growth retardation, abnormal skin pigmentation, deafness, and renal, ocular, genital, and cardiac defects. The cardinal clinical feature is a severe progressive pancytopenia.
Application: Detection and characterization of Fanconi's syndrome anemia.
Implementation: Evaluate the potential to detect conditions of FA rapidly and accurately applying alkylating agents as components of a screening methodology. In this context, NTM and DEB tests are performed in concert with the determination of baseline chromosomal breakage analyses.
Human Model: Cases screened for evidence of FA (n = 271).
Interpretation and Analysis: Chromosomal breakage analyses can facilitate the detection of cases with FA (e.g., n = 72) in contrast to cases unaffected by the condition (e.g., n = 136). Studies of family members of affected FA cases (n = 63 individuals) contribute to the conclusion that NTM seems more specific for identifying chromosomal breakages in FA parents than is possible with DEB.

Differential diagnosis of Fanconi's anemia by nitrogen mustard and diepoxybutane. Deviren A, Yalman N, Hacihanefioglu S. Ann Hematol 2003 Apr; 82 (4), pp. 223–7.

Organophosphate Intoxication: Red Blood Cells
Acetylcholine Analogs

Molecular Mechanism of Action: Organophosphate insecticides inhibit the biochemical activity of acetylcholine esterase. Red blood cells contain a substantial amount of acetylcholine esterase.
Application: Detection of organophosphate exposure.
Implementation: Evaluation of erythrocytes (RBC) acetylcholine esterase concentrations using acetylcholine analogs as a substrate diagnostic agent.
Interpretation and Analysis: Exposure to organophosphate insecticides is suggested by the detection of suboptimal enzymatic degradation of the test substrate reagent.

Stress-Susceptible and Stress-Resistant Swine: Red Blood Cells (RBCs)
Hydrogen Peroxide

Physiological Mechanism of Action: In part, hydrogen peroxide imposes a degree of oxidative stress on RBC populations.
Application: Detection of stress-susceptible swine.
Route of Administration: *In vitro* incubation of RBCs.
Implementation: Evaluation of erythrocyte (RBC) generation of by-products from lipid peroxidation (measured as thiobarbituric acid-reactive substances [TBARS]) in response to incubation in hydrogen peroxide formulations.
Animal Model: Swine (stress-resistant and stress-susceptible).
Interpretation and Analysis: When incubated with solutions of hydrogen peroxide, erythrocytes of stress-susceptible pigs produce more by-products of lipid peroxidation than do erythrocytes from stress-resistant pigs. Using this technique, discrimination between the 2 swine types is absolute at hydrogen peroxide concentrations of 0.9 and 1.5%. Such findings are in contrast to other methods of identifying stress-susceptible pigs, such as osmotically induced erythrocyte lysis and determination of plasma pyruvate kinase and creatine kinase activities, for which considerable overlap of data can be observed between the 2 swine types. Increased TBARS production by erythrocytes is further evidence for the existence of an antioxidant abnormality in stress-susceptible pigs. However, because there are no discernible differences in the major blood antioxidant-related values between stress-susceptible and stress-resistant pigs, the nature of the defect remains unclear. The production of TBARS by erythrocytes when incubated with hydrogen peroxide provides an improved method for identifying stress-susceptible pigs.

MUSCULOSKELETAL: *EX VIVO*

Malignant Hyperthermia (MH)
Caffeine/Diffusible CO_2

Background Information: Susceptibility to MH can be diagnosed applying a contracture screening test, which requires an open muscle biopsy sample.
Route of Administration: Caffeine (intramuscular).
Dosage: Caffeine (500 μl of an 80 mmol/L formulation).
Implementation: Evaluate the influence of intramuscular caffeine injection on local carbon dioxide pressure (pCO_2) in conditions of MH compared to normal negative reference controls. Measurements for pCO_2 are obtained from the rectus femoris muscle during local stimulation with injected caffeine.
Human Model: Cases susceptible to MH (n = 12) and cases classified as non-susceptible (n = 8) and 7 healthy/normal controls.
Interpretation and Analysis: In susceptible individuals, pCO_2 temporarily increases to a maximum of 63 mm Hg (IQR 61–69) compared with 44 mm Hg (43–47) for non-susceptible participants ($p = 0.003$) and 42 mm Hg (41–46) in controls ($p = 0.003$). Systemic side effects usually cannot be appreciated. Metabolic monitoring of the highly diffusible CO_2 stimulated by caffeine might allow for the development of a minimally invasive test for susceptibility to malignant hyperthermia.

Diagnosis of susceptibility to malignant hyperthermia by use of a metabolic test. Anetseder M, Hager M, Müller CR, Roewer N. Lancet 2002 May 4; 359 (9317), pp. 1579–80.

Malignant Hyperthermia (MH)
Caffeine/Halothane/Calcium-Induced Calcium Release (CICR)

Application: Detection and characterization of MH conditions.
Route of Administration: Topical application (emersion) of muscle biopsy specimens.
Implementation: Comparison of results of the *in vitro* caffeine-halothane contracture test (CHCT) according to the protocols of the North American Malignant Hyperthermia Group (NAMHG) and the European Malignant Hyperthermia Group (EMHG) with the CICR rate test. Muscle biopsies are usually obtained from the musculus vastus lateralis. Diagnostic cutoff points and procedures for CHCT protocols are as described in the original and renewal versions of NAMHG and EMHG. The CICR rate test can be performed according to previously reported protocols.
Human Model: Cases of suspected MH (n = 16; n = 5 normal negative reference controls).

Interpretation and Analysis: All 5 normal controls (e.g., n = 5), and some cases with abortive MH (e.g., n = 2), postoperative hyperthermia (n = 2), and high serum creatine kinase levels (e.g., n = 3) may be observed to have normal values for each of the 3 tests. Patients with MH reactions (e.g., n = 3) or with a history of masseter spasm (e.g., n = 1) may be classified as MH-positive according to NAMHG criteria and MH-susceptible and MH-equivocal according to EMGH criteria. Some cases may display discordant findings between CHCT and CICR rate test results (e.g., n = 5). It has therefore been proposed that a muscle biopsy for diagnosis of MH susceptibility should be used (combine the CHCT with the CICR rate test), which may identify the defective site of Ca^{++} release channels.

Comparison of the *in vitro* caffeine-halothane contracture test with the Ca-induced Ca release rate test in patients suspected of having malignant hyperthermia susceptibility. Oku S, Mukaida K, Nosaka S, Sai Y, Maehara Y, Yuge O. J Anesth 2000 Jan 20; 14 (1), pp. 6–13.

Malignant Hyperthermia (MH) *4-Chloro-m-Cresol (4-CmC)/Caffeine/Halothane*

Background Information: The *in vitro* contracture test (IVCT) for diagnosis of MH in the laboratory has a sensitivity of 100% and a specificity of 93%. Results are equivocal in 10–15%, and supplementary tests may thus be required.
Application: The agents 4-chloro-*m*-cresol, caffeine, and halothane can be applied to detect and characterize conditions of MH.
Implementation: Evaluation of muscle tissue collected as a biopsy specimen applying an *in vitro* contracture test performed by using topical exposure to increasing concentrations of 4-CmC (25, 50, 75, 100, 150, and 200 μmol/l). The corresponding force profiles are recorded as an objective measure of 4-CmC effectiveness. Diagnosis of MH susceptibility can be established with standard halothane and caffeine tests and classified as MHS (MH-susceptible), MHN (MH-negative), or MHE (equivocal result).

Human Model: Cases subjected to skeletal muscle biopsy (n = 41 from n = 7 families).
Interpretation and Analysis: At all concentrations of 4-CmC, the increase in baseline force is significantly greater in the MHS group compared to the MHN group ($p < 0.05$). Muscle from MH-susceptible cases (e.g., n = 15) responds to 4-CmC with increasing force at a threshold concentration of 75 μmol/l or less, whereas muscle tissues from cases classified as MH-non-susceptible (e.g., n = 23) have thresholds of 100 μmol/l or more. The accuracy of the chlorocresol test is approximately 100% (95% confidence limits 90.75–100%) at a threshold of 75 μmol/l. Amplitude of contractures at 2 mmol/l caffeine is not different from contractures at 75 μmol/l of 4-CmC in either the MHS or the MHN group ($p > 0.05$). *In vivo* concentrations of chlorocresol from clinical use of insulin and somatropin are estimated to be 20 times less than the threshold concentration and thus these drugs seem safe in MH cases. Therefore, 4-CmC may be a suitable aid to clarify puzzling results of standard testing of MH susceptibility.

4-Chloro-m-Cresol test—A possible supplementary test for diagnosis of malignant hyperthermia susceptibility. Ording H, Glahn K, Gardi T, Fagerlund T, Bendixen D. Acta Anaesthesiol Scand 1997 Sep; 41 (8), pp. 967–72.

Malignant Hyperthermia (MH) *Midazolam/Caffeine/Halothane*

Cellular/Molecular Mechanism of Action: Midazolam (water-soluble benzodiazepine).
Application: Premedicant for induction of anesthesia, but has occasionally been investigated to determine functionality as a screening agent for patients susceptible to the development of malignant hyperthermia. Midazolam is not an effective diagnostic pharmaceutical for the screening of patients susceptible to the development of malignant hyperthermia.
Dosage: Midazolam (ca 0.5 μg/ml.
Considerations: Midazolam has been applied to determine its influence on the resting tension of directly stimulated muscle biopsies. Interactions between midazolam and the 2 most commonly used MH diagnostic agents (halothane and caffeine) have also been compared.
Interpretation and Analysis: Midazolam, at maximum therapeutic concentrations (ca 0.5 μg/ml), has no detectable effects on muscle contraction in control and MH preparations. However, midazolam does elicit a contracture from control and MH-positive preparations when used within a concentration range between 160 and 1,280 μg/ml. There are no significant differences between control and MH-positive patients in minimum concentration of midazolam causing contracture or the strength of contracture at the respective eliciting concentration. There appears to be no interaction between midazolam and either halothane or caffeine on the resting tension of the directly stimulated muscle twitch preparation.

Effects of midazolam on directly stimulated muscle biopsies from control and malignant hyperthermia positive patients. Fletcher JE, Rosenberg H, Hilf M. Can Anaesth Soc J 1984 Jul; 31 (4), pp. 377–81.

Malignant Hyperthermia (MH)/Genomic Analyses and Provocative Testing Correlation/Arginine 614 → Cysteine (Arg614Cys: RYR1 C1840T) *Caffeine/Halothane*

Physiological Mechanism of Action: In conditions of MH, exposure of muscle tissue to caffeine/halothane promotes an exaggerated contractile response.
Background Information: Malignant hyperthermia is an important cause of anesthesia-induced death. The caffeine/halothane contracture test (CHCT) is highly invasive, expensive, and lacks 100% specificity. Genetic and biochemical evidence provide strong support for the view that the substitution of cysteine for arginine 614 (Arg614Cys) in the human ryanodine receptor gene is 1 of several mutations that are likely to cause human MH.
Application: Detection and characterization of MH conditions.
Route of Administration: Traditionally applied as a topical exposure for an *ex vivo* tissue-based challenge study.
Implementation: Delineation of a possible genetic basis for a predisposition to MH pertaining to the presence of Arg614Cys (RYR1 C1840T) mutations detected through a combination of polymerase chain reaction and restriction endonuclease digestion. Blood samples are utilized as a source of biological material for DNA analyses. Alternatively, detection of MH susceptibility can be established using the *in vitro* CHCT that employs fresh muscle biopsy specimens. Results from CHCT and DNA-based diagnostic parameters are then compared to determine their potential value in predicting MH susceptibility in large MH families that possess Arg614Cys mutations.

Human Model: Cases selected from a large Mennonite malignant hyperthermia kindred identified by an index patient that died at age 45 years of an MH crisis after general anesthesia. Case population: N = 68 family members, n = 19/68 muscle biopsies, n = 1/681 documented crisis without biopsy. Family members can be classified as MH-susceptible or MH-normal on the basis of the CHCT.

Interpretation and Analysis: In some family cohorts, as high as 33% of cases can be found to be heterozygous for the Arg614Cys mutation (n = 22/68) and slightly less than one-fourth have prior positive CHCT results (e.g., n = 5/22), while a small percentage may have an MH crisis without a biopsy procedure. Utilizing DNA testing, it is possible to detect a relatively large number of cases within a related kindred that are normal for the allele (e.g., n = 44). Of these, slightly less than 25% can be classified as MH-normal while some may be determined to be MH-susceptible (e.g., n = 5) on the basis of CHCT evaluations. Based on the interpretation of the data obtained in earlier CHCT diagnoses, the condition of the muscle is poor, with no twitch for approximately 60% of cases of homozygous for the normal allele but originally classified as MH-susceptible (e.g., n = 3/5) and for a small percentage that are homozygous for the normal allele and originally classified as MH-normal. Observations from CHCT results performed for such individuals (e.g., n = 4) can often be considered invalid. The twitch response is good for a small number of cases (e.g., n = 2 remaining) that are homozygous for the normal allele but classified as MH-susceptible if contracture can be observed with appropriately low levels of both caffeine and halothane.

An absolute correlation between DNA test results and CHCT assignment can often not be established. Possible explanations for discordance are that the Arg614Cys mutation is not linked to MH, that a 2nd MH mutation is segregating in the family, or that there are errors in the CHCT. Because there is strong evidence supporting the causal nature of the Arg614Cys mutation, the discordant cases are not closely related within the pedigree as they would be if a 2nd MH mutation was segregated, and the observed CHCT not 100% accurate. It has therefore been proposed that the observed discordance between DNA test results and CHCT assignment in 1 example kindred results from 2 false-positive diagnoses by the CHCT.

Comparison of the segregation of the RYR1 C1840T mutation with segregation of the caffeine/halothane contracture test results for malignant hyperthermia susceptibility in a large Manitoba Mennonite family. Serfas KD, Bose D, Patel L, Wrogemann K, Phillips MS, MacLennan DH, Greenberg CR. Anesthesiology 1996 Feb; 84 (2), pp. 322–9.

Malignant Hyperthermia (MH): Calcium Release (Sarcoplastic Reticulum [SR])/Calcium-Induced Calcium Release (CICR)
Cerivastatin / Dantrolene and Related Analogs

Cellular/Molecular Mechanism of Action: Cerivastatin (HMG-CoA) reductase inhibitors, which promote Ca^{2+} release from the SR.
Physiological Mechanism of Action: Induced release of intracellular calcium from the sarcoplasmic reticulum in skeletal muscle myocytes.
Application: Evaluation and characterization of skeletal muscle function and calcium ion physiology.
Route of Administration: *Ex vivo* (topical).
Semi-Synthetic Methods: Molecular design of specific photoaffinity probes based on the structural modification of dantrolene. Examples include GIF-0082 and GIF-0276 possessing azido- and trifluoromethyldiazirinyl-benzyl groups, respectively, at the hydantoin moiety. [^{125}I]GIF-0082 and [^{125}I]GIF-0276 are synthesized by substituting a stannyl group with [^{125}I] in the corresponding phenylstannane precursors.

The general synthesis of dantrolene analogues with various substituents on its phenyl ring has been developed via palladium-catalyzed cross-coupling reactions, employing the Stille or Suzuki reaction, as the key step. The effects of synthesized analogues have been evaluated by 2 kinds of Ca^{2+} release modes from SR of mouse skeletal muscle fibers based on (i) the measurement of twitch contraction caused by the physiological Ca^{2+} release (PCR) of intact skeletal muscle, and (ii) the rate of CICR in saponin-treated skinned muscle fibers.
Implementation: Evaluation of skeletal muscle function and calcium ion physiology with potential applications relevant to MH. In this manner, they can be applied to capture and identify key molecules that regulate the release of Ca^{2+} cation from the SR of skeletal muscle.

Animal Model: Rat (intact skeletal muscle and saponin-treated skinned muscle fibers).
Interpretation (Cerivastatin): The rates of Ca^{2+} release are increased by cerivastatin over a wide range of Ca^{2+} concentrations. Cerivastatin-induced Ca^{2+} release in the presence of Ca^{2+} is affected by adenosine monophosphate, Mg^{2+}, and procaine in essentially the same way as for caffeine-induced Ca^{2+} release. The Ca^{2+}-uptake capacity of the SR is reduced after co-treatment with ryanodine and cerivastatin at pCa 6.0 to a much greater extent than with ryanodine alone. Thus, cerivastatin-induced Ca^{2+} release in the presence of Ca^{2+} must be a result of the activation of the CICR mechanism of the ryanodine receptor. However, even when CICR is maximally inhibited by Mg^{2+} and procaine, or in the practical absence of Ca^{2+} (pCa > 8), cerivastatin still causes Ca^{2+} release. These results indicate that cerivastatin causes Ca^{2+} release also by activating some other mechanism(s) in addition to the activation of CICR. Either or both of these effects might be related to its adverse effect, rhabdomyolysis.
Interpretation (Dantrolene and Related Analogs): Although dantrolene, a lead compound, inhibits both twitch contraction and CICR, some structurally modified analogues exhibit 1 or the other of these effects. The methoxy congener, GIF-0185, potently inhibits the twitch contraction without affecting the CICR, while GIF-0166 and GIF-0248, the ortho-nitro regioisomer and ortho, ortho-dinitro substituted analogues, respectively, doubly potentiate the CICR exclusively.

The photoaffinity dantrolene analogs, GIF-0082 and GIF-0276, have a highly selective inhibitory effect on PCR without affecting CICR. Successful realization of the sharp discrimination between PCR and CICR has led to the creation of [^{125}I]GIF-0082 and [^{125}I]GIF-0276, which can be synthesized by substituting a stannyl group with [^{125}I] in the corresponding phenylstannane precursors.

Ca^{2+}-releasing effect of cerivastatin on the sarcoplasmic reticulum of mouse and rat skeletal muscle fibers. Inoue R, Tanabe M, Kono K, Maruyama K, Ikemoto T, Endo M. J Pharmacol Sci 2003 Nov; 93 (3), pp. 279–88.

Dantrolene analogues revisited: General synthesis and specific functions capable of discriminating two kinds of Ca^{2+} release from sarcoplasmic reticulum of mouse skeletal muscle. Hosoya T, Aoyama H, Ikemoto T, Kihara Y, Hiramatsu T, Endo M, Suzuki M. Bioorg Med Chem 2003 Mar 6; 11 (5), pp. 663–73.

[^{125}I]-N-[(3-azido-5-iodo)benzyl]dantrolene and [^{125}I]-N-[[3-iodo-5-(3-trifluoromethyl-3H-diazirin-3-yl)]benzyl]dantrolene: Photoaffinity probes specific for the physiological Ca^{2+} release from sarcoplasmic reticulum of skeletal muscle. Hosoya T, Aoyama H, Ikemoto T, Hiramatsu T, Kihara Y, Endo M, Suzuki M. Bioorg Med Chem Lett 2002 Nov 18; 12 (22), pp. 3263–5.

Malignant Hyperthermia (MH): Genetic Predisposition/(*Ex Vivo* Preparation of Skeletal Muscle Biopsies)
Caffeine/Caffeine and Halothane

Application: Caffeine and caffeine/halothane combinations can be applied as investigative probes to characterize certain intrinsic properties of skeletal muscle tissues. In this context, caffeine and caffeine plus halothane can serve as indicator agents for the detection of individuals with a genetic predisposition to the development of MH (potentially fatal complication of general anesthesia).

Implementation: Skeletal muscle *ex vivo* preparations are exposed to caffeine or caffeine plus halothane and the subsequent induction of contracture responses is measured for comparison with negative reference controls.

Interpretation and Analysis: In confirmed or suspect cases of MH and their genetic relatives, the responsiveness of the muscle specimens can often vary over 2 orders of magnitude. Frequency distribution curves suggest that the variation does not represent a continuum but rather is represented as 3 or more clusters of functional variants. Muscle specimens from males are on average more responsive to caffeine than is skeletal muscle collected from female subjects. Correlations within father-son and brother-brother pairs indicate complete heritability of responsiveness. Interestingly, there appears to be a lack of correlation between induced muscle contraction of muscle biopsies collected from mother-daughter pairs. There is an intermediate correlation in father-daughter pairs. The sex difference in heritability could be due to gender-related modifying genes or due to secondary modification of the muscle response in females by sex-related, perhaps hormonal factors. Among the effects of age appeared to be poor development in early childhood of the potentiation of caffeine-induced contracture by halothane.

Pharmacogenetics of caffeine and caffeine-halothane contractures in biopsies of human skeletal muscle. Kalow W, Sharer S, Britt B. Pharmacogenetics 1991 Dec; 1 (3), pp. 126–35.

Malignant Hyperthermia (MH): Skeletal Muscle *Caffeine/Halothane/Haloperidol/Succinylcholine/Caffeine*

Cellular/Molecular Mechanism of Action: Succinylcholine is an analog of the neurotransmitter acetylcholine.

Physiological Mechanism of Action: Droperidol and haloperidol are butyrophenone neuroleptic agents that induce a state of quiescence and reduced motor activity. Ketamine is a dissociative anesthetic agent.

Application: Screening for conditions or a predisposition to the development of MH.

Route of Administration: Halthane (inhalation); succinylcholine (intravenous). Many of the agents used to screen for malignant hyperthermia can also be incubated with *ex vivo* skeletal muscle preparations.

Considerations: If any agent augments the contracture response to the MH triggering or diagnostic agents, then the agent is considered unsafe for use in MH-susceptible patients.

Implementation: Utilized in the *in vitro* diagnostic screening of muscle strips from human patients being diagnostically assessed for malignant hyperthermia and muscle strips from the rat diaphragm where preparations are exposed to droperidol, haloperidol, or ketamine prior to challenge with halothane, succinylcholine, or caffeine (used to screen the MH potential of droperidol, heloperidol, ketamine).

Interpretation and Analysis: Droperidol (10 μmol/l) and ketamine (100 μmol/l) do not induce contractures in human or rat skeletal muscle when added alone, nor do they augment halothane, succinylcholine, or caffeine contractures. Therefore, these agents appear to be safe for use in patients susceptible to malignant hyperthermia. In contrast, haloperidol (10 μmol/l) augments the response to succinylcholine about 1.5-fold and may be contraindicated in MH-susceptible cases.

Effects of droperidol, haloperidol and ketamine on halothane, succinylcholine and caffeine contractures: Implications for malignant hyperthermia. Fletcher JE, Rosenberg H, Lizzo FH. Acta Anaesthesiol Scand 1989 Apr; 33 (3), pp. 187–92.

Rhabdomyolyis: Recurrent/Exertional (Equine)/Myoplasmic Calcium Concentration Estimation/Heritable Myopathy *Caffeine/4-Chloro-m-Cresol (4-CmC)*

Physiological Mechanism of Action: Caffeine promotes contraction of skeletal muscle tissue when exposed as an *in vitro* preparation.

Route of Administration: Topical (*in vitro*) bathing of muscle tissue biopsy specimens.

Implementation: Delineation of alterations in myoplasmic calcium regulation can be identified in muscle cell cultures (myotubes) and intact muscle fiber bundles derived from Thoroughbreds affected with recurrent exertional rhabdomyolysis (RER). Laboratory procedures that can be employed to address such an objective employ myotube cell cultures grown from satellite cells obtained from muscle biopsy specimens from horses affected by RER. Fura-2 fluorescence can be used to measure resting myoplasmic calcium concentration as well as caffeine- and 4-CmC-induced increases in myoplasmic calcium. In addition, intact intercostal muscle fiber bundles are prepared from both types of horses, and their sensitivities to caffeine and 4-CmC-induced contractures determined.

Animal Model: Equine cases of RER (n = 6 related Thoroughbreds with RER; n = 8 clinically normal control Thoroughbred or crossbred horses).

Interpretation and Analysis: Myotubes of RER-affected and control horses have identical resting myoplasmic calcium concentrations. Myotubes from RER-affected horses have significantly higher myoplasmic calcium concentrations than myotubes from control horses following the addition of ≥ 2mM caffeine; however, there is no difference in their response to 4-CmC (≥ 1 mM). Caffeine contracture thresholds for RER and control intact muscle cell bundles (2 vs 10mM, respectively) are significantly different, but 4-CmC contracture thresholds of muscle bundles from RER-affected and control horses (500 μM) do not differ. An increase in caffeine sensitivity of muscle cells derived from a family of related RER-affected horses has been detected *in vitro* by use of cell culture with calcium imaging and by use of fiber bundle contractility techniques. An alteration in muscle cell calcium regulation is a primary factor in the cause of this type of heritable myopathy.

Myoplasmic calcium regulation in myotubes from horses with recurrent exertional rhabdomyolysis. Lentz LR, Valberg SJ, Herold LV, Onan GW, Mickelson JR, Gallant EM. Am J Vet Res 2002 Dec; 63 (12), pp. 1724–31.

NEOPLASTIC DISEASE: *EX VIVO*

Somatostatin Receptor Complexes: Neoplastic Disease/Neuroendocrine Cancers/Gliomas/Mammary Carcinoma/Melanoma/Small Cell Lung Cancer *[^{99m}Tc]-Tricine-(HYNIC-TOC)/[^{99m}Tc]-Ethylenediaminediacetic Acid-(HYNIC-TOC)/[^{99m}Tc]-Tricine-Nicotinic acid-(HYNIC-TOC)/[^{111}In]-Diethylenetriaminepentaacetic Acid (DTPA)-Octreotide/[^{111}In]-DTPA-D-Phe1-Octreotide*

Cellular/Molecular Mechanism of Action: Recognizes and physically binds to somatostatin receptor complexes.

Background Information: Indium-111-labeled DTPA-D-Phe1-octreotide (DTPA-OC, OctreoScan) has been introduced into clinical routine for the detection of somatostatin receptor (SSTR)-positive tumors, which are predominantly of neuroendocrine origin. Potential applications also exist for other SSTR-positive cancers (e.g., small cell lung cancer, breast cancer, melanoma), but such evaluations

have been limited mainly due to restricted availability and high radionuclide costs. Previous attempts to introduce [^{99m}technetium]-labeled analogues of octreotide have not been entirely successful in terms of developing effective labeling procedure, variations in *in vivo* biodistribution patterns, and/or tumor detection capabilities.

Application: Early, rapid imaging-based diagnosis of somatostatin receptor positive neoplastic lesions.

Route of Administration: [^{99m}Tc]-HYNIC-D-Phe1-Tyr3-octreotide and [^{111}In]-DTPA-octreotide (intravascular).

Semi-Synthetic Methods: Production of high specific activity [^{99m}Tc]-radiolabeled hydrazinonicotinyl-Tyr3-octreotide (HYNIC-TOC) activities using tricine, ethylenediaminediacetic acid (EDDA), and tricine-nicotinic acid as co-ligand systems.

Implementation: Evaluation of the *in vitro* properties and *in vivo* biodistribution of [^{99m}Tc]-tricine-HYNIC-TOC, [^{99m}Tc]-EDDA-HYNIC-TOC, and [^{99m}Tc]-tricine-nicotinic acid-HYNIC-TOC compared to [^{111}In]-DTPA-octreotide. Receptor binding analyses can be performed using AR42J rat pancreatic tumor cell membranes. Complementary investigations can involve the characterization of internalization processes and protein binding studies, in addition to establishing *in vivo* biodistribution within pancreatic tumors.

***Ex Vivo* Model:** Rat AR42J pancreatic tumor cell membrane receptor binding analyses.

Animal Model: Murine (AR42J pancreatic tumor-bearing nude mice).

Interpretation and Analysis: The [^{99m}Tc]-labeled HYNIC peptides possess retained somatostatin-receptor binding affinities (Kd $<$ 2.65 nM). Protein binding and internalization rates are dependent on the co-ligand used. Specific tumor uptake between 5.8 and 9.6% of the injected dose per gram (%ID/g) can be appreciated for [^{99m}Tc]-labeled peptides, compared with 4.3 %ID/g for [^{111}In]-DTPA-octreotide. Tricine applied as a co-ligand shows higher activity levels in muscle, blood, and liver, whereas tricine-nicotinic acid produces significant levels of activity in the gastrointestinal tract. Ethylenediaminediacetic acid displays the most promising overall biodistribution profile, with tumor-to-liver and tumor-to-gastrointestinal tract ratios similar to those obtained with [^{111}In]-DTPA-octreotide, lower ratios in blood and muscle, but considerably higher tumor-to-kidney ratios. Molecular TOC can be radiolabeled to high specific activities using HYNIC as a bifunctional chelator. The high specific tumor uptake, rapid blood clearance, and predominantly renal excretion make [^{99m}Tc]-EDDA-HYNIC-TOC a promising candidate for an alternative to [^{111}In]-DTPA-octreotide for tumor imaging.

^{99m}Tc-HYNIC-[Tyr3]-octreotide for imaging somatostatin-receptor-positive tumors: Preclinical evaluation and comparison with ^{111}In-octreotide. Decristoforo C, Melendez-Alafort L, Sosabowski JK, Mather SJ. J Nucl Med 2000 Jun; 41 (6), pp. 1114–9.

RESPIRATORY: *EX VIVO*

Immune Hypersensitivity: Allergic Rhinitis (Nasal Biopsy)/Type Th2 Cytokine Expression Responses/Interleukin (IL)-13 Expression
Grass Pollen Extract/Fluticasone Propionate

Cellular/Molecular Mechanism of Action: Grass pollen extract can function as an allergen in immunehypersensitivity reactions. Fluticasone proprionate can inhibit phospholipase A biochemical activity.

Physiological Mechanism of Action: Fluticasone proprionate can promote immunesuppressive responses and anti-inflammatory properties in part due to its ability to inhibit both protein synthesis and leukocyte degranulation events.

Background Information: The allergen-induced late nasal response (LNR) is associated with high expression of IL-4 and IL-5 messenger RNA (mRNA) in the nasal mucosa, suggesting a role for Th2-type cytokines in the development of the LNR. Moreover, topical corticosteroid-mediated inhibition of the LNR is accompanied by inhibition of IL-4, but not IL-5, mRNA expression. Interleukin-13 shares a number of functions with IL-4, including IgE switching and vascular cell adhesion molecule-1 (VCAM-1) upregulation.

Application: Grass pollen extract can be used to initiate inflammatory and immune responses in conditions of hyperactive immune responses associated with this source of allergen. Fluticasone can be used as a probe to study alterations in the production of endogenous mediators of inflammatory and immune responses to allergen-induced responses.

Implementation: Evaluate the influence of fluticasone propionate on the expression of IL-4 and IL-13 mRNA sequences and immunoreactivity in nasal mucosa tissue biopsy specimens. Design of investigations can involve administration of either topical or inhaled fluticasone propionate in conditions of allergic rhinitis. Nasal biopsies are harvested before treatment and 24 hours after local nasal allergen provocation with a grass pollen extract.

Human Model: Nasal biopsy tissue specimens in conditions of allergic rhinitis (n = 20; n = 10 normal healthy reference controls; n = 10/20 inhaled fluticasone propionate; n = 10/20 topical fluticasone propionate BID for 6 weeks).

Interpretation and Analysis: No significant difference between the allergic rhinitis cases and controls in the expression of IL-13 mRNA and immunoreactivity can be detected prior to treatment.

After allergen provocation/challenge, significant increases in IL-13 mRNA-positive and immunoreactive cells at 24 hours can be detected in the absence of corticosteroid exposure (placebo $p < 0.001$). Inhibition of the LNR after corticosteroid treatment is associated with a marked decrease in allergen-induced IL-13 mRNA-positive ($p < 0.001$) and immunoreactive cells ($p < 0.001$). In the absence of steroid exposure (e.g., placebo), 76.9 + 5.5% of IL-13 mRNA-positive cells after allergen are CD3+, whereas 11.2 ± 2.7% co-express immunoreactivity for mast-cell tryptase. In these cases, increases in cells expressing IL-13 mRNA are greater than for IL-4 mRNA ($p = 0.001$), and double *in situ* hybridization studies reveal that 100% of the IL-4 mRNA-positive cells co-express IL-13 mRNA, whereas 66.6 + 10.5% of IL-13 mRNA-positive cells co-express IL-4 transcripts after allergen challenge. Such findings suggest that IL-13 expression is a prominent feature of the LNR, and that inhibition of the LNR following steroid therapy may be partly attributable to inhibition of IL-13 expression.

IL-13 mRNA and immunoreactivity in allergen-induced rhinitis: Comparison with IL-4 expression and modulation by topical glucocorticoid therapy. Ghaffar O, Laberge S, Jacobson MR, Lowhagen O, Rak S, Durham SR, Hamid Q. Am J Respir Cell Mol Biol 1997 Jul; 17 (1), pp. 17–24.

Immune Hypersensitivity: Asthma Alveolar Macrophages/Blood Monocytes/Septic Inflammatory Responses/Cytokine Responses: Interleukin (IL)-10, Macrophage Inflammatory Protein-1alpha (MIP-1α), Interferon-Gamma (IFN-γ), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)
IL-1β/Dexamethasone/Lipopolysaccharide (LPS)

Cellular/Molecular Mechanism of Action: Dexamethasone inhibits phospholipase A2 biochemical activity. Lipopolysaccharide is also referred to as bacterial endotoxin. Systemically, LPS can bind

to lipopolysaccharide-binding protein and membrane-associated LPS receptor complexes (CD14).

Physiological Mechanism of Action: Dexamethasone functions as a immunosuppressive agent due to its ability to inhibit normal protein synthesis and stabilization of cell membranes in a manner the inhibits cell activation and degranulation phenomenon. Bacterial lipopolysaccharide fractions (LPS, endotoxin) promote inflammatory responses in primarily leukocyte and endothelial cell populations. The cytokine IL-1β functions as an endogenous mediator of inflammation.

Application: Lipopolysaccharide (LPS, bacterial endotoxin) can be utilized to induce septic inflammatory responses. Dexamethasone can be used as a probe to study alterations in the production of endogenous mediators associated with inflammatory and immune responses during allergen-induced reactions.

Implementation: Evaluation of the effect of inhaled corticosteroid budesonide on the release of the anti-inflammatory cytokine IL-10 and of proinflammatory cytokines MIP-1α, IFN-γ, and GM-CSF from blood monocytes and alveolar macrophages.

Human Model: Cases of mild asthma used as donors for alveolar macrophages obtained by bronchoalveolar lavage at the end of each treatment phase (double blind, crossover, placebo-controlled study).

Interpretation and Analysis: Budesonide reduces bronchial hyperresponsiveness and improves baseline FEV_1. Alveolar macrophages obtained by bronchoalveolar lavage performed at the end of each treatment phase. Interleukin-10 from blood monocytes is not altered, but both IL-10 mRNA and protein expression from alveolar macrophages stimulated by lipopolysaccharide and IL-1β are increased after corticosteroid therapy. By contrast, alveolar macrophages release significantly less MIP-1α, IFN-γ, and GM-CSF after steroid challenge. In comparison to alveolar macrophages from normal non-asthmatic reference controls, those from cases of asthma release more MIP-1α, IFN-γ, and GM-CSF but lower amounts of IL-10, particularly at baseline and after IL-1β stimulation. The ability of steroids to inhibit proinflammatory cytokines but to enhance the anti-inflammatory cytokines such as IL-10 may contribute to their beneficial actions in asthma. Asthma is characterized by alveolar macrophages exhibiting both an enhanced capacity to release proinflammatory cytokines and a reduced capacity to produce IL-10.

Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein-1alpha, granulocyte-macrophage colony-stimulating factor, and interferon-gamma release from alveolar macrophages in asthma. John M, Lim S, Seybold J, Jose P, Robichaud A, O'Connor B, Barnes PJ, Chung KF. Am J Respir Crit Care Med 1998 Jan; 157 (1), pp. 256–62.

Immune Hypersensitivity: Bronchial Hyperresponsiveness (BHR)/Bronchial Lavage Fluid Eosinophils, Lymphocytes, and Neutrophils/Allergic Airway Eosinophilia Reactions/Type Th1 and Th2 Cytokine Expression Profiles

Ovalbumin/Dexamethasone/Cyclosporine

Cyclosporin A is an immunomodulatory agent that acts specifically at an early stage in the activation of T-lymphocytes. In this context, in contrast to azothioprine and other cytotoxic agents, cyclosporin suppresses T-lymphocyte mediated cellular immune responses without causing major effects on antibacterial defenses.

Cellular/Molecular Mechanism of Action: Ovalbumin is the avian equivalent of mammalian serum albumin and can be applied as an immunogenic protein fraction. Dexamethasone is a corticosteroid that has an ability to inhibit phospholipase A2 biochemical activity.

Physiological Mechanism of Action: Dexamethasone is a corticosteroid that promotes immunosuppressive properties in part due to inhibition of leukocyte degranulation phenomenon and general inhibition of protein synthesis.

Background Information: Cyclosporin A and dexamethasone exhibit different effects on allergic airway eosinophilia and BHR.

Application: Characterization of the molecular pathogenesis of normal and hypersensitivity immune responses including alteration of cytokine expression such as changes in the profile of Th1- vs Th2-derived cytokines. The agent can be applied as part of an investigative strategy to delineate the efficacy of various therapeutic agents possessing anti-inflammatory and immunosuppressive properties.

Implementation: Evaluation of the influence of cyclosporine or dexamethasone on the development of immune hypersensitivity reactions to ovalbumin aerosol challenge. Bronchial responsiveness is measured 18–24 hours after aerosol exposure. Airway cellular influx can be determined by bronchoalveolar lavage and tissue immunohistochemistry. Expression of Th1 and Th2 cytokine messenger RNA (mRNA) are then analyzed by reverse transcriptase-PCR.

Animal Model: Rat (Brown Norway rats sensitized to ovalbumin).

Interpretation and Analysis: Ovalbumin exposure induces significant BHR reactions, with increases in eosinophils, lymphocytes, and neutrophils in bronchoalveolar lavage fluid and an increase in eosinophils, CD2+ and CD8+, but not CD4+ T cells, in the airway submucosa. Interleukin-2, IFN-γ, IL-4, and IL-5 mRNA expression in ovalbumin-sensitized lungs increases ($p < 0.05$) compared with controls. Cyclosporin A does not significantly affect BHR and neutrophil accumulation but reduces the number of bronchoalveolar lavage eosinophils ($p < 0.002$), airway submucosal eosinophils, and CD4+ and CD8+ T cells ($p < 0.02$). It also suppresses the induction of mRNA expression of IL-2, IL-4, IL-5, and IFN-γ. By contrast, the inflammatory cell influx and mRNA expression of IL-2, IL-4, and IL-5, as well as BHR, is suppressed by dexamethasone. However, increases in IL-10 and IFN-γ mRNA expression can sometimes not be detected depending on the analytical system evaluated. The differential activities of cyclosporin A and dexamethasone on inflammatory cell influx, particularly neutrophils, or cytokine expression such as IL-10 and IFN-γ may underlie their contrasting effects on BHR.

Diagnostic pharmacology: Differential effect of corticosteroids and cyclosporin differential regulation of cytokine expression after allergen exposure of sensitized rats by cyclosporin A and corticosteroids: Relationship to bronchial hyperresponsiveness. Huang TJ, Newton R, Haddad EB, Chung KF. J Allergy Clin Immunol 1999 Sep; 104 (3 Pt 1), pp. 644–52.

Inflammatory Response: Nasal Mucosa Responses/Nasal Mucosa Cyclooxygenase (COX)-1 and COX-2 Synthesis

Dexamethasone/Interleukin (IL)-10/Actinomycin-D/Cyclohexamide/Proinflammatory Mediators (Interferon-Gamma [IFN-γ], IL-1β, and Tumor Necrosis Factor-Alpha [TNF-α])

Cellular/Molecular Mechanism of Action: Actinomycin-D intercalates between DNA strands within the DNA helix. Dexamethasone inhibits phospholipase A2 biochemical activity.

Physiological Mechanism of Action: Dexamethasone functions as an immunosuppressive and anti-inflammatory agent due to an ability to inhibit normal protein synthesis and stabilize cell membranes in a manner sufficient to inhibit cell activation and leukocyte

degranulation events. Actinomycin-D inhibits translation events while cyclohexamide inhibits transcription. Interleukin-10 exerts relatively profound anti-inflammatory properties.

Background Information: Cyclooxygenase converts arachidonic acid in prostanoids. Cyclooxygenase exists in 2 isoforms: COX-1 is the constitutive, whereas COX-2 is the inducible isoform. The regulation of COX-1 and COX-2 expression in nasal mucosa has not been extensively investigated.

Application: Characterization of the molecular pathogenesis of disease states, immune reactions/responses, and efficacy of various forms of therapeutic intervention. Dexamethasone, therefore, can be utilized as a probe for delineating the role of endogenous inflammatory mediators in immune responses and inflammatory reactions.

Implementation: Evaluation of alterations in endogenous COX-1 and COX-2 production *in vitro* in nasal biopsy tissues applying agents that promote proinflammatory and anti-inflammatory properties in addition to inhibitors of transcription and translation events as molecular probes. In this manner, it is possible to gain greater understanding about mechanisms involved in the expression and regulation of COX-1 and COX-2 by cytokines and corticosteroids. The specific design of such investigations can entail employing the use of cultured normal/healthy human nasal explants exposed/incubated with individual agents known to promote (i) proinflammatory properties (IFN-γ, IL-1β, and TNF-α); (ii) anti-inflammatory properties (dexamethasone, IL-10) and inhibitors of translation (actinomycin-D) and transcription (cycloheximide) events. Tissue culture preparations are subsequently analyzed for the detection of alterations in COX-1 and COX-2 expression by semi-quantitative competitive PCR and Western blot analyses.

Human *Ex Vivo* Model: Nasal mucosa biopsy tissues.

Interpretation and Analysis: Baseline expression of COX-2 mRNA is higher than COX-1 mRNA. In a tissue culture environment, there is a slight spontaneous upregulation of COX-1 and a strong COX-2 mRNA and protein upregulation. Incubation of nasal explants with proinflammatory cytokines increases the expression of COX-2 mRNA and protein, from 1 to 24 hours of incubation in a dose-related manner. Regulation of these effects occurs at the levels of both transcriptional and posttranscriptional phases. Dexamethasone and IL-10 abrogate cytokine-induced COX-2 mRNA and protein expression. Proinflammatory cytokines, dexamethasone and IL-10 have no effect on COX-1 mRNA expression. Prostanoids, therefore, appear to have an important regulatory effect on immunologically (cytokine)-mediated inflammatory responses.

Regulation of cyclooxygenase-1 and -2 expression in human nasal mucosa. Effects of cytokines and dexamethasone. Fernández-Morata JC, Mullol J, Fuentes M, Pujols L, Roca-Ferrer J, Pérez M, Xaubet A, Picado C. Clin Exp Allergy 2000 Sep; 30 (9), pp. 1275–84.

RANTES Production: Airway Smooth Muscle
Tumor Necrosis Factor-Alpha (TNF-α) and Interferon-Gamma (IFN-γ)/Interleukin (IL)-4, IL-10, and IL-13/Dexamethasone

Cellular/Molecular Mechanism of Action: RANTES is a basic 8-kDa polypeptide of the C-C chemokine subfamily that promotes strong chemotactic response activity in eosinophils, lymphocytes, and monocytes.

Implementation: Evaluation of mechanisms that regulate RANTES production by human airway smooth muscle cells in culture.

Interpretation and Analysis: While TNF-α, but not IFN-γ, increases RANTES mRNA expression and protein release, the combination of TNF-α and IFN-γ causes a greater degree of expression and release in a time- and dose-dependent manner. Sequential treatment of airway smooth muscle cells with TNF-α and IFN-γ reveals that IFN-γ sensitizes cells to the stimulatory effect of TNF-α. Using a modified Boyden chamber technique, RANTES separated by reverse-phase liquid chromatography from cell culture supernatants of airway smooth muscle cells stimulated by TNF-α and IFN-γ shows a strong chemoattractant effect on human eosinophils, an effect that is inhibited by an anti-RANTES antibody RANTES production induced by TNFα and IFN-γ is inhibited partly by the Th2-derived cytokines, IL-4, IL-10, and IL-13, as well as by dexamethasone. Studies indicate that, in addition to contractile responses and mitogenesis, airway smooth muscle cells have synthetic and secretory potential with the release of RANTES. They may participate in chronic airway inflammation by interacting with both Th1- and Th2-derived cytokines to modulate chemoattractant activity for eosinophils, activated T lymphocytes, and monocytes/macrophages.

Human airway smooth muscle cells express and release RANTES in response to T helper 1 cytokines: Regulation by T helper 2 cytokines and corticosteroids. John M, Hirst SJ, Jose PJ, Robichaud A, Berkman N, Witt C, Twort CH, Barnes PJ, Chung KF. J Immunol 1997 Feb 15; 158 (4), pp. 1841–7.

6 Hepatic System

BILIARY AND HEPATIC DIAGNOSTICS

BILIARY

Biliary Atresia (Infant): Jaundice/Frail and Compromised Patients
[^{131}I]-Rose Bengal (Fecal Excretion Test)/[^{99m}Tc]-Pyridoxylidene Glutamate (^{99m}Tc-PG)

Cellular/Molecular Mechanism of Action: Pyridoxylidene glutamate and rose bengal are both removed from the plasma by viable hepatocytes.
Application: Assess postoperative bile drainage with repeat scans in infants with biliary atresia. Scintigraphic imaging with [^{99m}Tc]-PG may also be useful as a screening test in difficult cases of jaundice, especially in frail patients, and cases with complications.
Route of Administration: Intravenous.
Implementation: Evaluation of hepatic function in cases of systemic jaundice applying [^{99m}Tc]-PG for nuclear scan imaging.
Human Model: Cases of systemic jaundice (n = 24 adults with mean total bilirubin = 255 μmol/l; n = 11 infants with prolonged obstructive jaundice and mean total bilirubin = 165 μmol/l).
Interpretation and Analysis: Absence of radioactivity in the gastrointestinal tract is interpreted as complete biliary obstruction and assumed to be the cause of jaundice (e.g., n = 10/24 adult and n = 6 infant jaundice cases). Using this criterion, occlusion or patency of the bile ducts can be correctly determined in a high percentage of cases (e.g., n = 21/24 adults; and n = 6/11 infants). A low percentage of jaundice cases display either false-negative gut images (e.g., n = 1/21 adults; and n = 2/11 infants) or inconclusive results (e.g., n = 2/24 adults; and n = 1/11 infants). Scans are unable to show details about the site of obstruction or pathology but the technique is simple, atraumatic, and safely performed in cases with serious complications (e.g., renal failure, coagulation defects, septicmia). In infants the [^{99m}Tc]-PG scan compares well with the [^{131}I]-rose bengal fecal excretion test and with liver biopsy in the investigation of prolonged obstructive jaundice.

Hepatobiliary imaging: The use of ^{99m}Tc-pyridoxylidene glutamate scanning in jaundiced adults and infants. Jenner RE, Howard ER, Clarke MB, Barrett JJ. Br J Radiol 1978 Nov; 51 (611), pp. 862–6.

Biliary Colic/(Sphincter of Oddi Spasm)
Codeine/(Ultrasound)

Background Information: Biliary colic from spasm of the sphincter of Oddi is difficult to diagnostically prove.
Application: Codeine may be a useful provocative agent and ultrasound measurements of changes in common bile duct diameter may provide a useful method in diagnostic approaches for the detection of sphincter of Oddi spasms.
Implementation: Biliary colic.
Interpretation and Analysis: Diagnosis can be established by (i) using a pharmacological provocative test employing codeine to reproduce pain; (ii) detection of changes in liver and pancreatic serum enzymes; and (iii) ultrasound imaging to demonstrate a transient increase in common bile duct diameter. Implemenation of such procedures prior to surgical sphincteroplasty reproduces symptoms and brings about marked elevations in pancreatic and liver enzyme fractions and increases in common bile duct diameter measured by ultrasound examination (e.g., 0.4 to 1.0 cm). Such events often cannot be appreciated after sphincteroplasty.

Ultrasound in the diagnosis of sphincter of Oddi spasm. Berezny GM, Beck IT, DaCosta LR, O'Brien PG, Paloschi GB. J Clin Gastroenterol 1985 Dec; 7 (6), pp. 528–32.

Biliary Dyskinesis/Cholecystography: Postevacuation
Kinevac/Sincalide/Secretin

Cellular/Molecular Mechanism of Action: Sincalide is a synthetic analog of cholecystokinin.
Physiological Mechanism of Action: Sincalide causes a prompt contraction of the gallbladder as compared to the stimulus of a fatty meal, which causes progressively more pronounced contractions that become maximal in about 40 minutes. Kinevac accelerates transit time through the lumen of the small intestine.
Application: Sincalide is used in postevacuation cholecystography. Kinevac, due to its physiological properties, can be applied to decrease the extent of radiation exposure associated with fluoroscopy and length of radiographic examinations of the intestinal tract.
Considerations: Serious reactions to sincalide have not been reported.
Implementation: Facilitation of duodenal fluid collection that is high in bile content.
Interpretation and Analysis: Duodenal aspirations obtained after the administration of Kinevac provide a sample of concentrated bile entirely suitable for analysis of cholesterol, bile salts, phospholipids, and crystals. When used in conjunction with secretin to stimulate pancreatic secretion, an aspirate can be readily obtained for analysis of enzyme activity, composition, and cytology. As the development of endoscopic manometry affords a modality to measure and record sphincter of Oddi pressures, the paradoxical responses noted to the intravenous administration of cholecystokinin (CCK) during manometric evaluation support the diagnostic value of Kinevac used as a provocative agent in the evaluation of biliary dyskinesia.

Kinevac (sincalide for injection)/Squibb Diagnostics. Maher KA. Gastroenterol Nurs 1991 Oct; 14 (2), pp. 98–100.

Biliary Tract: Sphincter of Oddi Spasms and Biliary Colic/Sphincter of Oddi Spasm Induction/ Ultrasound Imaging: Detection of Changes in Diameter
Codeine

Physiological Mechanism of Action: Codeine can promote alterations in the function of the sphincter of Oddi in conditions prone to spasms.
Background Information: Biliary colic from spasm of the sphincter of Oddi has been a difficult diagnosis to prove.
Application: Provocative test for the diagnosis of biliary colic due to spasms of the sphincter of Oddi in a manner that can be detected on ultrasound examination.
Implementation: Evaluation of conditions of possible biliary colic due to spasms of the sphincter of Oddi.
Interpretation and Analysis: Codeine can be applied as a valuable provocative testing agent for reproducing episodes of biliary colic (pain) due to spasms of the sphincter of Oddi. Affected cases may also promote changes in liver and pancreatic serum enzymes. A 2nd diagnostic benefit of codeine is its ability to demonstrate before surgical sphinteroplasty transient increases in common bile duct diameter that can be detected by ultrasound imaging (e.g., 0.4 cm → 1.0 cm). Such events cannot be recognized after successfully performing sphincteroplasty procedures. Codeine may be useful as a provocative testing agent, and ultrasound measurements of changes in common bile duct diameter may provide a useful method as a diagnostic approach for detecting sphincter of Oddi spasms.

Ultrasound in the diagnosis of sphincter of Oddi spasm. Berezny GM; Beck IT; DaCosta LR; O'Brien PG; Paloschi GB. J Clin Gastroenterol 1985 Dec; 7 (6), pp. 528–32.

HEPATOBILIARY

Gilbert's Syndrome (GS)/Hepatic Dysfunction
Rifampicin

Cellular/Molecular Mechanism of Action: Rifampicin is removed from the plasma, excreted into the bile, partially metabolized by gastrointestinal bacterial flora, and then absorbed within the small intestine (e.g., enterohepatic circulation route).
Background Information: Gilbert's syndrome is characterized by the existence of chronic mild unconjugated hyperbilirubinemia.
Application: Provocative test for GS but also causes increases in unconjugated bilirubin in some cases of chronic hepatic disease.
Dosage: Rifampicin (600 mg).
Implementation: Evaluation of the relative value of rifampicin as a provocative test for the diagnosis of GS compared to a fasting-based test. In the fasting test, subjects are given a 400-calorie/day diet for 24 hours; in the rifampicin test, unconjugated bilirubin levels are measured before and 4 hours after administration.

Human Model: Cases of GS (n = 22 GS; n = 15 chronic liver disease; n = 20 healthy reference controls). All cases evaluated with both provocative test procedures.
Interpretation and Analysis: Both tests achieve a significant increase in mean unconjugated bilirubin levels in patients with GS but not in the controls. The sensitivity and specificity of a rifampicin test in the diagnosis of GS is comparable with the fasting test. However, both tests cause a significant increase in unconjugated bilirubin levels in nearly half the patients with chronic liver disease. A rifampicin test may be used in the diagnosis of suspected GS instead of a fasting test, as it is simpler and more practical. However, its specificity for GS is not sufficient, because it also causes an increase in unconjugated bilirubin levels in some patients with chronic liver disease.

Rifampicin test in the diagnosis of Gilbert's syndrome. Erdil A, Kadayifci A, Ates Y, Bagci S, Uygun A, Dagalp K. Int J Clin Pract 2001 Mar; 55 (2), pp. 81–3.

Gilbert's Syndrome (GS)
Rifampicin/(Fasting Test Alternative)

Cellular/Molecular Mechanism of Action: Following oral ingestion, rifampicin undergoes multiple enterohepatic cycles.
Application: Gilbert's syndrome is characterized by the existence of chronic mild unconjugated hyperbilirubinemia. The specificity of the rifampicin test for GS is not sufficient, because it also causes an increase in unconjugated bilirubin levels in some patients with chronic liver disease.
Route of Administration: Oral (per os).
Dosage: Rifampicin 600 mg.
Implementation: Rifampicin and fasting tests can be applied to identify cases of GS. In the fasting test, cases are given a 400-calorie/day diet for 24 hours. Alternatively in the rifampicin test, unconjugated bilirubin levels are measured before and 4 hours after rifampicin (600 mg per os).
Interpretation and Analysis: Both tests achieve a significant increase in mean unconjugated bilirubin levels in patients with GS but not in normal subjects. The sensitivity and specificity of a rifampicin test in the diagnosis of GS are comparable with the fasting test. However, both tests cause a significant increase in unconjugated bilirubin levels in nearly half the patients with chronic liver disease. A rifampicin test may be used in the diagnosis of suspected GS instead of a fasting test, especially because it represents a simpler and more practical methodology.

The utility of rifampicin in diagnosing Gilbert's syndrome. Murthy GD, Byron D, Shoemaker D, Visweswaraiah H, Pasquale D. Am J Gastroenterol 2001 Apr; 96 (4), pp. 1150–4.

Hepatic Bile Acid Transport/Visual Detection and Monitoring
Lysyl Fluorescein Conjugated Bile Acid Analogues (LFCBAA)

Background Information: Lysyl fluorescein conjugated bile acid analogues closely parallel their natural counterparts.
Application: Lysyl fluorescein conjugated bile acid analogues can be applied as molecular tools or probes for visualizing bile acid transport within hepatic tissues.
Route of Administration: Intravenous or retrograde injection into the biliary tree.
Semi-Synthetic Methods: Conjugation of lysyl fluorescein to bile acid analogues.
Implementation: Physiological concentrations of the primary bile acid analogue cholyllysyl fluoroscein (CLF) and of the secondary bile acid analogue lithocholyllysyl fluorescein (LLF) are administered systemically, followed by the collection of serial liver biopsies at fixed timed intervals. Both compounds are also injected retrogradely into the biliary tree. Frozen sections are then examined by fluorescence microscopy.

Animal Model: Rat (Wistar).
Interpretation and Analysis: Both CLF and LLF are rapidly taken up from sinusoidal blood but differ significantly in their hepatic handling. Cholyllysyl fluoroscein is rapidly transported into the bile, whereas LLF transport is slower and produces significantly more bile duct fluorescence. Lithocholyllysyl fluorescein clearance involves a

lobular gradient with the last remaining bile acid being confined (largely zone 3). Both compounds are avidly taken up by cholangiocytes after intravenous or retrograde injection into the biliary tree. Visualization of LFCBAA by fluorescence microscopy may yield further information regarding hepatobiliary bile acid localization during studies designed to characterize physiological and pathological mechanisms involved in the transport of bile acids. The presence of both compounds within cholangiocytes strongly suggests that they may undergo a degree of chole-hepatic recirculation.

Instrumentation: Fluorescence microscopy.

Visualization of the transport of primary and secondary bile acids across liver tissue in rats: *In vivo* study with fluorescent bile acids. Milkiewicz P, Mills CO, Hubscher SG, Cardenas R, Cardenas T, Williams A, Elias E. J Hepatol. 2001 Jan; 34 (1), pp. 4–10.

HEPATIC BLOOD/PLASMA FLOW AND PERFUSION

Hepatic Cirrhosis: Alterations in Functional Blood Flow/Perfusion and Metabolic Capacity/Volume
D-Sorbitol

Cellular/Molecular Mechanism of Action: D-sorbitol is a carbohydrate molecule that is normally removed from the intravascular compartment by viable hepatocytes.

Application: Evaluation of the overall functional reserve of cirrhotic liver by assessing of hepatic functional blood flow, liver volume, Child-Pugh's classification, and discussion of its value for clinical application.

Implementation: Blood and urine samples are collected 4 times before and after intravenous D-sorbitol infusion. Hepatic clearance (CL_H) of D-sorbitol is then calculated according to an enzymatic spectrophotometric method while total blood flow (Q_{TOTAL}) and intrahepatic shunting (R_{INS}) can be detected by multicolor Doppler ultrasound, while liver volume is measured by spiral CT. Laboratory results are analyzed by t test, variance calculations, and chi^2 analysis. The relationships among all these parameters and different groups are then investigated according to Child-Pugh classification and postoperative complications, respectively.

Human Model: Cases of portal hypertension due to hepatic cirrhosis ($n = 92$ and all with a history of hematoemesis and hematochezia, esophageal and gastric fundus varices, splenomegaly and hypersplenia, with cases being followed during a 2-year monitoring period [$n = 22$ normal healthy volunteer controls]).

Interpretation and Analysis: Steady blood concentration is achieved 120 minutes after D-sorbitol intravenous infusion, which is (0.358 ± 0.064) mmol $\times L^{-1}$ in cirrhotic group and (0.189 ± 0.05) mmol $\times L^{-1}$ in control group ($p < 0.01$). $CL_H = (812.7 \pm 112.4)$ ml $\times$ min^{-1}, $Q_{TOTAL} = (1280.6 \pm 131.4)$ ml $\times$ min^{-1}, and $R_{INS} = (36.54 \pm 10.65)$ % in cirrhotic group and $CL_H = (1248.3 \pm 210.5)$ ml $\times$ min^{-1}, $Q_{TOTAL} = (1362.4 \pm 126.9)$ ml $\times$ min^{-1}, and $R_{INS} = (8.37 \pm 3.32)$ % in control group ($p < 0.01$). The liver volume of cirrhotic group is approximately 1057 ± 249 cm^3, 851 ± 148 cm^3, and 663 ± 77 cm^3 in Child A, B, and C groups, respectively, with significant difference ($p < 0.001$). The average volume of cirrhotic liver in Child B, C group is significantly reduced in comparison with that of control subjects ($p < 0.001$). Cases that have a decrease in liver volume of 40% and CLH below 600 ml $\times$ min^{-1} may have a higher incidence of postoperative complications. There is no strict correspondent relationship among CL_H, liver volume, and Child-Pugh's classification.

The hepatic clearance of D-sorbitol and CT measured liver volume can be reliably used for the evaluation of hepatic functional blood flow and liver metabolic volume. Combined with the Child-Pugh's classification, it could be very useful for further understanding the liver functional reserve, therefore helping to determine a reasonable therapeutic plan and choose surgical procedures and determining the appropriate time to operate.

Evaluation of liver functional reserve by combining D-sorbitol clearance rate and CT measured liver volume. Li YM, Lv F, Xu X, Ji H, Gao WT, Lei TJ, Ren GB, Bai ZL, Li Q. World J Gastroenterol 2003 Sep; 9 (9), pp. 2092–5.

Hepatic Plasma Flow
Sorbitol/Indocyanine Green (ICG)

Cellular/Molecular Mechanism of Action: Sorbitol is an organic compound that is removed from the plasma by the liver.

Application: Evaluation of whole-body removal kinetics of sorbitol using extrarenal sorbitol clearance to estimate hepatic plasma flow in humans and to compare measurements of liver flow by Fick's principle using either ICG or sorbitol.

Route of Administration: Sorbitol (intravenous bolus).

Dosage: Sorbitol (5 mmol/kg) or sorbitol infusion (287 μmol/min in liver patients and controls).

Implementation: Determination of the sorbitol elimination capacity (SEC) and distribution volume, $V_{sorb}d$, following bolus administration or sorbitol infusion. Extrarenal sorbitol clearance ($V_{sorb}x$) is calculated as a function of infusion rate (corrected for renal excretion and accumulation in $V_{sorb}d$) divided by arterial concentration. Liver flow (Q_{ICG}) is calculated from the ICG infusion and arterial and hepatic venous ICG concentrations according to Fick's principle.

Human Model: Healthy cases (bolus infusion in $n = 6$ controls, compared to infusion administration in $n = 17$ hepatic cases and $n = 11$ controls).

Interpretation and Analysis: Average SEC is 73 μmol/min/kg, $V_{sorb}d$ is approximately 0.16 L plasma per kilogram, and *in vivo* $V_{sorb}d$ is 3 mmol/L. Renal sorbitol excretion rate is 0.03–0.31 of infusion rate. Extrahepatic extrarenal removal is not significantly different from zero but varies considerably. Hepatic extraction fraction of sorbitol, (E_{sorb}), measured by liver vein catheterization, is 0.35–1.04 (median, 0.86) in cirrhotic patients and 0.90–0.98 (0.86) in controls. The requirement for using $Cl_{sorb}x$ as an estimate of Q_{ICG} is not violated by the data in controls, $Cl_{sorb}x/Q_{ICG}$ 0.70–1.55 (median, 1.08), whereas there is a systematic underestimation in cirrhotic patients (0.72–1.08 [0.85]). Liver flow calculated by Fick's principle using either sorbitol or ICG agrees well. $E_{sorb} > E_{ICG}$ in the vast majority of cases. Curvilinear relationships between E_{sorb} and E_{ICG} are in agreement with different kinetic parameters for sorbitol and ICG, and do not require additional assumption of intrahepatic shunts.

Sorbitol as a test substance for measurement of liver plasma flow in humans. Keiding S, Engsted E, Ott P. Hepatology 1998 Jul; 28 (1), pp. 50–6.

HEPATIC EXCRETORY FUNCTION

Hepatic Excretory and Biochemical Function/ Provocative Hepatic Probe Interference by Adenosine
Indocyanine Green (ICG) Disappearance/Galactose Elimination Capacity (GEC)/Lidocaine Monoethylglycinxylidide (MEGX) Metabolite

Physiological Mechanism of Action: Indocyanine green is removed from the plasma compartment and excreted into the bile but is not metabolized by hepatocytes. Galactose is removed from the

intravascular compartment by the liver. An extremely high percentage of lidocaine is removed from the portal circulation as it perfuses through the hepatic sinusoids and is subsequently metabolized by hepatocytes to MEGX.

Background Information: In cirrhosis, hepatic arterial vasodilatation occurs in response to reduced portal venous blood flow. However, although the hepatic arterial flow reserve is high in patients with cirrhosis, its impact on hepatic function is unknown.

Application: Indocyanine green disappearance, GEC, and formation of lidocaine metabolite MEGX can be used to assess hepatic function.

Route of Administration: Adenosine (intravenous infusion). Indocyanine green, lidocaine, and galactose are administered intravenously.

Dosage: Adenosine (2–30 μg/min/kg body wt). Indocyanine green, lidocaine, and galactose are administered at doses of 0.5, 1.0, and 500 mg/kg body weight, respectively.

Implementation: Evaluate the effect of adenosine-induced hepatic arterial vasodilatation on different markers for liver function. Adenosine is infused into the hepatic artery and hepatic arterial average peak flow velocities (APV), pulsatility indices (PI), and blood flow volumes (HABF) are measured using digital angiography and intravascular Doppler sonography. Indocyanine green, lidocaine, and galactose can be administered in the presence of adenosine-induced hepatic arterial vasodilatation, and again on a separate study day, without adenosine. Indocyanine green disappearance, GEC, and formation of the lidocaine metabolite MEGX are then assessed.

Human Model: Cases of hepatic cirrhosis (n = 20; Child-Pugh class A/B/C: n = 2/7/11).

Interpretation and Analysis: Adenosine markedly increases hepatic arterial APV and HABF, while markedly decreasing PI. Serum MEGX concentrations are approximately 63.7 ± 18.2 (median, 62; range, 36–107) and 99.0 ± 46.3 (82.5; 49–198) ng/mL in the absence and presence of adenosine infusion, respectively ($p = 0.001$). Adenosine-induced changes in MEGX concentrations correlate inversely to changes in hepatic arterial average peak flow velocities (APV $r = -0.5$, $p = 0.02$) and PI ($r = -0.55$, $p = 0.01$) and are more marked in Child-Pugh Class C compared with Child-Pugh Class A patients (57.4 ± 49.9 [44; −14–140] vs 8.4 ± 16.5 [13; −11–35] ng/mL, $p < 0.01$). Therefore, hepatic arterial vasodilatation provides substantial functional benefit in cases with cirrhosis. The effect does not depend directly on hepatic arterial macroperfusion and is observed preferentially with decompensated disease.

Functional significance of hepatic arterial flow reserve in patients with cirrhosis. Zipprich A, Steudel N, Behrmann C, Meiss F, Sziegoleit U, Fleig WE, Kleber G. Hepatology 2003 Feb; 37 (2), pp. 385–92.

Hepatic Excretory Function
Bromosulfophthalein (BSP)/Comparison to Bile Acids

Cellular/Molecular Mechanism of Action: In the intravascular compartment, the vast majority of the BSP becomes bound to serum albumin fractions. However, within the liver, it readily diffuses across the endothelium that lines the hepatic sinusoids because they are uniquely permeable to protein fractions. Bromosulfophthalein dissociates from its protein fraction, diffuses across the intact hepatocyte membrane, and then becomes reassociated with a protein fraction within the intracellular environment of hepatocytes. The majority of intracellular BSP within the hepatocyte then subsequently becomes conjugated to glutathione. Excretion of both conjugated and unconjugated forms of BSP occurs in essentially an exclusive manner into the biliary canaliculi.

Hepatic extraction of BSP from the systemic circulation (intravascular compartment) is maximal during the period immediately following intravenous injection. Compromised hepatic function results in delayed systemic clearance of BSP.

Diagnostic Application: Diagnosis of compromised hepatic function.

Route of Administration and Dosage: Injected intravenously at a dosage of 2 mg/kg. Blood samples should be collected from a vein different than the 1 used for administration.

Considerations: Clearance of BSP should be delayed if direct and indirect bilirubin levels are already elevated. Although BSP is less expensive than indocyanine green, it has a relatively higher degree of renal excretion and at the present time it is not available on a commercial basis.

Implementation: Following BSP administration by injection, venous blood samples are collected at 4-minute intervals. Examples of acceptable time intervals include 5 and 9 minutes, and 7 and 9 minutes postinjection.

Interpretation and Analysis: In conditions of compromised hepatic function, the plasma BSP clearance is delayed.

Based upon results obtained from a standard reference curve, individual measurements are then plotted on a vertical axis on semi-log paper with time (minutes) on the horizontal axis. Estimated $T_{1/2}$ is then calculated from this curve.

Normal values:

canine	≤ 5% retention at 30 minutes
equine	2.8% +/− 0.50 minutes
bovine	
-mature dairy cow	3.1% +/− 0.60 minutes
-yearling	4.5% +/− 0.32 minutes
ovine	2.0% +/− 0.30 minutes

Laboratory Instrumentation: Quantitative measurement of BSP in blood samples is achieved by adding plasma (2.0 ml) to tubes containing NaOH (0.1 N, 3.0 ml). Blank tubes should contain double distilled water (1.0 ml) and HCl (0.1 N, 3.0 ml). Spectrophotometric transmission is then measured at 565 nm and values plotted against time.

Hepatic Excretory Function
Indocyanine Green (ICG)/Sulfobromophthalein (BSP)

Physiological Mechanism of Action: Elimination of BSP via the biliary system following intravenous injection requires dissociation from albumin in plasma, translocation across the sinusoidal membrane, conjugation with glutathione within the hepatocyte, translocation across the bile canalicular membrane, and excretion in bile.

Application: Evaluation of hepatic excretion.

Route of Administration: Sulfobromophthalein and ICG are given as a single-dose IV injection.

Dosage: Sulfobromophthalein (5 mg/kg); ICG (1.5 mg/kg).

Implementation: Evaluation of hepatic viability as a function of BSP and ICG plasma disappearances. The half-life, disappearance rate (percent per minute), and clearance (ml/min/kg) are determined during the initial phase of plasma dye elimination.

Animal Model: Feline (example).

Interpretation and Analysis: The half-life for BSP is 2.2 ± 0.7 minutes, and for ICG, 3.8 ± 0.9 minutes. The disappearance rate for BSP is 34.7 ± 10.1, and for ICG, 19.2 ± 4.5. The clearance for BSP is 26.3 ± 23.1, and for ICG, 8.6 ± 4.1. The 30-minute percentage retention for BSP is 0.6 ± 0.8%, and for ICG, 7.3 ± 2.9%. In the cat,

ICG is cleared from the plasma less rapidly than BSP. There are no significant differences between males and females for these values. For both BSP and ICG, a significantly shorter half-life, faster disappearance rate, and lower 30-minute percentage retention is observed in the cat, compared with values observed in the dog given similar dye dosages. Clearance curves for BSP and ICG in the cat are similar to those of the dog, indicating that the 2 species may have similar volumes of dye distribution and excretory mechanisms. A measurement of a 30-minute plasma level following a 5 mg/kg dose of BSP in normal cats is often too low for measurement, whereas measurement of a 30-minute plasma concentration of ICG following a 1.5 mg/kg dose is adequate. For the 30-minute dye retention test in the cat, ICG may be a preferred diagnostic agent.

Binding of unconjugated and conjugated sulfobromophthalein to rat liver plasma membrane fractions *in vitro*. Reichen J, Blitzer BL, Berk PD. Biochim Biophys Acta 1981 Jan 8; 640 (1), pp. 298–312.

Comparison of sulfobromophthalein and indocyanine green clearances in the cat. Center SA, Bunch SE, Baldwin BH, Hornbuckle WE, Tennant BC. Am J Vet Res 1983 Apr; 44 (4), pp. 727–30.

Comparison of sulfobromophthalein and indocyanine green clearances in the dog. Center SA, Bunch SE, Baldwin BH, Hornbuckle WE, Tennant BC. Am J Vet Res 1983 Apr; 44 (4), pp. 722–6.

Hepatic Excretory Function/Cirrhosis Conditions/SPECT Correlation with Hepatic Function Tests
Indocyanine Green (ICG)

Cellular/Molecular Mechanism of Action: Indocyanine green is removed from the intravascular compartment by the liver without being metabolized and is subsequently excreted into the bile.
Background Information: Accurate quantitative determination of liver function is critical in cirrhotic patients in order to predict outcome, particularly in patients who undergo hepatic resection or nonhepatic surgery. As colloid uptake by perfused Kupffer cells is proportional to perfused hepatocyte mass, quantitative liver spleen scans may be used as an index of perfused hepatocyte mass.
Considerations: Single photon emission computed tomography (SPECT) of [^{99m}Tc]-phytate colloid uptake by the liver can be applied as a test for hepatic function in cirrhotic patients.
Application: Profiles for systemic ICG concentrations can be correlated with findings observed with SPECT for the purpose of assessing liver function in various disease states, including hepatic cirrhosis.
Route of Administration: Indocyanine green (intravenous).
Implementation: Indocyanine green can be applied in concert with quantitative SPECT to assess liver function in conditions of hepatic cirrhosis. Single photon emission computed tomography can be used to measure liver volume, quantitative colloid uptake by the liver, and percentage of injected dose/ml of liver tissue.

Human Model: Cases of hepatic cirrhosis (n = 75); noncirrhotic patients with chronic liver disease (n = 52); and patients without liver disease (n = 36).
Interpretation and Analysis: Although liver volume is similar among cirrhotic, non-cirrhotic, and chronic liver disease states, cases with cirrhosis have significantly lower total quantitative uptake and quantitative uptake/ml compared to hepatic disease group types ($p < 0.001$). Quantitative liver uptake in cirrhotic patients highly correlates with Child-Pugh scores ($r = -0.64$, $p < 0.0001$) and with ICG retention at 15 minutes ($r = -0.84$, $p < 0.0001$). Quantitative SPECT of the liver may be an additional, useful, non-invasive quantitative test for assessment of hepatic function and severity of liver disease in cirrhotic patients.

Quantitative liver-spleen scan using single photon emission computed tomography (SPECT) for assessment of hepatic function in cirrhotic patients. Zuckerman E, Slobodin G, Sabo E, Yeshurun D, Naschitz JE, Groshar D. J Hepatol 2003 Sep; 39 (3), pp. 326–32.

Hepatic Excretory Function/Example: Cadmium Hepatotoxic Injury
Bromosulfophthalein (BSP)

Cellular/Molecular Mechanism of Action: Bromosulfophthalein is removed from the intravascular compartment through the functions of the hepatic biliary system.
Physiological Mechanism of Action: Elimination of BSP via the biliary system following intravenous injection requires dissociation from albumin in plasma, translocation across the sinusoidal membrane, conjugation with glutathione within the hepatocyte, translocation across the bile canalicular membrane, and excretion in bile.
Application: Bromosulfophthalein is a relatively non-toxic organic anion used as an *in vivo* indicator of liver performance.
Implementation: Characterization of the *in vivo* hepatotoxicant effects of cadmium (Cd) on BSP kinetics in isolated perfused rat liver (IPRL) to determine the degree of association between liver toxicity and BSP kinetics. After a 30-minute period for acclimation to the IPRL system, livers are dosed with cadmium acetate in the presence of 0.25% bovine serum albumin, sufficient to achieve initial concentrations of 10 and 100 μM. Sixty minutes after Cd dosing, the IPRL system is dosed with BSP to give an initial concentration of 150 μM and the elimination kinetics of BSP from the perfusion medium monitored. Cadmium concentrations in livers at the end of the experiments are 60 ± and 680 ± 210 μmol/kg for the 10 and 100 μM doses, respectively.

Animal Model: Rat (Fisher strain, n = 344 males; isolated perfused liver preparations).
Interpretation and Analysis: Exposure to 10 μM Cd for 60 minutes results in a reduction in bile flow, no significant effect on lactate dehydrogenase (LDH) leakage, and slight effects on BSP clearance. Similar studies following exposure to 100 μM Cd show dramatic decreases in bile flow with complete cholestasis 60 minutes after Cd addition. Total leakage of LDH into perfusion medium is < 10%, indicating that Cd affects bile production well before the liver shows significant signs of necrosis. Clearance of BSP from the perfusion medium is dramatically reduced. Taken together, the data indicate that Cd has a significant effect on the kinetics of BSP in the IPRL and the dominant effects are mediated through Cd-mediated cholestatic effects.

Effect of cadmium on bromosulfophthalein kinetics in the isolated perfused rat liver system. Soto A, Foy BD, Frazier JM. Toxicol Sci 2002 Oct; 69 (2), pp. 460–9.

Hepatic Excretory Function/Lipidosis Example/ Correlation with Sulfobromophthalein (BSP) $T_{1/2}$ and Serum Bile Acids (No Diagnostic Relationship)
BSP

Cellular/Molecular Mechanism of Action: Sulfobromophthalein functions as a substrate for hepatic conjugation and excretion into the bile.
Physiological Mechanism of Action: Sulfobromophthalein is eliminated via the biliary system following intravenous injection. The excretion process requires dissociation of BSP from albumin fractions in the plasma, translocation across intact sinusoidal membranes, conjugation to glutathione intracellularly within the hepatocyte,

translocation across the bile canalicular membrane, and then excretion into the bile.

Application: Hepatic excretion evaluation.

Route of Administration: Sulfobromophthalein (intravenous).

Implementation: Evaluation of liver function and/or hepatic fat infiltration in dairy cattle as a function of BSP half-life ($T_{1/2}$), serum bile acid concentrations, and hepatic fat content assessed by histopathological analysis of liver biopsy specimens. Serum bile acid concentrations are measured in healthy dairy cattle at different stages of lactation after fasting or feeding.

Animal Model: Bovine (healthy dairy cattle potentially suffering from hepatic fat infiltration).

Interpretation and Analysis: Serum bile acid concentrations are higher in cows in early lactation and with higher daily milk production. Compared with prefasting values, bile acid concentrations are decreased at 8, 14, and 24 hours of fasting. Blood samples from fed cows at 1- to 2-hour intervals have wide and inconsistent variations in bile acid concentration. Because serum bile acids correlate well with BSP $T_{1/2}$, it is suggested that both measurements evaluate a similar aspect of liver function. Neither bile acids nor BSP $T_{1/2}$ correlates with differences in liver fat content among cows. Because of large variability in serum bile acid concentrations in fed cows and the lack of correlation of measured values with liver fat content, bile acid determinations do not appear useful for showing changes in hepatic function in fed cows with subclinical hepatic lipidosis nor for serving as a screening test for this condition.

Serum bile acid concentrations in dairy cattle with hepatic lipidosis. Garry FB, Fettman MJ, Curtis CR, Smith JA. J Vet Intern Med 1994 Nov–Dec; 8 (6), pp. 432–8.

Hepatic Excretory Function/Pharmaceutical Evaluation/Pharmaceutical Modification of Hepatic Blood Flow
Indocyanine Green (ICG)/[Diltiazem (DTZ)]

Cellular/Molecular Mechanism of Action: Indocyanine green is removed from the intravascular compartment by the liver when the reagent enters the hepatic sinusoids and is then excreted into the bile.

Application: Indocyanine green is a hepatic function test marker molecule that is not metabolized in the liver, but rather only excreted into the bile. The *in vivo* pharmacokinetic profile of ICG at steady state can be applied to detect pharmaceuticals that alter hepatic blood flow in a manner that can change hepatic blood clearance parameters.

Route of Administration: Indocyanine green (intravenous at a constant infusion rate).

Dosage: Indocyanine green (3.78 mg/kg bolus and constant rate infusion of 10,100 μg/kg/hr is administered until a steady state concentration of Css = 10 μg/ml is achieved at 20, 25, and 30 minutes).

Implementation: Evaluation of the influence of DTZ on the hepatic blood clearance of ICG as a function of its pharmacokinetic profile at steady state. Following development of a steady state concentration of ICG of 10 μg/ml and a subsequent 90-minute washout period, an intravenous bolus injection (108, 430, 860, and 1,720 μg/kg) and a constant-rate infusion (108, 433, 866, and 1,730 μg/kg/hr) of test pharmaceutical (e.g., DTZ) is introduced into the right femoral vein for the purpose of achieving steady state plasma concentrations (e.g., Css = 50, 200, 400, and 800 ng/ml DTZ) confirmed at 60, 70, and 80 minutes. During the steady state phase for the test pharmaceutical (e.g., DTZ), the intravenous bolus injection and the constant-rate infusion of ICG into the left femoral vein are made and also the steady state plasma concentration of ICG measured at 20, 25, and 30 minutes. Plasma concentrations of DTZ and ICG are determined by high-performance liquid chromatography analysis.

Animal Model: Rat (left femoral vein injection site).

Interpretation and Analysis: At steady state, the hepatic blood clearance of ICG is derived from values for ICG plasma concentration and the blood-to-plasma concentration ratio (RB). An example of a pharmaceutical that does not influence ICG hepatic clearance is DTZ. Pretreatment with various doses of DTZ does not influence plasma concentrations, RB, and plasma free fraction (fp) of ICG. So the hepatic blood clearance of ICG is independent of DTZ concentration. The hepatic blood clearance of ICG can possibly be affected by both hepatic blood flow and hepatic intrinsic clearance. However there is no change in the hepatic blood clearance of ICG between the control and DTZ-pretreated rats. Diltiazem therefore may not influence hepatic blood flow.

Instrumentation: High-performance liquid chromatography analytical system.

No effect of diltiazem on the hepatic clearance of indocyanine green in the rats. Joo EH, Lee YB. Arch Pharm Res 1998 Aug; 21 (4), pp. 411–7.

Hepatic Excretory Function/Insufficiency
Indocyanine Green (ICG)

Cellular/Molecular Mechanism of Action: Excreted by hepatic transport mechanisms into the bile.

Diagnostic Application: Detection of hepatic dysfunction.

Route of Administration: Intravenous.

Dosage: Indocyanine green (0.8–1.1 mg/kg.).

Considerations: Indocyanine green is more accurate in detecting subtle changes in hepatic conjugation function than is BSP because significant amounts of the latter reagent are eliminated from the systemic circulation by renal excretory mechanisms. Unfortunately, ICG is relatively more expensive than BSP, which can be of particular concern when applied as a diagnostic pharmaceutical in large animal species such as the horse.

Interpretation and Analysis: Delayed elimination of ICG from the vascular compartment indicates the presence of hepatic dysfunction. Biopsy specimens and serial collections of enzymology profiles are necessary to determine the extent of hepatic dysfunction, possible cause, and if resolution or degradation occurs over time.

Normal $T_{1/2}$ in fed and fasted horses is 3.5 ± 0.67 and 1.6 ± 0.57 ml/min/kg, respectively.

Hepatocellular Excretory Function
Indocyanine Green (ICG) Dye

Cellular/Molecular Mechanism of Action: Indocyanine green is removed from the vascular compartment by the function of normal hepatocytes.

Background Information: Although depression in hepatocellular function occurs early after trauma and severe hemorrhage and persists despite fluid resuscitation, it remains unknown whether reactive oxygen species (ROS) play any role in the initiation of hepatocellular depression and damage under those conditions. It has been hypothesized that administration of an ROS scavenger at the beginning of resuscitation will attenuate organ injury after severe shock.

Application: Evaluation of hepatic function.

Implementation: Male Sprague-Dawley rats following laparotomy are bled and then maintained at a mean arterial pressure of 40 mm Hg until 40% of the maximal bleed-out volume is returned in the form of Ringer's lactate (RL). Animals are then resuscitated with 4 times the volume of maximal bleed-out with RL over a 60-minute

period. The ROS scavenger 2-mercaptopropionyl glycine (30 mg/kg) or vehicle is then administered intravenously as a bolus at the beginning of resuscitation. At 2 hours after completion of crystalloid resuscitation or the equivalent interval after sham-operation, the cardiac index is measured using a dye-dilution technique. Hepatocellular function, that is, the maximum velocity of ICG clearance (Vmax) and efficiency of the active transport (Km), can be determined using an *in vivo* hemoreflectometer. In addition, serum levels of tumor necrosis factor-alpha (TNF-α) and alanine aminotransferase can be determined with ELISA and colorimetrically, respectively.

Animal Model: Male Sprague-Dawley rats (275–325 g) subjected to laparotomy (i.e., induction of soft tissue trauma).

Interpretation and Analysis: At 2 hours following trauma, hemorrhage and resuscitation, cardiac index, and hepatocellular function are markedly depressed with concomitant increases in serum levels of TNF-α and alanine aminotransferase ($p < 0.05$). Administration of 2-mercaptopropionyl glycine, however, restores depressed cardiac and hepatic function and markedly attenuates liver enzyme release and serum levels of TNF-α ($p < 0.05$). Reactive oxygen species therefore play a role in the induction of depressed organ function after severe hemorrhagic shock. Adjuncts that attenuate the detrimental effects of ROS may be useful for improving the depressed cardiac and hepatocellular functions after trauma hemorrhage and resuscitation.

Critical role of oxygen radicals in the initiation of hepatic depression after trauma hemorrhage. Jarrar D, Wang P, Cioffi WG, Bland KI, Chaudry IH. J Trauma. 2000 Nov; 49 (5), pp. 879–85.

Hepatocyte Excretory Function/Sinusoidal Endothelial Cell Function/[Experimental Model of Acute and Chronic Liver Failure]
Indocyanine Green (ICG)/Hyaluronic Acid

Cellular/Molecular Mechanism of Action: Indocyanine green and hyaluronic acid are removed from the peripheral circulation by the normal function of hepatocytes and sinusoidal endothelial cells.

Background Information: Experimental models of liver transplantation use normal recipients, although most patients undergoing liver transplantation suffer from acute or chronic liver failure.

Application: Indocyanine green and hyaluronic acid uptake can be utilized to evaluate the *in vivo* function of hepatocytes and sinusoidal endothelial cells.

Implementation: Evaluation of the outcome for orthotopic liver transplant in compromised hosts. Hepatic injury/dysfunction can be evaluated by collectively monitoring enzymes, bilirubin, ammonia levels, prothrombin, thrombin time, and cytokines. The *in vivo* function of hepatocytes and sinusoidal endothelial cells can be evaluated applying ICG and hyaluronic acid uptake.

Animal Model: Rat (D-galactosamine-induced acute liver failure or chronic liver failure secondary to common bile duct ligation). Transplantation performed in normal, acute, and chronic liver failure rats at different time points using either freshly harvested or cold-preserved syngeneic livers.

Interpretation and Analysis: Survival with fresh grafts decreases significantly when transplants are performed 48 hours after the induction of acute liver failure. Rats with acute liver failure almost invariably do not survive transplantation with grafts stored for 12 or 24 hours, but in chronic failure survival is more than 80%. Survival of acute liver failure rats receiving grafts preserved for 6 hours is 16.6% compared with 83.3% observed with fresh grafts transplanted at the same time point after D-galactosamine exposure. Elevated tumor necrosis factor-alpha and interleukin-1β levels as well as impaired sinusoidal endothelial cell function can be detected in acute liver failure rats with grafts preserved for 6 hours. Speculation therefore suggests that preoperative status and different host factors have a significant effect on outcome and graft function after liver transplantation in rats.

Graft function and survival depend primarily on host factors in compromised recipient models of orthotopic liver transplantation in the rat. Wang L, Roayaie S, Basile J, Florman S, Liu J, Tarcsafalvi A, Boros P, Miller CM. Transplantation 2001 Jan 15; 71 (1), pp. 14–20.

HEPATOCYTE PHASE I METABOLISM PATHWAYS

Hepatic Biochemical Cytochrome P450 Metabolism Capacity
Cimetidine/Indocyanine Green (ICG)/Lidocaine

Cellular/Molecular Mechanism of Action: Indocyanine green serves as a global marker of function, while lidocaine and its metabolite, MEGX, have utility as specific markers of the CYP450 enzyme system. Cimetidine functions as a CYP450 enzyme system inhibitor.

Application: Indocyanine green can be applied to assess general hepatic function, while lidocaine and its metabolite, MEGX, can be used as specific markers of the CYP450 enzyme system.

Route of Administration: Infusion of ICG and lidocaine via the portal vein after a 40-minute stabilization period until steady state concentrations are reached in the venous outflow.

Dosage: Cimetidine (80 mg/kg daily for 3 days); ICG and lidocaine are infused after a 40-minute stabilization period into the portal vein until steady state concentrations are reached in the venous outflow.

Considerations: Endotoxin administration and cecal ligation and puncture produce significant hepatocellular dysfunction when studied *in vivo*. Specific factors that are present *in vivo* after endotoxin administration and cecal ligation and puncture, such as alterations in liver blood flow, circulating mediators, and hypoxia, can alter hepatic function.

Implementation: Delineation of hepatic clearance and CYP450 function during fabricated conditions of endotoxemia. Indocyanine green (control, $n = 6$; endotoxin, $n = 5$) or lidocaine (control, $n = 8$; endotoxin, $n = 8$) is infused into livers of control and endotoxin-exposed rat populations. Cimetidine (CYP450 enzyme system inhibitor) can be employed as a reference control (e.g., $n = 6$ rats).

Animal Model: Rats exposed to endotoxin (*Escherichia coli* lipopolysaccharide@45 mg/kg i.p.) followed by a 6-hour monitoring period and subsequent isolation of an *in situ* perfused liver preparation. Livers are perfused via the portal vein using a single-pass system with a balanced salt solution 6 hours after receiving either endotoxin or saline or 24 hours after receiving the last dose of cimetidine.

Interpretation and Analysis: Total hepatic clearance and intrinsic hepatic clearance for ICG and lidocaine are unchanged in the livers obtained following endotoxin treatment. Such animal models can adequately detect CYP450 inhibition because cimetidine-treatment results in significantly lower initial MEGX concentrations (0.63 ± 0.03 mg/L) compared with control (0.77 ± 0.03 mg/L) and endotoxin-treated (0.74 ± 0.04 mg/L) test subjects (e.g., rat animal model). Septic livers have significantly higher initial hepatic oxygen consumption (HVO_2) than do control livers (45 ± 3 μL/min/g vs 82 ± 9 μL/min/g). The HVO_2 remains higher in the septic livers and significantly increases throughout the course of observation and demonstrates that livers remain viable and functional. These data indicate that there is no detectable hepatocellular dysfunction after endotoxin shock using ICG, lidocaine, and MEGX in the isolated

perfused liver; therefore the dysfunction reported from *in vivo* studies may be reversible when the liver is removed from the shocked environment.

Effect of endotoxin shock on the clearance of lidocaine and indocyanine green in the perfused rat liver. McKindley DS, Chichester C, Raymond R. Shock 1999 Dec; 12 (6), pp. 468–72.

Hepatic Metabolism/Age-Related Changes in Biochemical Capacity/Geriatric Metabolism Alterations
[^{13}C]-Methacetin Breath Test

Cellular/Molecular Mechanism of Action: [^{13}C]-methacetin is a radioactive analog of methacetin, which can be metabolized in the liver, yielding [$^{13}CO_2$].
Background Information: The issue of whether or not liver function is compromised in the healthy elderly population remains unsolved. However, it is known that endogenous CO_2 production changes with age, motor activity, and nutrition.
Application: Evaluation of the biochemical capacity of the liver. In this manner it can be used to investigate the putative age-related modifications of hepatic function in the form of the [^{13}C]-methacetin breath test.
Implementation: Evaluation of hepatic biochemical capacity by assessment of CO_2 production following [^{13}C]-methacetin administration. Trans-abdominal echosonography with Doppler pulsed wave analysis of the celiac axis and portal vein can be used to complement the [^{13}C]-methacetin breath test.

Human Model: Geriatric (n = 29 elderly cases; n = 17/12 female/male ratio; mean age = 79.8 ± 7.9 years); and adults (n = 28 adults; n = 13/15 female/male ratio; mean age = 40.6 ± 12.3 years).
Interpretation and Analysis: Although the [$^{13}CO_2$] peak occurs within 15 to 30 minutes in both elderly and adult subjects, it is significantly decreased in the former (30.66% ± 9.2% vs 38.33% ± 6.05%; $p < 0.001$), as is the cumulative excretion (33.07% ± 7.06% vs 39.81% ± 5.68%; $p < 0.001$). When correcting for the effects of CO_2 excretion by age, the age-related modification of the cumulative dose becomes more evident (elderly group 30.15% ± 6.46% vs adult group 37.97% ± 5.92%; $p < 0.0001$). Elderly cases also show an increase in the intrahepatic resistance index using Doppler pulsed wave analysis that inversely correlates with results from breath tests. Hepatic function is not well preserved in healthy humans throughout life and may be due to an increase in vascular resistance.

Study of liver function in healthy elderly subjects using the [^{13}C]-methacetin breath test. Ciccocioppo R, Candelli M, Di Francesco D, Ciocca F, Taglieri G, Armuzzi A, Gasbarrini G, Gasbarrini A. Aliment Pharmacol Ther 2003 Jan; 17 (2), pp. 271–7.

Hepatic Metabolism/Carbohydrate Biochemical Metabolism/Hepatic Residual Metabolizing Capacity Posthepatectomy
[^{13}C]-Labeled Glycerol/6,6 [$^{2}H_2$]Glucose

Cellular/Molecular Mechanism of Action: [^{13}C]-labeled glycerol can function as a biochemical substrate precursor for the hepatic synthesis of [^{13}C]-glucose (gluconeogenesis pathway).
Physiological Mechanism of Action: Glycerol is a substrate molecule that can be utilized by hepatocytes within the liver to synthesize glucose.
Background Information: Major liver resection can be used in the treatment of liver cancer. The functional capacity of liver parenchyma needs to be evaluated preoperatively because it determines outcome.
Application: [^{13}C]-labeled glycerol may function as a suitable simple probe for assessing liver biochemical capacity as a function of hepatic [^{13}C]-glucose synthetic capacity (e.g., gluconeogenesis pathway).
Route of Administration: [^{13}C]-labeled glycerol (intravascular).
Dosage: [^{13}C]-labeled glycerol (16 μmol/kg/min) during a 120-minute infusion phase.
Implementation: Assessment of hepatic metabolizing capacity as a function of whole-body glycerol clearance. Glycerol, in the form of [^{13}C]-labeled glycerol, can be administered systemically during a 120-minute infusion phase following a 150-minute basal period. Whole-body glycerol clearance is calculated from changes in plasma glycerol concentration. Whole-body glucose production is measured with 6,6 [$^{2}H_2$]glucose infused as a tracer in the basal state and during glycerol infusion. In addition, [^{13}C]-glucose synthesis can be monitored to quantitate gluconeogenesis from glycerol.

Human Model: Cases of major hepatectomy (n = 7); colectomy (n = 6); and healthy normal controls (n = 12). Patients are assessed on the 1st day after surgery.
Interpretation and Analysis: Postoperative liver resection cases have higher plasma glycerol concentrations and lower whole-body glycerol clearance than normal healthy reference controls or cases subjected to colectomy. Postoperative liver resection cases also have higher plasma glucagon concentrations. Their fasting glucose production is mildly elevated in the fasting state and does not change after glycerol infusion, indicating normal hepatic autoregulation of glucose production. The whole-body glycerol clearance can be simply determined from plasma glycerol concentrations during exogenous glycerol infusion. It is significantly reduced following major hepatectomy surgical procedures.

Measurement of the whole body clearance of infused glycerol as a test of liver function after major hepatectomy. Tappy L, Cayeux MC, Gillet M, Koestinger A, Matter M, Revelly JP, Berger M, Vallet C, Chioléro R. Clin Physiol Funct Imaging 2002 Jul; 22 (4), pp. 266–70.

Hepatic Metabolism/Cirrhosis Biochemical Capacity/Hepatic Cirrhosis Staging/Metabolic Probes Compared to Clinical Pathology Parameters
Lidocaine [Monoethylglycinexylidide]/Antipyrine

Cellular/Molecular Mechanism of Action: Lidocaine is rapidly removed from the systemic circulation and metabolized to monoethylglycinexylidide (MEGX).
Application: Evaluation of hepatic metabolic capacity as in conditions of cirrhosis of the liver.
Route of Administration: Lidocaine (intravenously); antipyrine (oral).
Dosage: Lidocaine (1 mg/kg); antipyrine (single 1,000-mg dose).
Implementation: Evaluation of liver function in conditions of hepatic cirrhosis as a function of lidocaine metabolism (e.g., MEGX metabolite) and antipyrine complemented by analysis of standard biochemical parameters used for assessment of liver function in cirrhotic patients. Each affected case can be evaluated with each of the 2 dynamic liver tests (e.g., MEGX and antipyrine in a crossover schedule with at least a 5-day interval). Characterization of the formation of MEGX metabolite involves the administration of a bolus dose and collection of blood samples 15 minutes postadministration for MEGX assays. Antipyrine concentrations are measured in samples collected over a 24-hour period and antipyrine concentrations measured spectrophotometrically. Standard biochemical parameters used for liver assessment are measured by means of routine laboratory methods.

Human Model: Case of hepatic cirrhosis (n = 30 Child-Pugh's score to subgroups A [n = 11], B [n = 12],and C [n = 7]) in addition to healthy reference controls (n = 14).
Interpretation and Analysis: In cases of hepatic cirrhosis, liver dynamic tests appear to be better predictors of hepatic function. The MEGX test is more feasible in a clinical setting, but the antipyrine test is more sensitive in staging liver cirrhosis.
Instrumentation: Fluorescence polarization immunoassay instrumentation (MEGX assay) and a spectrophotometer (antipyrine assay).

Comparison of MEGX (monoethylglycinexylidide) and antipyrine tests in patients with liver cirrhosis. Wojcicki J, Kozlowski K, Drozdzik M, Wojcicki M. Eur J Drug Metab Pharmacokinet 2002 Oct–Dec; 27 (4), pp. 243–7.

Hepatic Metabolization/Age-Related Biochemical Capacity/Age-Related Declines/Urea Cycle
Alanine

Cellular/Molecular Mechanism of Action: Alanine is 1 of the biochemical substrates for urea synthesis.
Background Information: Studies have shown that hepatic function, quantitatively measured by dynamic liver function tests, progressively declines with aging. Urea synthesis is a specific process taking place in the liver; a reduced urea synthesis in response to a protein-rich metal has previously been demonstrated in the elderly, but the process has never been standardized in relation to amino acid supply.
Application: Characterization of chronological declines in functional capacity in aging hepatic parenchyma.
Implementation: Evaluation of the influence of alanine on the hepatic conversion of α-amino nitrogen to urea.

Human Model: Cases in good health (e.g., n = 32) with normal routine liver and renal function tests and without evidence of previous hepatic disorders (n = 3 age groups; ≤ 55 years vs 56–70, vs ≥ 71).
Interpretation and Analysis: Functional hepatic nitrogen clearance is reduced on average by 20% in subjects aged 56–70 years, and by 30% in subjects over 70 years old in comparison to the age group under 55 years (ANOVA: $p = 0.0001$), and significantly correlates with age ($r = -0.684$). No sex differences can be appreciated on the effects of age on hepatic clearance. Also, liver volume, measured by ultrasonography, is reduced with advancing age, but the age-related decrease in hepatic nitrogen conversion is not primarily dependent on decreased liver volume. The measurement of functional hepatic nitrogen clearance has already been validated as a quantitative liver function test in clinical hepatology. In keeping with previous studies, the age-related decline in hepatic nitrogen conversion points to a decrease in functional capacity of the aging hepatic parenchyma.

Kinetics of hepatic amino-nitrogen conversion in aging man. Fabbri A, Marchesini G, Bianchi G, Bugianesi E, Zoli M, Pisi E. Liver 1994 Dec; 14 (6), pp. 288–94.

Hepatic Metabolization/Carboxylic Acid Biochemical Transformation/Nitrogen Metabolism/Gastrectomy, Hepatic Failure, Renal Failure, Myotonic Dystrophy
1-[^{14}C]-α-Ketoisovaleric Acid (KIV)

Cellular/Molecular Mechanism of Action: 1-[^{14}C]-α-ketoisovaleric acid is a keto analogue of valine and can function as a biochemical substrate for mechanisms of carboxylic acid metabolism.
Background Information: The keto analogues of essential amino acids represent a promising therapeutic modality in hereditary and acquired disorders of nitrogen metabolism. The utilization of these substances in humans has been assayed primarily by nitrogen balance studies.
Application: Endogenous biochemical metabolism of KIV can be measured using the [$^{14}CO_2$] breath excretion test. In this context, the agent can be applied as a tool for evaluating dietary formulation.
Route of Administration: Oral.
Implementation: Assessement of the *in vivo* decarboxylation of KIV by measurement of [$^{14}CO_2$] using a simple and accurate breath excretion test. Protein diets (5 g) supplemented with essential amino acids and KIV (substituted for valine) are then evaluated. Diets of 120 g of protein/day are then reevaluated in gastrectomy cases.

Human Model: Normal volunteers and cases suffering from assorted disease states (n = 6 diseased; gastrectomy, hepatic failure, renal failure, and myotonic dystrophy).
Interpretation and Analysis: In situations of low protein intake, 13–32% of ingested KIV undergoes rapid decarboxylation, and this proportion appears to correlate inversely with damage to organ systems containing branched-chain keto acid dehydrogenase. With high protein intake, the proportion of decarboxylated substrate increases from 44% to 53%. Such findings confirm that the decarboxylation of KIV in man varies under different conditions of dietary intake and metabolic disease. The [$^{14}CO_2$] breath excretion test is applicable to other related analyses of carboxylic acid metabolism in human subjects.

Decarboxylation of α-ketoisovaleric acid after oral administration in man. Epstein CM, Chawla RK, Wadsworth A, Rudman D. Am J Clin Nutr 1980 Sep; 33 (9), pp. 1968–74.

Hepatic Metabolization/Cirrhosis Biochemical Capacity/Assessment of Postoperative Risk Following Hepatic Resection/Hepatic Insufficiency/Cirrhosis/Hepatocellular Carcinoma
Lidocaine [Monoethylglycinexylidide (MEGX)]

Cellular/Molecular Mechanism of Action: Lidocaine is rapidly removed from the systemic circulation and metabolized to MEGX.
Application: Preoperative risk assessment.
Route of Administration: Lidocaine (intravenously).
Dosage: Lidocaine (1 mg/kg).
Implementation: Evaluation of the potential for MEGX to function as a preoperative risk assessment parameter for predicting the potential for development of postoperative complications in cases of cirrhosis and hepatocellular carcinoma that are candidates for curative liver resection.

Human Model: Cases in postoperative phase of hepatic resection evaluated with lidocaine test (e.g., n = 51; n = 22 with MEGX < 25 ng/ml; and n = 29 with MEGX > 25 ng/ml).
Interpretation and Analysis: Each type of case group is comparable for preoperative clinical parameters and surgical procedures. Cases with MEGX < 25 ng/ml have a significantly higher rate of postoperative complications (73% vs 28%, $p < 0.005$) and a tendency toward a longer hospital stay, compared to cases with an MEGX > 25 ng/ml concentration. The lidocaine value is an effective index of hepatic function. A preoperative MEGX value lower than 25 ng/dL in cirrhotic patients is related to a significantly higher risk of liver insufficiency and postoperative complications after hepatic resection.

Operative risk by the lidocaine test (MEGX) in resected patients for HCC on cirrhosis. Ravaioli M, Grazi GL, Principe A, Ercolani G, Cescon M, Gardini A, Varotti G, Del Gaudio M, Cavallari A. Hepatogastroenterology 2003 Sep–Oct; 50 (53), pp. 1552–5.

Hepatic Metabolization/Cirrhosis and Gastric Mucosa Integrity
Lysine Acetylsalicylate

Application: Evaluate the functional disruption of the gastric mucosa in conditions of cirrhosis by measuring the gastric potential difference.

Background Information: Lysine acetylsalicylate–induced bronchoconstriction can be used to evaluate the efficacy of various pharmaceutical agents pertaining to their ability to alleviate induced bronchoconstrictive responses. Additionally, it has been applied to characterize pharmaceutical efficacy and physiological processes in the kidney that are mediated by prostaglandin synthesis.

Route of Administration: Local installation of lysine acetylsalicylate.

Dosage: Lysine acetylsalicylate (500 mg).

Implementation: Potential differences are measured in the morning, on an empty stomach, and at least 3 days after endoscopy, employing a method that uses a double channel gastric perfused probe placed under fluoroscopy 10 cm above the cardia, and a subcutaneous reference, both connected to a millivoltimeter via gelose agar-KCl bridges. Potential differences are recorded in each case 20 minutes before (baseline) and after local instillation of lysine acetylsalicylate (500 mg) as a provocative test.

Human Model: Cases of hepatic cirrhosis ($n = 28$, consecutive conditions with endoscopically proven congestive gastropathy) and negative reference controls ($n = 12$).

Interpretation and Analysis: Cirrhotic patients have significantly lower basal potential differences than controls (-28.3 ± 1.5 mV vs -33.8 ± 1.3mV, $p = 0.007$). Potential differences are significantly lower in cases with severe gastropathy than in conditions with mild gastropathy (-20.5 ± 2.1 and -28.9 ± 1.6 mV, respectively, $p < 0.01$). After stimulation with acetylsalicylate, the area under the curve and the irritability index are greater in patients with gastropathy (81.4 ± 12.8 vs 41.2 ± 8.6 mV.min, $p = 0.032$ and 0.935 ± 0.19 vs 0.290 ± 0.07 mV.mV.min, $p = 0.022$, respectively). These differences are explained by a higher drop in potential difference (delta DPmax/baseline; 28.1 ± 3 vs 16.1 ± 3 p. 100, $p = 0.006$), whereas basal return time remains unchanged (16.2 ± 2.1 vs 13.7 ± 2.2 minutes).

Functional study, in man, of congestive gastropathy in cirrhosis by measurement of potential difference. Pienkowski P, Payen JL, Calès P, Monin JL, Gerin P, Pascal JP, Frexinos J. Gastroenterol Clin Biol 1989 Oct; 13 (10), pp. 763–8.

Furosemide: Progress in understanding its diuretic, anti-inflammatory, and bronchodilating mechanism of action, and use in the treatment of respiratory tract diseases. Prandota J. Am J Ther 2002 Jul–Aug; 9 (4), pp. 317–28.

Hepatic Metabolization/Decarboxylation Biochemical Capacity (Mitochondrial)/ Mitochondrial Respiration Uncoupling
[^{14}C]-α-Ketoisocaproic Acid/Exhaled [$^{14}CO_2$]/ Ethanol/Sodium Salicylate

Cellular/Molecular Mechanism of Action: α-ketoisocaproic acid is a substrate for enzyme decarboxylation mechanisms mediated through the function of hepatocyte mitochondria. Ethanol suppresses these biochemical processes. Sodium salicylate uncouples mitochondrial respiration and can therefore promote increased decarboxylation processes.

Application: α-ketoisocaproic acid can be applied in a breath test to assess *in vivo* hepatic mitochondrial function. Ethanol can be applied as a control reagent due to its ability to inhibit hepatic mitochondrial decarboxylation mechanisms.

Route of Administration: 1-[^{14}C]-α-ketoisocaproic acid (intraperitoneal).

Implementation: Evaluation of *in vivo* hepatic mitochondrial metabolic capacity as a function of [$^{14}CO_2$] exhalation following 1-[^{14}C]-α-ketoisocaproic acid administration.

Animal Model: Rat.

Interpretation and Analysis: Following administration of 1-[^{14}C]-α-ketoisocaproic acid, exhaled [$^{14}CO_2$] reaches a peak within 10 to 20 minutes and then declines exponentially, with a half-life of 14.3 minutes. In controls, approximately 38.6% of the administered radioactivity is exhaled within 1 hour. In functionally anhepatic animals, [$^{14}CO_2$] in breath amounted to 23% of that in control animals, indicating that α-ketoisocaproic acid decarboxylation reflects mainly *in vivo* hepatic mitochondrial function. Ethanol (3 gm/kg) significantly decreases α-ketoisocaproic acid decarboxylation (21.8% of the dose appearing in breath in 1 hour), probably due to the ethanol-induced shift in the NAD+:NADH ratio. In contrast, uncoupling of mitochondrial respiration with sodium salicylate (375 mg/kg), increases decarboxylation of α-ketoisocaproic acid (56.3% of the dose recovered as [$^{14}CO_2$] in 1 hour). Mitochondrial damage induced by 4-pentenoic acid decreases the decarboxylation of α-ketoisocaproic acid but does not affect the microsomal metabolism of antipyrine. The present data indicate that the α-ketoisocaproic acid breath test provides a non-invasive estimate of hepatic mitochondrial function *in vivo* that, when applied to man, might yield clinically useful information.

Assessment of mitochondrial function *in vivo* with a breath test utilizing α-ketoisocaproic acid. Michaletz PA, Cap L, Alpert E, Lauterburg BH. Hepatology 1989 Nov; 10 (5), pp. 829–32.

Hepatic Metabolization/Demethylation Biochemical Capacity
[^{13}C]-Aminopyrine

Cellular/Molecular Mechanism of Action: [^{13}C]-aminopyrine is demethylated by biochemical fractions residing within hepatocytes.

Application: Detection or confirmation of cases suspected of suffering from various stages of hepatic disease.

Route of Administration: [^{13}C]-aminopyrine.

Dosage: [^{13}C]-aminopyrine (2 mg/kg).

Implementation: Evaluation of suspected hepatic disease states using the [^{13}C]-aminopyrine demethylation blood test as a potential diagnostic parameter. A baseline blood sample is collected, followed by the administration of [^{13}C]-aminopyrine, followed by the collection of another blood sample at 45 minutes postinjection. Carbon dioxide is extracted from blood samples and analyzed using fractional mass spectrometry. Results from the [^{13}C]-aminopyrine demethylation blood test are then compared to clinical data and histologic findings.

Animal Model: Canine ($n = 6$ dogs initially suspected of having hepatic disease based on their history, physical examination, imaging studies, general laboratory parameters, or any combination).

Interpretation and Analysis: Intravenous administration of [^{13}C]-aminopyrine leads to a decrease in the percent dose of [^{13}C] recovered from dogs with histologically confirmed liver disease. Based on these results, a full-scale investigation of the potential clinical usefulness of a [^{13}C]-aminopyrine demethylation blood test for assessment of hepatic function in dogs is warranted.
Instrumentation: Fractional mass spectrometry system.

Use of a ^{13}C-aminopyrine blood test: First clinical impressions. Chiaramonte D, Steiner JM, Broussard JD, Baer K, Gumminger S, Moeller EM, Williams DA, Shumway R. Can J Vet Res 2003 Jul; 67 (3), pp. 183–8.

Hepatic Metabolization/Pharmacokinetic Profile Prediction with Cirrhosis Biochemical Changes/ Example: Cefoperzone/Correlation of Pharmacokinetic Alterations with Hepatic Function Profiles
Galactose

Cellular/Molecular Mechanism of Action: Galactose is normally removed from the intravascular compartment by viable hepatocytes.
Application: Galactose can be utilized to detect and characterize alterations in hepatic functional capacity. In this fashion, it can serve as a valuable probe that can be used to correlate changes in the pharmacokinetic/excretion profiles for various agents (e.g., cefoperzone) with alterations in hepatic viability. In this fashion, galactose can potentially be used to design administration strategies prior to the administration of therapeutic agents in various disease states.
Dosage: Galactose (0.5 g/kg).
Implementation: Evaluation of residual hepatic function using the galactose single point (GSP) technique. The method involves measuring galactose blood concentration 1 hour after galactose administration. The GSP method, the galactose elimination capacity (GEC) test, and the modified galactose elimination capacity (MGEC) test can each be applied to compare their relative ability to measure residual liver function.

Human Model: Characterization of cefoperazone pharmacokinetics in cases of hepatic cirrhosis. Cases of hepatic cirrhosis ($n = 12$) and healthy volunteers ($n = 11$) are administered intravenous doses of cefoperazone (1 g). Cefoperazone concentrations are then measured in blood and urine samples collected at appropriate intervals after drug administration. All blood and urine samples are preserved at $-30°C$ prior to quantitative analysis.
Interpretation and Analysis: Cefoperazone plasma concentrations are much higher in cirrhosis patients than in normal reference controls at all time intervals. The elimination half-life, hepatic clearance, mean residence time, and renal clearance of cirrhosis patients differ significantly from those of healthy volunteers. Plasma protein binding is not altered in either normal or cirrhosis cases. Urinary excretion of cefoperazone is significantly increased in cirrhosis patients ($23.95 \pm 5.06\%$ for normal men and $51.09 \pm 11.50\%$ in cirrhosis patients). Hepatic clearance, fraction excreted in urine, and total clearance significantly correlates with GSP, GEC, and MGEC ($p < 0.001$).
Instrumentation: High-performance liquid chromatography analysis.

Novel galactose single point method as a measure of residual liver function: Example of cefoperazone kinetics in patients with liver cirrhosis. Hu OY, Tang HS, Chang CL. J Clin Pharmacol 1995 Mar; 35 (3), pp. 250–8.

Hepatic Metabolization/Protein Biochemical Synthetic Function/Synthesis of Plasma and Hepatic Proteins/Vitamin D Deficiency
[^{14}C]-Labeled Amino Acids/[Bromosulphthalein (BSP)]

Cellular/Molecular Mechanism of Action: [^{14}C]-labeled amino acids are synthetically incorporated into biological protein fractions during intracellular translation events.
Application: Detection and characterization of *de novo* protein synthesis.
Route of Administration: [^{14}C]-labeled amino acids (variable but ideally intravenously).
Implementation: Evaluation of *de novo* hepatic and plasma protein synthesis in response to induced vitamin D deficiency. Liver function and protein metabolism is assessed by BSP clearance, liver histology, plasma transaminases, plasma alkaline phosphatase, and [^{14}C]-labeled amino acid incorporation into liver and plasma proteins.

Animal Model: Rats that are rendered vitamin D–deficient by housing them in a room free of ultraviolet light and maintained for 20 weeks on a diet devoid of only vitamin D. The vitamin D–deficiency state is confirmed by the undetectable levels of circulating vitamin D metabolites, severe hypocalcalcemia, and significantly reduced intestinal calcium transport.
Interpretation and Analysis: Subtle alterations in hepatic function are manifested by delayed BSP clearance and elevated levels of plasma transaminases and alkaline phosphatase. Liver histology reveals changes consistent with periportal necrosis. Synthesis of liver and plasma proteins is reduced by 26 to 34% ($p < 0.01$) without affecting the circulating levels of plasma proteins, suggesting reduced protein turnover with vitamin D deprivation. Alterations in hepatic function, therefore, appear to be directly and/or indirectly mediated by the properties of vitamin D.

Altered hepatic function in vitamin D–deprived rats. Pahuja DN, Deshpande UR, Soman CS, Nadkarni GD. J Hepatol 1989 Sep; 9 (2), pp. 209–16.

Hepatic Metabolization: Urea Cycle Biochemical Function/Congenital Portosystemic Vascular Anomalies
Ammonia Tolerance Test

Cellular/Molecular Mechanism of Action: Hepatocytes normally convert free ammonia into urea as a detoxification process. In conditions of compromised hepatic function, there is a delay or absence of ammonia detoxification by hepatocytes, resulting in higher than normal blood ammonia concentrations.
Application: Detection of conditions associated with hepatic disease.
Considerations: Contraindicated in patients with encephalomyelopathy or severe hepatic disease due to the risk of creating additional CNS dysfunction.
Implemenation: Evaluation of hepatic function based on the capacity to detoxify ammonia through conversion in the urea cycle. A postprandial ammonia tolerance test (PPATT) is performed in concert with trans-colonic scintigraphy, liver biopsy, or both and then classified as extrahepatic disease, primary hepatocellular, or congenital portosystemic vascular anomaly (PSVA) groups. Each dog is fed a chicken and rice diet providing 25% of its estimated daily metabolizable energy requirement (MER) as an ammonia challenge. This is practical in cases with liver disease because ammonium

chloride administration often causes vomiting or ammonia toxicity. Venous ammonia concentrations are measured before feeding and every 2 hours after feeding for 8 hours.

Animal Model: Canine (normal and with suspected hepatic disease),

Interpretation and Analysis: No difference in mean ammonia concentrations between dogs with extrahepatic disease and control dogs can be found. Therefore, the specificity of the PPATT is 100%. Dogs with hepatocellular disease show no change in mean ammonia concentration at any time point, before or after feeding, but sensitivity is greatest when venous ammonia is measured 6 hours after feeding (sensitivity before feeding, 28%, and after feeding, 36%). Among dogs with congenital PSVA, mean ammonia concentrations are higher than the reference range at all time points before and after feeding, and peak mean ammonia concentration occur 6 hours after feeding. In such cases, the sensitivity of the PPATT is 81% before feeding and 91% 6 hours after feeding. Measurements of venous ammonia concentration are a useful test to detect congenital PSVA, and the sensitivity of the test may be improved by sampling 6 hours after feeding. The PPATT has poor sensitivity in detecting primary hepatocellular disease.

Postprandial venous ammonia concentrations in the diagnosis of hepatobiliary disease in dogs. Walker MC, Hill RC, Guilford WG, Scott KC, Jones GL, Buergelt CD. J Vet Intern Med 2001 Sep–Oct; 15 (5), pp. 463–6.

Hepatic Metabolization/Warning for Administration Sequelae with Function Tests
Hepatotropic Diagnostic Media

Considerations: Hepatotropic diagnostic media when combined with protein solutions have been noted to create precipitates. The examination of different proteins and media has allowed the coagulative mechanism to be characterized and has revealed the development of electrostatic interactions between di- and polyvalent ligands. Positively charged protein groups play the major role and it has been suggested that this particular phenomenon contributes to the nephrotoxicity of some drug groups.

Hepatic Metabolization Capacity/Biochemical Marker/Cardiovascular Surgery Influence
Antipyrine

Cellular/Molecular Mechanism of Action: Antipyrine is a substrate for biochemical/metabolizing enzyme systems found in hepatic tissues.

Application: Molecular probe for assessing changes in hepatic drug metabolization.

Implementation: Evaluation of the effect of open-heart surgery on hepatic drug metabolism using the rate of antipyrine elimination as a reference index.

Human Model: Cases of open-heart surgery (n = 17).

Interpretation and Analysis: A degree of correlation exists between preoperative heart size and antipyrine elimination rate. In cases with a markedly dilated heart, the plasma antipyrine half-life is prolonged and apparent clearance significantly impaired. Immediately postoperatively, antipyrine elimination is impaired in all patients. Later, drug metabolism improves in cases with atrial septal defect, changes temporarily in patients with aortic valve replacement, and remains unchanged in patients with mitral valve replacement. The results indicate that adaptive changes in drug metabolizing capacity occur in cases undergoing cardiac surgery. The changes are related to the type of lesion corrected, the preoperative functional capacity of the liver, and the time lapse after surgery.

Liver drug metabolism in patients undergoing open-heart surgery. Kairaluoma MI, Sotaniemi EA, Mokka RE, Nuutinen L, Kärkölä P, Larmi TK. Scand J Thorac Cardiovasc Surg 1979; 13 (3), pp. 281–5.

HEPATOCYTE PHASE II BIOCHEMICAL METABOLISM PATHWAYS

Hepatic N-Methylation: Nicotinamide N-Methyltransferase (NNMT)/Pyrimidines Structurally Similar to Nicotinamide
Nicotinamide

Cellular/Molecular Mechanism of Action: Nicotinamide N-methyltransferase catalyses the N-methylation of nicotinamide and structurally related pyrimidines.

Background Information: Nicotinamide N-methyltransferase enzymatic activity in human liver varies over a 5-fold range with a bimodal frequency distribution. This observation raises the possibility that the expression of this enzyme fraction is regulated by genetic polymorphism.

Application: Substantial variations in NNMT biochemical activity (hepatic origin) are not associated with genetic polymorphisms associated with the 3 exons or 5′-flanking regions of the NNMT gene sequence.

Implementation: Initially, Northern blot analysis can be applied to confirm the expression of NNMT in liver biopsy specimens. Quantitative immunodetection (Western blot) analysis can then be applied to measure the amount of immunoreactive NNMT contained in tissue samples. Values for laboratory reference controls can be determined through analysis of liver biopsy samples collected from cases previously determined to have "low" or "high" levels of NNMT biochemical activity. The 3 exons for the NNMT gene can be amplified applying polymerase chain reaction (PCR) methodologies using hepatic biopsy specimens as a source of genetic material. Genetic polymorphism can then be detected applying DNA sequence analysis in order to determine if there is a degree of correlation with the "nicotinamide N-methyltransferase phenotype."

Interpretation and Analysis: Based on Northern blot analysis, NNMT is highly expressed in human liver tissue. There is a highly significant correlation ($r^s = 0.96$, $p < 0.0001$) between NNMT activity and immunoreactive protein in these samples. A potent promoter is located within the initial 700 bp of the 5′-flanking region of the human NNMT gene sequence. The gene consists of 3 exons, with an initial 1,240 bp intron and a 2nd intron that is approximately 14 kb in length. Analysis of DNA samples isolated from hepatic tissues associated with "low," "intermediate," or "high" levels of NNMT activity (e.g., n = 27 human biopsies) reveals that the 3 exons are all 1,240 bp of intron 1 and approximately 700 bp of the 5′-flanking region of the NNMT gene. No single-nucleotide polymorphisms (SNPs) or insertion/deletion events have been detected within either the exons or 5′-flanking regions of NNMT (e.g., n = 27 human hepatic biopsy samples). Although some single nucleotid polymorphism (e.g., n = 8/27) exists within intron 1, none has been detected to be systematically related to the level of NNMT biochemical activity. These results indicate that the exons and 5′-flanking region of the NNMT gene display little or no sequence variation.

Human nicotinamide N-methyltransferase pharmacogenetics: Gene sequence analysis and promoter characterization. Yan L, Otterness DM, Weinshilboum RM. Pharmacogenetics 1999 Jun; 9 (3), pp. 307–16.

Hepatocyte Conjugation/Phase II Biotransformation Capacity
Paracetamol

Cellular/Molecular Mechanism of Action: Paracetamol is biotransformed into glucuronide and sulfate metabolites that are ultimately excreted into the urine.
Application: Determination of phase II metabolism capacity.
Considerations: The use of wavelength-switching UV detection for the simultaneous detection of paracetamol and its glucuronide and sulfate metabolites in urine allows for minimal sample manipulation but maximal specificity in the analysis of biohazardous biological fluids.
Instrumentation: Reverse-phase high-performance liquid chromatography. The solvent system involves a simple isocratic elution with a composition of 50 mM sodium acetate buffer, pH adjusted to 3.5, and acetonitrile (96:4 v/v) modified with 0.35% trifluroacetic acid. The validated method is highly reproducible with an interassay variation of $< 7\%$. This method also shows good precision and sensitivity, making it an ideal assay for phenotyping studies to determine the extent of glucurondiation and sulfation activities.

A validated method for the determination of paracetamol and its glucuronide and sulfate metabolites in the urine of HIV+/AIDS patients using wavelength-switching UV detection. Di Girolamo A, O'Neill WM, Wainer IW. J Pharm Biomed Anal 1998 Sep 1; 17 (6–7), pp. 1191–7.

Hepatocyte Trans-Sulferation Capacity/Alcohol-Induced Cirrhosis
[$^2H_3$]-Methyl-1-[^{13}C]Methionine/[$^{13}CO_2$] Exhalation

Cellular/Molecular Mechanism of Action: [$^2H_3$]-methyl-1-[^{13}C]methionine is a radiolabeled analog of methionine that is metabolized within the liver along the same pathways as are similar amino acids. In this context, methionine can function as a substrate for both trans-sulfuration and remethylation metabolic pathways. Trans-sulfuration through decarboxylation of [$^2H_3$]-methyl-1-[^{13}C] methionine to yield [$^{13}CO_2$] is followed by remethylation involving the replacement of the labeled methyl group by an unlabeled methyl group.
Background Information: Disturbances of the methionine cycle may result in liver injury. Patients with alcohol-induced liver disease often exhibit hypermethioninemia and a delayed clearance (CL) of methionine, but the extent to which trans-sulfuration and remethylation pathways of the cyclic methionine metabolism are affected is largely unknown.
Application: Assessment of liver-associated biochemical metabolizing function, which can serve as a indicator of hepatic functional capacity.
Route of Administration: [$^2H_3$]-methyl-1-[^{13}C]methionine (oral).
Dosage: [$^2H_3$]-methyl-1-[^{13}C]methionine (2 mg/kg).
Implementation: Evaluation of methionine metabolism in conditions of alcohol-induced cirrhosis using [$^2H_3$]-methyl-1-[^{13}C]methionine as a metabolic probe. Specifically, the application of this agent permits monitoring trans-sulfuration by decarboxylation to [$^{13}CO_2$] and remethylation by replacement of the labeled methyl group by an unlabeled "1" molecule.

Human Model: Cases of alcohol-induced cirrhosis ($n = 6$ cases; $n = 7$ normal healthy volunteers).
Interpretation and Analysis: Basal plasma concentrations of endogenous methionine (50 ± 5 vs 25 ± 2 μmol/L, mean ± SEM, $p < 0.001$) are significantly higher in patients with cirrhosis and its clearance is significantly decreased (774 ± 103 vs $2{,}050 \pm 141$ mL/min, $p < 0.001$). Methionine turnover is much slower in conditions of cirrhosis compared to healthy controls (e.g., 27 ± 3 vs 42 ± 4 μmol/kg/hour, $p < 0.05$) respectively. The fraction of administered methionine undergoing remethylation is lower in cirrhosis (7.6 ± 1.5 vs $14.1 \pm 1.1\%$, $p < 0.005$). However, because of the larger pool of circulating methionine, the total flux of methionine through the remethylation pathway is similar in both cirrhosis and normal reference controls. A significantly lower fraction of the administered dose appears in the form of [$^{13}CO_2$] in the breath from patients with cirrhosis (2.2 ± 0.4 vs $11.0 \pm 0.8\%$, $p < 0.001$). Cirrhosis of the liver compensates for a decreased activity of remethylating enzymes by operating at higher concentrations of methionine. In contrast, trans-sulfuration is impaired in patients with alcohol-induced cirrhosis such that an assessment of trans-sulfuration by a simple breath test may provide a clinically useful estimate of hepatic function.

Remethylation and trans-sulfuration of methionine in cirrhosis: Studies with L-[H_3-methyl-1-C]methionine. Russmann S, Junker E, Lauterburg BH. Hepatology 2002 Nov; 36 (5), pp. 1190–6.

HEPATIC PATHOLOGY AND DISEASE STATES

Hepatic Inflammation in Hepatitis: Alcohol-Induced/H_2O_2/L-Selectin/IL-8/Tumor Necrosis Factor-Alpha (TNF-α)/IL-10
Corticosteroids

Background Information: Several observations point to an important role of interactions between polymorphonuclear neutrophils and cytokines in severe alcoholic hepatitis. The polymorphonuclear neutrophil activation status and the local and systemic pro- and anti-inflammatory cytokine responses are quantified. The effect of corticosteroids, widely used in this setting, is evaluated using these parameters.
Application: Potential differentiation of alchohol-induced hepatitis and alcohol-induced cirrhosis without hepatitis and normal reference (healthy) controls.
Implementation: Evaluation of blood polymorphonuclear neutrophil functions pertaining to L-selectin and β_2-integrin expression, H_2O_2 production, and IL-8 and TNF-α synthetic capacity. Complementary analysis includes measurement of plasma and hepatic IL-8, TNF-α, and IL-10 levels. The impact of a 28-day course of corticosteroids on blood neutrophils activation status and cytokine levels can be of benefit in evaluating patients with alcoholic hepatitis.

Human Model: Cases of alcoholic hepatitis ($n = 15$; compared to $n = 15$ conditions of alcoholic cirrhosis without alcoholic hepatitis; compared to $n = 10$ healthy volunteers).
Interpretation and Analysis: Blood polymorphonuclear neutrophils in conditions of alcoholic hepatitis are activated, as shown by increases in H_2O_2 production (48 ± 6 vs 29 ± 6 MFI in healthy controls), and decreases in L-selectin expression (300 ± 61 vs 449 ± 59 in healthy controls). Upon stimulation, polymorphonuclear neutrophils synthesize large amounts of IL-8 (21.7 ± 9.2 ng/ml vs 8.8 ± 10 ng/ml in healthy controls) and TNF-α (524 ± 132 pg/ml vs 79 ± 144 pg/ml in healthy controls). Tumor necrosis factor-alpha and IL-8 plasma and tissue levels markedly increase in contrast to IL-10, which is barely detectable in alcoholic hepatitis patients, compared to cirrhotic patients and healthy controls. During steroid therapy, plasma levels of the proinflammatory cytokine IL-8 fall as

early as day 14, while levels of the anti-inflammatory cytokine IL-10 increase on day 21. Finally, polymorphonuclear neutrophil functions return to normal after treatment. Severe alcoholic hepatitis appears to be associated with polymorphonuclear neutrophil activation and an imbalance between pro- and anti-inflammatory cytokines; during steroid therapy a normalization of these parameters is observed.

Blood neutrophil functions and cytokine release in severe alcoholic hepatitis: Effect of corticosteroids. Taïeb J, Mathurin P, Elbim C, Cluzel P, Arce-Vicioso M, Bernard B, Opolon P, Gougerot-Pocidalo MA, Poynard T, Chollet-Martin S. J Hepatol 2000 Apr; 32 (4), pp. 579–86.

Hepatic Neoplastic Disease: Metastatic Midgut Carcinoid
Pentagastrin (PG)/Calcium Infusion/Ketanserin

Cellular/Molecular Mechanism of Action: Pentagastrin is a synthetic pentapeptide that normally stimulates gastric function. Ketanserin binds to serotonin receptors and blocks binding of serotonin to these complexes.
Application: Pentagastrin has been applied successfully to detect conditions of carcinoid syndrome.
Implementation: Evaluation of hepatic midgut carcinoid metastatic conditions by measuring the clinical and hormonal response to PG compared to calcium infusion. Peripheral blood samples are then collected for measurement of serotonin (5-HT) and substance P (SP) concentrations. Ketanserin can be co-administered to delineate the molecular pathogenesis of gastrointestinal systems associated with carcinoid syndrome.
Interpretation and Analysis: Pentagastrin causes flushing and gastrointestinal symptoms in addition to promoting elevated levels of circulating serotonin (5-HT), but not SP. Pretreatment with a 5-HT2 receptor blocking agent (ketanserin) alleviates gastrointestinal symptoms but has no influence on either 5-HT release or PG-induced flushing. Calcium infusion can induce carcinoid symptoms in approximately 30% of affected cases (e.g., n = 2/6) that have associated elevations in 5-HT levels. Elevations in SP may be seen in only a limited number of cases. Serotonin (5-HT) appears to be important for the development of gastrointestinal symptoms but not flushing. Ketanserin may alleviate gastrointestinal symptoms but does not influence pentagastrin-induced release of 5-HT. Substance P and 5-HT do not seem to share a common release mechanism. Pentagastrin testing is superior to calcium infusion as a provocative test in patients with the carcinoid syndrome.

The pentagastrin test in the diagnosis of the carcinoid syndrome. Ahlman H, Dahlström A; Grönstad K, Jaffe BM, Nilsson O, Oberg K. J Cardiovasc Pharmacol 1985; 7 Suppl 7, pp. S86–8.

Hepatic Neoplastic Disease: Metastatic Midgut Carcinoid
Pentagastrin (PG)/Calcium Infusion/Ketanserin

Cellular/Molecular Mechanism of Action: Pentagastrin is a synthetic pentapeptide that normally stimulates gastric function. Ketanserin binds to serotonin receptors and blocks binding of serotonin to these complexes.
Application: Pentagastrin has been applied successfully to detect conditions of carcinoid syndrome. Ketanserin can be used as a validation pharmaceutical agent that blocks gastrointestinal signs precipitated by elevations in systemic serotonin concentrations.
Route of Administration: Pentagastrin (intravenous).
Implementation: Evaluation of carcinoid syndrome by characterizing the clinical response and measurment of changes in 5-hydroxytryptamine (serotonin, 5-HT) and substance P (SP) concentrations in response to intravenous PG and calcium infusion.
Human Model: Cases of known midgut carcinoid (n = 17 midgut carcinoid; n = 16/17 hepatic metastasis; n = 17/17 with supranormal basal levels of 5-HT and SP).
Interpretation and Analysis: Pentagastrin administration in conditions of midgut carcinoid syndrome causes (i) flushing in a high percentage of affected cases (e.g., n = 17/17); and (ii) gastrointestinal symptoms in most cases affected with hepatic involvement (e.g., n = 15/16); while it also universally elevates circulating 5-HT levels. Pretreatment with the 5-HT2-receptor blocking agent, ketanserin, abolishes the gastrointestinal effects but has virtually no influence on either 5-HT levels or flushing induced by intravenous pentagastrin. In contrast, calcium infusion induces carcinoid symptoms in only approximately 30% of cases (e.g., n = 2/6) and is a feature that is consistently associated with stimulation of circulating serotonin levels. Therefore, (i) 5-HT may be responsible for the gastrointestinal symptoms in carcinoid patients, but it does not seem to play any role in flushing; (ii) ketanserin may be a useful therapeutic agent in alleviating gastrointestinal symptoms in carcinoid patients; (iii) differential responses to PG suggests that SP is released from a site different from that of serotonin (5-HT); (iv) it is possible that SP may contribute to the mediation of flushing, but it cannot be the sole agent causing this symptom; and (v) the pentagastrin test with measurements of 5-HT levels in peripheral blood seems to be superior to calcium infusion as a provocative test in documenting the diagnosis of carcinoid disease.
Instrumentation: High-performance liquid chromatography-electron capture and radioimmunoassay methods.

Characterization of hepatic cytochrome P450 3A activity in patients with end-stage renal disease. Dowling TC, Briglia AE, Fink JC, Hanes DS, Light PD, Stackiewicz L, Karyekar CS, Eddington ND, Weir MR, Henrich WL. Clin Pharmacol Ther 2003 May; 73 (5), pp. 427–34.

Hepatic Organophosphate Metabolism/ Cytochrome P450/Genetic Suceptibility: Oxytremorine/Nicotine: Genetic Susceptibility
Diisopropylfluorophosphate (DFP)

Cellular/Molecular Mechanism of Action: The organophosphate DFP binds to acetylcholine esterase, thereby preventing the degradation of acetylcholine neurotransmitter.
Physiological Mechanism of Action: Inhibition of acetylcholine esterase causes an accumulation of acetylcholine neurotransmitter in a manner that promotes over-stimulation of the parasympathetic segment of the autonomic nervous system (e.g., diarrhea, salivation, altered cardiac function).
Application: Detection of genetic parameters that influence vulnerability or resistance to organophosphate intoxication (e.g., DFP). Examples could include variations in the genetic expression of certain microsomal enzyme P450 fractions within the liver.
Implementation: Inbred stains of mice (e.g., C57BL, DBA, C3H) can be applied to investigate relative differences in a battery of physiological and locomotor tests including respiratory rate, heart rate, body temperature, Y-maze activity, and rotarod performance in response to DFP exposure.
Interpretation and Analysis: In dose-response and time-course studies, marked affects are noted approximately 15 minutes after injection with maximal effects occurring approximately 2 hours after initial exposure. Strain comparisons made at the 2-hour time point reveal that males and females are affected about equally except for respiratory rate and rotarod performance in which females are slightly more affected. Strain comparisons reveal that for most of the tests the C57BL mice are most affected by the DFP in contrast to C3H mice that are least affected. In contrast, DBA mice strains are

most vulnerable to DFP-mediated alterations in heart rate. Analogous studies have demonstrated a similar rank ordering of inbred strains of mice in their responses to oxotremorine and nicotine. The strain differences in response to these agents is not easily explained by differences in number or affinity of brain muscarinic or nicotinic receptors. The genetic influence on cholinergic drug response may involve receptor coupling mechanisms.

A strain comparison of physiological and locomotor responses of mice to diisopropylfluorosphosphate. Smolen A, Smolen TN, Oh EI, Collins AC. Pharmacol Biochem Behav 1986 Apr; 24 (4), pp. 1077–82.

Hepatic Vascular Malformation: Portosystemic Shunt/Non-Invasive Detection of Portosystemic Shunt Patency/Postoperative Shunt Anastomosis Occlusion
D-Xylose/[Urinary Excretion]

Physiological Mechanism of Action: D-xylose is normally assimulated by hepatocytes. In circumstances where the plasma concentration of D-xylose exceeds the renal threshold, significant amounts are excreted into the urinary filtrate.
Application: The D-xylose excretion test appears to be a useful method of monitoring shunt patency after a portasystemic venous anastomsis for portal hypertension.
Implementation: Evaluation of cases of hepatic portosystemic shunt using a D-xylose 5-hour urinary excretion test.

Human Model: Cases of hepatic portosystemic shunt.
Interpretation and Analysis: A 5-hour D-xylose urinary excretion test yielding a total greater than 6 g appears to be an acceptable indicator that a patent shunt is present. A 2nd criterion for patency is an increase of 4 g or more over the result of preoperative tests. If the excretion rate falls in the postoperative period or remains $<$ 4–2 g/5 hours, the anastomosis may be assumed to be occluded.

Assessment of portasystemic shunt patency by estimation of D-xylose excretion. Smith RC, Macpherson AI. Br J Surg 1976 Jun; 63 (6), pp. 435–7.

EXTRAHEPATIC DISEASE CAUSED BY LIVER DYSFUNCTION

Hepatic Encephalopathy/Acute Hepatic Encephalopathy/Encephalopathy Associated with Hemorrhage/Potential Prognostic Indicators
Flumazenil/Pharmaco-Electroencephalography

Background Information: Randomized controlled trials testing flumazenil in hepatic encephalopathy have shown conflicting results.
Application: Evaluate the effects of flumazenil on conditions of hepatic encephalopathy in patients with liver cirrhosis and the potential for therapeutic response to correlate with prognosis.
Route of Administration: Flumazenil (intravenous).
Dosage: Flumazenil (2 mg).
Implementation: Evaluation of the response to flumazenil in conditions of encephalopathy associated with hepatic cirrhosis. Administration of flumazenil or placebo is accompanied by the co-administration of conventional treatments (e.g., branched-chain amino acid, saline, glucose, and lactulose). Alterations in clinical signs are assessed over a 24-hour observation period. Clinical improvement is defined as a 3-point decrease in the Glasgow Coma Score at any time within 24 hours.

Human Model: Cases of encephalopathy associated with hepatic cirrhosis (n = 54 cases with hepatic encephalopathy grade III–IV). For each end point, heterogeneity and treatment efficacy are assessed.

Study Design: Double blind randomized controlled trials (n = 6 with n = 641 patients; n = 326/641 treated with flumazenil, n = 315/641 placebo); the treatment duration can range between 5 minutes and 3 days. (Note: Heterogeneity tests between control groups not significant.)
Interpretation and Analysis: In trials (e.g., n = 5), the mean percentage of clinical improved is approximately 27% with pharmaceutical challenge and about 3% with placebo reference controls (Peto methodology: odds ratio = 6.15; 95% confidence interval, 4.0–9.5; $p < 0.001$; Der Simonian methodology: mean rate difference, 29%; 95% confidence interval, 17–41; $p < 0.001$). The mean percentage of cases with electroencephalographic improvement is approximately 19% with flumazenil but 2% with placebo. The difference is significant only with the Peto method (odds ratio = 5.8; 95% confidence interval, 3.4–9.7; $p < 0.001$). Sensitivity analysis reveals similar findings. Meta-analysis of this type reveals that flumazenil induces clinical and electroencephalographic improvement of hepatic encephalopathy in patients with cirrhosis.

In related investigations, clinical improvements have been appreciated in a large percentage of affected cases (e.g., n = 22/28 with flumazenil; n = 14/26 placebo@$p < 0.05$). Improvement is observed within the 1st 6 hours in the vast majority of cases given flumazenil (e.g., n = 21/22 patients vs n = 3/14 placebo). Unfortunately, the mortality rate is not different between case groups receiving either flumazenil challenge or placebo. However, a large percentage of non-responders subjected to flumazenil challenge died within 24 hours (e.g., n = 6/6 or 100%) in contrast to only slightly less than half of placebo-treated cases (e.g., n = 5/12 non-responders).

In conditions of postbleeding encephalopathy, over half of affected cases challenged with flumazenil improve (n = 11/17) compared to a very low number of placebo cases (e.g., n = 2/14@$p < 0.001$). Flumazenil therefore exerts a beneficial effect in a subset of patients with acute hepatic encephalopathy; encephalopathy associated with bleeding is more likely to respond to flumazenil; and responders to the treatment usually improve within the 1st 6 hours, while lack of response usually represents a bad prognostic sign.

Flumazenil in the treatment of acute hepatic encephalopathy in cirrhotic patients: A double blind randomized placebo controlled study. Laccetti M, Manes G, Uomo G, Lioniello M, Rabitti PG, Balzano A. Dig Liver Dis 2000 May; 32 (4), pp. 335–8.

Flumazenil vs placebo in hepatic encephalopathy in patients with cirrhosis: A meta-analysis. Goulenok C, Bernard B, Cadranel JF, Thabut D, Di Martino V, Opolon P, Poynard T. Aliment Pharmacol Ther 2002 Mar; 16 (3), pp. 361–72.

PHARMACOGENETICS

PHARMACOGENETICS CONCEPTS AND DISEASE EXAMPLES

Amitriptyline: Individualization of Dosage Regimens
Hamilton Score/Multiple Points Method/Modified Bayesian Method

Background Information: Individualization of an amitriptyline dose regimen offers substantial advantages over non-individualized treatment.
Application: Individualization of amitriptyline dose regimens for the purpose of maximizing therapeutic effect and minimizing adverse side effect.
Implementation: In patients with disorders of major depression and receiving amitriptyline therapy, the assessment of clinical effects, adverse effects, and plasma steady state concentrations can be

applied to evaluate cases within the following 3 categories: (i) patients in Group A taking non-individualized doses of amitriptyline; (ii) patients in Group B taking doses of amitriptyline individualized by the modified Bayesian method; and (iii) patients in Group C taking doses of amitriptyline individualized by the multiple point method.

Interpretation and Analysis: Individuals within Group A take significantly higher doses throughout the treatment course; the initial doses for cases in Group B are higher than doses for the patients in Group C, but after corrections based on measured steady state plasma concentrations they are found to be similar.

While the Hamilton score descends uniformly in all 3 groups, both adverse effects and steady state plasma concentrations of amitriptyline are higher in the non-individualized group during the whole treatment course. The multiple points method is considered to be the most precise but the most tedious and sometimes impractical technique. The modified Bayesian method with corrections based on 1st measured plasma steady state concentrations of amitriptyline offers similar therapeutic outcome and adverse effects scores combined with low cost and convenient and easy-to-use protocols.

Comparison of 2 approaches to amitriptyline dose individualization. Jankovic SM, Timotijevic I, Mihajlovic GS, Dukic-Dejanovic S. Eur J Drug Metab Pharmacokinet 1999 Apr–Jun; 24 (2), pp. 163–8.

Arylamine N-Acetyltransferase Genetic Polymorphism

Application: Human acetylation polymorphism, which confers marked interindividual variation in the effect and toxicity of numerous drugs, chemicals, and potential carcinogens.

Considerations: Human acetylation polymorphism confers marked interindividual variation related to the effect and toxicity of numerous drugs, chemicals, and potential carcinogens.

Animal Model: New Zealand white rabbit.

Implementation: Genetically polymorphic arylamine N-acetyltransferase (NAT; acetyl-CoA: arylamine N-acetyltransferase, EC 2.3.1.5) from rabbit liver can be successfully expressed in monkey kidney COS-1 cell populations. Applying the genetic clones and anti-NAT antibodies, it is possible to investigate the relationship among NAT activity, liver enzyme protein, and the level of mRNA in livers from *in vivo* phenotyped rapid-acetylator and slow-acetylator rabbits.

Interpretation and Analysis: Livers from slow acetylators are devoid of both immunodetectable NAT protein and its corresponding mRNA sequence. Analysis of genomic DNA with a panel of restriction enzymes reveals a loss of specific hybridizing bands in the DNA of slow-acetylator rabbits. Defective arylamine N-acetylation in certain strains of rabbits is believed to be caused by a gene deletion resulting in an absence of specific mRNA and NAT enzyme protein.

N-acetylation pharmacogenetics: A gene deletion causes absence of arylamine N-acetyltransferase in liver of slow acetylator rabbits. Blum M, Grant DM, Demierre A, Meyer UA. Proc Natl Acad Sci U S A. 1989 Dec; 86 (23), pp. 9554–7.

Azothioprine/6-Mercaptopurine Inflammatory Bowel Disease (IBD): Crohn's Disease/Sugar Permeability Test
Pharmacogenetics: Dosage Individualization

Background Information: The diagnosis and management of IBD in children and adolescents is a challenge to patients, to their families, and to the health-care team. Among the most prominent advances are new diagnostic serologic assays that can help screen for IBD in the absence of physical signs of disease and help discriminate between ulcerative colitis and Crohn's disease.

Application: Individualization of pharmaceutical dosage in conditions of IBD/Crohn's disease.

Interpretation and Analysis: Other tests that have been identified as potential non-invasive markers of disease activity include Doppler abdominal ultrasound. Recent advances in pharmacogenetics concepts afford the ability to optimize and individualize therapy using azathioprine or 6-mercaptopurine. Finally, bone health has come forth as a major issue in the complete management of pediatric IBD.

Recent advances in the diagnosis and treatment of pediatric inflammatory bowel disease. Seidman EG. Curr Gastroenterol Rep 2000 Jun; 2 (3), pp. 248–52.

Barbiturate Elimination: Genetic Variation
Amobarbital

Cellular/Molecular Mechanism of Action: Amobarbital is a barbiturate agent.

Application: Delineation of the influence of genetics on the pharmacokinetic profiles of amobarbital. Diagnostically, amobartital is frequently selected as the preferred agent for performing the Wada test in conditions affecting the central nervous system (e.g., selective delivery by arterial catheter to localize abnormal/dysfunctional regions of the brain as in intractable cases of epilepsy).

Route of Administration: Amobarbital (intravenous).

Dosage: Amobarbital (125 mg).

Implementation: Evaluation of the time course profile of plasma amobarbital sodium concentrations following intravenous administration (125 mg).

Human Model: Cases of dizygotic twins (n = 7 pairs) and monozygotic twins (n = 7 pairs).

Interpretation and Analysis: The number of detectable compartments varies from between 1, 2, or 3 but is consistent within a given subject. The terminal slope of the semilogarithmic concentration-time plot (biologic half-life of 22.8 hours) does not represent the elimination rate constant even in cases with apparently single-compartmental profiles. The redistribution of amobarbital is relatively more rapid compared to the elimination phase. The rate of the latter (characterized by k_{el} = 0.051 hr-1, plasma clearance = 37.7 ml/min) can be closely correlated with the rate of metabolism. Results obtained from twin subjects reveal that genetic factors influence the kinetic parameters characterizing the rate of amobarbital elimination and, therefore, the rate of its metabolism. Correlation analysis suggests that genetic factors function in a manner that is independent of body size (strongly heritable phenotype). Based on these observations, it is recommended that during the course of performing pharmacogenetic studies, it is important to assess model-independent kinetic parameters, such as plasma clearance with or without adjustments for body weight.

Genetic study of amobarbital elimination based on its kinetics in twins. Endrenyi L, Inaba T, Kalow W. Clin Pharmacol Ther 1976 Dec; 20 (6), pp. 701–14.

Carvedilol: Hepatic Metabolization Polymorphism/ Extrapolation from Debrisoquin and Mephenytoin
Mixed-Adrenergic Receptor Antagonists/Carvedilol/ Debrisoquin/Mephenytoin

Cellular/Molecular Mechanism of Action: Carvedilol is a mixed α-adrenergic and β-adrenergic receptor antagonist agent. Although the 2 isomers are equally potent as α1-blockers, the S(-)-isomer is principally responsible for the beta blockade of carvedilol.

Application: Utilization of metabolization profiles for debrisoquin and mephenytoin to determine their value as predictive molecular probes for the estimation of carvedilol metabolization profiles.
Dosage: Administered as a racemic mixture.
Implementation: Determination of the role of pharmacogenetics in the metabolism of carvedilol through the investigation of (i) individuals that extensively metabolize both debrisoquin and mephenytoin; (ii) individuals that poorly metabolize debrisoquin but are extensive metabolizers of mephenytoin; and (iii) poor metabolizers of mephenytoin but extensive metabolizers of debrisoquin.
Interpretation and Analysis: The clearance of R-carvedilol is significantly lower than S-carvedilol in both debrisoquin phenotypes. Poor metabolizers of debrisoquin have a significantly lower clearance of R-carvedilol than extensive metabolizers of debrisoquin. The partial metabolic clearance of carvedilol to the 2 ring-hydroxylated metabolites 4- and 5-hydroxyphenyl carvedilol is significantly reduced in poor metabolizers of debrisoquin. No effect of mephenytoin phenotype on carvedilol kinetics can be appreciated. Carvedilol is therefore stereoselectively metabolized in humans, and the clearance of S-carvedilol is higher than that of R-carvedilol. In poor metabolizers of debrisoquin the clearance of R-carvedilol is further reduced, resulting in higher plasma concentrations and perhaps greater levels of alpha-blockade.

Stereoselective disposition of carvedilol is determined by CYP2D6. Zhou HH, Wood AJ. Clin Pharmacol Ther 1995 May; 57 (5), pp. 518–24.

Pharmacogenetics Attributes and Perspectives

Pharmacogenomics is an emerging scientific discipline examining the genetic basis for individual variations in response to therapeutics. Genetic polymorphisms are a major cause of individual differences in drug response. Metabolic phenotyping can be accomplished by administering a probe drug or substrate and measuring the metabolites and clinical outcomes. However, this approach tends to be labor intensive and requires repeated sample collection from the individual being tested. Alternatively, genotyping allows determination of individual DNA sequence differences for a particular trait. Commonly used genotyping methods include gel electrophoresis-based techniques, such as polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism analysis, multiplex PCR, and allele-specific amplification. Fluorescent dye-based high-throughput genotyping procedures are increasing in popularity, including oligonucleotide ligation assay, direct heterozygote sequencing, and TaqMan (Perkin Elmer, Foster City, CA) allelic discrimination. High-density chip array and mass spectrometry technologies are the newest advances in the genotyping field, but their wide application is yet to be developed. Novel mutations/polymorphisms also can be identified by conformation-based mutation screening and direct high-throughput heterozygote sequencing. Rapid and accurate detection of genetic polymorphisms has great potential for application to drug development, animal toxicity studies, improvement of human clinical trials, and postmarket monitoring surveillance for drug efficacy and toxicity.

Technologies for detecting genetic polymorphisms in pharmacogenomics. Shi MM, Bleavins MR, de la Iglesia FA. Mol Diagn 1999 Dec; 4 (4), pp. 343–51.

Pharmacogenetics Attributes and Perspectives

The majority of the research and clinical-oriented manuscripts published in the field of pharmacogenetics, genetic defects, and predictive maternal genotype assays between 1985 and 1999 have primarily addressed the topics of (i) polymorphisms, (ii) genotyping, (iii) cytochrome P450 enzyme systems, (iv) epoxide hydrolase, (v) folate metabolism, metabolism of anticonvulsant medications, (vi) molecular genetics of neural tube defects, (vii) variations in drug metabolism, and (viii) environmental exposures. Collectively, this body of work has emphasized that individuals vary in how they metabolize drugs and handle toxic environmental exposures. In an ideal pregnancy, there is no or limited exposure to medications and environmental agents. However, in women with chronic medical conditions such as heart disease and seizures, this is often not possible. Unfortunately, no techniques have been developed to identify those at risk in this treatment subpopulation. Gene polymorphisms for a specific enzyme may result in an absence or reduction in the level of enzyme activity or in no change at all, with little effect on the structure/function of the gene product(s); they are not associated with clinical phenotypes in either the mother or the fetus. Other polymorphisms may be only markers. Thus, developing genotyping assays for women that are predictive of phenotype expression in the fetus is the key to screening for polymorphisms. As more mutations are identified and clinical, pharmacologic, biologic, and pharmacokinetic relationships are established, using these polymorphisms to develop a genotyping assay for women may become a clinical reality, possibly leading to preventive prepregnancy or prenatal treatment that may play an increasingly effective role in maternal care.

Pharmacogenetic screening for susceptibility to fetal malformations in women. Van Dyke DC, Ellingrod VL, Berg MJ, Niebyl JR, Sherbondy AL, Trembath DG. Ann Pharmacother 2000 May; 34 (5), pp. 639–45.

Pharmacogenetics Attributes and Perspectives

Genomics, particularly high-throughput sequencing and characterization of expressed human genes, has created new opportunities for drug discovery. Knowledge of all the human genes and their functions may allow effective preventive measures and change drug research strategy and drug discovery development processes. Pharmacogenomics is the application of genomic technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. It applies the large-scale systematic approaches of genomics to speed the discovery of drug response markers, whether they act at the level of the drug target, drug metabolism, or disease pathways. The potential implication of genomics and pharmacogenomics in clinical research and clinical medicine is that disease could be treated according to genetic and specific individual markers, selecting medications and dosages that are optimized for individual patients. The possibility of defining patient populations genetically may improve outcomes by predicting individual responses to drugs, and could improve safety and efficacy in therapeutic areas such as neuropsychiatry, cardiovascular medicine, endocrinology (diabetes and obesity), and oncology. Ethical questions need to be addressed and guidelines established for the use of genomics in clinical research and clinical medicine. Significant achievements are possible with an interdisciplinary approach that includes genetic, technological, and therapeutic measures.

Impact of genomics on drug discovery and clinical medicine. Emilien G, Ponchon M, Caldas C, Isacson O, Maloteaux JM. QJM 2000 Jul; 93 (7), pp. 391–423.

Pharmacogenetics Attributes and Perspectives

Pharmacogenetics is the science of the influence of heredity on pharmacological response. One issue related to this consideration is the cost of severe adverse drug reactions in individuals, which has

been estimated in the United States alone to be in excess of $4 billion. It has been argued that in a significant proportion of cases, the efficacy and toxicity profiles of drug therapy would be substantially improved in individuals if characteristics due to genetic variation are taken into account. Methods are now available that make screening for susceptibility feasible. There are several therapeutic areas in which screening may give rise to significant improvements in outcome with cost benefits to both the individual and the community. However, there is currently a lack of data on which cost-benefit analysis can be based. The challenge is to provide this information for new drugs and for drugs with established therapeutic roles.

Pharmacogenetic screening and therapeutic drugs. Steimer W, Potter JM. Clin Chim Acta 2002 Jan; 315 (1–2), pp. 137–55.

Pharmacogenetics Attributes and Perspectives

Genetic variability in drug response occurs as a result of molecular alterations at the level of drug-metabolizing enzymes, drug targets/receptors, and drug transport proteins. Reports have discussed the possibility that therapeutic drug monitoring (TDM) in the future will involve not the mere measurement and interpretation of drug concentrations but will include both traditional TDM and pharmacogenetics-oriented TDM. In contrast to traditional TDM, which cannot be performed until after a drug is administered to the patient, pharmacogenetics-oriented TDM can be conducted even before treatment begins. Other advantages of genotyping over traditional TDM include, but are not limited to, the following: (i) it does not require the assumption of steady state conditions (or patient compliance) for the interpretation of results; (ii) it can often be performed less invasively (with saliva, hair root, or buccal swab samples); (iii) it can provide predictive value for multiple drugs (e.g., a number of cytochrome P450 [CYP] 2D6, CYP2C 19, or CYP2C9 substrates) rather than a single drug; (iv) it provides mechanistic, instead of merely descriptive, information; and (v) it is constant over an individual's lifetime (and not influenced by concurrent drug administration, alteration in hormonal levels, or disease states). Pharmacogenetic information can be applied as a priority for initial dose stratification and identification of cases where certain drugs are simply not effective. However, traditional TDM will still be required for various reasons. In current clinical practice, pharmacogenetic testing is performed for only a few drugs (e.g., mercaptopurine, thioguanine, azathioprine, trastuzumab, and tacrine) and in a limited number of teaching hospitals and specialist academic centers. It has been proposed that other drugs (e.g., warfarin, phenytoin, codeine, oral hypoglycemics, tricyclic antidepressants, aminoglycosides, digoxin, cyclosporine, cyclophosphamide, ifosfamide, theophylline, and clozapine) are potential candidates for pharmacogenetics-oriented TDM. However, prospective studies of pharmacogenetics-oriented TDM must be performed to determine its efficacy and cost-effectiveness in optimizing therapeutic effects while minimizing toxicity. In the future, in addition to targeting a patient's drug concentrations within a therapeutic range, pharmacists are likely to be making dosage recommendations for individual drugs on the basis of the individual patient's genotype. Upon entering the era of personalized drug therapy, it will be possible to identify not only the best drug to be administered to a particular patient but also the most effective and safest dosage from the outset of therapy.

Pharmacogenetics: The therapeutic drug monitoring of the future? Ensom MH, Chang TK, Patel P. Clin Pharmacokinet 2001;40 (11), pp. 783–802.

Pharmacogenetics Attributes and Perspectives

In recent years intense activity in both academic and industrial sectors has provided a wealth of information on the human genome with an associated impressive increase in the number of novel gene sequences deposited in sequence data repositories and patent applications. This genomic industrial revolution has transformed the way in which drug target discovery is now approached. Previous reports have discussed how various differential gene expression (DGE) technologies are being utilized for cardiovascular disease (CVD) drug target discovery. Other approaches such as sequencing cDNA from cardiovascular-derived tissues and cells coupled with bioinformatic sequence analysis are used with the aim of identifying novel gene sequences that may be exploited toward target discovery. Additional leverage from gene sequence information is obtained through identification of polymorphisms that may confer disease susceptibility and/or affect drug responsiveness. Pharmacogenomic studies are described wherein gene expression-based techniques are used to evaluate drug response and/or efficacy. Industrial-scale genomics supports and addresses not only novel target gene discovery but also the burgeoning issues in pharmaceutical and clinical cardiovascular medicine relative to polymorphic gene responses.

Application of industrial scale genomics to discovery of therapeutic targets in heart failure. Mehraban F, Tomlinson JE. Eur J Heart Fail 2001 Dec; 3 (6), pp. 641–50.

Pharmacogenetics Attributes and Perspectives

The 1st observations of inherited differences in drug effects in the 1950s led to the recognition of a genetic basis for drug response. With the development of genetics and molecular biology, it became clear that certain drug responses could be associated with specific genetic variations or polymorphisms. There are now examples of polymorphisms that affect response to drugs ranging from common analgesics to chemotherapeutics. The goal of pharmacogenetics is to identify polymorphisms that can serve as predictive markers of drug response. Reviews have summarized how existing pharmacogenomic technologies can be applied advantageously throughout drug development to bring drugs successfully to market along with diagnostic tests that ensure their appropriate use.

Integrating pharmacogenomics into drug development. Ferentz AE. Pharmacogenomics 2002 Jul; 3 (4), pp. 453–67.

Pharmacogenetics Attributes and Perspectives

The introduction of targeted therapeutics into clinical practice has created major opportunities for further development of the molecular diagnostics industry. Emerging genomic and proteomic technologies and information are now resulting in the molecular subclassification of disease as the basis for diagnosis, prognosis, and therapeutic selection. The ultimate goals of personalized medicine are to take advantage of a molecular understanding of disease, both to optimize drug development and direct preventive resources and therapeutic agents at the right population of people while they are still well. Single nucleotide polymorphisms identification and genotyping have uncovered predisposition markers from cancer and heart disease as well in the prediction of both drug efficacy and toxicity. Pharmacogenomic and pharmacodynamic assays are being developed to enhance the speed and decrease the cost of drug development, as

well as reduce side effects and increase response rates in a variety of diseases. The traditional trial and error practice of medicine is progressively eroding in favor of more precise marker-assisted diagnosis and safer and more effective molecularly guided treatment of disease. For the diagnostics industry this represents an unprecedented opportunity for integration, increased value, and commercial opportunities for molecularly derived tests.

Integration of molecular diagnostics with therapeutics: Implications for drug discovery and patient care. Ross JS, Ginsburg GS. Expert Rev Mol Diagn 2002 Nov; 2 (6), pp. 531–41.

Pharmacogenetics Attributes and Perspectives

It is widely anticipated that during the next 5 years the molecular diagnostic industry will continue to grow at a double-digit pace to meet increasing demand for personalized medicine. A wide variety of drugs in late preclinical and early clinical development are being targeted to disease-specific gene and protein defects that will require co-approval of diagnostic and therapeutic products by regulatory agencies. An increasingly educated public will demand more information about their predisposition for serious diseases and how these potential illnesses can be detected in an early stage when they can be arrested or cured with new therapies custom designed for their individual clinical status. To respond to this demand, major pharmaceutical companies will partner with diagnostics companies or develop their own in-house capabilities that will permit efficient production of more effective and less toxic integrated personalized medicine drug and test products. For clinical laboratories and pathologists, this integration of diagnostics and therapeutics represents a major new opportunity to emerge as leaders of the new medicine, guiding the selection, dosage, route of administration, and multidrug combinations and producing increased efficacy and reduced toxicity of pharmaceutical products.

The integration of molecular diagnostics with therapeutics: Implications for drug development and pathology practice. Ross JS, Ginsburg GS. Am J Clin Pathol 2003 Jan; 119 (1), pp. 26–36.

Pharmacogenetics Ethics and Practices

Pharmacogenomics holds much promise for the application of genetic information to the improvement of clinical care. Ethical issues for pharmacogenomics arise at the intersection of the spheres of drug development and genetic testing. Clinical drug trial designs that use subject selection based on genotype must consider the features of scientific validity, social value and risk-benefit ratio, and, later, the impact of this strategy on postmarket studies and clinical use of drugs. Although the testing context for pharmacogenomic tests is different from other genetic tests, decisions to use any new clinical tests in medical practice will require evaluation of not only the benefit linked to improved drug use but also the risks arising in part from the scale of testing, predictive value, and collateral potential of the genetic test. Integration of pharmacogenomic information into clinical practice will require clinical trials to assess clinical usefulness, including the impact of tests on therapeutic outcomes. Trials will also be needed to demonstrate the effectiveness of education, informed consent, and counseling.

Emerging ethical issues in pharmacogenomics: From research to clinical practice. Freund CL, Wilfond BS. Am J Pharmacogenomics 2002; 2 (4), pp. 273–81.

Pharmacogenetics of Antidepressants in Females

Background Information: In the medical literature, there is a lack of sex-specific information regarding the efficacy, metabolism, and side effects associated with psychopharmacologic treatment. In part, this lack results from the historic underinclusion of women in clinical trials during early drug development, but it also occurs because investigators of treatment and metabolic studies do not routinely analyze results according to sex. In 1993, the U.S. Food and Drug Administration (FDA) announced changes that encourage the inclusion of women in early pharmacokinetic studies and emphasize the need for subset analyses using sex and age parameters. In conjunction with advances in basic science regarding drug metabolism, these modifications have led to modest increases in information regarding sex differences in drug metabolism and efficacy. In the referenced article, current information regarding potential sex differences in the pharmacotherapy of Major Depressive Disorder is reviewed.
Data Sources: A MEDLINE search using the terms "antidepressants," "sex-factors," "gender differences," and "women" for the years 1966–2000.
Data Synthesis and Conclusions: There are data supporting sex differences in the activity of various antidepressant-metabolizing enzymes. However, there is a paucity of investigation regarding how these differences might translate into differences in clinical efficacy. Notably, there is little work using existing databases to perform the subgroup analyses recommended by the FDA. The widespread dissemination of such work is needed, and, if conducted, investigations in this area have the potential to enhance psychopharmacologic treatment for both men and women.

The pharmacologic treatment of depression: Is gender a critical factor? Yonkers KA, Brawman-Mintzer O. J Clin Psychiatry 2002 Jul; 63 (7), pp. 610–5.

Pharmacogenetics of Antidepressants and Mood Disorders

Organization: Pharmacologic and Somatic Treatments Section of the National Institute of Mental Health Strategic Plan for Mood Disorders Committee on advancing novel pharmacologic and somatic treatments for mood disorders.
Topic: Current pharmacologic treatments and new directions in the field.
Discussion: Specific research initiatives exist that can advance the current therapeutics for mood disorders, including new basic and clinical research in *in vivo* human imaging procedures, somatic therapeutics, and the vast new area of pharmacogenetics. New scientific and technical opportunities are available today based on advances in basic neuroscience, opportunities in clinical testing, industry interest in advancing central nervous system therapeutics, and active consumer advocacy groups. The question of how to bring all of these positive forces together to accelerate discovery in mood disorder therapeutics is of major importance.

Developing novel treatments for mood disorders: Accelerating discovery. Tamminga CA, Nemeroff CB, Blakely RD, Brady L, Carter CS, Davis KL, Dingledine R, Gorman JM, Grigoriadis DE, Henderson DC, B Innis RB, Killen J, Laughren TP, McDonald WM, M Murphy GM Jr, Paul SM, Rudorfer MV, Sausville E, Schatzberg AF, Scolnick EM, Suppes T. Biol Psychiatry 2002 Sep 15; 52 (6), pp. 589–609.

Pharmacogenetics of Chemotherapeutic Agents

Background Information: Cancer chemotherapy doses are empirical in that the majority are administered at a fixed dose (mg/m^2 or mg/kg). One reason for this is that the intrinsic sensitivity of the tumor or host cells to 1 particular chemotherapy agent is unknown. Therefore, the likelihood of response or toxicity is unpredictable. This contrasts with antimicrobial chemotherapy, where sensitivity (minimum inhibitory concentration) can be determined for a specific bacterium. The pharmacokinetics of cancer chemotherapy agents is also highly variable between patients. In addition, the small therapeutic index of these drugs, combined with the lack of good surrogate markers of toxicity or response, adds to the empiricism of the administration of cancer chemotherapy. In the past few years, numerous studies have established good relationships between systemic exposure to cancer chemotherapy and both response and toxicity. These relationships have been used to individualize chemotherapic dose administration.

Interpretation and Analysis: Examples of some pharmaceuticals that are currently individualized based on their pharmacokinetics include methotrexate, busulfan, and carboplatin. Other examples of antineoplastic agents that may eventually be individualized based on their pharmacokinetics are mercaptopurine, fluorouracil, etoposide and teniposide, and topotecan and suramin. New strategies are being investigated to improve the therapeutic index of cancer chemotherapy agents such as biomodulation, pharmacogenetics, circadian administration, and the modification of drug scheduling. Pharmacokinetic studies have also played a major role in these areas. Thus, despite the empiricism associate with cancer chemotherapy administration, some progress has been made and shown to have an impact on outcome. However, more studies are needed to improve cancer chemotherapy administration.

Pharmacokinetic optimization of cancer chemotherapy. Effect on outcomes. Masson E, Zamboni WC. Clin Pharmacokinet 1997 Apr; 32 (4), pp. 324–43.

Pharmacogenetics of Epilepsy

Despite the recent development of new antiepilepsy drugs, a significant number of children are still unable to achieve seizure freedom without side effects. Understanding the factors behind individual variability in antiepilepsy drug tolerability and dose response and incorporating these factors into a treatment plan would represent an important advance in epilepsy pharmacotherapy. A more thorough understanding of the epileptogenic process may allow clinicians to select antiepilepsy drugs that interrupt or modify various steps in the epileptogenic progression (i.e., disease modification). Additionally, advances in the understanding of human genetics may allow for selection of antiepilepsy drugs and dosage regimens based on a patient's potential improvement in the medical treatment of patients with epilepsy. Such methods of tailoring antiepilepsy drug therapy would be preferable to the trial and error system that is currently used.

Advancing the medical management of epilepsy: Disease modification and pharmacogenetics. Glauser TA. J Child Neurol 2002 Jan; 17 Suppl 1, pp. S85–93.

Pharmacogenetics of Infectious Disease/ Polymorphism of Pathogen and Host

The impact of pharmacogenomics on the prevention, diagnosis, and treatment of infectious diseases has been previously described. The application of pharmacogenomics to infectious diseases requires consideration of the genomes of both the pathogen and the host. The pathogen's genome may be used for antigen identification, to identify infecting organisms, and to determine antimicrobial resistance. Diagnostic tool development and vaccine design can be aided by knowing which portions of a pathogen are important antigenic determinants. The unique genetic makeup of a pathogen can facilitate its identification as an augmentation to the traditional culture. Important genes conferring resistance to antibiotics can be detected, and this information can be used to choose appropriate antibiotic therapy. The genome of the host may reveal susceptibility genes and new drug targets that may be used in the treatment of infectious diseases. Thus far, polymorphisms in genes of the host immune system have been associated with susceptibility to infections and response to treatment. Examples of these findings are described in the referenced paper. Pharmacogenomics has the potential to revolutionize the prevention, diagnosis, and treatment of infectious diseases.

Pharmacogenomics and infectious diseases: Impact on drug response and applications to disease management. Hayney MS. Am J Health Syst Pharm 2002 Sep 1; 59 (17), pp. 1626–31.

Pharmacogenetics of Malignant Hyperthermia/ Long Q-T Syndrome/Venous Thromboembolic Disease/Tardive Dyskinesia/Drug Addiction

In the last decades, advances in molecular biology have led to modern pharmacogenetics, which started as a science that focused on investigating drug metabolizing enzymes and genetic determinants of pharmacokinetic variability. As more evidence has become available on the structure of drug targets and the genes coding for them, increasing attention has been directed toward pharmacodynamic explanations of variability in therapeutic response as well as in the risk for adverse drug reactions. Traditionally, genetic drug safety research has focused on variations in single genes whose functions are known to be related to given adverse drug reactions. Relevant examples include malignant hyperthermia, the long Q-T syndrome, venous thromboembolic disease, tardive dyskinesia, and drug addiction. In the future, results from the Human Genome Project, together with tools such as DNA microarray technology, high-output screening systems, and advanced bioinformatics, will permit a more thorough elucidation than is currently possible of the genetic components of adverse drug reactions. By screening for a large number of single-nucleotide polymorphisms (SNPs), SNP patterns associated with adverse drug reactions can be discovered even though the functions of the SNPs as such are completely unknown. On the basis of these findings, it can be expected that pharmacogenetic research will identify situations where a drug should be avoided in certain individuals in order to reduce the risk for adverse drug reactions. If so, it will be feasible to use molecular diagnostics to select drugs that are safe for the individual patient.

Genotyping of drug targets: A method to predict adverse drug reactions? Guzey C, Spigset O. Drug Saf 2002; 25 (8), pp. 553–60.

Pharmacogenetics of the Hepatic Cytochrome P450 Enzyme Systems

Background Information: Drug-metabolizing enzymes or xenobiotics-metabolizing enzymes (DMEs or XMEs) play central roles in the biotransformation, metabolism, and/or detoxification of xenobiotics or foreign compounds that are introduced to the human body. In general, DMEs protect or defend the body against the potential harmful insults from the environment. Once in the body, many

xenobiotics may induce signal transduction events either specifically or non-specifically leading to various cellular, physiological, and pharmacological responses including homeostasis, proliferation, differentiation, apoptosis, or necrosis. For the body to minimize the insults caused by these xenobiotics, various tissues/organs are well equipped with diverse DMEs including various Phase I and Phase II enzymes, which are present in abundance either at the basal level and/or increased/induced after exposure. Better understanding of the pharmacogenomic/gene expression profile of DMEs and the underlying molecular mechanisms after exposure to xenobiotics or drugs can be gained by review of current knowledge of DNA microarray technology in gene expression profiling and the signal transduction events elicited by various xenobiotics mediated by either specific receptors or non-specific signal transduction pathways. Pharmacogenomics is the study of genes and the gene products (proteins) essential for pharmacological or toxicological responses to pharmaceutical agents. In order to assess the battery of genes that are induced or repressed by xenobiotics and pharmaceutical agents, cDNA microarray or oligonucleotide-based DNA chip technology can be a powerful tool to analyze, simultaneously, the gene expression profiles that are induced or repressed by xenobiotics. The regulation of gene expression of the various phase I DMEs such as the cytochrome P450 (CYP) as well as phase II DMEs generally depends on the interaction of the xenobiotics with the receptors. For instance, the expression of CYP1 genes can be induced via the aryl hydrocarbon receptor (AhR), which dimerizes with the AhR nuclear translocator (ARNT), in response to many polycyclic aromatic hydrocarbons (PAHs). Similarly, the steroid family of orphan receptors, the constitutive androstane receptor (CAR) and pregnane X receptors (PXR), heterodimerize with the retinoid X receptor (RXR) and transcriptionally activate the promoters of CYP2B and CYP3A gene expression by xenobiotics such as phenobarbital-like compounds (CAR) and dexamethasone and rifampicin-type of agents (PXR). The peroxisome proliferator activated receptor (PPAR), which is 1 of the 1st characterized members of the nuclear hormone receptor, also dimerizes with RXR, and it has been shown to be activated by lipid lowering agent fibrate-type of compounds leading to transcriptional activation of the promoters on the CYP4A genes. The transcriptional activation of these promoters generally leads to the induction of their mRNA. The physiological and the pharmacological implications of common partner of RXR for CAR, PXR, and PPAR receptors largely remain unknown and are under intense investigations. For the phase II DMEs, phase II gene inducers such as phenolic compounds butylated hydroxyanisol (BHA), tert-butylhydroquinone (tBHQ), green tea polyphenol (GTP), (-)-epicatechin-3-gallate (EGCG), and the isothiocyanates (PEITC, sulforaphane) generally appear to be electrophiles. They can activate the mitogen-activated protein kinase (MAPK) pathway via electrophilic-mediated stress response, resulting in the activation of bZIP transcription factors Nrf2, which dimerizes with Mafs and binds to the antioxidant/electrophile response element (ARE/EpRE) enhancers that are found in many phase II DMEs as well as many cellular defensive enzymes such as thioredoxins, gammaGCS, and HO-1, with the subsequent induction of gene expression of these genes. It appears that in general, exposure to phase I or phase II gene inducers or xenobiotics may trigger a cellular "stress" response leading to the increase in the gene expression of these DMEs, which ultimately enhance the elimination and clearance of the xenobiotics and/or the "cellular stresses" including harmful reactive intermediates such as reactive oxygen species (ROS), so that the body will remove the "stress" expeditiously. Consequently, this homeostatic response of the body plays a central role in the protection of the organism against environmental insults such as xenobiotics. Advances in DNA microarray technologies and mammalian genome sequencing will soon allow quantitative assessment of expression profiles of all genes in the selected tissues. The ability to predict phenotypic outcomes from gene expression profiles is currently in its infancy, however, and will require additional bioinformatic tools. Such tools will facilitate information gathering from literature and gene databases as well as integration of expression data with animal physiology studies. The study of pharmacogenomic/gene expression profile and the understanding of the regulation and the signal transduction mechanisms elicited by pharmaceutical agents can be of potential importance during drug discovery and the drug development.

Pharmacogenomics, regulation and signaling pathways of phase I and II drug metabolizing enzymes. Rushmore TH, Kong AN. Curr Drug Metab 2002 Oct; 3 (5), pp. 481–90.

Pharmacogenetics/Acute Lymphoblastic Leukemia (ALL)

Malignancies in childhood occur with an incidence of 13 to 14 per 100,000 children under the age of 15 years. Acute lymphoblastic leukemia with an incidence of 29% is the most common pediatric malignancy, whereas acute myeloid leukemias account for about 5%. The treatment of acute leukemias consists of sequential therapy cycles (induction, consolidation, intensification, maintenance therapy) with different cytostatic drugs over a time period of up to 1.5 to 3 years. Over the last 25 years of clinical trials, a significant rise in the rate of complete remissions as well as an increase in long-term survival has been achieved. Therefore, growing attention is now focused on the long-term effects of antileukemic treatment. Several cytostatic drugs administered in the treatment of acute leukemia in childhood are known to cause long-term adverse effects. Anthracyclines may induce chronic cardiotoxicity, alkylating agents are likely to cause gonadal damage, and secondary malignancies and the use of glucocorticoids may cause osteonecrosis. Most of the long-term adverse effects have not been analyzed systematically. Approaches to minimizing long-term adverse effects without jeopardizing outcome have included the design of new drugs such as a liposomal formulation of anthracyclines; the development of anthracycline-derivates with lower toxicity; the development of cardioprotective agents, or, more recently, the use of targeted therapy; alternative administration schedules like continuous infusion or timed sequential therapy; and risk group stratification by the monitoring of minimal residual disease. Several attempts have been made to minimize the cardiotoxicity of anthracyclines: decreasing concentrations delivered to the myocardium by either prolonging infusion time or using liposomal formulated anthracyclines or less cardiotoxic analogues, or the additional administration of cardioprotective agents. The advantage of these approaches is still controversial, but there are ongoing clinical trials to evaluate the long-term effects. The use of new diagnostic methods, such as diagnosis of minimal residual disease, which allow reduction or optimization of dose, offers potential advantages compared with conventional treatment in terms of reducing the risk of severe long-term adverse effects. Most options for minimizing long-term adverse effects have resulted from theoretical models and *in vitro* studies, but only some of the modalities, such as the use of dexrazoxane, the continuous infusion of anthracyclines, or timed sequential therapy, have been evaluated in prospective, randomized studies in patients. Future approaches to predict severe toxicity may be based upon pharmacogenetics and gene profiling.

Minimizing the long-term adverse effects of childhood leukemia therapy. Langebrake C, Reinhardt D, Ritter J. Drug Saf 2002; 25 (15), pp. 1057–77.

Pharmacogenetics/Acute Lymphoblastic Leukemia (ALL)
Therapeutic Response

Pharmacogenomics is a burgeoning field that aims to elucidate inherited differences in drug disposition and treatment response toward individualizing therapy to enhance efficacy and reduce toxicity. Approximately 80% of children with ALL can be cured with modern therapy. Despite this success, the number of cases of relapsed ALL remains greater than the number of new cases of most childhood cancers. New strategies are needed to develop curative therapy for the 20% of patients who are not being cured today and to develop less toxic and less onerous treatment for ALL patients. Molecular genetics investigations have provided important insights to the mechanisms of leukemogenesis and are now routinely used to define the prognosis and guide treatment intensity for childhood ALL. As a result, substantial progress has been made recently in this field of study pertaining to conditions of childhood ALL that will hold great promise in further enhancing the treatment of this most common cancer in children.

Pharmacogenomics of childhood acute lymphoblastic leukemia. Brenner TL, Pui CH, Evan WE. Curr Opin Mol Ther 2001 Dec; 3 (6), pp. 567–78.

Pharmacogenetics/Genetics and Human Genome Project

The explosion of information generated by large-scale functional genomics technologies has resulted in an exponential increase in the number of potential genes and proteins available for pharmaceutical and diagnostic research development. In order to tap this potential, the primary challenge is to develop a strategy to effectively integrate and extract meaning from the human genomic sequence information that has been generated since the start of the Human Genome Project. Strategies have been applied by academics and by the biotechnology sector to sort and triage this information with the ultimate goal of identifying future therapeutic targets for cancer and other diseases.

Implications of oncogenomics for cancer research and clinical oncology. Herrmann JL, Rastelli L, Burgess CE, Fernandez EE, Rothberg BE, Rothberg JM, Shimkets RA. Cancer J 2001 Jan–Feb; 7 (1), pp. 40–51.

Pharmacogenetics/Microarray Technology Platforms

Background Information: High-density microarrays are ideally suited for analyzing thousands of genes against a small number of samples. The next step in the discovery process is to take the resulting genes of interest and rapidly screen them against thousands of patient samples, tissues, or cell lines to further investigate their involvement in disease risk or the response to medication.
Implementation: Microarray technology platforms can be applied for the detection of both single-nucleotide polymorphisms (SNPs) and alterations in protein expression. Each microarray contains up to 250 elements that can be customized for each application. Slides are usually designed in either a 16- or 96-microarray format (4,000–24,000 elements per slide), thereby allowing the corresponding number of samples to be rapidly processed in parallel (simultaneously).
Interpretation and Analysis: Findings for SNP genotyping and protein profiling agree with results of restriction fragment length polymorphism (RFLP) analysis or ELISA, respectively. Genotyping analyses, using the microarray technology, on large sample sets over multiple polymorphisms in the NAT2 gene are in full agreement with traditional methodologies, such as sequencing and RFLP analysis. The multiplexed protein microarray provides correlation coefficients of 0.82 to 0.99 (depending on analyte) compared with ELISAs. The integrated microarray technology platform is adaptable and versatile, while offering the high-throughput capabilities needed for drug development and discovery applications.

High-throughput genomic and proteomic analysis using microarray technology. Huang JX, Mehrens D, Wiese R, Lee S, Tam SW, Daniel S, Gilmore J, Shi M, Lashkari D. Clin Chem 2001 Oct; 47 (10), pp. 1912–6.

Toborinone Pharmacokinetic/Pharmacodynamic Prediction/Hepatic Substrate Metabolism and Clearance/Congestive Heart Failure (CHF) Renal Glomerular Filtration Rate
Caffeine/Indocyanine Green (ICG)/Iothalamate

Cellular/Molecular Mechanism of Action: Caffeine is a substrate for hepatic cytochrome P450 1A2 (CYP1A2) and the biochemical activity of this enzyme system yields a metabolite that can be measured or quantified. Indocyanine green is not metabolized by the liver but instead is removed from the intravascular compartment by hepatocytes and eliminated in the bile. Iothalamate is simply eliminated from the intravascular compartment by mechanisms of glomerular filtration and excreted in the urinary filtrate.
Application: Plasma clearance of iothalamate can be used to evaluate glomerular filtration. Caffeine metabolism is useful as a molecular probe for evaluating hepatic metabolic capacity while ICG can serve as a marker molecule for characterizing hepatic clearance parameters.
Implementation: Delineation of the pharmacokinetic profile of toborinone in disease states affecting mutiple organ systems that influence circulation, metabolism, and excretion. In this context, objective evaluation of the glomerular filtration rate can be established by measuring iothalamate clearance, while hepatic function can be assessed by delineating caffeine metabolism profiles and clearance of indocyanine green.
Human Model: Cases of CHF complicated by concomitant renal and/or hepatic disease. Cases can be divided into groups based on estimated creatinine clearance and serum bilirubin concentrations.
Interpretation and Analysis: No significant differences can be observed for mean toborinone pharmacokinetic parameters among the 4 study groups. Positive correlations can be appreciated between toborinone clearance and measured indices for renal and hepatic function: creatinine clearance, iothalamate renal clearance, paraxanthine/caffeine ratio, and ICG clearance. Toborinone clearance decreases with decreasing creatinine clearance, decreasing glomerular filtration rate, decreasing demethylation metabolic activity, and decreasing hepatic bloodflow, although no significant differences are observed in any mean toborinone pharmacokinetic parameters evaluated among the 4 study groups.

The pharmacokinetics of toborinone in subjects with congestive heart failure and concomitant renal impairment and/or concomitant hepatic impairment. Tammara B, Trang JM, Kitani M, Miyamoto G, Bramer SL. J Clin Pharmacol 2002 Dec; 42 (12), pp. 1318–25.

PHARMACOGENETICS: GENERAL HEPATIC BIOCHEMICAL METABOLIZATION

Acyclovir: Optimization of Individual Pharmacokinetic Profiles/Renal Dialysis: Induced Pharmacokinetic Variability
Bayesian Method

Cellular/Molecular Mechanism of Action: Acyclovir is a synthetic purine nucleoside analog [9-[(2-hydroxy-ethoxy)methyl] guanine] that functions as a false (decoy)analog corresponding to guanosine. Viral thymidine kinase has a much higher affinity for acyclovir than does mammalian thymidine kinase.
Physiological Mechanism of Action: Acyclovir functions as an antiviral agent (herpes viruses).
Background Information: The therapeutic regimen for intravenous acyclovir in pediatric patients can often be complicated by evolving renal impairment directly related to intraindividual pharmacokinetic variability linked to the patient's clinical condition.
Application: Acyclovir is traditionally applied as an antiviral agent.
Route of Administration: Acyclovir (intravenous).
Dosage: Acyclovir.
Implementation: Acquisition of therapeutic drug monitoring data can be utilized to assess acyclovir intraindividual pharmacokinetic variability during several types of renal replacement therapy. In this manner, Bayesian adaptive control of acyclovir dosage regimens can be performed in pediatric patients with bone marrow transplants that subsequently develop severe renal impairment. Acyclovir pharmacokinetic parameter values corresponding to different techniques and periods of renal replacement therapy can be estimated using USCPACK PC Clinical Programs and therapeutic drug monitoring data.
Interpretation and Analysis: Clinical experience has shown that a wide variation in intraindividual pharmacokinetic profiles exists during the application of different dialysis techniques (e.g., CAVH, CAVHDF, CVVHD). Such findings reflect not only the performance (effectiveness) of each dialysis technique but also the difficulty in their clinical utilization. The acyclovir elimination rate constant is higher during CVVHD compared to CAVH or CAVHDF. Bayesian method appears to be valuable in assessing intraindividual pharmacokinetic variability, as it allows the clinician to deal with sparse routine patient data.

Assessment of acyclovir intraindividual pharmacokinetic variability during continuous hemofiltration, continuous hemodiafiltration, and continuous hemodialysis. Bleyzac N, Barou P, Massenavette B, Contamin B, Maire P, Berthier JC, Aulagner G. Ther Drug Monit 1999 Oct; 21 (5), pp. 520–5.

Pharmacogenetics and Pharmacogenomics/ Proteomics Technologies: 2D-PAGE/Mass Spect/ Microarray Analysis Platforms: Investigative Methodologies/Human Tumor Gene Index/Laser Capture Microdisection (LCM)

Genomics has expanded the field of molecular oncology, and proteomics is complementing genomics in the fields of elucidation of pathophysiology, gene function, molecular diagnosis, and anticancer drug discovery. This trend is reflected in the establishment of the Human Tumor Gene Index by the National Cancer Institute (NCI), which is now followed by the Tissue Proteomics Initiative. Laser capture microdissection provides an ideal method for extraction of cells from specimens in which the exact morphologies of both the captured cells and the surrounding tissue are preserved. Proteomic technologies can be applied for the further characterization and analysis of proteins. Laser capture microdissection can also be combined with the protein chip technology. Proteomic technologies have been used for the study of cancer of various organs including the liver, prostate, breast, bladder, and esophagus. Some of the anticancer strategies are directed against proteases that facilitate several steps in cancer progression. Proteomic mapping of blood vessels in normal and malignant tissues can be used to identify tissue-specific markers on the endothelium that serve as potential targets for *in vivo* drug delivery. Studies of global protein expression in human tumors have led to the identification of various polypeptide markers, potentially useful as diagnostic tools. Genes that encode proteins that are overexpressed in tumors are being identified. Demonstration of tissue or cell-type specific expression of some nuclear matrix proteins has led to the search for tumor-specific nuclear matrix proteins. There is considerable activity in the commercial sector to develop diagnostic tests, as well as to facilitate anticancer drug discovery using proteomic technologies. Continued refinement of techniques and methodologies to determine the abundance and status of proteins *in vivo* holds great promise for future study of normal cells and associated neoplasms.

Applications of proteomics in oncology. Jain KK. Pharmacogenomics 2000 Nov; 1 (4), pp. 385–93.

Pharmacogenetics and Pharmacogenomics: Genomic Polymorphism as a Function of Protein Biological Activity

There is a rapidly growing body of evidence linking genetic polymorphisms with functional changes in proteins that are responsible for the metabolism and disposition of many medications. Likewise, polymorphisms in genes encoding the targets of medications (e.g., receptors) can alter the pharmacodynamics of the drug response by changing receptor sensitivity. As a result, the inherited basis of drug effects is often polygenic in nature, and thus more challenging to define. However, technological advances, coupled with new insights into the molecular pharmacology of medications and the functional consequences of polymorphisms in the human genome, are providing the tools needed to elucidate genetic determinants of drug response and translate functional genomics into personalized medicine.

Molecular diagnostics as a predictive tool: Genetics of drug efficacy and toxicity. Johnson JA, Evans WE. Trends Mol Med 2002 Jun; 8 (6), pp. 300–5.

Pharmacogenetics and Pharmacogenomics: Human Proteome/Genome

Now that the sequencing of the human genome has been completed, the basic challenges are finding the genes, locating their coding regions, and predicting their functions. This will result in a new understanding of human biology as well as in the design of new molecular structures as potential novel diagnostic or drug discovery targets. The assessment of gene function may be performed using the tools of the genome program. These tools represent high-throughput methods used to evaluate changes in the expression of many or all genes of an organism at the same time in order to investigate genetic pathways for normal development and disease. This will lead to a shift in the scientific paradigm: In the preproteomics era, functional assignments were derived from hypothesis-driven experiments designed to understand specific cellular processes. The new tools describe proteins on a proteome-wide scale, thereby creating a new

way of doing cell research that results in the determination of 3-dimensional protein structures and the description of protein networks. These descriptions may then be used for the design of new hypotheses and experiments in the traditional physiological, biochemical, and pharmacological sense. The evaluation of genetically manipulated animals or newly designed biomolecules will require a thorough understanding of physiology, biochemistry, and pharmacology and the experimental approaches will involve many new technologies, including *in vivo* imaging with single photon emission computed tomography and positron emission tomography. Nuclear medicine procedures may be applied for the determination of gene function and regulation using established and new tracers or using *in vivo* reporter genes such as enzymes, receptors, antigens, or transporters. Pharmacogenomics will identify new surrogate markers for therapy monitoring that may represent potential new tracers for imaging. Also, drug distribution studies for new therapeutic biomolecules are needed, at least during preclinical stages of drug development. Finally, new biomolecules will be developed by bioengineering methods that may be used for isotope-based diagnosis and treatment of disease.

Functional genomics and proteomics—The role of nuclear medicine. Haberkorn U, Altmann A, Eisenhut M. Eur J Nucl Med Mol Imaging 2002 Jan; 29 (1), pp. 115–32. Epub 2001 Nov.

Pharmacogenetics and Pharmacogenomics: Mass Spectrometry

Single-nucleotide polymorphisms (SNPs) are currently being identified and mapped at a remarkable pace, providing a rich genetic resource with vast potential for disease gene discovery, pharmacogenetics, and understanding the origins of modern humans. High-throughput, cost-effective genotyping methods are essential in order to make the most advantageous and immediate use of these SNP data. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) can be incorporated as a tool for differentiating genotypes based on the mass of the variant DNA sequence, and this method has been utilized for production scale SNP genotyping. A 4-μl polymerase chain reaction (PCR) amplification reaction using 3 ng of genomic DNA can be combined with a secondary enzymatic reaction (mini-sequencing) containing oligonucleotide primers that anneal immediately upstream of the polymorphic site, dideoxynucleotides, and a thermostable polymerase used to extend the PCR product by a single base pair. Mass spectrometry (MS) analysis of mini-sequencing reactions is performed using a MALDI-TOF instrument (Voyager-DE, Perseptive Biosystems, Framingham, MA). Both single and multiplex PCR can be applied along with mini-sequencing reactions, for genotyping different variant sites in random samples (e.g., n = 989 individuals). Genotypes generated with MS methods are then compared with genotypes produced using a 5′ exonuclease fluorescence-based assay (Taqman, Applied Biosystems, Foster City, CA) and a gel-based genotyping protocol. Because multiple polymorphisms can be detected in a single reaction, the MS technique provides a cost-effective and efficient method for high-throughput genotyping.

High-throughput multiplex SNP genotyping with MALDI-TOF mass spectrometry: Practice, problems and promise. Bray MS, Boerwinkle E, Doris PA. Hum Mutat 2001 Apr; 17 (4), pp. 296–304.

Pharmacogenetics and Pharmacogenomics: Mass Spectrometry

The use of liquid chromatography-mass spectrometry (LC-MS) has recently exploded in various analytic fields, including toxicology and therapeutic drug monitoring (although still far behind pharmacokinetics). There is no doubt that LC-MS is currently competing with gas chromatography (GC)-MS for the status of the reference analytic technique in toxicology. The principles, advantages, and drawbacks have been described for LC-MS systems using atmospheric pressure interfaces. Overviews have also reported the analytic methods for xenobiotics that could be set up with these instruments for clinical or forensic toxicology. In particular, as far as quantitative techniques are concerned, reviews have highlighted the large number and variety of drugs or classes of drugs (drugs of abuse, therapeutic drugs) or toxic compounds (e.g., pesticides) that can be readily determined using such instruments, the respective merits of the different ionization sources, and the improvements brought about by tandem MS. New applications have also been addressed for LC-MS in the field of toxicology, such as "general unknown" screening procedures and mass spectral libraries using LC-atmospheric pressure ionization (API)-MS or MS-MS, presenting the different solutions proposed to overcome the naturally low fragmentation power of API sources. Finally, the opportunities afforded by the most recent or proposed instrument designs have been clarified.

Progress of liquid chromatography-mass spectrometry in clinical and forensic toxicology. Marquet P. Ther Drug Monit 2002 Apr; 24 (2), pp. 255–76.

PHARMACOGENETICS: PHASE I METABOLIZING ENZYME SYSTEMS

MICROSOMAL ENZYME METABOLIZING CAPACITY (GENERAL)

Hepatic Microsomal Enzyme Metabolizing Capacity/Phenobarbital (PB) Induction of Cytochrome P450
Antipyrine

Application: Assessment of hepatic function. Antipyrine total body clearance directly correlates with hepatic cytochrome P450 activity. In this context, antipyrine as been applied as an indicator of decreasing hepatic function in dogs with experimentally-induced progressive liver disease (e.g., hepatotoxin: dimethylnitrosamine).
Route of Administration: Dogs: rapid intravenous administration.
Dosage: Canine: 20 mg/kg of body weight, and serum samples are obtained at intervals for determination of at least 5 terminal-phase drug half-lives.
Considerations: Disposition of antipyrine has been used as an indicator of decreasing hepatic function in dogs with experimentally-induced progressive liver disease.
Implementation: Evaluation of hepatic injury/insult caused by the hepatospecific toxin dimethylnitrosamine as a function of antipyrine plasma concentration that reflects rate and extent of liver metabolization.
Animal Model: Canine hepatic injury model induced through the oral administration of dimethylnitrosamine (n = 16 dogs with 6 negative controls). Disposition of antipyrine is studied 3 weeks after the last dose of toxin in each dog.
Interpretation and Analysis: Clearance and mean residence times vary among different histiological groups (e.g., mild, moderate, and severe) observed with dimethyletirosamine treated dogs ($p \leq 0.01$). Clearance of antipyrine is decreased in moderate and severe groups compared with negative controls and mild histiologically affected dogs. Antipyrine mean residence times are longer in moder-

ately affected dogs, compared with controls and mildly affected animals. Correction of antipyrine clearances for hepatic weight increases the ability to discriminate between moderately and severely affected dogs. Comparison of the clearance and mean residence time ratios of antipyrine calculated for each group with values for negative reference controls reveals there is no difference in the ratios among untreated and mildly affected dogs. Experimental findings also reveals that the disposition of antipyrine and caffeine appears to change similarly with progression of (dimethylnitrosamine-induced) liver disease.

In qualitative and quantitative evaluations of canine hepatic pharmaceutical metabolizing capacity, most of these enzyme systems reach maximal concentrations or biochemical activity by the 5th to 8th week after birth; they tended to decrease after weaning. Examples in this regard include (i) cytochromes P450 and b5 in addition to the NADPH and NADH-dependent reductases associated with each of these cytochromes; (ii) cytochrome P450-mediated enzymes (e.g., aniline and coumarin hydroxylases, aminopyrine N-demethylase, and 7-ethoxycoumarin O-deethylase); (iii) activity of a uridine diphosphoglucuronic acid glucuronyl transferase; and (iv) glutathione-S-transferase activities. The activity of dichloronitrobenzene-glutathione transferase in geriatric dogs (312 to 525 weeks old) is approximately 2-fold greater than that of 8-week-old puppies. There appear to be no gender-related differences in any of the enzyme concentrations or biochemical activities. Individual variation is pronounced even in homogeneous canine populations.

Antipyrine total body clearance has been applied to evaluate the effect of oral PB (antiepileptic therapeutic dose $\times$ 35 days) on hepatic cytochrome P450 function and plasma α1-acid glycoprotein concentrations. In this context, antipyrine total body clearance directly correlates with hepatic cytochrome P450 activity. Phenobarbital promotes increases in antipyrine total body clearance that approach a 3-fold elevation compared to preadministration levels. In concert with these observations, α1-acid glycoprotein levels increase approximately 4-fold. Following 35 days of PB administration, canine hepatic cytochrome P450 content and enzyme kinetics reveal values that are approximately 2-fold higher than that from untreated dogs. The Vmax values for cytochrome P450 1A-like activity (ethoxyresorufin O-deethylation); cytochrome P450 2B-like activity (ethoxycoumarin O-deethylation); cytochrome P450 2C-like activity (tolbutamide hydroxylation); and cytochrome P450 3A-like activity (midazolam 4-hydroxylation) are higher (2- to 4-fold) than analogous values observed in dogs not receiving PB therapy. In summary, a therapeutic dose of PB for antiepileptic therapy significantly induces hepatic cytochrome P450 enzymes and plasma α1-acid glycoprotein in dogs. As a consequence, it is important during PB antiepileptic therapy that precautions be taken to recognize the potential effect this may have on the pharmacokinetic profile of drugs simultaneously administered.

In sheep, the amount of aminopyrine N-dimethylease and most other hepatic metabolizing enzyme systems progressively increases up until and past the time of weaning (0 to 12 weeks). However, cytochrome b5 and uridine diphosphoglucuronic acid transferase activity are not affected by age, whereas NADPH cytochrome c (P450) reductase activity actually decreases after weaning. In some instances (e.g., coumarin hydroxylase, cytochrome P450, and dinitrochlorobenzene-glutathione-S-transferase), differences from preweaning hepatic pharmaceutical metabolizing capacity can only be observed in sheep that are 6 months of age or older. The simultaneous occurrence of sore mouth, pneumonia, foot rot, parasitism, and systemic bacterial infections has a minimal effect on the metabolizing capacity of hepatic (i) cytochromes P450 and b5 in addition to the NADPH and NADH-dependent reductases associated with each of these cytochromes; (ii) cytochrome P450-mediated reactions (e.g., aniline and coumarin hydroxylases); (iii) aminopyrine N-demethylase; (iv) 7-ethoxycoumarin 0-deethylase; (v) uridine diphosphoglucuronic acid glucuronyl transferase; and (vi) glutathione-S-transferase.

In young (4–6 years) and older (15–20) Cebus monkeys (Cebus albifrons), vitamin E supplementation significantly ($p < 0.05$) increases antipyrine clearance consistent with decreased N-demethylation of this indicator pharmaceutical. The effect of dietary vitamin E on the decreased half-life and increased clearance of antipyrine is most prominent when results from both age groups are evaluated collectively. The implication of these findings is that dietary antioxidants appear to consistently enhance the clearance of diagnostic pharmaceuticals that undergo hepatic oxidative metabolism.

In obese rats subjected to very-low-energy diet with essential nutrients to cause declines in liver mass and total liver protein, the administration of 9.25 k Bq (0.25 μcurie) [dimethylamine-^{14}C] aminopyrine reveals an increased half-life of exhaled $^{14}CO_2$ ($p < 0.01$) and a decreased rate constant of aminopyrine elimination ($p < 0.01$). These findings imply that these dietary changes decrease the N-demethylation of aminopyrine. Furthermore, identification of decreases in hepatic glutathione suggests a reduced capacity of the liver to detoxify drugs through this particular conjugation pathway. Animals on very-low-energy diets are therefore likely to experience a decreased capacity to metabolize various pharmaceuticals and toxins through oxidative mechanisms.

Hepatic Microsomal Enzyme Metabolizing Capacity *Caffeine/β-Naphthoflavone/Tolbutamide/ Sulphaphenazole/Sulphadimethoxine/Quinine/ Antipyrine/Phenobarbital*

Cellular/Molecular Mechanism of Action: Caffeine is metabolized by the hepatic P450 cytochrome oxidase system.

Application: The clearance of caffeine can function as an excellent quantitative test of hepatic function in human beings and domestic animals (mammals). It is currently used in much the same way that creatinine clearance is used to assess renal function in that caffeine can be used as an indicator of decreasing hepatic function. Caffeine has been applied in this manner in cattle and in canine models of experimentally-induced progressive liver disease (e.g., hepatotoxin: dimethylnitrosamine).

Route of Administration: Intravenous (canine studies have applied a rapid IV infusion rate).

Dosage: Caffeine sodium benzoate administered at a dose of 2 mg of caffeine/kg of body weight has been successfully applied to measure hepatic function in lactating dairy cattle. In canine subjects, it can be administered at 20 mg/kg of body weight with serum samples obtained at intervals that span the duration of at least 5 terminal-phase drug half-lives.

Considerations: In lactating dairy cattle, no adverse effects have been observed following caffeine administration.

Cytochrome P450 activities *in vivo* and *in vitro* and enzyme induction by phenobarbital, β-naphthoflavone, isoniazid, and triacetyloleandomycin have been investigated in female dwarf goats.

Disposition of caffeine has been used as an indicator of decreasing hepatic function in dogs with experimentally-induced progressive liver disease. Disposition of caffeine can be studied 3 weeks after the last dose of the hepatospecific toxin, dimethylnitrosamine, in individual dogs.

Interpretation and Analysis: In lactating dairy cattle (n = 6), serum caffeine concentration-time curves best fit an open 2-compartment pharmacokinetic model. Harmonic mean elimination half-life is 3.8 (range, 2.6–6.9) hours, and total clearance is 0.118 (range, 0.090–0.197) L/kg/h. Milk caffeine concentration is similar to serum concentration 1.5–24 hours after caffeine administration. Adverse effects are not observed in cows given caffeine. In the bovine, antipyrine and sulphadimidine do not affect the pharmacokinetic profile of caffeine.

In β-naphthoflavone treated goat, the microsomal ethoxyresorufin-O-deethylation rate becomes markedly increased. Beta-naphthoflavone also induces caffeine plasma clearance but does not affect microsomal caffeine 1- and 3-demethylation rates. Phenobarbital also increases caffeine plasma clearance. In contrast to β-naphthoflavone, phenobarbital increases microsomal caffeine 1- and 3-demethylation rates. Goat hepatic microsomes are able to hydroxylate tolbutamide (predominantly a CYP2C9 activity in man) and debrisoquine (a CYP2D activity in various species). These activities are not affected by either β-naphthoflavone or phenobarbital. Sulphaphenazole is a more potent inhibitor of tolbutamide hydroxylation than sulphadimethoxine, and quinine is a more potent inhibitor of debrisoquine hydroxylation than is quinidine. As might be anticipated, microsomal aniline-4-hydroxylation rate (CYP2E) increases after isoniazid treatment. The microsomal testosterone 6 β-hydroxylation rate (CYP3A) is increased after phenobarbital and triacetyloleandomycin treatment. Antipyrine plasma clearance is also increased after phenobarbital treatment. As cytochrome P450 activities and inducibility in the dwarf goat are similar to patterns appreciated in man, they may be of value as a model for human biotransformation research.

In dogs, the clearance of caffeine is decreased in conditions of moderate and severe hepatic disease, compared with negative controls and mild histiologically affected animals. Caffeine mean residence times are longer in moderately affected dogs, compared with controls and mildly affected animals. Correction of caffeine clearances for hepatic weight increases the ability to discriminate between moderately and severely affected dogs. Comparison of the clearance and mean residence time ratios of caffeine calculated for each group with values for negative reference controls revealed there is no difference in ratios among untreated and mildly affected dogs. Experimental findings also revealed that the disposition of antipyrine and caffeine appears to change similarly with progression of (dimethylnitrosamine-induced) liver disease.

Instrumentation and Data Analysis: Caffeine concentration can be analyzed utilizing automated enzyme immunoassay techniques. Such analytical systems have a lower limit of detection of the assay for caffeine in serum of approximately 0.079 μg/ml.

In vivo kinetics of antipyrine, sulphadimidine, and caffeine are studied separately and as a combination ("cocktail"). After establishing a lack of interaction among these compounds the effects of the inducing agents can be investigated. *In vitro,* hepatic microsomal enzyme activities and apoprotein levels are then determined.

MICROSOMAL CYTOCHROME P450 ENZYME SYSTEMS (GENERAL)

Hepatic Cytochrome P450: Microsomal Fractions
Doxifluridine (5′-DFUR)/Tegafur (FT)

Cellular/Molecular Mechanism of Action: Doxifluridine (5′-DFUR) and tegafur (FT) are 2 fluoropyrimidine prodrugs that are metabolized to 5-fluorouracil (5-FU) by hepatic microsomal enzyme fractions.

Application: Characterization of differences in the *in vitro* metabolic profiles for chemotherapeutic agents including doxifluridine (5′-DFUR) and tegafur (FT).

Implementation: Microsomal and cytosolic fractions processed from hepatic tissue (e.g., human liver) are incubated with doxifluridine (5′-DFUR) and tegafur (FT) preparations (500 μM final concentration). Conversion of these 2 fluoropyrimidine prodrugs to 5-FU is measured by high-performance liquid chromatography. The degree of correlation between the 5-FU-forming activities is determined using various cytochrome P450-dependent reactions.

Interpretation and Analysis: Liver microsomes catalyze 5-FU formation from 5′-DFUR at rates of 10.0 to 160.1 pmol/min per mg protein. These findings correlate well with CYP2A6-dependent coumarin 7-hydroxylase activity. The rates of microsomal 5-FU formation from FT range from 44.9 to 808.3 pmol/min per mg protein and similarly correlate with coumarin 7-hydroxylase activity. The cytosol fractions catalyze 5-FU formation from 5′-DFUR at rates of 3,164.6 to 6,026.6 pmol/min per mg protein, or almost 2 orders of magnitude higher than the rates of cytosolic 5-FU formation from FT (46.8–219.0 pmol/min per mg protein). The cytosolic enzymes in hepatic tissue appear to be important for 5-FU formation from 5′-DFUR. Both cytosolic and microsomal enzymes are involved almost equally in 5-FU formation from FT. The increased formation of 5-FU from 5′-DFUR might provide an answer to the question of why similar blood 5-FU levels are retained despite lower blood levels of 5′-DFUR compared to FT concentrations.

Cytosolic and microsomal activation of doxifluridine and tegafur to produce 5-fluorouracil in human liver. Ozawa S, Hamada M, Murayama N, Nakajima Y, Kaniwa N, Matsumoto Y, Fukuoka M, Sawada J, Ohno Y. Cancer Chemother Pharmacol 2002 Dec; 50 (6), pp. 454–8.

Hepatic Cytochrome P450 (16-α)/Genetic Alteration of Metabolic Capacity
Estradiol/Progesterone/Testosterone

Molecular Mechanism of Action: The enzyme C-P450(16-α) hydroxylates testosterone, progesterone, and estradiol specifically at the 16-α position.

Application: Characterization and profiling the metabolizing capacity for testosterone, progesterone, and estrogen by hydroxylation at the 16-α position. Using conventional transfection procedures, it is theoretically possible to introduce and promote the expression of C-P450(16-α) hydroxylase biochemical activity within mammalian cell populations.

Considerations: It is possible to construct and express chimeras of different P450 gene sequences such as 16-α-hydroxylase [C-P450(16-α)]. Experience with C-P450(16-α) has revealed that the presence of about two-thirds of the corresponding peptide molecule from its C-terminus is necessary for the chimeric cytochrome to maintain steroid 16-α-hydroxylase activity.

In male mice, the expression of C-P450(16-α) is both growth hormone dependent and androgen dependent.

Interpretation and Analysis: A broad steroid substrate specificity with high regio-selectivity and stereoselectivity at 16-α position is a biochemical characteristic of C-P450(16-α).

Functional characterization of 2 cytochrome P450s within the mouse, male-specific steroid 16-α-hydroxylase gene family: Expression in mammalian cells and chimeric proteins. Ichikawa T, Itakura T, Negishi M. Biochemistry 1989 May 30; 28 (11), pp. 4779–84.

Hepatic Cytochrome P450(15-α): Steroid Hydroxylation/Steroid 15-α-Hydroxylase Metabolizing Capacity: Female Hormone Specific
Female Hormone/Growth Hormone

Cellular/Molecular Mechanism of Action: Steroid 15-α-hydroxylase (P450(15-α)) is a female-specific enzyme expressed in the livers of many inbred mice (e.g., strain DBA/2J).
Application: Delineation of the expression of female-specific steroid 15-α-hydroxylase (P450(15-α)) biochemical activity.
Interpretation and Analysis: Run-on assays using liver nuclei from GH-deficient Little mice reveal that the P450(15-α) gene is transcriptionally repressed by growth hormone in male mice. BALB/cJ is a variant strain in which the gene is expressed in the males as well as in the females. Genetic crosses between DBA/2J and BALB/cJ indicate that expression of the P450(15-α) gene in BALB/cJ males is inherited as a recessive trait and is regulated by a single locus. The parental origin of the P450(15-α) gene can be determined by characterizing any restriction site polymorphism that may exist at the exons of P450(15-α) genes. This facilitates the division of F2 males expressing the P450(15-α) gene into 3 phenotypes at a ratio of 1:1:2, including (i) individuals expressing the gene from only BALB/cJ or (ii) DBA/2J; and (iii) individuals expressing the genes from both BALB/cj and DBA/2J parents. Repression of the P450 (15-α) gene in male mice appears to be regulated by a trans-acting regulatory locus between the DBA/2J and BALB/cJ pairs. Because hypophysectomy derepresses the P450(15-α) gene in F1 males and GH represses the gene in hypophysectomized F1 males, the hormone appears to regulate gene repression through a trans-acting locus, referred to as GH-dependent repression (e.g., GDR).

A trans-acting locus regulates transcriptional repression of the female-specific steroid 15-α-hydroxylase gene in male mice. Aida K, Negishi M. J Mol Endocrinol 1993 Oct; 11 (2), pp. 213–22.

Hepatic Cytochrome P450: Steroid 7-α-Hydroxylase/Cytochrome P450: Steroid 15-α-Hydroxylase
Steroid Hormones/Corticosteroids/Testosterone

Cellular/Molecular Mechanism of Action: The murine steroid 7-α-hydroxylase P450(7-α), also referred to ascytochrome P450(7-α), hydroxylates steroid hormones (e.g., testosterone). Similary, 15-α-hydroxylase also converts steroid hormones to a hydroxylated analog.
Application: Corticosteroids can modify the metabolizing capacity of P450(7-α) and P450(15-α) depending on the identity of the amino acid residue located at the 209 position.
Interpretation and Analysis: The leucine amino acid residue at 209 of P450(15-α) is the most important residue in determining the steroid hydroxylase activity of this particular P450 enzyme system. The P450(7-α) contains Asn at the position corresponding to the Leu-209 of P450(15-α), although both P450s hydroxylate testosterone. The CO-reduced P450(7-α) complex is unstable and is quickly converted into inactive P420, whereas P450(15-α) is very stable. The P450(7-α), however, is stabilized either by addition of testosterone or by a mutation of Asn-209 to Leu. Mutant P450(7-α) displays a 17-fold lower Vmax value than the wild-type enzyme. Unexpectedly, it also has 3-fold lower Km and Kd values. Residue 209 in P450(7-α), therefore, appears to be located at a critical site of the haem-substrate-binding pocket. Corticosterone inhibits the testosterone 7-α-hydroxylase activity of wild-type P450(7-α), whereas it does not inhibit the mutant P450(7-α). Interestingly, P450(15-α) activity is inhibited by corticosterone upon the replacement of Leu-209 by Asn. In addition, this mutation increases the corticosterone 15-α-hydroxylase activity of P450(15-α) at least 20-fold. Whereas the inhibition by corticosterone depends on the presence of Asn at position 209, deoxycorticosterone inhibits the activities of the P450s regardless of the type of residue at 209. The results indicate, therefore, that the identity of residue 209 determines the affinity as well as specificity of steroid binding to both P450(7-α) and P450(15-α).

Site-directed mutagenesis of mouse steroid 7-α-hydroxylase (cytochrome P450(7-α)): Role of residue-209 in determining steroid-cytochrome P450 interaction. Iwasaki M, Lindberg RL, Juvonen RO, Negishi M. Biochem J 1993 Apr 15; 291 (Pt 2), pp. 569–73.

Hepatic Cytochrome P450: Genetic Variability of Expression Profiles/(Neoplastic Disease Susceptibility)
Polycyclic Aromatic Hydrocarbons/N-Nitrosamines/Benzene

Background Information: Several cytochrome P450 enzymes, responsible for metabolically activating carcinogens and medications, express wide interindividual variation whose genetic coding has now been identified as polymorphic and linked to cancer risk. For example, a restriction fragment-length polymorphism for cytochrome P450 1A1, which metabolizes polycyclic aromatic hydrocarbons, and cytochrome P450 2E1, which metabolizes N-nitrosamines and benzene, is linked to lung cancer risk. Cytochrome P450 2D6, responsible for metabolizing many clinically important medications, also is linked to lung cancer risk. The frequency for each of these genetic polymorphisms varies among different ethnic and racial groups. In addition to inherited factors for the detection of sensitive populations, determining the biologically effective doses for carcinogenic exposures also should quantitatively and qualitatively enhance the risk assessment process. Levels of carcinogen-DNA adducts reflect the net effect of exposure, absorption, metabolic activation, detoxification, and DNA repair. These effects are genetically predetermined, inducibility notwithstanding. The combination of adduct and genotyping assays provides an assessment of risk that reflects recent exogenous exposure as well as one's lifetime ability to activate and detoxify carcinogens.
Considerations: Risk assessment models strive to predict risks to humans from toxic agents. Safety factors and assumptions are incorporated into these models to allow a margin of error. In the case of cancer, substantial evidence shows that the carcinogenic process is a multistage process driven by the interaction of exogenous carcinogenic exposures, genetic traits, and other endogenous factors. Current risk assessment models fail to consider genetic predispositions that make people more sensitive or resistant to exogenous exposures and endogenous processes.

Pharmacogenetics: Detecting sensitive populations. Shields PG. Environ Health Perspect 1994 Dec; 102 Suppl 11, pp. 81–7.

Hepatic Cytochrome System/Susceptibility to 2nd-Stage Hepatocarcinogenesis
Phenobarbital (PB)/N-Nitrosodiethylamine (DEN)

Cellular/Molecular Mechanism of Action: The metabolite of N-nitrosodiethylamine is a hepatocarcinogenic agent.
Application: N-nitrosodiethylamine can function as a model for the analysis of the pharmacogenetics related to susceptibility to hepatocellular tumor promotion.
Route of Administration: N-nitrosodiethylamine (intraperitoneal).
Dosage: N-nitrosodiethylamine (90 mg/kg body weight).

Implementation: Certain selected strains of inbred mice can be utilized as an animal model for investigating susceptibility to 2-stage liver carcinogenesis. The carcinogen DEN (90 mg/kg body weight) is given as a single dose (e.g., 5-week-old male mice of strains C57BL/6NCr [C57], C3H/HeNCrMTV- [C3H], and DBA/2NCr [DBA]). Reference controls receive intraperitoneal injections of tricaprylin solvent (10 ml/kg). At 2 weeks postinjection, phenobarbital in drinking water (0.05%) is administered to mice. Data points are collected at 12, 24, 36, and 52 weeks of age (5, 17, 29, and 45 weeks for murine subjects exposed to phenobarbital).

Interpretation and Analysis: Phenobarbital significantly increases both the number of hepatocellular foci/cm^2 and the incidence of hepatocellular tumors after 17 weeks of treatment in 24-week-old DEN-initiated mice of strains C3H (0.11 ± 0.07 vs 2.9 ± 0.3 foci/cm^2 and 20 vs 70% incidence of hepatocellular tumors) and DBA (0.09 ± 0.09 vs 3.72 ± 0.6 foci/cm^2 and 0 vs 90% incidence of hepatocellular tumors) but is ineffective in C57 mice (0.04 ± 0.04 vs 0.07 ± 0.07 foci/cm^2). At 36 weeks of age the incidence of liver cell tumors in mice given DEN but not PB is 10 (DBA), 10 (C57), and 50% (C3H); the incidence is increased by PB to 90% in DBA and 100% in C3H mice, but there is no increase in C57 mice. Even at 52 weeks, the low incidence of hepatocellular tumors in C57 mice given DEN only (20%) is not significantly increased by subsequent exposure to PB. Serum PB levels observed at 12, 24, and 36 weeks of age are significantly higher in DBA mice than in C57 or C3H mice. Similar results can be appreciated when PB is administered in drinking water to 7-week-old male mice of these 3 strains for 20 days, during which period serum PB levels are measured at more frequent intervals. DBA mice thus appear to be unable to metabolize PB, which itself rather than its metabolites is probably responsible for tumor-promoting effects. DBA mice are especially sensitive, while C57 mice are refractory to the promotion of hepatocarcinogenesis facilitated by PB. The DBA and C57 strains of mice provide a model for analysis of the pharmacogenetics of susceptibility to hepatocellular tumor promotion. Note that DBA and C57 strains of mice differ with respect to other significant parameters for chemical carcinogenesis including inducibility for aryl hydrocarbon hydroxylase and susceptibility to promotion of hydrocarbon-initiated skin tumors by 12-O-tetradecanoylphorbol-13-acetate.

Interstrain differences in susceptibility to liver carcinogenesis initiated by N-nitrosodiethylamine and its promotion by phenobarbital in C57BL/6NCr, C3H/HeNCrMTV- and DBA/2NCr mice. Diwan BA, Rice JM, Ohshima M, Ward JM. Carcinogenesis 1986 Feb; 7 (2), pp. 215–20.

MICROSOMAL CYTOCHROME P450 1A1 AND P450 1A2

Hepatic Cytochrome P450 1 (CYPI) Metabolic Capacity/(Tropical Chronic Pancreatitis) *Theophylline*

Cellular/Molecular Mechanism of Action: Theophylline is a substrate for cytochrome P450 1.

Application: Measurement of the *in vivo* biochemical activity of cytochrome CYPI as a relevant avenue for determining the ability of this enzyme system to metabolize pharmaceuticals, optimize dosage schemes, determine individual susceptibility to pro-toxin biotransformation, and/or detect therapeutic non-compliance. In this context, theophylline can be applied as an *in vivo* probe to determine kinetic profiles of this pharmaceutical as a parameter that reflects the potentially toxic cytochrome P450 1 pathway of drug metabolism.

Examples of Clinical Implementation: Theophylline clearance is accelerated in patients suffering from conditions of calcific chronic pancreatitis compared to healthy/normal controls (median 69 [range 39–114] vs 45 [33–56] ml/hr/kg, $p = 0.003$). Review of clinical histories reveals that such conditions are frequently accompanied by exposure to xenobiotics known to be inducers of cytochrome P450 1 and/or yield reactive metabolites subsequent to functioning as a biochemical substrate. However, the concentration of D-glucaric acid in urine, as a marker of phase II conjugating pathways of drug metabolism, is oddly similar in both affected cases or normal/healthy control subjects. Such patterns of drug metabolism could predispose to oxidant stress and serve to support the practice of administering micronutrient antioxidant supplements that may have therapeutic and possibly prophylactic value in conditions of tropical chronic pancreatitis.

Evidence for induction of cytochrome P450 1 in patients with tropical chronic pancreatitis. Chaloner C, Sandle LN, Mohan V, Snehalatha C, Viswanathan M, Braganza JM. Int J Clin Pharmacol Ther Toxicol 1990 Jun; 28 (6), pp. 235–40.

Hepatic Cytochrome P450 1A1 (CYP1A1) and NQO1 Genetic Polymorphism/Acute Lymphoblastic Leukemia (ALL): Childhood/Genetic Prognostic Marker

Background Information: The origin of ALL, the most common pediatric cancer, can be explained by a combination of genetic factors and environmental exposure. The environmental toxicants to which an individual is exposed are biotransformed and eliminated from the body after metabolic conversion mediated by Phase I and Phase II xenobiotic-metabolizing enzymes. Phase I enzymes catalyze hydroxylation, reduction, and oxidation reactions of xenobiotics (carcinogens/drugs), often converting them into more active or toxic compounds. Phase II enzymes catalyze conjugation reactions (glucuronidation, acetylation, methylation), thereby converting the metabolites into non-reactive, water-soluble products that are eliminated from the organism.

Application: Expression of P450 1A1 and NQO1 variants can be applied in pharmacogenetic and prognostic-related decision making processes pertaining to the childhood variant of ALL.

Implementation: The genetic polymorphism underlying the variation in enzyme activity can modify susceptibility to diverse adult cancers, probably by influencing the activation and removal of toxicants or drugs. Genetic variants of certain Phase I and Phase II enzymes have a role in the development of childhood ALL and represent a viable model for such studies (e.g., short latency period). The genetic contribution to the development of ALL has been examined by association studies that analyze the loci of Phase I enzymes (cytochrome P450, myeloperoxidase) and Phase II enzymes (quinone-oxidoreductase, glutathione-S-transferase, N-acetyltransferase).

Interpretation and Analysis: The loci of the enzyme variants CYPlA1, CYP2E1, NQO1, GSTM1, GSTP1, and NAT2 are associated with disease development, and evidence of gene-gene interactions also appears to exist. Despite the improvements in treatment, resistant cases of ALL remain a leading cause of cancer-related death in children. Although the underlying mechanism of drug resistance is not well understood, differences in the capacity of ALL patients to process drugs and environmental carcinogens could play a role by modifying the risk of recurrent malignancy, as well as the response to therapy. Therefore, polymorphic genes encoding carcinogen- and drug-metabolizing enzymes may not only increase the risk of ALL but also influence the risk of relapse in patients. The prognosis of patients with CYPlA1 and NQO1 variants is worse than that of patients who lack these variants. Therefore, the genotyping of ALL cases for functional polymorphisms of candidate genes can become an important tool in predicting disease outcome.

Childhood acute lymphoblastic leukemia: Genetic determinants of susceptibility and disease outcome. Krajinovic M, Labuda D, Sinnett D. Rev Environ Health 2001 Jul–Sep; 16 (4), pp. 263–79.

Hepatic Cytochrome P450 1A1 and P450 1A2 Metabolic Capacity
R-Warfarin

Cellular/Molecular Mechanism of Action: Warfarin (R-warfarin enantiomer) is metabolized as a substrate by cytochrome P450 1A1 (CYP1A1) and P450 1A2 (CYP1A2) enzyme systems.
Application: Phenotyping of P450 1A1 and P450 1A2 for the purpose of assessing procarcinogen susceptibility. Hydroxylation rates for R-warfarin can be applied as a marker system for the detection and measurement of hepatic P450 1A2 metabolizing capacity (human). In addition, the calculation of 6-hydroxylation/8-hydroxylation ratios can be used *in vitro* as a marker system for the detection and measurement of P450 1A1 enzyme fractions.
Considerations: Cytochrome P450 enzyme systems P450 1A1 and P450 1A2 are heterogeneously distributed within human populations. Each 1 is induced by a number of environmental factors including various procarcinogens.
Interpretation and Analysis: The recombinant P450 1A1 and P450 1A2 from cDNA clones are regioselective for R-warfarin 6- and 8-hydroxylation and possess very similar Km values (1.4 mM 6-hydroxylation; 1.2 mM 8-hydroxylation; 1.6 mM 6-hydroxylation; 1.4 mM 8-hydroxylation, respectively). The R-warfarin enantiomer may therefore exhibit binding competition between the 2 forms. However, when comparing 6- and 8-hydroxylation, P450 1A1 shows weak regioselectivity for 8-hydroxylation, whereas P450 1A2 displays a strong regioselectivity for 6-hydroxylation, with 6-hydroxylation/8-hydroxylation ratios of 0.6 and 5.0, respectively. Analyses utilizing microsomal fractions isolated from (i) HepG2 and MCF-7 cells expressing only P450 1A1 (ratios of 0.7) exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin; and (ii) hepatic cells expressing only P450 1A2 (average ratios of 4.0) confirm this perception. Immunoblot densitometry scans of P450 1A2 in liver preparations correlate with R-warfarin 6-hydroxylation rates ($r^2 = 0.83$) and caffeine 3-demethylation rates ($r^2 = 0.67$), but not with R-warfarin 8-hydroxylation rates. The biotransformation of R-warfirin to 6-hydroxy-warfarin or 8-hydroxy-warfarin is not recognized for P450 enzyme fractions CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4.

Human cytochromes P450 1A1 and P450 1A2: R-warfarin metabolism as a probe. Zhang Z, Fasco MJ, Huang Z, Guengerich FP, Kaminsky LS. Drug Metab Dispos 1995 Dec; 23 (12), pp. 1339–46.

Hepatic Cytochrome P450 1A2 (CYP1A2) Metabolic Capacity
Caffeine

Cellular/Molecular Mechanism of Action: Caffeine is a substrate for cytochrome P450 1A2 and the biochemical activity of this enzyme system yields a metabolite that can be measured or quantified.
Application: Measurement of the *in vivo* biochemical activity of cytochrome P450 1A2 relevant to the ability of this enzyme system to metabolize pharmaceuticals, optimize dosage schemes, determine individual susceptibility to protoxin biotransformation, and/or detect therapeutic non-compliance.
Route of Administration: The distribution/activity of this enzyme is reported to be reflected by metabolic ratios.
Considerations: Several metabolic ratios using different combinations of urinary metabolites have been used to measure cytochrome P450 1A2. Utilization of this biochemical probe has resulted in some uncertainty about the distribution of this enzyme system.

Comparisons of 5 mathematical models that calculate the metabolic ratio for caffeine and therefore represent an estimate of CYP1A2 reveal that all of the methods produce a symmetrical distribution. Of these 5 ratio estimates, 3 different parameters are approximated and none of the 5 ratios correlate closely within 1 another. Based on the interpretation of findings generated by the 5 mathematical models, the complex metabolism of caffeine together with different parameters controlling the renal clearance of each metabolite, the criticism has been made that caffeine is an inaccurate probe for assessing the distribution of CYP1A2 activity in populations applying calculations for estimating urinary metabolic ratios.

Caffeine as a metabolic probe: A comparison of the metabolic ratios used to assess CYP1A2 activity. Notarianni LJ, Oliver SE, Dobrocky P, Bennett PN, Silverman BW. Br J Clin Pharmacol 1995 Jan; 39 (1), pp. 65–9.

Hepatic Cytochrome P450 1A2 (CYP1A2) Metabolizing Capacity
Caffeine

Application: Estimation of hepatic cytochrome CYP1A2 metabolizing capacity.
Route of Administration: Caffeine (oral).
Dosage: Caffeine (100 mg).
Considerations: A caffeine-free diet must be enforced prior to the initiation of the diagnostic procedure.
Diagnostic Strategy: Establishing correlations between different parameters related to plasma, saliva, and urine caffeine clearance/excretion with 2 measures of caffeine clearance, CL(oral) and CL(137X $\rightarrow$ 17X), functioning as reference standards.
Examples of Clinical Implementation: Both plasma and saliva total clearances of caffeine highly correlate with each other ($r^s = 0.97$, $p < 0.0001$). The ratio 17X/137X restricted to 1 sampling point taken 4 hours after administration reveals a high degree of correlation (r^s) with CL(oral) and CL(137X $\rightarrow$ 17X) in plasma (0.84/0.83) and saliva (0.82/0.77) ($p < 0.0001$ for all the correlation values) where 17X is 1,7-dimethylxanthine (paraxanthine) and 137X is 1,3,7-trimethylxanthine (caffeine). Additionally, the ratio (AFMU + 1U + 1X + 17U + 17X)/137X in 0 to 24 hours urine samples highly correlate with CL(137X $\rightarrow$ 17X) ($r^s = 0.85$, $p < 0.001$) where AFMU is 5-acetylamino-6-formylamino-3-methyluracil, 1U is 1-methyluracil, 1X is 1-methylxanthine, and 17U is 1,7-dimethyluric acid. The major estimates of CYP1A2 activity are significantly less in non-smoking females and are probably related to the use of oral contraceptives in this subpopulation.
Interpretation and Analysis: The biochemical conversion of caffeine to its metabolite directly corresponds to hepatic cytochrome CYP1A2 metabolizing capacity. Among caffeine-based approaches for CYP1A2, it is recommend that either plasma or saliva 17X/137X ratio and the urinary (AFMU + 1U + 1X + 17U + 17X)/137X) ratio be delineated for sampling intervals of at least 8 hours (T_0 = time of caffeine intake). These indices are simple, reliable, and relatively inexpensive estimates of (human population) CYP1A2 activity.

Evaluation of caffeine as an *in vivo* probe for CYP1A2 using measurements in plasma, saliva, and urine. Carrillo JA, Christensen M, Ramos SI, Alm C, Dahl ML, Benitez J, Bertilsson L. Ther Drug Monit 2000 Aug; 22 (4), pp. 409–17.

Hepatic Cytochrome P450 1A2 Metabolic Capacity
Caffeine

Application: Determining certain forms of hepatic cytochrome P450 biochemical function that corresponds to human P450 1A2 activity.
Considerations: Primary steps in the metabolism of caffeine and theophylline are cleavage of methyl groups and/or hydroxylation at position 8, mediated by cytochromes P450. Chinese hamster cells (V79) genetically engineered for stable expression of single forms of rat cytochromes P450 IA1, P450 1A2, and P450 IIBI and human P450 1A2 and rat liver epithelial cells expressing murine P450 1A2 can be used to overcome problems arising from the proper allocation of metabolic pathways to specific isoforms by conventional techniques.
Interpretation and Analysis: Caffeine is metabolized by human, rat, and murine P450 1A2, resulting in the formation of 4 primary demethylated and hydroxylated metabolites. However, there are differences in the relative amounts of the quantities of individual metabolites generated. The human and the mouse P450 1A2 isoforms predominantly mediate 3-demethylation of caffeine. The rat cytochrome P450 1A2 mediates both 3-demethylation and 1-demethylation of caffeine to a similar extent. Theophylline is metabolized mainly via 8-hydroxylation. The vast majority of these cell lines is capable of carrying out this reaction, with highest activities in cell lines expressing rat or human P450 1A2 or rat P450 1A1.
Instrumentation: High-performance liquid chromatography for the analysis and measurement of caffeine and/or theophylline concentrations.

Biotransformation of caffeine and theophylline in mammalian cell lines genetically engineered for expression of single cytochrome P450 isoforms. Fuhr U, Doehmer J, Battula N, Wölfel C, Kudla C, Keita Y, Staib AH. Biochem Pharmacol 1992 Jan 22; 43 (2), pp. 225–35.

MICROSOMAL CYTOCHROMES P450 2A6, 2C9, 2C19, 2D6, AND 2E1

Distinguishing Cytochrome P450 2A6 (CYP2A6) Metabolizing Capacity/CYP2E1
Disulfiram/Coumarin

Cellular/Molecular Mechanism of Action: Disulfiram and its principal metabolite (diethyldithiocarbamate) directly inhibit the biochemical activity of cytochrome P450 2E1 (CYP2E1) isotype. Diethyldithiocarbamate *in vitro* also inhibits hepatic CYP2A6 (human).

Coumarin is selectively catalyzed *in vivo* by cytochrome P450 isotype CYP2A6 to 7-hydroxycoumarin.
Application: Single-dose administration of disulfiram can be applied to delineate the role of P450 2E1 in the metabolism of specific pharmaceutical agents or prevent CYP2E1-mediated biotransformation. Disulfiram can be used to distinguish between CYP2E1 and CYP2A6 cytochrome P450 isotype metabolism capacity.
Route of Administration: Disulfiram (oral) followed 10 hours later by coumarin (oral).
Dosage: Disulfiram (500 mg) followed 10 hours later by the ingestion of coumarin (50 mg).
Examples of Clinical Implementation: The combination of disulfiram and coumarin have been utilized to determine if disulfiram and its metabolite, diethyldithiocarbamate, inhibit the *in vivo* biochemical activity of both CYP2E1 and CYP2A6 cytochrome P450 isotypes. The basis for this consideration is based on *in vitro* observations that have noted that disulfiram inhibits CYP2E1 while its metabolite, diethyldithiocarbamate, suppresses the biochemical activity of CYP2A6 cytochrome P450 isotype.
Interpretation and Analysis: The area under the plasma 7-hydroxycoumarin vs time curve (2.69 $\pm$ 0.90 μg.hr/ml) is not decreased after disulfiram pretreatment (3.33 $\pm$ 0.93 μg.hr/ml). Furthermore, maximum plasma concentration (Cmax) of 7-hydroxycoumarin (1.4 $\pm$ 0.5 vs 1.8 $\pm$ 0.6 μg/ml) and time to reach Cmax (1.0 $\pm$ 0.2 and 1.0 $\pm$ 0.4 hour) are unchanged by disulfiram pretreatment. Urinary 7-hydroxycoumarin excretion over a 24-hour period (38.9 $\pm$ 10.8 mg) is also not diminished by disulfiram pretreatment (45.2 $\pm$ 6.6 mg). Based on the interpretation of preliminary *in vivo* research findings, single-dose disulfiram does not appear to inhibit *in vivo* CYP2A6 activity. When single-dose disulfiram is used as an *in vivo* probe, the detection of inhibited drug metabolism suggests involvement of the CYP2E1 but not CYP2A6 cytochrome P450 isotype
Instrumentation: High-performance liquid chromatography analysis for the measurement of urine 7-hydroxycoumarin and plasma coumarin concentrations.

Single-dose disulfiram does not inhibit CYP2A6 activity. Kharasch ED, Hankins DC, Baxter PJ, Thummel KE. Clin Pharmacol Ther 1998 Jul; 64 (1), pp. 39–45.

Hepatic and Adrenal Metabolization: Microsomal CYP2D/(Inhibition of CYP2D Biochemical Activity)
Adenocorticotropic Hormone (ACTH)

Application: Induction of cytochrome CYP2D in the adrenal cortex and liver hepatocytes.
Route of Administration: ACTH.
Dosage: ACTH for 1, 3, or 7 days.
Animal Model: Guinea pigs treated with ACTH for 1, 3, or 7 days. In addition, some animals receive ACTH for 7 days and then untreated for an additional 3 or 7 days to test for reversibility of ACTH actions.
Interpretation and Analysis: ACTH treatment causes a time-dependent decrease in the rates of adrenal microsomal bufuralol metabolism, a CYP2D-catalyzed reaction; hepatic bufuralol metabolism is unaffected by ACTH. Adrenal enzyme activity is significantly reduced by ACTH within 1 day and decreases by 80% after 7 days. Western blotting and *in situ* hybridization analyses reveal corresponding declines in adrenal CYP2D16 protein and mRNA concentrations. Nuclear runoff assays indicate that ACTH treatment inhibits CYP2D16 expression at the transcriptional level. Adrenal 17 α-hydroxylase activities are increased by ACTH treatment, but CYP17 protein concentrations are not affected. Following the cessation of ACTH administration, the rates of adrenal bufuralol metabolism and CYP2D16 protein and mRNA concentrations return to control levels within 7 days. In summary, ACTH has a relatively rapid and reversible effect on the inhibition of adrenal CYP2D16 transcription, thereby decreasing adrenal xenobiotic metabolism. Thus, the actions of ACTH on CYP2D16 expression are opposite to those on other adrenal P450 isozymes, indicating unique regulatory mechanisms.

Downregulation of CYP2D16 by ACTH in the guinea pig adrenal cortex: Time course, reversibility, and mechanism of action. Colby HD, Longhurst PA, Burczynski JM, Hayes JR, Yuan BB, Voigt JV. Pharmacology 2003 Mar; 67 (3), pp. 121–7.

Hepatic Cytochrome P450 2A6 (CYP2A6) Metabolizing Capacity/Fadrozole/ Nitrosoamines/Aflatoxin B1
Coumarin/Nicotine/Cotinine

Cellular/Molecular Mechanism of Action: Coumarin undergoes 7-hydroxylation by high-affinity hepatic cytochrome CYP2A6 (human) in liver microsomes. Several mutated or deleted CYP2A6 alleles have been characterized. Although CYP2A6 represents up to 15% of human microsomes P450 proteins, it is still 1 of the less well characterized cytochrome P450 enzymes.
Application: Selective evaluation of cytochrome CYP2A6 metabolizing capacity in the context that coumarin can be applied as an *in vitro* and *in vivo* probe for detecting the activity of this biochemically-mediated metabolizing system. CYP2A6 may be inducible by antiepileptic drugs and it is decreased in alcohol-induced severe liver cirrhosis. Several mutated or deleted CYP2A6 alleles have been characterized.
Considerations: CYP2A6 is a major contributor to the oxidative metabolism of nicotine and cotinine, and it also contributes, to a larger or smaller extent, to the metabolism of a few pharmaceuticals (e.g., fadrozole), nitrosamines, other carcinogens (e.g., aflatoxin B1) and a number of coumarin-type alkaloids.

CYP2A6: A human coumarin 7-hydroxylase. Pelkonen O, Rautio A, Raunio H, Pasanen M. Toxicology 2000 Apr 3; 144 (1–3), pp. 139–47.

Hepatic Cytochrome P450 2C9 (CYP2C9) Metabolizing Capacity
Flurbiprofen/Sulfrapenazole/Piroxicam

Cellular/Molecular Mechanism of Action: Flurbiprofen is a non-steroidal anti-inflammatory drug (NSAID) that is metabolized by both oxidation to a 4-hydroxy-flurbiprofen metabolite (cytochrome P450 2C9) and conjugation (glucuronidation). Alternatively, sulfraphenazole functions as an inhibitor of cytochrome P450 2C9. Piroxicam functions as a non-steroidal anti-inflammatory agent that inhibits the biochemical activity of cyclooxygenase and the generation of prostaglandin species from arachidonic acid substrate.
Application: Flurbiprofen can potentially functions as both an *in vitro,* and possibly an *in vivo* probe for the detection and measurment of cytochrome P450 2C9 biochemical activity.
Interpretation and Analysis: Increases in the extent and rate of flurbiprofen biotransformation to 4′-hydroxy-flurbiprofen directly corresponds to the metabolizing capacity of hepatic P450 2C9 and its R144C variant.

The 4′-hydroxylated metabolite of flurbiprofen is not generated by CYP1A2, CYP2C8, CYP2E1, or CYP3A4 at physiologically relevant concentrations of this NSAID agent. Conversely, inhibition of (R)- and (S)-flurbiprofen 4′-hydroxylation occurs with the inhibitors of P450 2C9, sulfaphenazole (Ki = 0.07 and 0.06 μM, respectively), and the NSAID piroxicam (Ki = 10 and 7 μM, respectively). Furthermore, cytochromes CYP1A1, CYP2A6, CYP2B6, CYP2C19, and CYP2D6 are not involved in flurbiprofen hydroxylation at physiological relevant flurbiprofen concentrations. Such findings are particularly important due to the sequence homology and potential substrate overlap of P450 2C9 and 2C19. Conversely, inhibition of (R)- and (S)-flurbiprofen 4′-hydroxylation occurs with the P450 2C9 inhibitors, sulfaphenazole (Ki = 0.07 and 0.06 μM, respectively), and the NSAID piroxicam (Ki = 10 and 7 μM, respectively). Collective evaluation of research investigations conducted to date strongly suggests that P450 2C9 may be the only isoform involved to any substantial degree in flurbiprofen 4′-hydroxylation.

Studies of flurbiprofen 4′-hydroxylation. Additional evidence suggesting the sole involvement of cytochrome P450 2C9. Tracy TS, Marra C, Wrighton SA, Gonzalez FJ, Korzekwa KR. Biochem Pharmacol 1996 Oct 25; 52 (8), pp. 1305–9.

Hepatic Cytochrome P450 2C9 (CYP2C9) Metabolizing Capacity
Diclofenac (Ineffective)

Cellular/Molecular Mechanism of Action: Diclofenac is biochemically metabolized by the cytochrome CYP2C9 enzyme system to 4′-hydroxydiclofenac.
Application: *In vivo* cytochrome CYP2C9 probe based on either the measurment of the urinary 4′-hydroxydiclofenac/diclofenac metabolic ratio (MR) compared to the apparent partial metabolic clearance of diclofenac through its biochemical conversion to 4′-hydroxydiclofenac.
Route of Administration: Diclofenac (oral).
Dosage: Diclofenac (50 mg). Blood and urinary pharmacokinetics of diclofenac are studied over a 48-hour time period.
Considerations: Identification of the CYP2C9 alleles (CYP2C9*1, CYP2C9*2, and CYP2C9*3) can be established by performing genomic DNA sequence analysis.
Interpretation and Analysis: Dramatic interindividual variability in the delay of diclofenac intestinal absorption can often be appreciated because its 1st detectable blood concentration ranges from between 0.5 hours to more than 12 hours after a single dose. Oral dosage forms of diclofenac creates results that suggest that no correlation exists between the metabolic clearance of diclofenac into 4′-hydroxydiclofenac and the different urinary 4′-hydroxydiclofenac/diclofenac metabolic ratios calculated at 0 to 4 hours, 0 to 8 hours, 0 to 12 hours, 0 to 24 hours, and 0 to 48 hours urinary collections. In conclusion, diclofenac, in its enteric-coated form, is not a useful *in vivo* CYP2C9 probe probably because of its highly variable intestinal absorption rate.

Is diclofenac a valuable CYP2C9 probe in humans? Morin S, Loriot MA, Poirier JM, Tenneze L, Beaune PH, Funck-Brentano C, Jaillon P, Becquemont L. Eur J Clin Pharmacol 2001 Jan–Feb; 56 (11), pp. 793–7.

Hepatic Cytochrome P450 2C9 (CYP2C9) and P450 2C19 (CYP2C19) Metabolizing Capacity/(Genetic Polymorphisms)
Tolbutamide/Glucose

Cellular/Molecular Mechanism of Action: Tolbumamide can function as a selective probe for assessing and measurement of hepatic cytochrome metabolizing capacity (e.g., human CYP2C9).
Application: Evaluation of hepatic cytochrome CYP2C19 and CYP2C9 xenobiotic metabolizing capacity.
Route of Administration: Tolbutamide (oral).
Dosage: Tolbutamide (500 mg). Serum glucose concentrations are measured before and after oral intake of 100 g dextrose. Pharmacokinetic parameters are estimated from plasma and urine concentrations of tolbutamide and 4-hydroxytolbutamide.
Examples of Clinical Implementation: Several recent *in vitro* investigations have revealed that human CYP2C19 and CYP2C9 are involved in 4-methylhydroxylation of tolbutamide.

Distinguishing between predominantly CYP2C19 or CYP2C9 mediated cytochrome metabolization of pharmaceuticals and toxins can be established utilizing tolbutamide as a biochemical probe. In subjects heterozygous for the CYP2C9*3 allele, the C_{max} and AUC for tolbutamide are significantly greater and the plasma half-life significantly longer than in homozygous CYP2C9*1 subjects. No pharmacokinetic differences can be found between CYP2C19 EM and PM genotype subjects. The estimated AUC of the increase in serum glucose after oral intake of 100 g dextrose is 2.7-fold higher with the CYP2C9 vs the CYP2C9*1/*3 genotypes than in those with CYP2C9*1/*3. However, CYP2C19 genetic polymorphism does not alter the blood glucose lowering effect of tolbutamide. The plasma AUC for 4-hydroxytolbutamide and the ratio of 4-hydroxytolbutamide/tolbutamide does not differ significantly between CYP2C19 that demonstrate either poor or extensive metabolizing capacity. Conversely, plasma AUC for 4-hydroxytolbutamide and the ratio of 4-hydroxytolbutamide/tolbutamide are about twice as high in the CYP2C9 wild genotype than in CYP2C9*3 heterozygotes ($p < 0.05$). Results from these investigations strongly suggest that the disposition and hypoglycemic effect of tolbutamide are affected mainly by CYP2C9 genetic polymorphism, but not by CYP2C19 polymorphism. The *in vivo* contribution of CYP2C19 to tolbutamide 4-methylhydroxylation appears to be minor in humans.

Human Model: Normal healthy volunteers (Korean descent).
Interpretation and Analysis: Tolbutamide promotes declines in blood glucose levels. In this context, the level of 4-methylhydroxylation of tolbutamide by hepatic cytochrome systems is indirectly proportional to plasma glucose concentrations. This suggests that, at least *in vivo*, tolbutamide remains a selective probe for measuring CYP2C9 activity in humans.

Effects of CYP2C19 and CYP2C9 genetic polymorphisms on the disposition of and blood glucose lowering response to tolbutamide in humans. Shon JH, Yoon YR, Kim KA, Lim YC, Lee KJ, Park JY, Cha IJ, Flockhart DA, Shin JG. Pharmacogenetics 2002 Mar; 12 (2), pp. 111–9.

Hepatic Cytochrome P450 2C19 (*2 & *3): Genotype Polymorphism/Cytochrome P450 3A4 (*1B, *2 & *3)
Omeprazole/(Helicobacter pylori Eradication Therapy Outcome Prediction Variable)

Cellular/Molecular Mechanism of Action: Cytochrome P450 2C19 (*2 & *3) and P450 3A4 (*1B, *2 & *3) function as hepatic enzyme metabolizing systems.
Application: Characterize the potential efficacy of *Helicobacter pylori* eradication therapy as a function of cytochrome P450 (CYP) genotype polymorphism.
Implementation: Evaluation of human genomic DNA, CYP2C19 (*2 and *3) and CYP3A4 alleles (*1B, *2, and *3) by polymerase chain reaction-restriction fragment length polymorphism assays (confirmed by amplicon sequencing) can be applied to diagnose and characterize cases of *H. pylori* infections and their treatment with 1-week triple therapy (European Helicobacter Pylori Study Group guidelines; n = 143 Italian Caucasians).
Interpretation and Analysis: Most diagnosed cases of *H. pylori* infection can be irradicated of the microorganism according to a endoscope "gold-standard" (e.g., n = 93/143). Essentially all of the cases that remain infected with *H. pylori* and are of the CYP2C19 genotype can also be be found to be either homozygous or heterozygous extensive metabolizers (e.g., n = 50). Homozygous extensive metabolizers experience a significantly lower level of eradication than do heterozygous extensive metabolizers. Furthermore, the homEM genotype has value as a predictive variable for failure (univariate/multivariate analyses). Carriers of CYP3A4 polymorphisms achieved favorable eradication rates similar to cases with the CYP2C19 genotype. Cases with single CYP3A4*2 polymorphism (e.g., n = 4) achieve eradication, and only 29% (5/17) of all CYP3A4*1B carriers do not achieve eradication. A high percentage of carriers with CYP3A4 polymorphisms within CYP2C19 hetEM subgroups are cured (e.g., n = 9/9), suggesting a possible positive synergism between CYP3A4 and CYP2C19. Similar to Asian populations, different expression patterns for the CYP2C19 genotype are probably relevant in Caucasians receiving *H. pylori* eradication regimens that include omeprazole. The possibility of a favorable drug interaction mediated by CYP2C19 and CYP3A4 requires further research in future investigations.

The clinical role of cytochrome P450 genotypes in Helicobacter pylori management. Sapone A, Vaira D, Trespidi S, Perna F, Gatta L, Tampieri A, Ricci C, Cantelli-Forti G, Miglioli M, Biagi GL, Paolini M. Am J Gastroenterol. 2003 May; 98 (5), pp. 1010–5.

Hepatic Cytochrome P450 CYP2C9/CYP2C19/CYP2D6/Drug-Induced Adverse Reactions: Increased Risk/Genetic Polymorphism of "Poor Metabolizers"
Psychiatric Agent/Anticoagulant Agent

Background Information: Genetic polymorphism has been characterized for the drug-metabolising cytochrome P450 (CYP) enzymes CYP2C9, CYP2C19 and CYP2D6.
Application: Profiling genetic polymorphism for the drug-metabolising cytochrome P450 (CYP) enzymes CYP2C9, CYP2C19 and CYP2D6 can serve as an avenue for predicting increased susceptibility to the development of pharmaceutical-induced adverse reactions. Presently, CYP genotyping (CYP2C9/2C19/2D6) is most frequently requested in psychiatry and anticoagulant therapy, but the field is expanding. Important factors for implementation of pharmacogenetic methods are accuracy of diagnosis, quality of reports, response time, and cost.
Interpretation and Analysis: Several variations in the expression of cytochrome P450 (CYP) metabolizing enzymes CYP2C9, CYP2C19 and CYP2D can lead to reduced or absent biochemical activity. Occurance of such genetic-based deficiencies is of clinical importance mainly in patients having 2 non-functional alleles, phenotypically characterized as "poor metabolizers" (1–10% of Caucasians). Because most pharmaceuticals are transformed into inactive or less active metabolites, "poor metabolizers" are at increased risk of developing drug-induced adverse reactions. In practice, pharmacogenetic analyses will significantly contribute to reducing treatment costs for drug-induced adverse reactions and costs of sick leave, by predicting the best drug and the most effective and safest dosage.

Individualized pharmacotherapy based on cytochrome P450 (CYP) genotyping. (Article in Norwegian.) Johansen PW, Bergan S, Rootwelt H, Kvittingen EA, Rugstad HE. Tidsskr Nor Laegeforen 2002 Nov 30; 122 (29), pp. 2781–3.

Hepatic Cytochrome P450 2C9 (CYP2C9) Metabolizing Capacity/NAT2 Inborn Errors and Acetylator Polymorphism
Sulfonamides/Hydroxylamines

Background Information: Since the discovery of polymorphic N-acetylation of drugs nearly 40 years ago, great progress has been made in understanding the molecular genetics of acetylation as well as the clinical consequences of being a rapid or slow acetylator.

Interpretation and Analysis: Inborn errors (several different alleles) at the NAT2 locus are responsible for the traditional acetylator polymorphism. Studies have revealed variant alleles at the NAT1 locus as well. The consequences of pharmacogenetic variation in these enzymes include (i) altered kinetics of specific drug substrates; (ii) drug-drug interactions resulting from altered kinetics; (iii) idiosyncratic adverse drug reactions. The latter have been extensively investigated for the arylamine-containing sulfonamide antimicrobial drugs. Individual differences in multiple metabolic pathways can increase the likelihood of covalent binding of reactive metabolites of the drugs to cell macromolecules with resultant cytotoxicity and immune response to neoantigens. This can result clinically in an idiosyncratic hypersensitivity reaction, manifested by fever, skin rash, and variable toxicity to organs including liver, bone marrow, kidney, lung, heart, and thyroid. Slow acetylation by NAT2 is a risk factor for such reactions to sulfonamides. Given the incidence of these severe adverse drug reactions (much $<$ 1/1,000), slow acetylation cannot be the sole mechanism of predisposition in the population. Differences in rates of production of hydroxylamine metabolites of the drugs by cytochrome P450 (CYP2C9), myeloperoxidase, and thyroid, roxidase, along with an inherited abnormality in detoxification of the hydroxylamines are critically important in determining individual differences in adverse reaction risk. Both NATs, particularly NAT1, also can further metabolize hydroxylamine metabolites to N-acetoxy derivatives. Intensive investigation of patients with these rare adverse reactions using a variety of tools from *in vitro* cell toxicity assays through molecular genetic analysis will help elucidate mechanisms of predisposition and ultimately lead to diagnostic tools to characterize individual risk and prevent idiosyncratic drug toxicity.

N-acetyltransferases: Pharmacogenetics and clinical consequences of polymorphic drug metabolism. Spielberg SP. J Pharmacokinet Biopharm 1996 Oct; 24 (5), pp. 509–19.

Hepatic Cytochrome P450 Polymorphism/P450 2C9*2 (CYP 2C9*2)/P450 2C9*1/*3 (CYP2C9*1/*3)/P450 2C9*4 (CYP2C9*4)
(S)-Warfarin/Delayed Pharmacokinetic Clearance/Interpatient Variability

Background Information: Warfarin is 1 of the most widely prescribed oral anticoagulants. However, optimal use of the drug has been hampered by its $>$ 10-fold interpatient variability in the doses required to attain therapeutic responses. Pharmacogenetic polymorphism of cytochrome P450 (CYP) may be associated with impaired elimination of warfarin and exaggerated anticoagulatory responses to the drug in certain patients. Clinically available warfarin is a racemic mixture of (R)- and (S)-warfarin, and the (S)-enantiomer has 3 to 5 times greater anticoagulation potency than its optical congener. Both enantiomers are eliminated extensively via hepatic metabolism with a low clearance relative to hepatic blood flow. CYP2C9 is almost exclusively responsible for the metabolism of the pharmacologically more active (S)-enantiomer.

Implementation: Several human allelic variants of CYP2C9 have been cloned, (e.g., designated CYP2C9*1 reference sequence or wild-type allele), CYP2C9*2, CYP2C9*3, and CYP2C9*4, respectively.

Interpretation and Analysis: The allelic frequencies for these variants differ considerably among different ethnic populations. Caucasians appear to carry the CYP 2C9*2 (8 to 20%) and CYP2C9*3 (6 to 10%) variants more frequently than do Asians (0% and 2 to 5%, respectively). In an *in vitro* environment the metabolic activities of the CYP2C9 variants reveal that the catalytic activity of CYP2C9*3 expressed from cDNA is significantly less than that of CYP2C9*1. Human liver microsomes obtained from individuals heterozygous for CYP2C9*3 show significantly reduced (S)-warfarin 7-hydroxylation compared with those obtained from individuals of the CYP2C9*1 genotyped.

Pharmacokinetic (*in vivo*) clearance of (S)-warfarin in the homozygous CYP2C9*3 genotype as well as the heterozygous CYP2C9*1/*3 genotype, have a significantly reduced pharmacokinetic clearance of (S)-warfarin (-90% and -60%, respectively) compared with those with the CYP2C9*1 homozygous genotype (e.g., Japanese patients). The maintenance dosages of warfarin required for Japanese patients with heterozygous and homozygous CYP2C9*3 mutations are significantly lower than those in patients with CYP2C9*1/*1. In addition, 86% of British patients exhibiting adequate therapeutic responses with lower maintenance dosages of warfarin ($<$ 1.5 mg/day) have either the CYP2C9*2 or CYP2C9*3 mutation singly or in combination, whereas only 38% of randomly selected patients receiving warfarin express the corresponding mutations. Furthermore, the former group shows more frequent episodes of major bleeding associated with warfarin therapy. Such observations indicate that the CYP2C9*3 allele may be associated with retarded elimination of (S)-warfarin and induce or prolong certain clinical effects. However, relationships between CYP2C9 genotype, enzyme activity, metabolism of probe substrates, dosage requirements and bleeding complications should be interpreted with caution, and further studies are required.

Pharmacogenetics of warfarin elimination and its clinical implications. Takahashi H, Echizen H. Clin Pharmacokinet 2001; 40 (8), pp. 587–603.

Hepatic Cytochrome P450 Metabolizing Enzymes/P450 2C19 (CYP2C19)/P450 2D6 (CYP2D6)
Tricyclic Antidepressants/Selective Serotonin Uptake Inhibitors

Background Information: Both noradrenaline (NA) and serotonin (5-HT) appear to be involved in depression. Evidence suggests that dual-acting antidepressants, that is, those that affect both monoamine systems, such as tricyclic antidepressants and the noradrenergic and specific serotonergic antidepressant mirtazapine, may have greater efficacy and a faster onset of action than drugs that act on a single monoamine system only, such as the selective serotonin reuptake inhibitors (SSRIs). Cell firing is reduced by SSRIs in the short-term, but is increased by mirtazapine, probably due to its actions on both NA (via $alpha_2$ antagonism) and 5-HT (via $alpha_1$-stimulation by NA). This may help to explain clinical evidence suggesting that mirtazapine has a faster onset of action than the more selective antidepressants.

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Application: Individualized design of dosage regimens for antidepressant therapies.

Implementation: Characterization of the cytochrome P450 enzymes CYP2D6 and CYP2C19 genotypes in a manner that complements clinical evaluation and therapeutic monitoring procedures.
Interpretation and Analysis: In literature searches reviewing 32 antidepressants marketed in Europe, Canada, and the United States, a number of studies have compared the pharmacokinetic parameters of antidepressants among cases with poor, intermediate, extensive, and ultrarapid levels of metabolization. Regarding 14 different antidepressants, distinct dose recommendations for extensive, intermediate and poor metabolizers of either CYP2D6 or CYP2C19 have been described. For the tricyclic antidepressants, dose reductions around 50% are generally recommended for poor metabolizers of substrates for CYP2D6 or CYP2C19, whereas differences are reportedly smaller for the selective serotonin reuptake inhibitors. Review of databases can facilitate the formulation of average dose suggestions based on the phenotype or genotype.

CYP2D6 and CYP2C19 genotype-based dose recommendations for antidepressants: A 1st step towards subpopulation-specific dosages. Kirchheiner J, Brosen K, Dahl ML, Gram LF, Kasper S, Roots I, Sjoqvist F, Spina E, Brockmoller J. Acta Psychiatr Scand 2001 Sep; 104 (3), pp. 173–92.

Pharmacological principles of antidepressant efficacy. Schatzberg AF. Hum Psychopharmacol 2002 Jun; 17 Suppl 1, pp. S17–22.

Hepatic Cytochrome P450 2D6 (CYP2D6) Biochemical Metabolization/β-Adrenergic Blockers/Tricyclic Antidepressants/Class 1C Antiarrhythmics
Debrisoquine

Cellular/Molecular Mechanism of Action: Debrisoquine (antihypertensive) is metabolized to 4-hydroxydebrisoquine (4-OHD) by hepatic cytochrome CYP2D6 fractions.
Application: Phenotyping of individuals to predict if they have either an extensive or poor capacity to metabolize various pharmaceutical agents including β-adrenergic blockers, tricyclic antidepressants, and class 1C antiarrhythmics.
Route of Administration: Debrisoquine sulfate (oral).
Dosage: Debrisoquine sulfate (10 mg). All the urine excreted during the following 8-hour period is collected for analysis of metabolite concentrations.
Interpretation and Analysis: The debrisoquine metabolic ratio (DMR) is calculated as a percentage of the dose excreted as debrisoquine vs the percent dose excreted as 4- hydroxydebrisoquine. The debrisoquine recovery ratio (DRR) is calculated as the percent dose excreted as 4-hydroxydebrisoquine vs the percentage of dose excreted as debrisoquine plus 4-hydroxydebrisoquine.
Examples of Clinical Implementation: Hypertensive patients displaying a debrisoquine metabolic ratio of < 12.6 or a debrisoquine recovery ratio higher than 0.12 are classified as individuals with an extensive metabolic capacity. A high degree of enantioselectivity in the 4-hydroxylation of debrisoquine favouring the S-(+) enantiomer occurs resulting in R-(-)-4-hydroxydebrisoquine not detected in the urine of hypertensive patients that possess extensive metabolizing capacity.
Instrumentation: Reversed-phase high-performance liquid chromatography (HPLC) for the selective quantification of debrisoquine, 4-hydroxydebrisoquine, S-(+) 4-hydroxydebrisoquine, and R-(-) 4-hydroxydebrisoquine in urine samples without the need for derivatization techniques. Debrisoquine and its metabolite can be detected in urine samples by HPLC using (i) a reversed-phase Select B LiChrospher column; (ii) acetate buffer (0.25 N) and acetonitrile (pH 5) reverse phase (9:1, v/v); and (iii) fluorescence detector. The limit of quantitation with this system is 25.0 ng/ml for debrisoquine and 18.75 ng/ml for 4-hydroxydebrisoquine. Intra-day and interday relative standard deviations (RSDs) are $< 10\%$.

Direct enantioselective separation on chiral stationary phase involved resolution of S-(+)-4-OHD and R-(-)-4-OHD on a Chiralcel OD-R column with a mobile phase of 0.125 N sodium perchlorate, pH 5-acetonitrile-methanol (85:12:3, v/v/v). The quantitation limit of each enantiomer is 3.75 ng/ml of urine. Intra- and interday RSDs are $< 10\%$ for each enantiomer.

Enantioselectivity of debrisoquine 4-hydroxylation in Brazilian Caucasian hypertensive patients phenotyped as extensive metabolizers. Cerqueira PM, Mateus FH, Cesarino EJ, Bonato PS, Lanchote VL. J Chromatogr B Biomed Sci Appl 2000 Dec 1; 749 (2), pp. 153–61.

Hepatic Cytochrome P450 2D6 (CYP2D6) and P450 3A (CYP3A)
Dextromethorphan (DM)/Dextrorphan/ 3-Methoxymorphinan

Cellular/Molecular Mechanism of Action: Dextromethorphan is O-demethylated to dextrorphan by the human cytochrome P450 designated as CYP2D6, while it is N-demethylated to 3-methoxymorphinan via P450 3A (CYP3A).
Application: Dextromethorphan can be used as a probe to detect and measure cytochrome CYP2D6 biochemical activity.
Implementation: Dextromethorphan and its 3 demethylated metabolites can be measured in 4-hour spot urine samples employing high-performance liquid chromatography (HPLC) analyses. The urinary dextromethorphan-dextrorphan molar ratio is used to assess CYP2D6 activity while dextromethorphan/3-methoxymorphinan is calculated to determine CYP3A biochemical/metabolic activity.
Interpretation and Analysis: DM-3MM ratios are sensitive to the co-administration of selective CYP3A inhibitors grapefruit juice and erythromycin. In addition, in healthy volunteers and cancer patients, the N-demethylation of DM correlated with the CYP3A-mediated metabolism of verapamil and tamoxifen. DM appears to be a promising way to simultaneously phenotype patients for CYP2D6 and CYP3As.
Instrumentation: Processing involving HPLC solid-phase extraction (C18 column); phenyl column analysis [mobile phase, methanol-acetonitrile-phosphate buffer (10 mM, pH 3.5, 20:25:55, v/v)] in concert with fluorescence detection (excitation at lambda=228 nm, no emission cut-off filter).

Dextromethorphan as an *in vivo* probe for the simultaneous determination of CYP2D6 and CYP3A activity. Ducharme J, Abdullah S, Wainer IW. J Chromatogr B Biomed Sci Appl 1996 Mar 29; 678 (1), pp. 113–28.

Hepatic Cytochrome P450 2D6 (CYP2D6) Metabolizing Capacity/Individualization of Antipsychotic Agent Dosage: Schizophrenia
Clozapine

Background Information: The reintroduction of clozapine, the prototype of atypical antipsychotics, in the late 1980s has led to significant advances in the pharmacological management of schizophrenia. Since then, there has been a rapid development of novel "atypical" antipsychotic agents that have been pharmacologically modeled, to a certain extent, after their predecessor clozapine.
Implementation: As with all antipsychotics, there is variability among individuals in their response to "atypical" antipsychotic agents. Pharmacogenetic concepts provide a valuable foundation for understanding interindividual variability observed with responses to antipsychotic agents. Delineation of pharmacokinetic and pharmaco-

dynamic parameters are integral factors that need to be addressed in the pursuit of this rationale.

Molecular biology (genetic) and proteomic laboratory techniques represent powerful laboratory methodologies that provide additional insight about mechanisms associated with interindividual variations in response to pharmaceuticals such as clozapine and establishing phenotype and genotype classification schemes (e.g., variable cytochrome P450 CYP2D6 expression). Findings observed during the course of such laboratory-based investigations provide a basis for formulating recommendations for optimizing the pharmacokinetic profiles of antipsychotic agents; designing optimum dosage/frequency regimens and establishing recommendations for patient monitoring. In this fashion, it will ultimately be possible in the field of psychiatric pharmacogenetics to individualize pharmacotherapy of schizophrenia, and may also lead to the development of newer, more efficacious antipsychotic agents.

Pharmacogenetic methods as a complement to therapeutic monitoring of antidepressants and neuroleptics. Dahl ML, Sjoqvist F. Ther Drug Monit 2000 Feb; 22 (1), pp. 114–7.

Pharmacogenetics of antipsychotic treatment: Lessons learned from clozapine. Masellis M, Basile VS, Ozdemir V, Meltzer HY, Macciardi FM, Kennedy JL. Biol Psychiatry 2000 Feb 1; 47 (3), pp. 252–66.

Hepatic Cytochrome P450 2D6 (CYP2D6) Metabolizing Capacity/Dopamine D1 Receptor Genotype: Treatment Resistant/Individualization of Antipsychotic Agent Dosage: Schizophrenia

Clozapine/[^{18}F]-Fluorodeoxyglucose

Cellular/Molecular Mechanism of Action: [^{18}F]-fluorodeoxyglucose: functions as a false glucose analog that can detect alterations in glucose metabolism within the brain.
Physiological Mechanism of Action: Potentiate the effects of GABA-mediated presynaptic and postsynaptic inhibitory pathways.
Background Information: A goal of pharmacogenetics is to clarify associations between allelic variation and risk factors in psychiatric illness.
Application: Characterization of different patterns in dopamine alleles that correlated with resistance to therapeutic management in conditions of treatment-resistant schizophrenia. A close relationship exists between regional brain metabolic activity and clinical response to clozapine that is related to the D1 receptor genotype.
Implementation: Positron emission tomography (PET) scans with [^{18}F]-fluorodeoxyglucose are performed on cases of treatment-resistant schizophrenic subjects after 5 weeks of clozapine treatment (vs placebo).
Interpretation and Analysis: Significant regional brain metabolic effects can be found for D1 receptor genotypes ($p < 0.05$: adjusted for multiple comparisons). Metabolic decreases for the 2,2 genotype but not the 1,2 genotype can be observed in all major sectors of the brain, with the exception of the ventral parts of the caudate and putamen. Frontal, temporal, parietal, and occipital neocortices show decreases in metabolism as do the cingulate juxtaallocortex and the parahippocampal allocortex. Decreases can also be observed in the thalamus, amygdala, and cerebellum bilaterally. No significant metabolic differences by genotype have been observed for D3, 5HT(2A), and 5HT(2C) polymorphisms. In terms of clinical response, the DRD1 2,2 genotype significantly improves with clozapine treatment, demonstrating a 30% decrease in the Brief Psychiatric Rating Scale positive symptoms in contrast to a 7% worsening for the 1,2 genotype ($p < 0.05$). Therefore, brain metabolic activity, and clinical response to clozapine appears to be related to the D1 receptor genotype.

D1 receptor alleles predict PET metabolic correlates of clinical response to clozapine. Potkin SG, Basile VS, Jin Y, Masellis M, Badri F, Keator D, Wu JC, Alva G, Carreon DT, Bunney WE Jr, Fallon JH, Kennedy JL. Mol Psychiatry 2003 Jan; 8 (1), pp. 109–13.

Hepatic Cytochrome P450 2D6 (CYP2D6)/CYP2D6 Genotype Polymorphism

Nortriptyline Pharmacokinetics/Antidepressant Therapy

Background Information: The administration of nortriptyline results in approximately a 21% total interindividual variance in oral clearance, and 34% of that in steady state plasma concentrations.
Application: The pharmacokinetic profile for nortriptyline is significantly influenced by the expression of the CYP2D6 genotype. Prior knowledge of CYP2D6 expression characteristics could realistically aid in attempts to predict plasma concentration of the drug and thus allow for the tailoring of patient-specific dosage regimens.
Route of Administration: Nortriptyline (oral/per os).
Implementation: Establish a quantitative clearance model for nortriptyline as a function of the cytochrome CYP2D6 genotype. Such information can in effect serve as an avenue for establishing to what extent the CYP2D6 genotype contributes to the interindividual variability in steady state plasma concentration and metabolic clearance. Such information can be gained in healthy subjects (e.g., $n = 20$) administered a single dose of nortriptyline for comparison with cases suffering from depression undergoing steady state oral treatment. Plasma concentrations are analyzed by standard non-linear regression and non-linear mixed effect models. All experimental cases are genotyped for CYP2D6 polymorphism prior to the initiation of the study.
Interpretation and Analysis: The pharmacokinetic model that appropriately fits the plasma concentration data sets is a 1-compartment model with 1st-order absorption and elimination characteristics. The pharmacokinetic model with the closest fit of plasma concentration profiles is 1 where the intrinsic clearance is a linear function of the number of viable genes expressing CYP2D6 and where hepatic blood flow is fixed (60 L/h). Stable estimates can ultimately be obtained for population pharmacokinetic parameters and interindividual variances. Assuming 100% absorption, the model allows systemic clearance and bioavailability to be estimated. Bioavailability usually varies between 0.17 and 0.71, depending on the genotype. Using the frequency distribution of the CYP2D6 genotype, it is possible to estimate the number of functional CYP2D6 genes in individuals on nortriptyline monotherapy.

The pharmacokinetic profile for nortriptyline is significantly influenced by the expression of the CYP2D6 genotype and such factors may account for the substantial interindividual variance recognized for nortriptyline. In this context, non-linear mixed-effects modelling can be used to quantify the influence of the number of functional CYP2D6 genes on the metabolic clearance and plasma concentration of drugs metabolized by this cytochrome enzyme system.

Quantitative pharmacogenetics of nortriptyline: A novel approach. Kvist EE, Al-Shurbaji A, Dahl ML, Nordin C, Alvan G, Stahle L. Clin Pharmacokinet 2001; 40 (11), pp. 869–77.

Hepatic Cytochrome P450 2E1 Metabolizing Capacity

Chlorzoxazone/Chlorzoxazone: 6-Hydroxychlorzoxazone Hydroxylation Index

Cellular/Molecular Mechanism of Action: Chlorzoxazone is as substrate for and metabolized by cytochrome P450 2E1 enzyme fractions to 6-hydroxychlorzoxazone.

Application: The urinary excretion of 6-hydroxychlorzoxazone is limited by the rate of formation. Chlorzoxazone may therefore be utilized as an *in vivo* probe for evaluating P450 2E1 function based on the measurement of urinary 6-hydroxychlorzoxazone excretion as a parameter for assessing the metabolic capacity of this enzyme system.
Route of Administration: Chlorzoxazone (oral).
Dosage: Chlorzoxazone (500 mg) and plasma is collected at 3.5, 4.5, 5.5, and 6.5 hours after dosing in addition to the collection of urine for an 8-hour period.
Interpretation and Analysis: Plasma and urine samples can be applied to define the absorption phase and plasma area under the concentration-time curve of chlorzoxazone and the urinary kinetics of the 6-hydroxy metabolite. A parameter that can be applied as a measure of CYP2E1 activity is the hydroxylation index (mmole oral chlorzoxazone dose/mmole 6-hydroxychlorzoxazone in 8-hour urine).

There is a significant positive correlation between the plasma elimination rate constant for chlorzoxazone (Ke) and urinary metabolite excretion ($n = 27$, $r = 0.42$, $p < 0.03$) while a significant negative correlation exists between plasma Ke and the hydroxylation index ($n = 27$, $r = -0.41$, $p < 0.04$). The mean absorption rate constant for chlorzoxazone of 3.11 ± 4.67/hr is 5-fold greater than the plasma Ke of 0.57 ± 0.17/hr for full kinetic studies. The formation clearance of 6-hydroxy metabolite is negative between the plasma Ke of the parent compound and the disposition rate constant for urinary excretion of the 6-hydroxy metabolite ($n = 15$, $r = 0.85$, $p < 0.0001$).
Instrumentation: Chlorzoxazone and the 6-hydroxy metabolite are measured by high-performance liquid chromatography.

Urinary excretion of 6-hydroxychlorzoxazone as an index of CYP2E1 activity. Dreisbach AW, Ferencz N, Hopkins NE, Fuentes MG, Rege AB, George WJ, Lertora JJ. Clin Pharmacol Ther 1995 Nov; 58 (5), pp. 498–505.

Hepatic Cytochrome P450 2E1 (CYP2E1) Metabolizing Capacity
Chlorzoxazone

Cellular/Molecular Mechanism of Action: Chlorzoxazone is a substrate for cytochrome CYP2E1 and the biochemical activity of this system produces the metabolite, 6-hydroxy-chlorzoxazone.
Application: Measurement of the *in vivo* biochemical activity of cytochrome CYP2E1 relevant to the ability of this enzyme system to metabolize pharmaceuticals, optimize dosage schemes; determine individual susceptibility to pro-toxin biotransformation and/or detection of therapeutic non-compliance.
Interpretation and Analysis: In a population reference controls, both oral (330 ± 111 ml/min, mean $\pm$ SD) and fractional (213 ± 86 ml/min) clearances can vary substantially (e.g., 4-fold to 5-fold). Simple visual inspection of "raw" data usually reveals normal unimodal distribution profiles.

Interestingly, some investigations have reported clearance values being one-third greater in men than in women, but such differences are less striking after normalization according to body weight. Attempts to develop a single-time-point measure of 6-hydroxylating ability on the basis of plasma levels or urinary excretion of chlorzoxazone or its metabolite have been unsuccessful.

Genetic polymorphisms (Pst I and Rsa I restriction fragment length polymorphisms) in the 5′-flanking region of (human) CYP2E1 deoxyribonucleic acid obtained from peripheral leukocytes are not associated with differences in the disposition of chlorzoxazone. Similarly, no major effects on 6-hydroxylation is associated with mutations in intron 6 associated with a Dra I restriction fragment length polymorphism. The interindividual variability in CYP2E1 activity as measured *in vivo* in normal reference controls is considerably less than that expected based on *in vitro* studies. How closely associated such forms of variability correlate with individual susceptibility to CYP2E1-mediated biotransformation of various pro-toxic agents remains to be determined.

Interindividual variability of chlorzoxazone 6-hydroxylation in men and women and its relationship to CYP2E1 genetic polymorphisms. Kim RB, O'Shea D. Clin Pharmacol Ther 1995 Jun; 57 (6), pp. 645–55.

Hepatic Cytochrome P450 2E1 (CYP2E1) Metabolism Capacity
Chlorzoxazone

Cellular/Molecular Mechanism of Action: Chlorzoxazone is a substrate for cytochrome CYP2E1 yielding the metabolite 6-hydroxy-chlorzoxazone.
Application: Measurement of the *in vivo* biochemical activity of cytochrome P450 2E1 (CYP2E1) relevant to the ability of this enzyme system to metabolize pharmaceuticals, optimize dosage schemes; determine individual susceptibility to pro-toxin biotransformation and/or detection of therapeutic non-compliance.
Route of Administration: Chlorzoxazone (oral).
Dosage: Chlorzoxazone (250 mg).
Examples of Clinical Implementation: Prolonged fasting produces a significant increase in circulating ketone bodies that occurs simultaneously with declines in the oral clearance of chlorzoxazone (human mean $\pm$ SD, 5.79 ± 1.04 to 3.69 ± 1.54 ml/min/kg; $p < 0.03$). The 0- to 24-hour urinary recovery of the 6-hydroxy metabolite is substantial (human: 50% to 80%), and reductions in clearance reflect a lower 6-hydroxylating ability after fasting. The elimination half-life of the drug is increased similar to clearance parameters (1.00 ± 0.09 vs 1.50 ± 0.42 hours; $p < 0.004$), but the apparent volume of distribution is unaffected by fasting. Alternatively, obesity tends to significantly increase the oral clearance and distribution of chlorzoxazone on both an absolute and weight-normalized basis; (e.g., 4.15 ± 0.81 vs 6.23 ± 1.72 ml/min/kg and 0.50 ± 0.28 vs 0.82 ± 0.19 L/kg). Elimination half-life values are similar in both groups of subjects. Measurement of 6-hydroxychlorzoxazone concentrations reveals that obesity increases estimates of the fractional clearance of this metabolite. The difference in the oral clearance and 6-hydroxylating ability of non-obese men and women is also statistically different.

In the comparison of experimental findings observed in rats and man, fasting appears to have a different affect in these 2 species. Such discrepancies may reflect an interspecies difference in CYP2E1 regulation or, more likely, destruction of the enzyme by lipid peroxidation resulting from prolonged periods of fasting. Conversely, extreme obesity in humans increases 6-hydroxylation of chlorzoxazone, consistent with induction of CYP2E1 biochemical activity. Accordingly, such individuals may be at increased risk of CYP2E1-mediated toxicities and adverse effects caused by the formation of CYP2E1-mediated metabolites of environmental agents. In addition, the efficacy of an active drug that is a CYP2E1 substrate may be reduced in obese patients.
Interpretation and Analysis: In animals (e.g., rat) levels of CYP2E1 are induced by both fasting and obesity. Studies have investigated whether these 2 physiologic factors are determinants of the metabolism and disposition of chlorzoxazone in humans.

Effect of fasting and obesity in humans on the 6-hydroxylation of chlorzoxazone: A putative probe of CYP2E1 activity. O'Shea D, Davis SN, Kim RB, Wilkinson GR. Clin Pharmacol Ther 1994 Oct; 56 (4), pp. 359–67.

Hepatic Cytochrome P450 2E1 (CYP2E1) Metabolizing Capacity
Sevoflurane/Chlorzoxazone

Cellular/Molecular Mechanism of Action: Sevoflurane is a metabolic substrate for hepatic cytochrome CYP2E1 resulting in the production of free fluoride ion levels within the plasma.

Application: Sevoflurane and chlorzoxazone can be utilized to assess hepatic cytochrome CYP2E1 metabolizing capacity. In situations where high preoperative chlorzoxazone metabolic ratios are detected, then there is significant merit to the evaluation of renal function in the postoperative period following sevoflurane anesthesia.

Route of Administration: Sevoflurane (inhalation) compared to chlorzoxazone (oral).

Dosage: Sevoflurane (inspired concentration 1–3%) compared to chlorzoxazone (500 mg).

Considerations: Chlorzoxazone and its 6-hydroxymetabolite concentrations are measured in plasma 2 hours after drug administration. Anesthesia can be induced with propofol, fentanyl, and atracurium intravenously and maintained with sevoflurane. Plasma fluoride concentrations are determined before the induction of anesthesia, at the cessation of sevoflurane, and 2, 4, 6, 10, and 24 hours thereafter.

Implementation: Evaluation and characterization of increases in plasma fluoride concentrations (sevoflurance) and chlorzoxazone metabolic ratio (6-hydroxy-chlorzoxazole/chlorzoxazole).

Interpretation and Analysis: The area under the plasma fluoride concentration-time curve (AUC) can be calculated up to 24 hours after sevoflurane cessation. The chlorzoxazone metabolic ratio correlates significantly with plasma fluoride AUC measurements ($r^2 = 0.28$, $p < 0.025$); the elimination constant calculated for the postanesthetic 10- to 24-hour period ($r^2 = 0.30$, $p < 0.025$); and plasma fluoride levels 24 hours after cessation of sevoflurane ($r^2 = 0.48$, $p < 0.05$). A comparison between subjects receiving sevoflurane vs chlorzoxazone reveals that the administration of chlorzoxazone itself does not alter postanesthetic fluoride kinetics. Therefore, the interindividual variability in perianesthetic plasma fluoride levels after sevoflurane anesthesia is reflected by differences in the chlorzoxazone metabolic ratio, and effectively detects interindividual variability in CYP2E1 activity. In the application of sevoflurane anesthesia, it has subsequently been concluded that there is significant merit for examining renal function in the postoperative period if high preoperative metabolic ratios of chlorzoxazone are detected.

The relationship between cytochrome P450 2E1 activity and plasma fluoride levels after sevoflurane anesthesia in humans. Wandel C, Neff S, Keppler G, Böhrer H, Stockinger K, Wilkinson GR, Wood M, Martin E. Anesth Analg 1997 Oct; 85 (4), pp. 924–30.

Hepatic Cytochrome P450 2E1 (CYP2E1) Metabolizing Capacity
Chlorzoxazone

Cellular/Molecular Mechanism of Action: Chlorzoxazone is a substrate for cytochrome CYP2E1 which biochemically creates its 6-hydroxy metabolite.

Application: Cytochrome CYP2E1 is considered to play an important role in the metabolic activation of procarcinogens such as N-nitrosoamines and low molecular weight organic compounds.

Considerations: An RsaI polymorphism is present in the 5′-flanking region of the CYP2E1 gene, which could possibly affect its transcription. However, the relationship between genotype and the phenotypic catalytic activity of the enzyme has not been defined. Also, the effects in humans of specific dietary factors, other than ethanol, which have been shown in animal and in *in vitro* studies to modulate CYP2E1 activity, remain largely unknown.

Examples of Clinical Implementation: The metabolism (oral clearance) of chlorzoxazone by CYP2E1 to its 6-hydroxy metabolite is substantially less in healthy Japanese-Americans compared to analogous values reported in European-Americans. Applying adjustments for age and sex, the oral clearance of chlorzoxazone significantly decreases with the number of variant c2 alleles, and its mean in the c2/c2 genotype (147 ml/min) is statistically lower ($p \leq 0.05$) than for either the homozygous wild type (238 ml/min) or the heterozygote (201 ml/min) genotypes. Body weight is a major contributor to interindividual variability in the oral clearance of chlorzoxazone, accounting for 43% of the variance when analyzed applying stepwise multiple regression analysis. Dietary consumption (e.g., lettuce, broccoli, black tea) contributes to the introduction of additional variability (7, 5, and 6%, respectively), as does medication use (3%), age (4%), and CYP2E1 genotype (5%). Overall, 73% of the variance can be accounted for by these variables. Body weight, lettuce, and use of medications are associated with increased CYP2E1 activity, and the other covariates are associated with reduced enzyme function. Because of the role that CYP2E1 plays *in* procarcinogen activation, especially of N-nitrosamines involved *in* lung cancer, the identified factors may account *in* part for observed differences in individual susceptibility to disease and may also have implications for cancer prevention.

Interpretation and Analysis: Alterations in the rate and extent of endogenous biochemical conversion of chlorzoxazone to its 6-hydroxy metabolite positively correspond to CYP2E1 associated metabolizing capacity of this cytochrome system.

Genetic and dietary predictors of CYP2E1 activity: A phenotyping study in Hawaii Japanese using chlorzoxazone. Marchand LL, Wilkinson GR, Wilkens LR. Cancer Epidemiol Biomarkers Prev 1999 Jun; 8 (6), pp. 495–500.

Hepatic Cytochrome P450 2E1 (CYP2E1) Metabolizing Capacity/(Posttransplant)
Chlorzoxazone

Application: Determination of phenotypic cytochrome CYP2E1 expression and establishing hepatic metabolic capacity (e.g., post liver transplant) as a measure of physiological integrity. Parameters generated from establishing the degree or extent of chlorzoxazone metabolism by CYP2E1 can be utilized to adjust dosage regimens for pharmaceuticals in certain disease states or following various surgical procedures. Importance of this consideration is based on the observation that drug metabolism is actually not uniformly depressed after liver transplantation.

Dosage: Chlorzoxazone (250 mg) with the collection of urine and blood samples over an 8-hour period. The chlorzoxazone metabolic ratio is calculated as the plasma concentration of 6-hydroxychlorzoxazone/chlorzoxazone at 4 hours after chlorzoxazone administration (phenotypic index). Additional blood samples can be collected to facilitate the calculation of chlorzoxazone pharmacokinetic profiles applying non-compartmental methods.

Examples of Clinical Implementation: The chlorzoxazone metabolic ratio in the 1st month following liver transplant (mean $\pm$ SD, 6.4 ± 5.1) is significantly higher than observed after 1 month postsurgery (2.1 ± 2.0), when the chlorzoxazone metabolic ratio is not different from control subjects (0.8 ± 0.5). These findings reveal that significant induction of CYP2E1, as indicated by the chlorzoxazone metabolic ratio, occurs during the 1st month following liver transplant procedures. The maximum observed 6-hydroxychlorzoxazone plasma concentration is more highly variable but statistically

greater compared to healthy normal controls ($p < 0.05$). The maximum observed concentration of chlorzoxazone, the chlorzoxazone apparent oral clearance, and the formation clearance of 6-hydroxychlorzoxazone are also significantly different following liver transplant procedures.

Interpretation and Analysis: The chlorzoxazone metabolic ratio (plasma concentration of 6-hydroxychlorzoxazone/chlorzoxazone at 4 hours) is directly proportional to hepatic metabolizing capacity.

Instrumentation: High-performance liquid chromatography.

Induction of CYP2E1 activity in liver transplant patients as measured by chlorzoxazone 6-hydroxylation. Burckart GJ, Frye RF, Kelly P, Branch RA, Jain A, Fung JJ, Starzl TE, Venkataramanan R. Clin Pharmacol Ther 1998 Mar; 63 (3), pp. 296–302.

Hepatic Cytochrome P450 2E1 (CYP2E1) Metabolizing Capacity
Chlorzoxazone

Cellular/Molecular Mechanism of Action: Chlorzoxazone is a metabolic substrate for hepatic cytochrome CYP2E1 metabolizing systems.

Dosage: Chlorzoxazone (reported range = 250–750 mg) and timed blood and urine samples are obtained for 10 hours in order to measure chlorzoxazone and 6-hydroxychlorzoxazone concentrations. Recent investigations indicate that a chlorzoxazone dose of 250 mg should be used and that a single plasma ratio obtained 2 to 4 hours after dosing is reflective of chlorzoxazone 6-hydroxylation and thus may serve as a measure of the cytochrome P450 2E1 phenotypic trait.

Considerations: Chlorzoxazone is being developed and proposed for use as a probe to measure *invivo* cytochrome P450 2E1 activity, but the phenotypic trait measures that are used vary. Although the doses proposed for phenotyping range from 250 mg to 750 mg, the effect of dose on chlorzoxazone hydroxylation has not previously been evaluated.

Interpretation and Analysis: Increases in the rate of chlorzoxazone metabolism directly (positively) correlate with relative hepatic P450 2E1 biochemical activity. A parameter used to reflect chlorzoxazone metabolism is the 6-hydroxychlorzoxazone-to-chlorzoxazone plasma concentration ratio measured at 2 to 4 hours post administration. Pharmacokinetic parameters are estimated using non-compartmental methods.

Evaluation of phenotypic trait measures show that 6-hydroxychlorzoxazone to chlorzoxazone plasma concentration ratios highly correlate with metabolite formation clearance measurements ($r = 0.9$; $p < 0.001$). Urine-based parameters (e.g., total recovery) are not significantly related to formation clearance ($r = 0.5$; $p > 0.05$). Dose dependency in chlorzoxazone metabolism is reflected by a 30% increase ($p < 0.05$) in the dose-normalized area under the concentration-time curve (AUC) of chlorzoxazone and lower incremental dose-normalized urinary recovery of 6-hydroxychlorzoxazone at early timepoints after administration of 750-mg doses of chlorzoxazone. In addition, the plasma ratio of 6-hydroxychlorzoxazone to chlorzoxazone at 4 hours is reduced by 48% in 5 of 6 subjects after 750-mg dosages ($p > 0.05$). Speculation suggests that at the higher dosage levels 6-hydroxylation biochemical mechanisms become saturated and further support the concern about the importance of cytochrome phenotyping as a basis for the design of optimal dosage regimens.

Use of chlorzoxazone as an *in vivo* probe of cytochrome P450 2E1: Choice of dose and phenotypic trait measure. Frye RF, Adedoyin A, Mauro K, Matzke GR, Branch RA. J Clin Pharmacol 1998 Jan; 38 (1), pp. 82–9.

Hepatic Cytochrome P450 2E1 (CYP2E1) Metabolizing Capacity
Chlorzoxazone/Disulfiram

Cellular/Molecular Mechanism of Action: Chlorzoxazone (CLZ) is a selective substrate for hepatic cytochrome P450 CYP2E1 enzyme systems. Disulfiram (DSF) is a mechanism-based inhibitor of cytochrome CYP2E1.

Application: Chlorzoxazone (CLZ) is a selective (*in vivo*) probe for detecting and measuring hepatic cytochrome P450 CYP2E1 biochemical activity. Disulfiram, has potential value as a strategy for preventing CYP2E1-mediated xenobiotic toxicity.

Route of Administration: Disulfiram (oral).

Dosage: Disulfiram (500 mg SID).

Examples of Clinical Implementation: Chlorzoxazone can be applied *in vivo* to determine baseline CYP2E1 activity (CLZ plasma clearance and 6-hydroxychlorzoxazone fractional metabolic clearance). Investigations are then complemented 1 week later by the subsequent administration disulfiram in concert with chlorzoxazone intake approximately 12 hours later and then again on days 3, 6, 8, 10, and 13. A terminal DSF metabolite, 2-thiothiazolidine-4 carboxylic acid, can also be measured in urine samples at 24-hour intervals. The mean CLZ clearance and 6-hydroxychlorzoxazone fractional metabolic clearance on day 1 declines to 10.2% and 5.5% of baseline values, which reflect rapid and profound CYP2E1 inhibition. On day 3, CYP2E1 activity usually has returned to half that of control and by day 8 has returned to baseline values. Assuming zero-order synthesis and 1st-order degradation, the *in vivo* CYP2E1 synthesis rate and degradation half-life is estimated to be 11 ± 5 nmol/hr and 50 ± 19 hr, respectively. Significant amounts of 2-thiothiazolidine-4 carboxylic acid are present only on day 1 suggesting that the return of *in vivo* CYP2E1 activity is not associated with inhibitor washout, but instead by enzyme resynthesis. Results regarding CYP2E1 disposition may be useful for modeling the effects of CYP2E1 inducers and inhibitors. For prevention of CYP2E1-mediated bioactivation, depending on protoxicant disposition, a 2nd DSF dose might be necessary to completely prevent toxicity.

Interpretation and Analysis: Results regarding CYP2E1 disposition may be useful for modeling the effects of CYP2E1 inducers and inhibitors. For prevention of CYP2E1-mediated bioactivation, depending on protoxicant disposition, a 2nd DSF dose might be necessary to completely prevent toxicity.

Duration of cytochrome P450 2E1 (CYP2E1) inhibition and estimation of functional CYP2E1 enzyme half-life after single-dose disulfiram administration in humans. Emery MG, Jubert C, Thummel KE, Kharasch ED. J Pharmacol Exp Ther 1999 Oct; 291 (1), pp. 213–9.

Hepatic Steroid 15-α-Hydroxylase (CYP2A4)/Steroid 16-α-Hydroxylase (CYP2D9)/Estrogen Receptor-Alpha (ERα)/Signal Transducers and Activators of Transcription (STAT)
Estrogen/Growth Hormone

Cellular/Molecular Mechanism of Action: CYP2A4 (steroid 15-α-hydroxylase) and CYP2D9 (steroid 16-α-hydroxylase) function as biochemical enzyme fractions that hydrolyze steroid hormone substrates.

Physiological Mechanism of Action: CYP2A4 (steroid 15-α-hydroxylase) and CYP2D9 (steroid 16-α-hydroxylase) function as female-specific and male-specific genes, respectively, that are regulated through estrogen receptor complexes (ERα) and the Stat5b form of STAT (signal transducers and activators of transcription).
Application: Potential modification of the expression of sex-specific CYP2A4 (steroid 15-α-hydroxylase) and CYP2D9 (steroid 16-α-hydroxylase) applying various hormonal agents (e.g., estrogen, growth hormone).
Interpretation and Analysis: The female-specific enzyme CYP2A4 (steroid 15-α-hydroxylase) is directly regulated by the binding of estrogen to ERα receptor complexes. Suppression of ERα expression decreases female (murine) CYP2A4 expression. Interestingly, the CYP2D9 gene continues to be expressed in ERα-deficient males and does not appear to be ERα regulated. Nuclear localization of Stat5b occurs in both sexes of ERα-deficient mice, although it is normally observed in only wild-type males. Nuclear localization of Stat5b correlates with the repression of CYP2A4 and expression of CYP2D9, respectively. Because Stat5b is not detectable in liver nuclear extracts prepared from hypophysectomized ERα-deficient females, the regulation by ERα appears to be mediated through a pituitary hormone (i.e., growth hormone). Thus, ERα appears to play a key role in the mechanism that inhibits nuclear localization of Stat5b in female mice, leading to feminization of the ERα-GH-Stat5b pathway and CYP expression. Defaulting to this ERα-dependent mechanism results in localization of Stat5b to nuclei, which masculinizes the expression of CYP genes in male mice.

Developmental action of estrogen receptor-alpha feminizes the growth hormone-Stat5b pathway and expression of CYP2A4 and CYP2D9 genes in mouse liver. Sueyoshi T, Yokomori N, Korach KS, Negishi M. Mol Pharmacol 1999 Sep; 56 (3), pp. 473–7.

MICROSOMAL CYTOCHROMES P450 3A

Hepatic Cytochrome P450 3A (CYP3A) Metabolic Capacity/Renal Dysfunction: End-Stage
Rifampicin/Erythromycin Breath Test

Background Information: The cytochrome P450 (CYP) oxidative enzyme system, located primarily in the liver and small intestine, is responsible for the metabolism and detoxification of numerous endogenous and exogenous substances. The most abundant CYP enzyme, CYP3A, is known to be involved in the metabolism of more than 200 commonly used medications. In experimental models of renal failure, both hepatic function and CYP enzyme content are reduced; however, direct evidence in humans is lacking. Evaluation of drug metabolism in patients with end-stage renal disease is important because these patients use a large number of medications and are at risk of adverse reactions and drug-drug interactions.
Implementation: Measurement of hepatic CYP3A activity at baseline and after rifampicin enzyme induction in 12 patients with end-stage renal disease and 12 healthy, age-matched controls. Hepatic CYP3A phenotype can be characterized with the erythromycin breath test (ERBT), and enzyme induction capacity is evaluated with a short course of rifampicin (600 mg/d for 6 days).
Interpretation and Analysis: In end-stage renal disease, baseline ERBT values are 28% lower than reference controls ($p < .05$). However, enzyme induction capacity after rifampicin administration is similar between groups ($p = 0.70$).

Characterization of hepatic cytochrome P450 3A activity in patients with end-stage renal disease. Dowling TC, Briglia AE, Fink JC, Hanes DS, Light PD, Stackiewicz L, Karyekar CS, Eddington ND, Weir MR, Henrich WL. Clin Pharmacol Ther 2003 May; 73 (5), pp. 427–34.

Hepatic Cytochrome P450 3A (CYP3A) Metabolic Capacity/Liver Biopsy
Midazolam (MDZ)/Cyclosporine

Cellular/Molecular Mechanism of Action: Midazolam is a substrate for cytochrome P450 3A and yields the metabolite 1′-hydroxy-midazolam.
Background Information: The clearance of MDZ in humans is principally facilitated by metabolic biotransformation catalyzed by P450 3A isoforms.
Application: Optimization of dosage schemes, identification of individuals with susceptibility to protoxin biotransformation, and/or detection of therapeutic non-compliance as a function of measuring the *in vivo* biochemical activity of cytochrome P450 3A (CYP3A) enzyme systems.
Route of Administration: Midazolam (IV), cyclosporine (IV).
Considerations: Midazolam 1′-hydroxylation can be measured in liver biopsy specimens collected within 24 hours of initiation of pharmacokinetic studies utilizing tissue supernants produced following ultra-centrifugation (e.g., 13,000 × g).
Examples of Clinical Implementation: The clearance of midazolam and cyclosporine approximately 10 days after liver transplant surgery reveals that the *in vitro* rate of 1′-hydroxy-MDZ formation closely correlates with the total CYP3A content in hepatic S-13 fractions ($r = .84$, $p < 0.01$).

Total midazolam clearance measured *in vivo* highly correlates with hepatic CYP3A content measured *in vitro* ($r = 0.93$, $p < 0.001$), and with *in vivo* cyclosporine clearance ($r = 0.81$, $p < 0.001$). The intrinsic clearance of midazolam to 1′-hydroxy-midazolam (Vmax/Km) *in vitro* measured in ultra-centrifugation supernatants can be scaled to accommodate for total liver mass and applied to the model of hepatic clearance to yield a prediction of *in vivo* midazolam clearance (e.g., data set of $n = 5$ cases). The mean value predicted for midazolam clearance based on the interpretation of *in vitro* 1′-hydroxylation data is identical to the mean clearance observed *in vivo* (0.60 ± 0.24 vs 0.59 ± 0.25 liter/min). Such results suggest that variability in hepatic CYP3A expression in liver transplant recipients, and possibly in other populations, can be determined by the measurement of midazolam metabolic clearance.

Use of midazolam as a human cytochrome P450 3A probe: I. *In vitro-in vivo* correlations in liver transplant patients. Thummel KE, Shen DD, Podoll TD, Kunze KL, Trager WF, Hartwell PS, Raisys VA, Marsh CL, McVicar JP, Barr DM, et al. J Pharmacol Exp Ther 1994 Oct; 271 (1), pp. 549–56.

Hepatic Cytochrome P450 3A (CYP3A) Metabolic Capacity/S-Mephenytoin Poor Metabolizer Phenotype
Proguanil/Benzo-a-Pyrene/Caffeine/Omeprazole/S-Mephenytoin (Inhibitor)/Troleandomycin (Inhibitor)/α-Naphthoflavone (Activator)

Cellular/Molecular Mechanism of Action: Proguanil is a substrate for isoenzymes in the cytochrome CYP3A superfamily. Proguanil is also a substrate for S-mephenytoin hydroxylase. One important metabolite produced as a product from these 2 hepatic enzyme systems is cycloguanil.
Application: Measurement of the *in vivo* biochemical activity of cytochrome P450 2E1 (CYP2E1) and S-mephenytoin hydroxylase as it correlates with the ability of these 2 enzyme systems to metabolize

pharmaceuticals, optimize dosage schemes, determine individual susceptibility to protoxin biotransformation, and/or detect therapeutic non-compliance.

Interpretation and Analysis: In hepatic microsome preparations (human), the apparent Km values for proguanil range between 35 μM to 183 μM (n = 17 livers, 6.3-fold range in activity). Importantly, the rates of proguanil activation correlate significantly with CYP3A activity assessed through the measurement of benzo[a] pyrene metabolism, caffeine 8-oxidation, and omeprazole sulfone formation, in addition to parameters such as CYP3A immunoreactive content. A significantly high level of correlation also is associated with the rates of hydroxyomeprazole formation. In contrast, correlations with activities selective for CYP1A2, CYP2C9/10, and CYP2E1 and with immunoreactive CYP1A2 content are not significant.

Proguanil activation is inhibited by R,S-mephenytoin, troleandomycin, anti-CYP3A antiserum, and anti-CYP2C IgG but is activated by α-naphthoflavone. Inhibitors selective for CYP1A2, CYP2E1, CYP2A6, or CYP2C9/10 have little or no effect on proguanil activation. The extents of inhibition by R,S-mephenytoin, troleandomycin, and the 2 antibodies vary with the immunoreactive CYP3A content of the microsomes used. It is concluded that proguanil activation to cycloguanil by human liver microsomes is mediated both by S-mephenytoin hydroxylase and isoforms of the CYP3A subfamily. This has implications for the use of proguanil as an *in vivo* probe for the S-mephenytoin poor metabolizer phenotype.

In vitro proguanil activation to cycloguanil by human liver microsomes is mediated by CYP3A isoforms as well as by S-mephenytoin hydroxylase. Birkett DJ, Rees D, Andersson T, Gonzalez FJ, Miners JO, Veronese ME. Br J Clin Pharmacol 1994 May; 37 (5), pp. 413–20.

Hepatic Cytochrome P450 3A (CYP3A) Metabolization
Erythromycin (Breath Test)/Midazolam

Cellular/Molecular Mechanism of Action: Erythromycin and midazolam both are biochemically metabolized by hepatic cytochrome CYP3A enzyme fractions.

Application: Erythromycin and midazolam can be applied to assess the cytochrome CYP3A metabolizing capacity of the liver. Parameters generated in this manner can be applied to estimate the relative extent certain xenobiotics will be metabolized. More recently, the hydroxylation of midazolam has been suggested as an alternative *in vivo* probe , because it allows for estimation of CYP3A activity in both the hepatic parenchyma and intestinal epithelium.

Route of Administration: [^{14}C-N-methyl]-erythromycin (intravenous), and midazolam (intravenously [1 mg] or orally [2 mg]).

Dosage: [^{14}C-N-methyl]-erythromycin (3 μCi), midazolam (1 mg intravenously, or 2 orally). An erythromycin breath test (ERBT) can be performed 60 minutes after administration, and clearances estimated after midazolam administration. In addition, an endogenous 0- to 4-hour urinary 6-beta-hydroxycortisol/cortisol ratio can be measured to complement the aquired data set.

Considerations: Cytochrome CYP3A activity exhibits considerable interindividual variability, and an *in vivo* probe to measure such differences would serve several purposes. The ERBT is an established approach that has proven useful in this regard, but it has several limitations. More recently, the hydroxylation of midazolam as an *in vivo* probe has been suggested as an alternative approach for estimating CYP3A activity in the intestinal epithelium as well as in the liver.

Examples of Clinical Implementation: All 3 measured drug trait values vary approximately 3-fold to 5-fold, whereas the endogenous phenotype measure exhibit far greater variability (> 100-fold). A relatively low degree of correlation ($p < 0.05$) exists between any of the trait values including the erythrocyte breath test parameter obtained through the intravenous administration of the radiolabeled probe and the systemic clearance of midazolam, expressed in terms of either total or unbound drug, or on an absolute or a body weight-corrected basis ($r = 0.03$ to $r = 0.24$; $p = 0.08$ to $p = 0.90$). Substratification according to sex generally does not improve correlation statistics.

Interpretation and Analysis: Despite the biochemical conversion of both erythromycin to its N-demethylated metabolite, and the transformation of midazolam to its hydroxylated metabolite by cytochrome CYP3A, there is little correlation in the parameters generated by these 2 agents when they are applied *in vivo* as diagnostic probes. Indirect measurements such as the ERBT may reflect CYP3A activity and be useful for some purposes, but the estimation of the oral and intravenous clearance of midazolam has additional advantages, and they may be more applicable and have broader usefulness as quantitative estimates of CYP3A activity.

Failure of erythromycin breath test to correlate with midazolam clearance as a probe of cytochrome P450 3A. Kinirons MT, O'Shea D, Kim RB, Groopman JD, Thummel KE, Wood AJ, Wilkinson GR. Clin Pharmacol Ther 1999 Sep; 66 (3), pp. 224–31.

Hepatic Cytochrome P450 3A (CYP3A) Metabolizing Capacity
Midazolam

Application: Functions as an *in vivo* probe for measuring cytochrome CYP3A activity in order to predict or quantify hepatic xenobiotic metabolic capabilities.

Interpretation and Analysis: Plasma concentration-time curves following intravenous and oral administration of midazolam can be generated applying non-compartmental pharmacokinetic analysis. Concentration at the mean residence time (MRT) represent the best predictor for calculated estimates of total area-under-the-curve (AUC) parameters according to the concepts of statistical moment theory. Following IV or oral midazolam administration, the average MRT is approximately 3.5 hours, suggesting that the optimal single sampling time to predict AUC is between 3 and 4 hours. The concentrations of midazolam measured 4-hours after an IV or oral dose accounts for 80 and 91% of the constitutive interindividual variability in midazolam AUC, respectively. The 4-hour midazolam measurement can also function as an excellent predictor of drug-drug interactions involving CYP3A induction and inhibition.

In vivo phenotyping for CYP3A by a single-point determination of midazolam plasma concentration. Lin YS, Lockwood GF, Graham MA, Brian WR, Loi CM, Dobrinska MR, Shen DD, Watkins PB, Wilkinson GR, Kharasch ED, Thummel KE. Pharmacogenetics 2001 Dec; 11 (9), pp. 781–91.

Hepatic Cytochrome P450 3A4
Alprazolam/Citalopram/Fluoxetine

Physiological Mechanism of Action: Citalopram is a selective serotonin reuptake inhibitor antidepressant.

Background Information: The selective serotonin reuptake inhibitor (antidepressant) fluoxetine decreases or prevents alprazolam metabolism *in vivo* through cytochrome P450 3A4 inhibition. Both *in vitro* and *in vivo* observations suggest that citalopram has a minimal effect on cytochrome P450 3A4.

Application: Alprazolam can be applied as a probe to evaluate the metabolism of other pharmaceutical agents through cytochrome P450 3A4 isoenzyme pathways.

Dosage: Alprazolam (1 mg).
Implementation: Evaluation of the potential for citalopram to be metabolized *in vivo* by cytochrome P450 3A4-mediated enzyme systems determined as a function of alprazolam metabolism. Theoretically, therapeutic doses of citalopram (20 mg/d), as compared with fluoxetine (20 mg/d), should create less impairment on the metabolism of the probe drug alprazolam (1 mg) through inhibition of the cytochrome P450 3A4 isozyme as measured by *in vivo* pharmacokinetic and pharmacodynamic parameters.
Interpretation and Analysis: Fluoxetine prolongs the half-life of alprazolam by 16% and increases the area under the curve 0-infinity of alprazolam by 32%, while citalopram does not affect these parameters, although the time of maximum concentration of alprazolam is prolonged by 30 minutes after citalopram administration. Neither selective serotonin reuptake inhibitor significantly affects the pharmacodynamic profile of alprazolam. This experiment suggests differential effects by citalopram and fluoxetine on alprazolam kinetics.

Pharmacokinetic and pharmacodynamic evaluation of the inhibition of alprazolam by citalopram and fluoxetine. Hall J, Naranjo CA, Sproule BA, Herrmann N. J Clin Psychopharmacol 2003 Aug; 23 (4), pp. 349–57.

Hepatic Cytochrome P450 3A4 (CYP3A4): Endobiotic Metabolism/Xenobiotic Metabolism: Pharmaceuticals and Environmental Toxins
Steroids

Background Information: Marked interindividual variability exists in the expression of the cytochrome P450 3A4 metabolizing enzyme system.
Application: Determination of the genetic variability of cytochrome CYP3A4 expression can serve as an avenue for determining the pharmacogenetic implications related to individual differences in the metabolization of endogenous substances and xenobiotics, including the metabolism of steroids, environmental toxins and therapeutic pharmaceuticals.
Implementation: Determination of the genetic variability of cytochrome CYP3A4 expression can be achieved through the analysis of DNA samples from hepatic tissue harvested from known cases of the CYP3A4 phenotype.

Human Model: Cases of the CYP3A4 phenotype (e.g., Caucasians n = 53; African-Americans n = 21; CYP3A4 metabolism "outliers" n = 7; poor nifedipine metabolizers n = 5; DNA Polymorphism Discovery Resource subset [Coriell Institute] n = 8; 9 ethnic group representatives n = 89).
Interpretation and Analysis: Five non-synonymous mutations in the coding region of CYP3A4 have been identified.. CYP3A4*14 (T44C) in exon 1 results in an L15P change; CYP3A4*15 (G14387A) in exon 6 resulted in a R162Q substitution; CYP3A4*10 (G14422C) in exon 6 results in a D174H substitution; CYP3A4*16 (C15721G) in exon 7 results in a T185S amino acid substitution; and CYP3A4*12 (C22002T) in exon 11 results in a L373F change in the CYP3A4 protein. An additional observation that can be appreciated is 6 single nucleotide polymorphisms (SNPs) in the 5′-UTR, 13 SNPs in the introns and 3 SNPs in the 3′-UTR. Extensive population differences exist in the frequencies of various CYP3A4 alleles. None of the 28 CYP3A4 SNPs identified in CYP3A4 phenotyped persons (most individuals being heterozygous for any CYP3A4 variant) is associated with low hepatic CYP3A4 protein expression or low CYP3A4 activity *in vivo.*

Common allelic variants of cytochrome P450 3A4 and their prevalence in different populations. Lamba JK, Lin YS, Thummel K, Daly A, Watkins PB, Strom S, Zhang J, Schuetz EG. Pharmacogenetics 2002 Mar; 12 (2), pp. 121–32.

Hepatic Cytochrome P450 3A (CYP3A) 1st-Pass Activity/Non-Pharmacokinetic (Miosis Measurement)
Alfentanil

Cellular/Molecular Mechanism of Action: Alfentanil is an opioid that is a metabolic substrate for hepatic cytochrome CYP3A that is extracted from the plasma on 1st-pass perfusion of the liver.
Application: Non-invasive molecular probe that can be utilized in the assessment of 1st-pass hepatic cytochrome P450 3A metabolizing capacity through the indirect evaluation of the influence of alfentanil on pupil size (promotes miosis). Plasma concentrations of alfentanil directly correlate with the degree of observed miosis.
Route of Administration: Alfentanil (oral).
Dosage: Efficacy trial evaluating escalating dosage of 23, 30, 43, and 75 μg/kg administered orally on separate days.
Interpretation and Analysis: Oral alfentanil is rapidly absorbed (time to maximum concentration T_{max} = 0.7 ± 0.5 hours). Mean values for area-under-the-plasma-concentration-time-curve extrapolated to infinity [$AUC_{infinity}$ (27 ± 14, 38 ± 22, 57 ± 31, and 105 ± 59 ng/hr/ml] and maximum concentration (16 ± 8, 23 ± 16, 31 ± 18, and 50 ± 22 ng/mL) reveal a linear relationship to dose, but are associated with a substantial degree of interindividual variability. Parameters that are independent of administered dose include T_{max}, elimination half-life (1.0 ± 0.2 hours), total body clearance after oral administration [20 ± 18 ml/kg/min], and dose-normalized AUC ($AUC_{infinity}$). In contrast, alfentanil deposition is dose-dependent and displays a direct relationship to changes in clinical effect. The miosis created by alfentanil is variable to the extent that it may not be detectable in some cases at the lower dosage range. Mean miosis AUC (AUEC) and peak miosis are log-dose linear and the effective half-life (1.3 ± 0.9 hours) parallels the pharmaceutical's plasma half-life.

Oral alfentanil is rapidly absorbed (time to maximum concentration T_{max} = 0.7 ± 0.5 hours), exhibits linear and dose-independent kinetics, and undergoes substantial 1st-pass metabolism. Oral alfentanil may be a suitable probe for 1st-pass CYP3A activity. Measurement of the biological effect of alfentanil (miosis) may be an acceptable surrogate for plasma alfentanil concentrations (pharmacokinetic profiles). Further studies are warranted to determine whether oral alfentanil and alfentanil effect kinetics may be a suitable non-invasive *in vivo* probe for 1st-pass hepatic CYP3A activity

Disposition and miotic effects of oral alfentanil: A potential non-invasive probe for 1st-pass cytochrome P450 3A activity. Kharasch ED, Hoffer C, Walker A, Sheffels P. Clin Pharmacol Ther 2003 Mar; 73 (3), pp. 199–208.

Hepatic Cytochrome P450 3A4 (CYP3A4) 1st-Pass Activity /Non-Pharmacokinetic (Miosis Measurement)
Alfentanil/Midazolam/Rifampil/Troleandomycin

Cellular/Molecular Mechanism of Action: The opioid alfentanil is a CYP3A4 substrate and its rate and extent of plasma clearance depends exclusively on hepatic CYP3A4 biochemical activity.
Application: Determination of the CYP3A4 biochemical metabolizing capacity of the liver. Results from such evaluations are potentially valuable in predicting drug-interaction phenomenon, sequelae, and optimizing plasma pharmacokinetic profiles.
Route of Administration: Midazolam (IV); alfentanil (IV).

Dosage: Medazolam (1 mg) followed 1 hour later by intravenous alfentanil (15 μg/kg). Induction of CYP3A4 is achieved with rifampicin, while CYP3A4 inhibition is produced with troleandomycin administration.
Considerations: Pharmacokinetic studies are invasive and time-consuming, and they require extensive analytical effort. Alternatively, alfentanil-induced miosis (drug effect) can be used as a surrogate measure for assessing alfentanil plasma concentrations. Alfentanil can therefore be utilized as a non-invasive probe for hepatic CYP3A4 xenobiotic metabolizing systems.
Examples of Clinical Implementation: Dark-adapted pupil diameter and dynamic light response are measured coincident with venous blood sampling for up to 8 hours. Plasma concentrations of alfentanil and midazolam (an additional CYP3A4 probe) and pupil diameter vs time data can be analyzed by use of non-compartmental modeling. Pupil diameter change can be analyzed analogously to determine the area under the alfentanil effect (miosis)-time curve (AUEC), effect clearance (CL_{miosis}), and effect half-time.
Interpretation and Analysis: Compared with control, CYP3A4 induction and inhibition significantly alters the clearances of alfentanil (2.8 ± 1.4, 5.3 ± 1.0, and 0.42 ± 0.1 ml/kg/min, respectively; $p < .05$ vs control) and midazolam. Dark-adapted resting diameter (in millimeters) is the best measure of alfentanil pupil effects. Alfentanil-dependent miosis is significantly altered by CYP3A4 modulation, and $\log(\text{diameter}_0 - \text{diameter}_t)$ vs time curves resemble alfentanil plasma disposition. $AUEC_{infinity}$ values after control, CYP3A4 induction, and inhibition are 280 ± 150, 120 ± 22, and 1030 ± 240 mm × min, respectively ($p < 0.05$ vs control). Effect clearances (CL_{miosis}) are approximately 4.2 ± 1.3, 8.8 ± 2.4, and 1.2 ± 0.8 μg/mm × min, respectively, and effect half-times are 62 ± 23, 34 ± 27, and 211 ± 35 minutes, respectively ($p < 0.05$ vs control). CL_{miosis} significantly correlates with plasma clearances of alfentanil ($r = 0.77$, $p < 0.001$) and midazolam ($r = 0.80$; $p < 0.001$).

Alfentanil effect (miosis) may be a sensitive and reliable surrogate for plasma alfentanil concentrations. Alfentanil kinetics profiles may be used as a non-invasive surrogate for conventional pharmacokinetic studies. Effective clearance as measured by miosis response appears to be a highly suitable non-invasive *in vivo* probe for hepatic CYP3A4 biochemical metabolizing capacity, and its merits warrant further investigation. In summary, alfentanil clearance is an excellent *in vivo* probe for assessing hepatic CYP3A4 biochemical activity and drug interactions in humans.
Instrumentation: Ophthalmascope for evaluation of papillary responses and a gas chromatography-mass spectrometry for quantifying midazolam and alfentanil contained in biological fluid samples.

A pilot evaluation of alfentanil-induced miosis as a non-invasive probe for hepatic cytochrome P450 3A4 (CYP3A4) activity in humans. Phimmasone S, Kharasch ED. Clin Pharmacol Ther 2001 Dec; 70 (6), pp. 505–17.

Hepatic Cytochrome P450 3A4 (CYP3A4) Metabolizing Capacity
Dexamethazone (CYP3A4)/Ketoconazole (−CYP3A4)/ Furafyllin (−CYP1A)/Tolbutamide (CYP2C)/ Sulfaphenazole (−CYP2C)

Cellular/Molecular Mechanism of Action: Dexamethazone is a synthetic glucocorticoid that can serve as an *in vitro* and *in vivo* probe for detecting and measuring cytochrome CYP3A4 biochemical activity. Ketoconazole functions as a inhibitor of cytochrome CYP3A4 biochemical activity. Furafylline is a CYP1A inhibitor, tolbutamide is a CYP2C substrate, and sulfaphenazole functions as a CYP2C inhibitor.
Interpretation and Analysis: In liver preparations (human), the following metabolites of dexamethazone can be identified; 6-beta-hydroxydexamethasone, 6-alpha-hydroxydexamethasone, 6-hydroxy-9-alpha-fluoro-androsta-1,4-diene-11 beta-hydroxy-16 alpha-methyl-3,17-dione (6-hydroxy-9 alpha-F-A) and 9 alpha-fluoro-androsta-1,4-diene-11 beta-hydroxy-16 alpha-methyl-3,17-dione (9 alpha-F-A). Dexamethasone undergoes side-chain cleavage to form 9 alpha-F-A, which then becomes a substrate for 6-hydroxylation. Interindividual variability in metabolic profiles is considerable. Mean (+/-S.D.) K_m values for 6-beta-hydroxydexamethasone and 6-alpha-hydroxydexamethasone formation are 23.2 ± 3.8 and 25.6 ± 1.6 μM ($n = 4$), respectively. The corresponding V_{max} values are 14.3 ± 9.9 and 4.6 ± 3.1 pmol/min/mg protein. Ketoconazole (3 μM) completely inhibits 6-alpha-hydroxylation and 6-beta-hydroxylation in a manner that suggests that the formation of both metabolites is catalyzed by CYP3A4. Further evidence validating this concept includes the degree of correlation between the rate of metabolite formation and the relative expression of CYP3A4 [6-beta-hydroxydexamethasone ($r = 0.74$ at $p = 0.003$); and 6-alpha-hydroxydexamethasone ($r = 0.70$ at $p = 0.006$)]. In addition to ketoconazole, both ellipticine and gestodene cause marked inhibition of 6-hydroxylation. Ellipticine is clearly not a selective CYP1A inhibitor as has been stated previously. However, furafylline (CYP1A inhibitor), tolbutamide (CYP2C substrate), and sulfaphenazole (CYP2C inhibitor) are essentially non-inhibitory. The relatively simple metabolic profile of dexamethasone compared to other steroids may point to this being a potentially useful *in vivo* probe for CYP3A4 in humans.
Instrumentation: Radiometric high-performance liquid chromatography and liquid-chromatography-mass spectrometry in concert with the identification of the major metabolite 6-beta-hydroxydexamethasone by co-chromatography with a chemically synthesized standard.

Dexamethasone metabolism by human liver *in vitro*. Metabolite identification and inhibition of 6-hydroxylation. Gentile DM, Tomlinson ES, Maggs JL, Park BK, Back DJ. J Pharmacol Exp Ther 1996 Apr; 277 (1), pp. 105–12.

Hepatic Cytochrome P450 3A4 (CYP3A4) Metabolizing Capacity
Erythromycin Breath Test (ERBT) (CYP3A4)/Phenyltoin (+CYP3A4)/Dexamethazone (+CYP3A4)

Cellular/Molecular Mechanism of Action: Erythromycin is a substrate for hepatic P450 isoenzyme CYP3A4 and radioactive carbon atoms from labeled analogs are expired by pulmonary mechanisms.
Application: Determination of the metabolizing capacity of hepatic P450 isoenzyme CYP3A4 in order to approximate the biochemical degradation rate of selected chemotherapeutic agents. Delineation of such parameters can faciliate optimal CYP3A4 metabolization of cytotoxic agents.
Route of Administration: [^{14}C]-erythromycin (intravenous) and erythromycin lactobionate (intravenous).
Dosage: [^{14}C]-erythromycin (4 μCi) followed on day 2 by 10-minute infusion of 100 mg of erythromycin lactobionate.
Implementation: Evaluation of hepatic cytochrome P450 3A4 microsomal enzyme activity. One experimental protocol that can be used for this purpose involves collection of breath samples on day 1 after the administation of [^{14}C]-erythromycin. The breath [$^{14}CO_2$] flux (CERt) is then estimated at 11 time points over a 2-hour period. On day 2, the ERBT is repeated midway through an infusion of erythromycin lactobionate, and the plasma pharmacokinetic profile for erythromycin established.

Interpretation and Analysis: Increases in the expiration of [$^{14}CO_2$] in the ERBT directly corresponds with the P450 isoenzyme CYP3A4 associated metabolizing capacity of the liver.

The infusion of 100 mg of erythromycin does not significantly modify the results generated by the ERBT. Measurements of the conventional ERBT parameter CER20 minutes obtained on day 1 are comparable for most subjects (0.03–0.06% dose/min) except in situations associated with the co-administration of dexamethazone and phenytoin (known CYP3A4 inducers: 0.14% dose/min). A significant correlation does not exist between any of the conventional ERBT parameters and erythromycin clearance. However, 2 parameters reflecting early emergence of breath radioactivity (1/TMAX and CER3 min/CERMAX) correlate significantly with erythromycin clearance ($p = 0.005$ and 0.006, respectively). Novel parameters derived from the ERBT significantly correlate with the clearance of erythromycin even in the presence of confounding factors, such as metastatic liver disease, altered protein binding, and therapeutic co-administration.

Optimizing the erythromycin breath test for use in cancer patients. Rivory LP, Slaviero K, Seale JP, Hoskins JM, Boyer M, Beale PJ, Millward MJ, Bishop JF, Clarke SJ. Clin Cancer Res 2000 Sep; 6 (9), pp. 3480–5.

Hepatic Cytochrome P450 3A4 (CYP3A4) Metabolizing Capacity
Alfentanil (CYP3A4)/Midazolam (CYP3A4)/Rifampicin (+CYP3A4)/Troleandomycin (−CYP3A4)

Cellular/Molecular Mechanism of Action: Alfentanil (metabolic substrate for P450 3A4); midazolam (reference CYP3A4 probe); rifampicin (induction of P450 3A4 activity); and troleandomycin (selective P450 3A4 inhibitor).

Application: Alfentanil can be applied *in vivo* to assess cytochrome P450 3A4 (CYP3A4) biochemical activity. Justification for performing alfentanil-based diagnostic testing procedures is supported by the (i) known population variability in P450 3A4 activity to the extent that it complicates the design of dosage regimens for alfentanil and other pharmaceutical agents metabolized by this cytochrome system; and (ii) propensity of certain pharmaceuticals to enhance or inhibit the biochemical activity of P450 3A4 that in turn can influence the metabolism of co-administered pharmaceutical agents. Lastly, the *in vitro* evaluation of alfentanil metabolism utilizing liver microsomal enzyme preparations (human) is an excellent supplemental model for predicting alfentanil metabolism *in vivo* (human).

Route of Administration: Alfentanil (intravenous) 30 minutes after midazolam (intravenously). Induction of high P450 3A4 activity can be achieved by the administration of rifampicin, while low P450 3A4 activity can be promoted with the selective inhibitor troleandomycin.

Dosage: Alfentanil (20 μg/kg) 30 minutes after midazolam (1 mg) on 3 occasions: control; high P450 3A4 activity (rifampicin induction); and low P450 3A4 activity (selective inhibition by troleandomycin). Venous blood samples are collected for a period of 24 hours and plasma concentrations of midazolam and alfentanil and their primary metabolites 1′-hydroxymidazolam and noralfentanil measured.

Considerations: There is considerable unexplained variability in alfentanil pharmacokinetics, particularly with regard to systemic clearance.

Interpretation and Analysis: Pharmacokinetic parameters are determined by 2-stage analysis using both non-compartmental and 3-compartment models.

Plasma alfentanil concentration-time profiles depend significantly on P450 3A4 activity. Alfentanil non-compartmental clearance is 5.3 ± 2.3, 14.6 ± 3.8, and 1.1 ± 0.5 ml/kg/min, while the elimination half-life is 58 ± 13; 35 ± 7; and 630 ± 374 minutes, respectively, in P450 3A4 activity classified as normal/controls; high/ rifampicin; and low/troleandomycin (means ± SD; $p < 0.05$ compared with controls). Multicompartmental modeling suggests a time-dependent inhibition-resynthesis model for the troleandomycin effects on P450 3A4 activity, characterized as $k10_t = k10[1\text{-}\pi\ e\text{-}\alpha(t\text{-}tzero)]$, where $k10_t$ is the apparent time-dependent rate constant, k10 is the uninhibited rate constant, π is the fraction of P450 3A4 inhibited, and α is the apparent P450 3A4 reactivation rate. Furthermore, a significant degree of correlation ($r = 0.97$, $p < 0.001$) exists between alfentanil systemic clearance and P450 3A4 activity. Alfentanil clearance is positively affected by rifampicin (13.2 ± 3.6 ml/kg/min), but negatively influenced by troleandomycin (1.5 ± 0.8 ml/kg/min) compared to reference controls (4.9 ± 2.1 ml/kg/min). Based on the influence of rifampicin and troleandomycin on alfentanil clearance and their known biological effect on CYP3A4, alfentanil metabolism and clearance is predominantly influenced by cytochrome P450 3A4 biochemical activity.

This observation, combined with the known population variability in P450 3A4 activity, provides a mechanistic explanation for the interindividual variability in alfentanil disposition. Furthermore, the known susceptibility of P450 3A4 (human) activity to induction and inhibition provides a conceptual framework for understanding and predicting clinical alfentanil drug interactions.

Instrumentation: Gas chromatography-mass spectrometry.

The role of cytochrome P450 3A4 in alfentanil clearance. Implications for interindividual variability in disposition and perioperative drug interactions. Kharasch ED, Russell M, Mautz D, Thummel KE, Kunze KL, Bowdle A, Cox K. Anesthesiology 1997 Jul; 87 (1), pp. 36–50.

Hepatic Cytochrome P450 3A4 (CYP3A4) Metabolizing Capacity
Quinine (CYP3A4)/ Erythromycin (Breath Test: CYP3A4)/Troleandomycin (−CYP3A4)

Cellular/Molecular Mechanism of Action: Quinine can be used as a substrate to assess hepatic CYP3A4 biochemical activity. Troleandomycin functions as a selective cytochrome P450 3A4 inhibitor. Erythromycin is a substrate (probe) for hepatic P450 isoenzyme CYP3A4.

Application: Detection of elevations or declines in the biochemical capacity of hepatic cytochrome P450 type 3A4 in response to xenobiotic substances. The agents can also be utilized to delineate individual variations in the capacity to metabolize pharmaceuticals or toxins.

Route of Administration: Quinine sulfate (oral).

Dosage: Quinine sulfate (600 mg), troleandomycin (TAO; 500 mg every 8 hours).

Implementation: Evaluate the potential of the antimalarial agent quinine to function *in vivo* as a molecular probe for hepatic cytochrome CYP3A4 activity. Plasma and urine samples are collected before the administration of quinine, and then over the next 48-hour period. Detection is achieved with the aid of high-performance liquid chromatography analysis for both quinine and its CYP3A4-generated metabolite, 3-hydroxyquinine. During both phases, the erythromycin breath test (ERBT) is administered at specific times to assess hepatic CYP3A4 activity.

Interpretation and Analysis: Compared with reference controls, troleandomycin significantly decreases the mean time-averaged ERBT result by 77% (95% CI, 68, 85%), the mean apparent

oral clearance of quinine (CL/F) by 45% (95% CI, 39, 52%), and the mean apparent formation clearance of 3-hydroxyquinine (CL3-OH) by 81% (95% CI, 76, 87%). There is apparently no correlation between the troleandomycin-mediated percent decrease in the time-averaged ERBT result and the percent decrease in mean apparent oral clearance of quinine CL/F or apparent formation clearance of 3-hydroxyquinine. When TAO and control treatments are analyzed separately, no significant correlation can be appreciated between the time-averaged ERBT result and CL/F, CL3-OH, or single plasma quinine concentration (12, 24, or 48 hours).

Prolonged plasma clearance of intact quinidine implies compromised cytochrome P450 metabolism. Quinine may be a useful probe for detecting inhibition of liver CYP3A4 activity within an individual. Further studies are needed to determine whether it can provide a quantitative measure of CYP3A4 activity suitable for intersubject comparison.

Instrumentation: High-performance liquid chromatography analyses.

Is quinine a suitable probe to assess the hepatic drug-metabolizing enzyme CYP3A4? Wanwimolruk S, Paine MF, Pusek SN, Watkins PB. Br J Clin Pharmacol 2002 Dec; 54 (6), pp. 643–51.

Hepatic Cytochrome P450 3A4/5 (CYP3A4/5) Metabolization

Dextromethorphan (Substrate)/Rifampicin (+CYP3A4)/Erythromycin (−CYP3A4)

Cellular/Molecular Mechanism of Action: Dextromethorphan is N-demethylated to 3-methoxymorphinan by CYP3A4/5 (human). Rifampicin can function as an inducer of P450 3A4 activity. Alternatively, erythomycin inhibits the biochemical activity of P450 3A4 biochemical activity.

Application: Dextromethorphan is used widely as an *in vivo* probe for phenotyping the polymorphical expression of cytochrome P450 2D6 (CYP2D6) biochemical activity. More recent investigations have concluded that dextromethorphan can be effectively applied as an *in vivo* marker system for the detection and measurement of both CYP3A and CYP2D6 biochemical activity. Dextromethorphan is N-demethylated *in vitro* to 3-methoxymorphinan by human CYP3A4/5.

Dosage: Rifampicin (300 mg b.i.d. for 7 days), erythromycin (250 mg q.i.d. for 7 days).

Implementation: Evaluate the potential for erythromycin to inhibit metabolism of dextromethorphan by cytochrome P450 3A4, and determine the ability of rifampicin to enhance dextromethorphan metabolism through induction of hepatic cytochrome P450 3A4 microsomal enzyme fractions.

Interpretation and Analysis: The urinary excretion of 3-methoxymorphinan is rate limiting when metabolization by CYP2D6 is extensive and necessitates a longer time period for urine collection (0–72 hours) to obtain true values for CYP3A metabolic ratios (3-methoxymorphinan/dextromethorphan). Conversely, urinary excretion of dextromethorphan and 3-methoxymorphinan is delayed when CYP2D6 metabolizing capacity is low but appears to be formation rate limiting. Delayed excretion in conditions of poor metabolizing capacity necessitates longer urine collection intervals (0–11 days) in order to estimate the true CYP3A metabolic ratio, while the necessary collection interval for establishing true CYP2D6 metabolic ratios is slightly shorter (0–8 days). However, a 72-hour collection in poor metabolizers can be used as an index of the true dextromethorphan/3-methoxymorphinan metabolic ratio.

Rifampicin (300 mg b.i.d. for 7 days) significantly reduces the 0-to-72-hour dextromethorphan/3-methoxymorphinan metabolic ratio consistent with an 830% (± 1,808%) induction of CYP3A activity (n = 8). Conversely, erythromycin significantly increases the dextromethorphan/3-methoxymorphinan metabolic ratio, corresponding to a 34% ± 44% inhibition of activity, respectively, in conditions where extensive CYP3A metabolization exists and in situations of low-level degradation. The changes in CYP3A activity are independent of CYP2D6 phenotype and can be observed after 24- and 48-hour urine collections in conditions of either extensive or suboptimal metabolization. In addition, metabolic ratios reflecting CYP2D6 and CYP3A do not significantly correlate with 1 another.

Determination of cytochrome P450 3A4/5 activity *in vivo* with dextromethorphan N-demethylation. Jones DR, Gorski JC, Haehner BD, O'Mara EM Jr, Hall SD. Clin Pharmacol Ther 1996 Oct; 60 (4), pp. 374–84.

Hepatic Cytochrome P450 (CYP3A4) Metabolization Capacity

Contraceptives (Male)/Potential Application of Dextromethorphan (Substrate), Rifampicin (+CYP3A4), and Erythromycin (−CYP3A4)/T ethanate

Cellular/Molecular Mechanism of Action: The CYP3A4 gene encodes for a major hepatic T-inactivating enzyme.

Background Information: Clinical trials of hormonal male contraceptive regimens have identified a consistent population polymorphism in susceptibility to hormone-induced azoospermia. Using identical hormonal regimens, fewer men of European origin (approximately two-thirds) become azoospermic compared with Asian men, who virtually all become azoospermic. This variation within and between populations remains unexplained but has been spectulated to be due to a pharmacogenetic difference in androgen metabolism.

Application: Delineate the possible existence of pharmacogenetic differences in androgen metabolism or action that may correlate with variable susceptibility to hormonal-induced azoospermia.

Considerations: Citation represents an example of an experimental approach to investigating the pharmacogenetic properties associated with a given biological observation.

Implementation: Evaluation of single nucleotide polymorphism in the CYP3A4 gene (encodes the major hepatic T-inactivating enzyme), and CAG and GGC triplet repeats in the AR gene have been characterized to delineate the pharmacogenetic differences in androgen metabolism in human males (e.g., n = 75 receiving hormonal contraceptives; and n = 106 negative reference controls). Identification of A/G variants in the promoter region of the CYP3A4 gene can be delineated applying a mutagenically differentiated polymerase chain reaction (PCR) methodology. Fluorescent-labeled DNA fragments containing either CAG or GGC repeats are amplified from genomic DNA, and their sizes determined based on capillary electrophoresis analysis.

Interpretation and Analysis: Human subjects can be classified into groups according to whether 6 months of weekly T enanthate injections produce azoospermia (n = 54), near-azoospermia (n = 7), and non-azoospermia (n = 14). The G allele of CYP3A4 gene is absent from the non-azoospermia and near-azoospermia groups, but overall this single nucleotide polymorphism distribution does not differ significantly between azoospermia males and reference contol populations. There is no significant difference in distribution of CAG and GGC triplet repeats among 3 groups or between them and population controls based on the maximum likelihood estimate of odds ratio and CLUMP II analyses. Therefore, neither genetic polymorphisms in the AR gene (CAG and GGC repeats) nor that in hepatic forms of the gene appear to be influenced.

Pharmacogenetic polymorphisms of the AR and metabolism and susceptibility to hormone-induced azoospermia. Yu B, Handelsman DJ. J Clin Endocrinol Metab 2001 Sep; 86 (9), pp. 4406–11.

Hepatic Cytochrome P450 3A4 (CYP3A4) Metabolizing Capacity
Steroids

Cellular/Molecular Mechanism of Action: The enzyme CYP3A4 has an important role in endogenous processes, most notably steroid catabolism.

Background Information: The enzyme CYP3A4 is the most abundant cytochrome P450 in human liver, comprising approximately 30% of the total liver P450 content and plays a fundamental role in the metabolism of more than half of the clinically used drugs currently prescribed. Majority of the substrates for CYP3A are also capable of upregulating CYP3A activity, mainly through transcriptional activation. The molecular mechanisms that underlie the transcriptional activation of CYP3A4 are complex, involving many steroid hormone nuclear receptors, including GR, PXR, VDR and CAR, which play a role in these mechanisms. However, the net result of transcriptional activation is an increase in the metabolism of the inducing compounds and, therefore, increased clearance. An important side effect of this transcriptional activation is that coadministered chemicals metabolized by CYP3A may also have their pharmacokinetics altered. Such changes can result in reduced clinical efficacy of drugs, resulting in poor patient response, or the development of adverse drug responses.

Evaluation of the toxicological relevance of CYP3A4 induction. Plant NJ, Gibson GG. Curr Opin Drug Discov Devel 2003 Jan; 6 (1), pp. 50–6.

Hepatic vs Intestinal Cytochrome P450 3A (CYP3A) Metabolizing Capacity/Pharmaceutical Metabolizing Capacity
Midazolam/Ketoconazole

Cellular/Molecular Mechanism of Action: Midazolam is a substrate for cytochrome isotype CYP3A while ketoconazole function as an inhibitor of CYP3A activity.

Application: Determine the intestinal and hepatic contribution to the bioavailability of midazolam with use of the CYP3A inhibitor ketoconazole.

Route of Administration: Midazolam (intravenous and oral) and ketoconazole (oral).

Dosage: Midazolam (2 mg IV and 6 mg oral) and ketoconazole (200 mg).

Considerations: The cytochrome P450 3A (CYP3A) isoforms are responsible for the metabolism of a majority of therapeutic compounds, and they are abundant in the intestine and liver. CYP3A activity is highly variable, causing difficulty in the use of therapeutics that are substrates for P450 3A enzyme systems.

Implementation: Midazolam can be employed as a practical *in vivo* probe for characterizing both intestinal and hepatic CYP3A activity. Previous reports have described the calculation of midazolam pharmacokinetic profiles utilizing single doses of 2 mg (IV) and 6 mg (oral).

Interpretation and Analysis: Area-under-the-concentration-vs-time-curve of midazolam increases 5-fold after intravenous midazolam administration ($p \leq 0.001$) and 16-fold after oral midazolam administration ($p \leq 0.001$). Comparison of the midazolam pharmacokinetic profile for this administration scheme compared to a schedule that also includes 3 doses of 200 mg oral ketoconazole results in decreases in intrinsic clearance (84%, $p = 0.003$) and increases in total bioavailability from 25% to 80% ($p < 0.001$). The intestinal component of midazolam bioavailability increases to a greater extent than the hepatic component (2.3-fold [$p = 0.003$] and 1.5-fold [$p \leq 0.001$], respectively). In reference controls, females have greater midazolam clearance values than male subjects. In summary, ketoconazole causes a marked inhibition of CYP3A activity that is greater in the intestine than the liver. Administration of single doses of oral and intravenous midazolam with and without oral ketoconazole exemplifies a practical method for differentiating intestinal and hepatic CYP3A activity.

Differentiation of intestinal and hepatic cytochrome P450 3A activity with use of midazolam as an *in vivo* probe: Effect of ketoconazole. Tsunoda SM, Velez RL, von Moltke LL, Greenblatt DJ. Clin Pharmacol Ther 1999 Nov; 66 (5), pp. 461–71.

HEPATIC CYTOCHROME P450 4 MICROSOMAL ENZYMES

Cytochrome P450 4B1 (CYP4B1)/Cytotoxicity Induction/Herpes Simplex Virus Type I Thymidine Kinase/Ganciclovir/Pharmacogene Therapy Systems: Bystander Effect/Biosafety Enhancers: Gene Marking or Replacement Studies/Biosafety Enhancers: Lymphocyte Infusions Postallogeneic Marrow Transplant
4-Ipomeanol (4-IM)/2-Aminoanthracene (2-AA)

Application: Determination of the time course of cytotoxicity induction and the bystander effect for (rabbit) cytochrome CYP4B1/4-IM or 2-AA pharmacogene therapy systems. A weak bystander effect together with a fast induction of cytotoxicity may provide marked advantages for the use of cytochrome CYP4B1 systems as biosafety enhancers for gene marking or replacement studies and donor lymphocyte infusions after allogeneic bone marrow transplantation.

Implementation: The time course of cytotoxicity induction and the bystander effect of the rabbit cytochrome CYP4B1/4-IM or 2-AA pharmacogene therapy systems has been characterized for comparison with the herpes simplex virus type 1 thymidine kinase/ganciclovir (HSV-tk/GCV) system.

Cellular Model: Rat 9L gliosarcoma cells stably expressing CYP4B1 (9L-4B1), HSV-tk (9L-tk), or their enhanced green fluorescent protein (EGFP) fusion genes.

Interpretation and Analysis: CYP4B1-mediated activation of 2-AA shows a high cell killing efficiency during only a 48-hour window and with an onset after only 15 minutes of prodrug exposure. Residual 9L-4B1 cells are mostly damaged sublethally upon 2-AA treatment, showing an S phase arrest by cell cycle analysis. 4-ipomeanol treatment of 9L-4B1 cells generates an overall weaker cell killing, especially after prodrug exposure periods of < 48 hours. Residual cells surviving 4-IM treatment show a G2/M arrest and restarted proliferation after prodrug treatment is stopped. HSV-tk/GCV pharmacogene therapy results in a slower cytotoxicity induction than CYP4B1/2-AA treatment with a significantly lower cell killing efficiency after 24 and 48 hours. HSV-tk/GCV-mediated cytotoxicity is similar to the cytotoxicity induced by CYP4B1/4-IM with the exception of a continuous 48-hour prodrug exposure where 4-IM treatment shows a significantly higher cell killing rate. Cells surviving HSV-tk/GCV suicide gene therapy are not viable and show an S-phase arrest. Whereas HSV-tk/GCV induce a strong bystander effect, only moderate bystander cell death depending on cell-to-cell contact is demonstrated in 9L/9L-4B1 co-cultures upon 2-AA treatment and is even absent with 4-IM, in contrast to previously

published reports. The absence of a strong bystander effect may limit the overall utility of the CYP4B1 systems for cancer gene therapy. Alternatively, a weak bystander effect together with the fast induction of cytotoxicity may provide marked advantages for the use of the CYP4B1 systems as biosafety enhancers for gene marking or replacement studies and donor lymphocyte infusions after allogeneic bone marrow transplantation.

Differential cytotoxicity and bystander effect of the rabbit cytochrome P450 4B1 enzyme gene by 2 different prodrugs: Implications for pharmacogene therapy. Frank S, Steffens S, Fischer U, Tlolko A, Rainov NG, Kramm CM. Cancer Gene Ther 2002 Feb; 9 (2), pp. 178–88.

Hepatic Cytochrome P450 4B1 (CYP4B1)/ Polymorphic Screening Aberrant Activating Enzyme Systems
4-Ipomeanol (4-IM): Antineoplastic Prodrug

Cellular/Molecular Mechanism of Action: 4-ipomeanol functions as a DNA alkylating agent once it has been converted to its active form by rabbit hepatic cytochrome P450 4B1 enzyme systems.
Physiological Mechanism of Action: 4-ipomeanol induces the development of glioblastoma in rabbits after it has been converted from a prodrug form to its biologically active metabolite.
Background Information: Gene therapy using vector-mediated transfer of prodrug activating genes is a promising treatment approach for malignant tumors. The novel prodrug activating gene coding for rabbit cytochrome CYP4B1 is able to induce tumor cell death at low micromolar concentrations in glioblastoma cells after *in vitro* and *in vivo* treatment with the prodrug 4-IM. The (rabbit) cytochrome P450 system, CYP4B1, converts this prodrug and other furane analogs and aromatic amines, such as 2-aminoanthracene, into highly toxic alkylating metabolites, whereas the human isoenzyme exhibits only minimal enzymatic activity.
Application: The prodrug 4-IM can potentially be applied in combination with appropriate enzyme systems capable of activating this therapeutic agent to its biologically active form. Pharmacogenetic screening could be employed to identify cases that idiosyncratically express aberrant enzyme systems capable of activating 4-IM in a manner that exerts cytotoxic properties against normal/health tissues and organ systems.
Dosage: 4-ipomeanol (0.5 to 2.0 μg/ml).
Implementation: Sequences of cDNA encoding for cytochrome P450 4B1 (rabbit CYP4B1) can be used for pharmacogene therapy of hepatocellular carcinoma (HCC). Cell clones derived from the human HCC cell lines Hep3B, HuH-7, and HepG2 that stably express the chimeric protein CYP4B1-EGFP (CYP4B1 coding sequence fused to the enhanced green fluorescent protein [EGFP] gene) are then identified for application in prodrug activation investigations. Appropriate reference controls can include HCC clones that express EGFP.
Interpretation and Analysis: The prodrug 4-IM rapidly induces tumor cell death in CYP4B1-EGFP-expressing clones at low concentrations (a 50% lethal dose of between 0.5 and 2 μg/mL). No signs of toxicity can be appreciated in control cells expressing EGFP even at high prodrug concentrations (20 μg/mL). Cell death occurs by apoptosis and independent of functional p53. A pronounced direct bystander effect can be observed in Hep3B cells, whereas bystander HepG2 and HuH-7 cells are highly resistant to toxic 4-IM metabolites. The CYP4B1/4-IM system efficiently and rapidly induces cell death in HCC cells, and a cell line-specific mechanism may exist that limits the extent of the bystander effect of this novel prodrug activating system.

Rabbit cytochrome P450 4B1: A novel prodrug activating gene for pharmacogene therapy of hepatocellular carcinoma. Mohr L, Rainov NG, Mohr UG, Wands JR. Cancer Gene Ther 2000 Jul; 7 (7), pp. 1008–14.

MICROSOMAL FLAVIN-BASED METABOLIZING SYSTEMS

Hepatic Flavin-Containing Monooxygenase Biochemical Activity
Benzydamine-N-Oxide/N-Benzylimidazole (CYP Inhibitor)/Methimazole

Cellular/Molecular Mechanism of Action: Benzydamine metabolized by hepatic flavin-containing monooxygenases (FMO) to an N-oxide metabolite. N-benzylimidazole is a non-specific CYP inhibitor, while methimazole is a competitive flavin monooxygenase inhibitor.
Application: Evaluate the influence of FMO on the disposition of xenobiotics.
Route of Administration: In preliminary investigations, benzydamine has been incubated with human liver microsomes and recombinant enzymes to evaluate the metabolism schemes that this pharmaceutical is vulnerable to in an effort to determine its suitability as a potential *in vivo* probe for determining hepatic flavin monooxygenases metabolic capacity.
Dosage: Not applicable.
Interpretation and Analysis: Benzydamine N-oxide formation rates in liver microsomes follow Michaelis-Menten kinetic patterns (mean Km = 64.0 μM, mean Vmax = 6.9 nmol/mg protein/min; n = 35). The non-specific CYP inhibitor, N-benzylimidazole, and selective inhibitors of various CYP isoforms do not affect benzydamine N-oxidation. Alternatively, formation of the N-oxide metabolite of benzydamine is almost abolished by heat treatment of microsomes in the absence of NADPH and is strongly inhibited by methimazole (competitive flavin monooxygenase inhibitor). Recombinant FMO3 and FMO1 (not expressed in human liver), but not FMO5, possess an ability to metabolize benzydamine to its N-oxidase metabolite. Respective Km values for FMO3 and FMO1 are 40.4 μM and 23.6 μM, and respective Vmax values for FMO3 and FMO1 are 29.1 and 40.8 nmol/mg protein/min. Based on the interpretation of findings from preliminary investigations, benzydamine could be used as a model substrate for human liver FMO3 activity *in vitro* and may be further developed as an *in vivo* probe reflecting FMO3 activity.
Instrumentation: Human liver samples can be genotyped using polymerase chain reaction (PCR)-restriction fragment length polymorphism methodologies.

Benzydamine N-oxidation as an index reaction reflecting FMO activity in human liver microsomes and impact of FMO3 polymorphisms on enzyme activity. Störmer E, Roots I, Brockmöller J. Br J Clin Pharmacol 2000 Dec; 50 (6), pp. 553–61.

Hepatic Flavin Monooxygenase Metabolizing Capacity
Benzydamine

Cellular/Molecular Mechanism of Action: Benzydamine is a substrate for flavin-containing monooxygenase isoenzymes FMO1 and FMO3.

Application: Detection and characterization of hepatic FMO metabolizing capacity.
Implementation: Determination of the FMO and P450 isoform selectivity for metabolism of benzydamine and caffeine in order to determine their individual potential to function as *in vivo* probes for flavin-containing monooxygenase isoenzyme fractions (e.g., human FMO3).
Route of Administration: Benzydamine.
Laboratory Methodology: Preliminary investigations have performed metabolic incubations at a physiological pH using substrate concentrations of 0.01 to 10 mM with either recombinant human flavin monooxydases, cytochrome P450 fractions, or human liver microsomes serving as sources of biochemical enzymes.
Considerations: Speculation suggests that it is likely that the metabolic capacity of hepatic FMO3 is a much greater contributor to plasma levels of the N-oxide metabolite *in vivo* than is the FMO1 extrahepatic isoenzyme.
Examples of Research Implementation: Caffeine is metabolized by CYP1A2 but not by any of the human flavin-containing monooxydases. In contrast, benzydamine can function as a substrate for human flavin-containing monooxygenases (isotypes FMO1, FMO3, FMO4, and FMO5). Apparent Km values for benzydamine N-oxygenation are 60 ± 8 μM, 80 ± 8 μM, > 3 mM, and > 2 mM, for FMO1, FMO3, FMO4, and FMO5, respectively. Corresponding Vmax values are 46 ± 2 min^{-1}, 36 ± 2 min^{-1}, < 75 min^{-1}, and < 1 min^{-1}. Small quantities of benzydamine N-oxide are also formed by CYPs 1A1, 1A2, 2C19, 2D6, and 3A4 isoenzymes.
Interpretation and Analysis: The rate and extent of conversion to metabolites is directly proportional to the concentration and biochemical capacity associated with hepatic cytochrome protein fractions. The rate and extent of caffeine metablism correlates to CYPA2 activity in contrast to benzydamine metabolism facilitated through 4 different flavin-containing monooxygenases (isotypes FMO1, FMO3, FMO4, and FMO5).

Flavin-containing monooxygenase isoenzymes FMO1 and FMO3 catalyse benzydamine N-oxygenation with the highest level of efficiency. However, it is likely that the metabolic capacity of hepatic FMO3 is a much greater contributor to plasma levels of the N-oxide metabolite *in vivo* than is extrahepatic FMO1. Therefore, benzydamine, but not caffeine, is a potential *in vivo* probe for human FMO3.
Instrumentation: Products of caffeine and benzydamine metabolism are analyzed by reversed-phase high-performance liquid chromatography in concert with UV and fluorescence detection systems.

In vitro evaluation of potential *in vivo* probes for human flavin-containing monooxygenase (FMO): Metabolism of benzydamine and caffeine by FMO and P450 isoforms. Lang DH, Rettie AE. Br J Clin Pharmacol 2000 Oct; 50 (4), pp. 311–4.

Hepatic Flavin Monooxygenase Metabolizing Capacity (FM01, FM02, FM03, FM05)/ Cytochrome P450 Hepatic Metabolizing Capacity *Ranitidine/Metimazol/Tranylcypromine (−CYP2C19)/a-Naphthoflavon (−CYP1A2)/Quinidine (−CYP2D6)*

Cellular/Molecular Mechanism of Action: Ranitidine is a substrate for hepatic microsomal oxidation to N-oxide (66–76%), S-oxide (13–18%), and desmethylranitidine (12–16%) metabolites. Metimazol is an inhibitor of flavin-containing monooxygenase. Alternatively, tranylcypromine, α-naphthoflavon, and quinidine inhibit P450 2C19, P450 1A2, and P450 2D6, respectively.
Application: Urinary contents of ranitidine N-oxide can be used as an *in vivo* probe to detect and measure hepatic FMO3 activity.
Examples of Research Implementation: The N-oxidation and S-oxidation of ranitidine is inhibited by metimazol (flavin-containing monooxygenase [FMO] inhibitor) 96–97% and 71–85%, respectively. Desmethylation of ranitidine is inhibited 71–95% by SKF525A (cytochrome P450 [CYP] inhibitor). Recombinant FMO isozymes FMO1, FMO2, FMO3 and FMO5 produce 39, 79, 2180, and 4 (pmol/min/nmol FMO) 4 ranitinine N-oxide, compared to 45, 0, 580, and 280 (pmol/min/nmol FMO) ranitinine S-oxide, respectively. Desmethyranitinine is not produced by recombinant FMOs. Production of desmethylranitidine by rat and human liver microsomes is inhibited by tranylcypromine, α-naphthoflavon and quinidine, which are known to inhibit CYP2C19, 1A2 and 2D6, repectively. Ranitidine N-oxide and S-oxide are produced at a 4-to-1 ratio by isoenzyme FM03 (major form in adult liver). Conversely, FM01 (major renal isoenzyme) is 55-fold and 13-fold less efficient than hepatic FM03 in producing ranitidine N-oxides and S-oxides respectively. Isoenzymes FMO2 and FMO5, although expressed slightly in human liver, kidney, and lung, are not efficient producers of ranitidine N- and S-oxides.
Interpretation and Analysis: Increases in the extent and rate that ranitidine is converted biochemically to ranitidine N-oxide directly corresponds to hepatic FM03 metabolic activity.

Oxidation of ranitidine by isozymes of flavin-containing monooxygenase and cytochrome P450. Chung WG, Park CS, Roh HK, Lee WK, Cha YN. Jpn J Pharmacol 2000 Oct; 84 (2), pp. 213–20.

MICROSOMAL MIXED FUNCTION OXIDASES

Hepatic Mixed Function Oxidases/Depression (Individualization of Dosage Regimens) *Amitriptyline/Hamilton Score/Multiple Points Method/Modified Bayesian Method*

Background Information: Individualization of an amitriptyline dose regimen offers substantial advantages over non-individualized treatments.
Application: Individualization of amitriptyline dose regimens for the purpose of maximizing therapeutic effect and minimizing adverse side effects.
Implementation: In patients with disorders of major depression and receiving amitriptyline therapy, the assessment of clinical effects, adverse effects, and plasma steady state concentrations can be applied to evaluate cases within the following 3 categories: (i) patients in Group A taking non-individualized doses of amitriptyline; (ii) patients in Group B taking doses of amitriptyline individualized by a modified Bayesian method; and (iii) patients in Group C taking doses of amitriptyline individualized by a multiple point method.
Interpretation and Analysis: Individuals within Group A take significantly higher doses throughout the treatment course; the initial doses for cases in Group B are higher than doses for the patients in Group C, but after corrections based on measured steady state plasma concentrations they can be found to be similar.

While the Hamilton score descends uniformly in all 3 groups, both adverse effects and steady state plasma concentrations of amitriptyline are higher in the non-individualized group during the whole treatment course. The multiple points method is considered to be the most precise technique, but it is a tedious and impractical technique. The modified Bayesian method with corrections based on 1st measured plasma steady state concentrations of amitriptyline

offers similar therapeutic outcome and adverse effects scores combined with low cost and convenient and an easy-to-use protocol.

Comparison of 2 approaches to amitriptyline dose individualisation. Jankovic SM, Timotijevic I, Mihajlovic GS, Dukic-Dejanovic S. Eur J Drug Metab Pharmacokinet 1999 Apr–Jun; 24 (2), pp. 163–8.

Mixed Function Oxidases/Fescue Endophyte Ingestion (Sheep)/Mental Depression
Cimetidine/Antipyrine

Cellular/Molecular Mechanism of Action: Cimetidine competitively inhibits binding of histamine to gastric H2 receptor complexes and may also influence the activity of hepatic mixed function oxidases. Antipyrine is believed to be metabolized through hepatic cytochrome P450 activity.
Application: The combined application of antipyrine and cimetidine can be used to delineate how a paticular toxin or pharmaceutical is metabolized or to detect abnormalities in hepatic function/integrity.
Route of Administration: Cimetidine (intravenous).
Dosage: Cimetidine (800 mg).
Implementation: Evaluation of the influence of endophyte-infected fescue (Kentucky 31) hay and cimetidine on respiration rates, rectal temperatures, and hepatic antipyrine uptake. Hepatic antipyrine uptake can be measured as an indirect indicator of hepatic mixed-function oxidase (MFO) activity.

Animal Model: Ovine (n = 3 mature ewe sheep exposed to endophyte-infected fescue [Kentucky 31]). Experimental Design: Ewes during a period of consuming endophyte-free (one exp/ewe, control), endophyte-infected (2 exp/ewe, EIF), and endophyte-infected fescue hay plus cimetidine (2 exp/ewe, EIFC).
Interpretation and Analysis: Respiration rates increase ($p < 0.05$) 2.6-fold after 11 days on EIF from 26 to 68 and decrease ($p < 0.025$) to 27 breaths/minute after 4 days of cimetidine treatment. Rectal temperatures increase ($p < 0.05$) 1.1°C after 8 days on EIF. Cimetidine lowers temperatures to control levels by day 4. Endophyte-infected fescue hay increases ($p < 0.05$) hepatic antipyrine uptake 70% after 11 days, but uptake is lowered to control levels by cimetidine by day 4. Increased respiration rates and rectal temperatures in animals consuming EIF seem to be related to increased MFO activity. Cimetidine reversed some of these effects.

Effects of endophyte-infected fescue and cimetidine on respiration rates, rectal temperatures and hepatic mixed function oxidase activity as measured by hepatic antipyrine metabolism in sheep. Zanzalari KP, Heitmann RN, McLaren JB, Fribourg HA. J Anim Sci 1989 Dec; 67 (12), pp. 3370–8.

PHASE II METABOLIZING ENZYME SYSTEMS

CONJUGATION SYSTEMS

Glutathione-S-Transferases (GST): Alzheimer's Therapy/Glutathione Conjugation: Isoenzymes GST M1 and GST T1/Prediction of Hepatotoxicity Susceptibility: Alzheimer's Therapy
Tacrine Reactive Metabolites

Background Information: Glutathione conjugation of tacrine reactive metabolites depends in part on the activity of GST, of which 2 isozymes (GST M1 and GST T1) are polymorphically expressed.
Application: Detection of combined alleles M1 and T1 deficiencies in glutathione-S-transferase genes serve as predictive paramaters related to an increased susceptibility to tacrine hepatotoxicity relevant to the therapeutic management of mild and moderate cases of Alzheimer's disease.
Implementation: Determination of the potential association between the parameters of (i) GST M1; (ii) GST T1; and (iii) the combined GST M1 and GST T1 null genotypes and the prediction of individual susceptibility to tacrine hepatotoxicity.
Interpretation and Analysis: In cases of mild to moderate Alzheimer's disease on tacrine therapy, some cases display elevations in alanine aminotransferase levels (e.g., n = 52/141) at least 3 times the upper limit of normal. The remaining subpopulation of mild to moderate Alzheimer's disease cases (e.g., n = 89/141) have normal alanine aminopeptidase values (≤ upper limit of normal).

Approximately 20% of such cases (e.g., 28/141 [95% CI]) are GST T1-negative while 48% (e.g., 68/141 (95% CI)] are GST M1-negative. The combined GST M1-T1 null genotype can be observed in approximately 13% of mild to moderate severity Alzheimer's disease cases (e.g., n = 18 [95% CI]) and large percentage of this subpopulation (e.g., n = 13/18 or 72%) display elevated plasma ALT levels that are at least 3 times the upper limit of normal. Although the cumulative percentage of elevated plasma ALT tends to be higher in the GST M1 null genotype, neither GST M1 nor GST T1 alone can predict individual susceptibility to tacrine hepatotoxicity. Multivariate Cox hazards model show that the association of the GST M1-T1 null genotype is an independent risk factor of hepatotoxicity. Presence of combined alleles M1 and T1 deficiencies in glutathione-S-transferase genes therefore increases susceptibility to tacrine hepatotoxicity.

Combined glutathione-S-transferase M1 and T1 genetic polymorphism and tacrine hepatotoxicity. Simon T, Becquemont L, Mary-Krause M, de Waziers I, Beaune P, Funck-Brentano C, Jaillon P. Clin Pharmacol Ther 2000 Apr; 67 (4), pp. 432–7.

Methylation/Catechol O-Methyltransferase (COMT)/ Genetically Regulated Thermal Stability/(Hepatic Biopsy Specimens)
Adrenergic Neurotransmitters

Cellular/Molecular Mechanism of Action: Catechol O-methyltransferase activity mediates the biochemical inactivation of epinephrine and norepinephrine neurotransmitters.
Application: Detection of COMT genotypes and characterization of COMT genetic polymorphism.
Considerations: The level of COMT activity and COMT thermal stability in human tissue is controlled by a common genetic polymorphism.
Implementation: Individual hepatic biopsies are collected for analysis (e.g., from individuals with high, low, or intermediate COMT biochemical activity and thermal stabilities) to determine if the molecular mass (M_r) and/or isoelectric point (pI) of COMT enzymes differs in tissue from subjects with different presumed genotypes for the COMT genetic polymorphism. Laboratory techniques employed entail the partial purification of COMT from individual hepatic tissue samples by (i) sequential ion exchange and gel filtration chromatography; (ii) photoaffinity labeling performed with [^{3}H-methyl]-S-adenosyl-L-methionine (functions as the methyl donor for the COMT enzymatic reactions); and (iii) 2-dimensional sodium dodecylsulfate polyacrylamide gel electrophoresis (2-D SDS-PAGE) analysis.
Interpretation and Analysis: Two-dimensional sodium dodecylsulfate polyacrylamide gel electrophoresis (2-D SDS-PAGE) analysis of individual samples consistently shows the presence of 3 [^{3}H-methyl]-S-adenosyl-L-methionine photoaffinity-labeled proteins

with pI values of 5.4, 5.5, and 5.7 (all 3 have M_r values of approximately 27.1 kDa). The same pattern can be observed in all samples irrespective of COMT phenotype. Western blot analysis of 2-D SDS-PAGE gels utilizing rabbit polyclonal antibodies to partially purified human kidney COMT reveals a pattern similar to that found with photoaffinity labeling. Once again, the same pattern is found in all samples irrespective of COMT phenotype. Therefore, neither photoaffinity labeling nor Western blot analysis reveal differences in either M_r or pI of cytoplasmic COMT in hepatic tissue from subjects selected on the basis of different phenotypic expression of the COMT genetic polymorphism.

Catechol O-methyltransferase pharmacogenetics: Photoaffinity labeling and Western blot analysis of human liver samples. Aksoy S, Klener J, Weinshilboum RM. Pharmacogenetics 1993 Apr; 3 (2), pp. 116–22.

Methylation/Catechol-O-Methyltransferase (COMT)/Pharmacogenetic Polymorphism: Lack of Relevance
Lithium

Application: Lithium is frequently used for the modification of mood disorders. However, the response outcome to lithium therapy does not appear to be a function of the genetic variation in the expression of COMT G158A, monoamine oxydase A (MAO-A) 30-bp repeat, G-protein beta 3-subunit (Gbeta3) C825T polymorphism.
Implementation: Delineation of the possible relationship between the prophylactic efficacy of lithium administration and genetic variations in the expression of COMT G158A, monoamine oxydase A (MAO-A) 30-bp repeat, and G-protein beta 3-subunit (Gbeta3) C825T. Polymorphism can be detected through the application of polymerase chain reaction (PCR) methodologies.
Interpretation and Analysis: In cases of psychological disorders (e.g., n = 201; bipolar n = 160/201; major depression n = 41/201) monitored prospectively for an average of 59.8 months reveals that COMT, MAO-A, and Gbeta3 variants are not associated with lithium outcome. Similarly, analysis of stratification effects such as sex, polarity, age at onset, duration of lithium treatment, and previous episodes does not reveal the existence of any relationship between lithium response and expression of genetic variants.

Pharmacogenetics of lithium prophylaxis in mood disorders: Analysis of COMT, MAO-A, and Gbeta3 variants. Serretti A, Lorenzi C, Lilli R, Mandelli L, Pirovano A, Smeraldi E. Am J Med Genet 2002 May 8; 114 (4), pp 370–9.

Methylation/Trans-Methylation, Trans-Sulfuration, Remethylation/Hyperhomocysteinemia: Endstage Renal Failure/Single-Carbon Group Transfer (5,10-Methylenetetrahydrofolate)/Hepatic Cytosol and Mitochondria
Carbon Dioxide Breath Tests/[$^2H_3$C-1(-13)C]-Methionine/[2,3,3-$^2H_3$] Serine

Cellular/Molecular Mechanism of Action: Substrates that can be applied as "probes" to detect alterations in the biochemical activity of specific enzyme systems involved in metabolic pathways.
Application: Estimation of trans-methylation, trans-sulfuration, and remethylation rates, and evaluation of carbon group transfer.
Route of Administration: Intravenous.
Implementation: Evaluation of whole-body trans-methylation, trans-sulfuration, and remethylation rates (e.g., [$^2H_3$C-1(-13)C]-methionine]. Following administration, carbon radioisotope contained in exhaled air as carbon dioxide is then measured. Preparations of [2,3,3-$^2H_3$] serine can be applied to evaluate the transfer of a 1-carbon group from serine via 5,10-methylenetetrahydrofolate in human hepatocyte cytosol and mitochondria, respectively.
Animal Model: Ovine.
Interpretation and Analysis: The amount of carbon radioisotope contained in exhaled air is directly proportional to the rate the test molecular is metabolized *in vivo* following injection. Tissue-free methionine enrichments after infusion of universally labeled [U-^{13}C] methionine show the highest remethylation activity in postmortem investigation of jejunum, liver, and kidney tissue samples, but no such activity can be appreciated in muscle and brain preparations.
Instrumentation: Methods to quantitate 1-carbon acceptor metabolism pathways and folate metabolism have recently become available.

In vivo stable isotope measurements of methyl metabolism: Applications in pathophysiology and interventions. de Meer K, van den Akker JT, Smulders Y, Stam F, Stehouwer CD, Finglas P. Food Nutr Bull 2002 Sep; 23 (3 Suppl), pp. 113–9.

Methylation/5,10-Methylenetetrahydrofolate Reductase (MTHFR) Gene (C677) Polymorphism/TT Genotype
Methotrexate (MTX)

Background Information: About 10% to 12% of the human population carries the MTHFR TT genotype (enzyme activity, 30% of wild type [CC]).
Application: Detection of the influence of MTHFR gene (C677) polymorphism on the response to MTX.
Implementation: Marrow allografts and short-course MTX administration regimens in cases suffering from chronic myelogenous leukemia (e.g., n = 220). Measures of MTX toxicity include the oral mucositis index (OMI), speed of engraftment (platelet and granulocyte counts), and bilirubin.
Interpretation and Analysis: Cases with lower MTHFR activity (TT genotype) have a 36% higher mean OMI during days 1–18 (+5.7, p = 0.046) and 20% higher OMI between days 6 and 12 (+3.8, p = 0.27). Platelet counts recover more slowly among patients with the TT genotype compared to wild type (24% slower recovery to 10,000 platelets/μL, p = 0.23; 34% slower to 20,000/μL, p = 0.08). Patients with decreased MTHFR activity appear at risk of higher MTX toxicity. Because of the high prevalence of the TT genotype, these results may have implications for guiding the design of MTX administration regimens.

Pharmacogenetics of methotrexate: Toxicity among marrow transplantation patients varies with the methylenetetrahydrofolate reductase C677T polymorphism. Ulrich CM, Yasui Y, Storb R, Schubert MM, Wagner JL, Bigler J, Ariail KS, Keener CL, Li S, Liu H, Farin FM, Potter JD. Blood 2001 Jul 1; 98 (1), pp. 231–4.

Methylation/5,10-Methylenetetrahydrofolate Reductase (MTHFR) Gene (C677) Polymorphism/TT/CT/CC Genotypes
Methotrexate (MTX)

Cellular/Molecular Mechanism of Action: Methylenetetrahydrofolate reductase C677T catalyzes reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate.

Background Information: Methylenetetrahydrofolate reductase C677T polymorphism is a common mutation of the gene encoding the enzyme that catalyzes reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a carbon donor in the metabolism of folate. Mutations of this type evoke a striking reduction in enzyme activity in carriers that have a homozygous mutation.
Application: Identification of cases with MTHFR C677T polymorphism that closely correlate with an increased risk of developing methotrexate toxicity. The TT genotype may indicate a need to reduce the dose of MTX during prolonged administration. Considering the high prevalence of homozygous individuals in the Italian population, pretreatment screening may be worthwhile.
Implementation: Characterization of the vulnerability to MTX intoxication (genotype) as a function of MTHFR C677T polymorphism in cases of acute leukemia undergoing maintenance chemotherapy. Toxicity to MTX is evaluated through assessement of bone marrow, liver, and mucosa integrity and function (e.g., n = 61 cases).
Interpretation and Analysis: The incidence of C677T mutation in the general Italian population corresponds to (i) 23.08% TT cases; (ii) 38.46% CT cases; and (iii) 38.46% of CC cases. The TT genotype is significantly associated with an increase of toxicity during periods of MTX administration. No specific pattern of toxicity can be appreciated; however, myelosuppression and liver toxicity are noticeably more pronounced with the TT genotype.

Preponderance of methylenetetrahydrofolate reductase C677T homozygosity among leukemia patients intolerant to methotrexate. Chiusolo P, Reddiconto G, Casorelli I, Laurenti L, Sora F, Mele L, Annino L, Leone G, Sica S. Ann Oncol 2002 Dec; 13 (12), pp. 1915–8.

Methylation/5,10-Methylenetetrahydrofolate Reductase Gene (C677) Polymorphism/G Allele (TT vs TG/GG Genotypes)/Rheumatoid Arthritis: Severe vs Mild-Moderate (Prediction of Disease Severity)/Tumor Necrosis Factor-Alpha I (TNF-α) Receptor Type II (TNFRII): Exon 6 Polymorphism
Methotrexate (MTX)/Anti-TNF-α Response

Application: Characterizing severe vs mild-moderate conditions of rheumatoid arthritis as a function of exon 6 TNFRII gene polymorphism. The G allele appears to have utility in predicting the degree of responsiveness to anti-TNF-α treatment while the GG genotype for the exon 6 TNFRII polymorphism may be useful in predicting the more aggressive form of rheumatoid arthritis.
Implementation: Characterization of exon 6 TNFRII gene polymorphism in cases of (i) mild-moderate synovitis classified as responders to MTX therapy (e.g., n = 97) for comparison with (ii) "non-responder" cases receiving anti-TNF-α treatment due to severe and aggressive disease (e.g., n = 78); and (iii) cases with severe rheumatoid arthritis monitored for 6 months during anti-TNF-α therapy. Results are evaluated pertaining to response according to genotype and compared to matched age, sex, and ethnic reference controls (e.g., n = 84).
Interpretation and Analysis: A distinct trend exists toward increases in the frequency of the GG genotype in patients with severe rheumatoid arthritis (6.4%) compared to cases of mild-moderate disease (3.1%) and reference controls (1.2%). Evaluation of the response to anti-TNF-α therapy after 12 weeks of treatment reveals that 37.8% of the TT genotype pass from high to medium-low disease activity. Such observations are in contrast to the 10.7% of the TG/GG genotype that pass from high to medium-low disease activity (p = 0.03).

Collective evaluation of laboratory results therefore suggests that in cases of rheumatoid arthritis classified according to response to the conventional therapy and by disease severity, there is a discernable trend toward a higher prevalence of the GG genotype for the exon 6 TNFRII polymorphism in less responsive cases displaying a more aggressive form of disease. In addition, the G allele tends to promote a lower degree of response to anti-TNF-α treatments.

Tumor necrosis factor-alpha receptor II polymorphism in patients from southern Europe with mild-moderate and severe rheumatoid arthritis. Fabris M, Tolusso B, Di Poi E, Assaloni R, Sinigaglia L, Ferraccioli G. J Rheumatol 2002 Sep; 29 (9), pp. 1847–50.

N-Acetyltransferase Metabolizing Capacity
Caffeine

Cellular/Molecular Mechanism of Action: Caffeine is a substrate for N-acetyltransferase.
Application: Detection and evaluation of N-acetyltransferase metabolic capacity and genetic polymorphism.
Route of Administration: Caffeine (oral).
Dosage: Caffeine (200 mg).
Examples of Clinical Implementation: Inactivation of the antituberculosis drug isoniazid occurs principally through metabolizing mechanisms that create an N-acetylated metabolite. Genetically, there exists a degree of polymorphism within a given population with regard to the expression of N-acetyltransferase. As a metabolic probe, caffeine has been applied to determine N-acetyltransferase polymorphism within Greek populations by means of the molar urine ratio of 5-acetylamino-6-formylamino-3-methyluracil and 1-methylxanthine determined following caffeine ingestion. Frequency distribution analysis of the metabolic ratios 5-acetylamino-6-formylamino-3-methyluracil/1-methylxanthine reveals that 2 distinct groups exist that are either slow (2/3) or fast (1/3) rapid caffeine acetylators and are in agreement with findings noted in populations from other regions of the world.
Interpretation and Analysis: The activity of N-acetyltransferase can be estimated from the urinary metabolic ratio of 2 caffeine metabolites, namely, 5-acetylamino-6-formylamino-3-methyluracil (AFMU) and 1-methylxanthine (1X) after the ingestion of caffeine. The use of caffeine as a metabolic probe is a reliable method for assessing N-acetyltransferase activity.

Determination of N-acetylation phenotyping in a Greek population using caffeine as a metabolic probe. Asprodini EK, Zifa E, Papageorgiou I, Benakis A. Eur J Drug Metab Pharmacokinet 1998 Oct–Dec; 23 (4), pp. 501–6.

Sulfate Conjugation: Phase II Biotransformation/ Pharmaceuticals and Xenobiotics
Sulfotransferase (SULT) Enzyme/Genetic Polymorphism

Cellular/Molecular Mechanism of Action: Sulfotransferase (SULT) enzymes catalyze an important phase II reaction in the biotransformation of many drugs and other xenobiotics.
Application: Evaluate the possible contribution of sulfotransferase polymorphisms to variation in the sulfate conjugation of drugs, other xenobiotics and/or disease pathophysiology.
Implementation: Cloning of human SULT1C1 cDNA as a step towards a foundation for establishing a pharmacogenetic database reference. The exons, portions of the introns flanking the exons and approximately 315 bp of the 5′ flanking region of SULT1C1 have been "resequenced" in Caucasian DNA samples (e.g., n = 89) in order to identify common genetic polymorphisms. Expression con-

structs can be created for all of the non-synonymous cSNPs observed, and those constructs used to transfect COS-1 cells.

Interpretation and Analysis: Nineteen separate polymorphisms have been recognized, including 4 non-synonymous coding region single nucleotide polymorphisms (cSNPs) and 5 insertions/deletions. These data can be used to determine and/or infer common SULT1C1 haplotypes. Three of the 4 non-synonymous cSNPs have allele frequencies greater than 1%, including 1 with a frequency of 6.7%. Three of the 4 SULT1C1 variant allozymes have significantly reduced enzyme activity when compared with the wild-type enzyme. Among the variant allozymes, apparent Km values for 3′-phosphoadenosine 5′-phosphosulfate (PAPS), the sulfate donor for the reaction, varies 7-fold. Quantitative Western blot analysis shows variable levels of immunoreactive protein when compared to the wild-type enzyme. Therefore, mechanisms responsible for decreased activity involve both alterations in enzyme protein levels and alterations in substrate kinetics. Therefore, application of a "genotype to phenotype" strategy has facilitated the identification of a series of functionally significant common genetic polymorphisms for SULT1C1.

Human sulfotransferase SULT1C1 pharmacogenetics: Gene resequencing and functional genomic studies. Freimuth RR, Eckloff B, Wieben ED, Weinshilboum RM. Pharmacogenetics 2001 Dec; 11 (9), pp. 747–56.

Sulfate Conjugation: Phase II Biotransformation/ Pharmaceuticals and Xenobiotics
Sulfotransferase (SULT) Enzymes/3′-Phosphoadenosine 5′-Phosphosulfate

Cellular/Molecular Mechanism of Action: 3′-phosphoadenosine 5′-phosphosulfate (PAPS) is the sulfate donor co-substrate for all SULT enzymes. Sulfotransferases catalyze the sulfate conjugation of many endogenous and exogenous compounds, including drugs and other xenobiotics. In humans, PAPS is synthesized from adenosine 5′-triphosphate (ATP) and inorganic sulfate (SO_4^{-2}) by 2 enzyme isoforms, PAPSS1 and PAPSS2.

Background Information: Rare mutations that inactivate PAPSS2 are associated with human spondyloepimetaphyseal dysplasia and murine brachymorphism.

Application: Delineation of the functional significance of PAPSS2 genetic polymorphisms pertaining to its potential contribution to *in vivo* variations in sulfate conjugation of pharmaceuticals and other xenobiotics.

Implementation: The human PAPSS2 gene has been cloned to aid in determining whether or not more common genetic polymorphisms that do not completely inactivate the enzyme might be 1 factor responsible for individual differences in sulfate conjugation. In part this entailed the "resequencing" of all twelve PAPSS2 exons and splice junctions, as well as approximately 500 bp of the 5′-flanking region (e.g., Polymorphism Discovery Resource [PDR] DNA samples from the Coriell Cell Repository; n = 90).

Interpretation and Analysis: Twenty-two single nucleotide polymorphisms (SNPs) have been identified including 4 non-synonymous coding region SNPs (cSNPs) that alter the following amino acids: Glu10Lys, Met281Leu,Val291Met and Arg432Lys. Four insertions/deletions, have also been observed including 1 sample that is homozygous for an 81-bp deletion in the 5′-flanking region 286 bp upstream from the site of transcription initiation. Transient expression studies show that 2 of the non-synonymous cSNPS (those that resulted in Glu10Lys and Val291Met alterations in encoded amino acids), show significant decreases in levels of PAPSS activity. In the case of Glu10Lys, decreased activity is paralleled by a decrease in immunoreactive protein, while the Val291Met allozyme displays a significant decrease in affinity for both ATP and Na_2SO_4 when compared to the "wild-type" enzyme, but without a significant alteration in the levels of immunoreactive protein.

Human 3′-phosphoadenosine 5′-phosphosulfate synthetase 2 (PAPSS2) pharmacogenetics: Gene resequencing, genetic polymorphisms and functional characterization of variant allozymes. Xu ZH, Freimuth RR, Eckloff B, Wieben E, Weinshilboum RM. Pharmacogenetics 2002 Jan; 12 (1), pp. 11–21.

Sulfotransferase (Hepatic SULT2A1)/Pharmacogenetic-Relevant Polymorphism
Steroid Sulfate Conjugation

Cellular/Molecular Mechanism of Action: SULT2A1 catalyzes the sulfate conjugation of dehydroepiandrosterone (DHEA) as well as other steroids.

Application: Characterization of the common genetic polymorphisms for SULT2A1 as it relates to *in vivo* variations (e.g., reductions) in levels of both activity and enzyme protein. Preliminary research suggests the possibility that ethnic-specific pharmacogenetic variation exists in SULT2A1-catalyzed sulfation of both endogenous and exogenous substrates for this phase II drug-metabolizing enzyme.

Implementation: The pharmacogenetic relevance of SULT2A1 can be discerned through the analysis of DNA preparations collected from different phenotype subpopulations (e.g., African-American n = 60; Caucasian-American n = 60). The "resequencing" of the SULT2A1 gene involves sequencing of all exons, splice junctions, and approximately 370 bp located 5′ of the site of transcription initiation.

Interpretation and Analysis: A total of 15 single nucleotide polymorphisms (SNPs) exist, including 3 non-synonymous coding SNPs (cSNPs) that are present only in DNA from African-American subjects. Linkage analysis reveals that 2 of the non-synonymous cSNPs are tightly linked. Expression constructs created for all non-synonymous cSNPs, including a "double variant" construct that includes 2 linked cSNPs, are expressed in COS-1 cells, which facilitates a more in-depth analysis of transcription/translation events. In this context, SULT2A1 activity is significantly decreased for 3 of the 4 variant allozymes. Western blot analysis demonstrates that decreased levels of immunoreactive protein appear to be the major mechanism responsible for decreases in activity, although apparent Km values also vary among the recombinant allozymes. In addition, the most common of the non-synonymous cSNPs disrupts the portion of SULT2A1 involved with dimerization, and this variant allozyme behaves as a monomer rather than a dimer during gel filtration chromatography.

Human sulfotransferase SULT2A1 pharmacogenetics: Genotype-to-phenotype studies. Thomae BA, Eckloff BW, Freimuth RR, Wieben ED, Weinshilboum RM. Pharmacogenomics J 2002; 2 (1), pp. 48–56.

Sulfoxidation/Hydroxylation Metabolization
S-Carboxymethyl-L-Cysteine/Debrisoquine

Cellular/Molecular Mechanism of Action: S-carboxymethyl-L-cysteine and debrisoquine are substrates for biochemical mechanisms that create sulfoxide and hydroxylated analogs.

Application: Measurement of the *in vivo* biochemical activity of hepatic hydroxylation and sulfoxidation mechanisms relevant to the

ability of these enzyme systems to metabolize pharmaceuticals, optimize dosage schemes, determine individual susceptibility to protoxin biotransformation, and/or detect therapeutic non-compliance. In this context, chlorpromazine can be applied as an *in vivo* probe to detect scenarios conducive to the development of chlorpromazine jaundice.

Route of Administration: S-carboxymethyl-L-cysteine (oral); debrisoquine (oral).

Dosage: S-carboxymethyl-L-cysteine (750 mg) and the unchanged compound and sulfoxide metabolites are measured in urine.

Implementation: Detection and evaluation of cases susceptible to chlorpromazine jaundice facilitated by the administration of S-carboxymethyl-L-cysteine and debrisoquine.

Interpretation and Analysis: The amount of sulfoxide S-carboxymethyl-L-cysteine analogue and hydroxylated debrisoquine (4-hydroxydebrisoquine) excreted into the urine directly (positively) correlates with hepatic sulfoxidation and hydroxylation metabolizing capacity. Individuals that are susceptible to chlorpromazine jaundice have a poor capacity to create sulfoxide-analogs of S-carboxymethyl-L-cysteine. However, this same pattern can also be recognized in some reference controls that do not have a history of chlorpromazine jaundice or liver disease (statistically significant difference between groups = $p < 0.001$). A history of chlorpromazine jaundice does not correlate with evidence of impaired hydroxylation capacity as assessed by recovery of 4-hydroxydebrisoquine in urine following oral debrisoquine administration. Susceptibility to the development of chlorpromazine jaundice appears to be an inherent metabolic disorder.

A proposed mechanism for chlorpromazine jaundice—Defective hepatic sulfoxidation combined with rapid hydroxylation. Watson RG, Olomu A, Clements D, Waring RH, Mitchell S, Elias E. J Hepatol 1988 Aug; 7 (1), pp. 72–8.

Sulfoxidation/Hydroxylation Metabolization
S-Carboxymethyl-L-Cysteine/Debrisoquine

Cellular/Molecular Mechanism of Action: S-carboxymethyl-L-cysteine and debrisoquine are substrates for biochemical mechanisms that create sulfoxide and hydroxylated analogs.

Application: Measurement of the *in vivo* biochemical activity of hepatic hydroxylation and sulfoxidation mechanisms relevant to the ability of these enzyme systems to metabolize pharmaceuticals, optimize dosage schemes, determine individual susceptibility to pro-toxin biotransformation, and/or detect therapeutic non-compliance. In this context, chlorpromazine can be applied as an *in vivo* probe to detect scenarios conducive to the development of chlorpromazine jaundice.

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Sulfurtransferase/Dehydroepiandrosterone Sulfotransferase (DHEA ST)
Dehydroepiandrosterone (DHEA)

Cellular/Molecular Mechanism of Action: Dehydroepiandrosterone sulfotransferase catalyzes the sulfation of DHEA and other hydroxysteroids.

Background Information: Dehydroepiandrosterone sulfotransferase enzymatic activity in individual human liver biopsy samples has been shown to vary over a 5-fold range, and frequency distribution histograms are bimodal, with approximately 25% of subjects included in a high activity subgroup.

Application: Identification of common genetic polymorphisms within the dehydroepiandrosterone sulfotransferase gene will now facilitate the testing of how genetic polymorphism alters the *in vivo* expression and/or function of this (human) steroid-metabolizing enzyme.

Implementation: Characterization of the molecular basis for variation within the human liver for dehydroepiandrosterone sulfotransferase biochemical activity can be delineated applying immunodetection methodologies (e.g., Western blot analysis). Performing quantitative Western analysis involves prepration of cytosol isolates from liver samples (e.g., n = 92 human liver specimens). Establishment of laboratory reference control values entails the analyis of liver speciments that have previously been phenotyped with regard to their level of DHEA ST enzymatic activity.

Characterization of genetic polymorphysim of dehydroepiandrosterone sulfotransferase can be determined through the isolation of DNA extracts of liver specimens obtained from cases that express "low" and "high" DHEA ST biochemical activity. Exons and the 5′-flanking region of the DHEA ST gene (STD) are then amplified by the polymerase chain reaction (PCR) for each tissue sample. Complementary laboratory procedures include sequence analysis of hepatic DNA from at exons 2 and 4 for the DHEA ST gene (STD) utilizing liver specimens obtained from individuals previously phenotyped for DHEA ST biochemical activity.

Interpretation and Analysis: There is a highly significant correlation ($r^s = 0.635$, $p < 0.0001$) between levels of DHEA ST activity and immunoreactive protein identified by Western Blot analysis. When compared with "wild type" STD sequence, some tissue samples contain a T → C transition at DHEA ST cDNA nucleotide 170, located within exon 2, resulting in a change at the Met 57 amino acid residue (Met 57 → Thr). Other samples can contain an A → T transversion at nucleotide 557 within STD exon 4 that results in a Glu 186 → Val change.

The allele frequency for the exon 2 polymorphism is 0.027, whereas that for the exon 4 polymorphism is 0.038, but neither polymorphism is systematically related to the level of enzyme activity.

Transient expression in COS-1 cells of cDNA that contain nucleotide 170 and 557 polymorphisms, either separately or together, result in decreased expression of both DHEA ST enzymatic activity and the level of immunoreactive protein, but only when the nucleotide 557 variant is present.

Human dehydroepiandrosterone sulfotransferase pharmacogenetics: Quantitative Western analysis and gene sequence polymorphisms. Wood TC, Her C, Aksoy I, Otterness DM, Weinshilboum RM J Steroid Biochem Mol Biol 1996 Dec; 59 (5–6), pp. 467–78.

Thiopurine Methyltransferase (TPMT): Genetic Polymorphism
Thiopurines

Cellular/Molecular Mechanism of Action: Thiopurine methyltransferase catalyzes the S-methylation of thiopurine drugs.
Background Information: Individual variation in the toxicity and therapeutic efficacy of thiopurine drugs is associated with a common genetic polymorphism that controls levels of TPMT activity and immunoreactive protein in human tissues. Because of the clinical significance of "pharmacogenetic" regulation of this enzyme, it would be important to clone the gene for TPMT in humans and to study the molecular basis for the genetic polymorphism.
Application: Molecular cloning and structural characterization of the TPMT gene as well as elucidation of the molecular basis for a common TPMT genetic polymorphism will help make it possible to develop DNA-based diagnostic tests for polymorphism and to determine the mechanism by which it results in decreased expression of this important drug-metabolizing enzyme.
Implementation: The cloning of the gene for TPMT can be achieved through the rapid amplification of genomic DNA ends to obtain a TPMT-specific intron sequence. Such a DNA sequence can be used as a template for designing primers for a polymerase chain reaction (PCR). In this fashion it is possible to establish the location of the active gene for TPMT on human chromosome 6. A TPMT-positive cosmid clone is then isolated from a human chromosome 6-specific genomic DNA library, and the gene sublocalized to chromosome band 6p22.3 by fluorescence *in situ* hybridization.
Interpretation and Analysis: The gene for TPMT has been determined to be approximately 34 kb in length and to consist of 10 exons and 9 introns. On the basis of the results of 5′-rapid amplification of cDNA ends, transcription initiation occurs at or near a point 89 nucleotides upstream from the translation initiation codon of previously reported TPMT cDNAs. Following the determination of the structure for TPMT gene, it becomes possible to perform PCR analysis with primers complementary to the sequences of introns flanking each exon that encodes enzyme protein with template DNA obtained from subjects with known phenotypes for the TPMT genetic polymorphism.

In studies where DNA is isolated from blood samples from unrelated subjects with genetically low TPMT activity (e.g., n = 4) and unrelated subjects with high TPMT activity (e.g., n = 4), it can be appreciated that all subjects with low TPMT activity are homozygous for 2 point mutations-a G → A transition at nucleotide 460 in exon 7 and an A → G transition at nucleotide 719 in exon 10. Both mutations result in alterations in amino acid sequence, with Ala-154 → Thr and Tyr-240 → Cys, respectively. All DNA samples isolated from the blood of subjects with high TPMT activity contain "wild-type" sequence. Results obtained with these blood samples can be confirmed when DNA from human liver samples (n = 4) with high TPMT activity have a wild-type sequence at nucleotides 460 and 719, while some liver samples with intermediate enzyme activity (i.e., samples presumed to be heterozygous for the polymorphism) are heterozygous for the exon 7 and exon 10 mutations present in the blood samples of homozygous low subjects. Transient expression in COS-1 cells of TPMT expression constructs that contain both of the mutations in exons 7 and 10, as well as each independently, demonstrate that each mutation, as well as both together, result in decreased expression of TPMT enzymatic activity and immunoreactive protein.

Thiopurine methyltransferase pharmacogenetics: Human gene cloning and characterization of a common polymorphism. Szumlanski C, Otterness D, Her C, Lee D, Brandriff B, Kelsell D, Spurr N, Lennard L, Wieben E, Weinshilboum R. DNA Cell Biol 1996 Jan; 15 (1), pp. 17–30.

Thiopurine Methyltransferase (TPMT): Genetic Polymorphism
Thiopurines

Background Information: Genetic polymorphism of TPMT has been shown to have a highly significant clinical impact, namely in the therapeutic efficiency of thiopurine drugs used in the treatment of a wide range of diseases. Available diagnostic methods, although reproducible and sensitive, are relatively laborious, thus population studies are still very scarce.
Application: Detection and characterization of genetic polymorphism for TPMT relevant to the therapeutic management of disease conditions (e.g., acute lymphoblastic leukemia) that require the administration of thiopurine-based pharmaceutical agents.
Implementation: A new polymerase chain reaction-single strand confirmational analysis-based protocol for specific TPMT detection has been developed that has been technically simplified. The method avoids the use of restriction enzyme treatment after polymerase chain reaction amplification. The use of this laboratory protocol allows the simultaneous detection of T474 to C substitutions that is known to be a frequent silent mutation (e.g., North Portuguese populations: TPMT*1S = 0.215).
Interpretation and Analysis: Approximately 4.8% (e.g., n = 15/310) of unrelated Northern Portuguese individuals are heterozygous for the TPMT*3A allele (defined by the presence of 2 transitions, G460 to A and A719 to G) that is associated with TPMT enzymatic deficiency (corresponding gene frequency estimate = 0.024). In children suffering from acute lymphoblastic leukemia (e.g., n = 24), some degree of correlation exists between molecular genotype for TPMT expression and their reaction/response to curative therapy with thiopurine chemotherapeutics. In this context, cases that are heterozygous for the TPMT*3A allele have a high incidence of exhibited signs for severe hepatic toxicity during treatment.

Thiopurine methyltransferase pharmacogenetics: Alternative molecular diagnosis and preliminary data from Northern Portugal. Alves S, Prata MJ, Ferreira F, Amorim A. Pharmacogenetics 1999 Apr; 9 (2), pp. 257–61.

Thiopurine Methytransferase (TPMT): Genetic Polymorphism/Leukemic Blast and Erythrocyte TPMT Biochemical Activity/Mercaptopurine: 6-Thioguanine Metabolite Accumulation
Thiopurines/Methotrexate

Background Information: The activity of TPMT exhibits genetic polymorphism, with approximately 1 in 300 individuals inheriting TPMT deficiency as an autosomal recessive trait, and about 11% having intermediate activity (i.e., heterozygotes). In situations of TPMT deficiency, excessive concentrations of 6-thioguanine nucleotides (TGNs) accumulate resulting in the development of severe toxicity when standard dosages of mercaptopurine

are administered. Conversely, high TPMT activity has been associated with lower concentrations of 6-thioguanine nucleotides yielding a higher risk of treatment failure in children with acute lymphoblastic leukemia (ALL).

Implementation: Investigation of the biochemical basis of the pharmacodynamic relationships between mercaptopurine plasma concentrations and TPMT genetic polymorphism through the analysis of red blood cell and lymphoblast TPMT activity in cases of ALL.

Interpretation and Analysis: A 58-fold range of erythrocyte TPMT biochemical activity can be observed among cases receiving ALL chemotherapy (0.6–34.9 U/mL packed erythrocytes), but relatively low intrapatient variability (coefficient of variation, 13.5%) is observed (e.g., over a period of 1 year; n = 119 cases). A 27-fold range in TPMT biochemical activity can be appreciated in leukemic blasts obtained from cases at initial diagnosis (e.g., n = 42 with 3.3 to 88.9 U/1 $\times$ 10^9 cells). Thiopurine methyltransferase activity in leukemic blasts at diagnosis significantly correlates with TPMT in erythrocytes before therapy (r^s = .75, $p < 0.0001$, n = 13). Therefore, extensive interpatient variability exists with respect to TPMT biochemical activity in ALL blasts. However, these findings correlate with polymorphic TPMT activity in erythrocytes, thereby providing a new mechanism by which erythrocytes serve as prognostic markers of mercaptopurine metabolism and TPMT activity in children with ALL.

Polymorphic thiopurine methyltransferase in erythrocytes is indicative of activity in leukemic blasts from children with acute lymphoblastic leukemia. McLeod HL, Relling MV, Liu Q, Pui CH, Evans WE. Blood 1995 Apr 1; 85 (7), pp. 1897–902.

Thiopurine Methyltransferase (Metabolism)/Acute Lymphocytic Leukemia (ALL): Response Variability/Prognosis: Prediction of Relapse
Methotrexate/6-Mercaptopurine

Background Information: The intensity of cancer chemotherapy has been reported to influence clinical responses to several drug-sensitive cancers.

Application: Delineation of the relationship between systemic exposure to high-dose methotrexate (HDMTX) and clinical response in childhood ALL.

Route of Administration: High-dose methyltrexate (intravenous); methotrexate (oral/per os); low-dose 6-mercaptopurine (oral/per os).

Dosage: High-dose methyltrexate (1,000 mg/m^2 over 24 hours weekly for 3 weeks, then every 6 weeks for 72 weeks); methotrexate (25 mg/m^2 per week); low-dose 6-mercaptopurine (50 mg/m^2 per day).

Implementation: Initiation of postinduction therapy with high-dose methyltrexate superimposed on conventional therapy with low-dose 6-mercaptopurine and methotrexate in untreated children with "standard-intermediate risk" (e.g., n = 108).

Interpretation and Analysis: The systemic clearance of HDMTX ranges from 40 to 131 ml/min/m^2 yielding MTX steady state plasma concentrations (Cpss) ranging from 9.3 to 25.4 μM during the infusion ("standard-intermediate risk" children). A higher probability of developing any type of relapse is observed with median MTX Cpss < 16 μM during the HDMTX infusion (e.g., n = 59) in contrast to cases with MTX Cpss greater than 16 μM (e.g., n = 49; $p < 0.05$). In univariate analysis, cases with MTX Cpss ≤ 16 μM are approximately 3.2 times more likely to relapse on therapy (p = 0.01) and 6.9 times more likely to have a hematologic relapse on therapy (p = 0.001). Multivariate and stepwise Cox's regression analyses indicate that MTX Cpss retains its prognostic importance even when other prognostic variables (i.e., DNA Index, WBC, hemoglobin) are considered.

Clinical pharmacodynamic studies of high-dose methotrexate in acute lymphocytic leukemia. Evans WE, Abromowitch M, Crom WR, Relling MV, Bowman WP, Pui CH, Ochs J, Dodge R. NCI Monogr 1987; (5), pp. 81–5.

Thiopurine Methyltransferase (TPMT): Homozygous Wild Type/Acute Lymphoblastic Leukemia (ALL): Childhood Form/Prediction of Event-Free Survival (EFS)
6-Mercaptopurine

Background Information: 6-mercaptopurine (6MP) and methotrexate are the backbone of continuation therapy for childhood ALL. In studies of oral 6MP and methotrexate, indices of chronic systemic exposure to active metabolites of these agents, namely, red blood cell (RBC) concentrations of methotrexate polygluta-mates (MTXPGs) and thioguanine nucleotides (TGNs), have been positively correlated with EFS.

Application: Determination of the potential to form RBC concentrations of methotrexate polyglutamates and thiguaninine nucleotides as a potential EFS prognostic marker.

Considerations: Increased dose intensity of oral 6MP is an important determinant of EFS in ALL, particularly among children with a homozygous wild-type TPMT phenotype.

Route of Administration: Methotrexate (parenteral); 6-mercaptopurine (oral).

Dosage: Methotrexate (40 mg/m^2 once/week).

Implementation: Delineation of the prognosis in conditions of childhood ALL based on analysis of dose intensity responses for methotrexate and 6-mercaptopurine administration. Parameters tabulated include dose intensity information (dose received divided by protocol dose per week; n = 19,046 weeks of 6-mercaptopurine and methotrexate). Measurement of complementary variables include the quantification of RBC MTXPGs; MTXPGs (n = 418); TGNs (n = 709); and TPMT (n = 267).

Protocol: Administration of methotrexate and 6-mercaptopurine is administered to cases in remission (e.g., n = 182) complemented by pulse chemotherapy every 6 weeks during the 1st 1-year period of management.

Interpretation and Analysis: Only higher dose intensity 6MP and weekly methotrexate are significant predictors of overall EFS (univariant analyses p = 0.006 and 0.039, respectively). The occurrence of neutropenia is associated with poorer case outcome (p = 0.040). Only higher dose intensity 6MP (p = 0.020) is a significant predictor of EFS, while lower TPMT activity (p = 0.096) tends to be associated with better outcome (multivariate analyses). 6-mercaptopurine dose intensity is also associated (p = 0.007) with EFS among patients with the homozygous wild-type TPMT phenotype. Lower 6-mercaptopurine dose intensity is primarily due to missed weeks of therapy and not to reductions in daily dose. Increased dose intensity of oral 6MP is an important determinant of EFS in ALL, particularly among children with a homozygous wild-type TPMT phenotype. However, increasing intensity of therapy such that neutropenia precludes chemotherapy administration may be counterproductive.

Prognostic importance of 6-mercaptopurine dose intensity in acute lymphoblastic leukemia. Relling MV, Hancock ML, Boyett JM, Pui CH, Evans WE. Blood 1999 May 1; 93 (9), pp. 2817–23.

Thiopurine Methyltransferase (TPMT) Genetic Polymorphism/Xanthine Oxidase: Variation in Genetic Expression/Toxic 6-Thioguanine Nucleotide Metabolites/Alternative Selection of Cyclosporine A, Mycophenolate Mofetil, or Tacrolimus
Azathioprine/Cyclosporine A

Physiological Mechanism of Action: Cyclosporine A and azathioprine function as immunosuppressive agents.
Background Information: Immunosuppressive regimens employed following solid organ transplantation commonly entail the use of cyclosporine A, azathioprine, and steroids. Azathioprine is known to carry the risk of severe myelosuppression and is catabolized *in vivo* by xanthine oxidase and TPMT, an enzyme that exhibits a common genetic polymorphism (e.g., 11% of Caucasians are heterozygous and 0.3% are homozygous with respect to TPMT deficiency). Toxicity and immunosuppressive effects have been attributed to the 6-thioguanine nucleotides generated from azathioprine.
Application: Characterization of variations in individual expression of TPMT activity in order to identify individuals at risk of metabolizing azathioprine to the toxic 6-thioguanine nucleotide metabolites.
Implementation: Measurement of TPMT activity and 6-thioguanine nucleotide concentrations in erythrocytes harvested to evaluate and monitor immunosuppressive therapeutic regimens (e.g., cardiac and renal transplant patients). Erythrocyte thiopurine methyl-transferase can be determined by a radioenzymatic assay and erythrocyte 6-thioguanine nucleotide concentration with high-performance liquid chromatography. Thiopurine methyltransferase activity (median [range, 10th–90th percentile]) is significantly ($p < 0.05$) higher in patients ($n = 39$) receiving azathioprine (285 [218–362] vs 262 [160–352] mU/I erythrocytes) than in healthy blood donor controls ($n = 120$). When stratified according to TPMT phenotype, a few cases homozygous for the low allele exhibit excessive erythrocyte 6-thioguanine nucleotide concentrations (2,210 pmol/0.8 $\times 10^9$ erythrocytes). Heterozygous patients have significantly higher 6-thioguanine nucleotide concentrations (median: 435 pmol/0.8 $\times 10^9$ erythrocytes) compared with concentrations for patients homozygous for the high allele (median: 86 pmol/0.8 $\times 10^9$ erythrocytes; $p < 0.01$), although the azathioprine dosage does not differ ($p = 0.66$). Erythrocyte TPMT determination therefore identifies patients at high risk of accumulating 6-thioguanine nucleotides. The monitoring of this enzyme may contribute to the safer management of immunosuppressive therapy with azathioprine. Alternative regimens such as cyclosporine A/mycophenolate mofetil or tacrolimus should also be considered for this patient group.

Azathioprine pharmacogenetics: The relationship between 6-thioguanine nucleotides and thiopurine methyltransferase in patients after heart and kidney transplantation. Schutz E, Gummert J, Armstrong VW, Mohr FW, Oellerich M. Eur J Clin Chem Clin Biochem 1996 Mar; 34 (3), pp. 199–205.

Thiopurine S-Methyltransferase (TPMT): Genetic Polymorphism/Rheumatoid Arthritis: Predictive Model for Cost-Effectiveness
Azathioprine (AZA)

Cellular/Molecular Mechanism of Action: Thiopurine S-methyltransferase catalyzes the inactivation of AZA.
Background Information: Thiopurine S-methyltransferase exhibits genetic polymorphism that results in dose-related, serious toxicities (mainly hematological cytopenias) in 10 to 15% of individuals treated with AZA. Polymerase chain reaction (PCR)-based methodologies provide a sensitive, specific means of prospectively identifying these patients before AZA therapy and minimizing toxicity through dosage reduction.
Application: Characterization of thiopurine S-methyltransferase genetic polymorphism in order to identify subpopulations vulnerable to developing dose-related toxicity associated with azothioprine administration. In this manner it is possible to identify cases that require a reduction in the conventional dose of azothioprine. In addition, an analytical model has been developed to delineate the cost-effectiveness of azothioprine therapy for the management of rheumatoid arthritis.
Implementation: Design of a decision analytic model for application in delineating the cost-effectiveness and outcomes of patients undergoing different azothioprine therapeutic strategies. Data applied to the model includes (i) positive and negative predictive values of PCR analyses for detecting TPMT genetic polymorphism; (ii) the probabilities of adverse events due to AZA; and (iii) the costs associated with management. Sources of data can include published clinical trials, diagnostic test evaluations, surveillance trials, and economic evaluations.
Interpretation and Analysis: Dose-related toxicities result in azothioprine discontinuation rates of 10 to 20%. The traditional dosing strategy costs $677 per patient, whereas the genotype directed dosing strategy costs $663 per patient (Canadian). In the genotype dosing strategy, the number needed to treat and avoid 1 adverse event over 6 months is 20. Thus, the genotype-based dosing strategy is superior to usual dosing strategies. One-way sensitivity analyses reveal that estimates are robust within ranges of $\pm$ 30% for the costs, the properties of the PCR test, and the probability of adverse events. The introduction of PCR testing to identify TPMT polymorphisms prior to AZA treatment may represent good value in certain health care settings.

Practical pharmacogenetics: The cost-effectiveness of screening for thiopurine s-methyltransferase polymorphisms in patients with rheumatological conditions treated with azathioprine. Marra CA, Esdaile JM, Anis AH. J Rheumatol 2002 Dec; 29 (12), pp. 2507–12.

Thiopurine S-Methyltransferase (Hepatic): Genetic Polymorphism/(UGT1A1)/(DPD)/(TS)/(MTHFR)/(FCGR3A)/(XPD)/(XRCC1)
Thiopurines

Background Information: Interindividual variability in the efficacy and toxicity of drug therapy is associated with polymorphisms in genes encoding drug-metabolizing enzymes, transporters, or drug targets. Pharmacogenetics aims to identify individuals predisposed to high risk of toxicity from conventional doses of cancer chemotherapeutic agents.
Interpretation and Analysis: Genetic polymorphisms in UGT1A1 and TPMT, as well as mutations in DPD, directly influence drug disposition and toxicity. Pharmacogenetic determinants may also influence treatment outcomes. The clinical significance of polymorphisms in TS, MTHFR, and FCGR3A, as well as the polymorphic DNA repair genes XPD and XRCC1, is related to their influence on chemotherapy response and survival outcomes. Several potential implications exist related to transporter pharmacogenetics pertaining to their influence on drug bioavailability.

Pharmacogenetics in cancer treatment. Nagasubramanian R, Innocenti F, Ratain MJ. Annu Rev Med 2003; 54, pp. 437–52. Epub 2001 Dec 03.

UDP-Glucuronosyl Transferase Polymorphism/ Parkinson's Disease: Susceptibility to Hepatotoxicity
Tolcapone (TASMAR)

Application: Characterization of UDP-glucuronosyl transferase 1A gene complex expression can be applied to identify increased susceptibility to the development of hepatotoxic responses associated with the administration of tolcapone (TASMAR) for the therapeutic management of Parkinson's disease.
Implementation: Delineation of the genotype for 30 previously described or newly characterized bi-allelic single nucleotide polymorphisms representing 12 candidate genes selected based on the known metabolic pathways involved in the tolcapone elimination. Samples of DNA can be processed from biological material collected from cases (e.g., n = 135) displaying elevations of ≥ 1.5 times the upper limit of normal and findings compared with matched reference control DNA samples obtained from cases administered tolcapone but without evidence of elevated transaminase levels.
Interpretation and Analysis: Single nucleotide polymorphisms located within the UDP-glucuronosyl transferase 1A gene complex coding for enzymes involved in the main elimination pathway of the tolcapone (TASMAR) are significantly associated with the occurrence of tolcapone-associated elevations in liver transferase concentrations.

Pharmacogenetic analysis of adverse drug effect reveals genetic variant for susceptibility to liver toxicity. Acuna G, Foernzler D, Leong D, Rabbia M, Smit R, Dorflinger E, Gasser R, Hoh J, Ott J, Borroni E, To Z, Thompson A, Li J, Hashimoto L, Lindpaintner K. Pharmacogenomics J 2002; 2 (5), pp. 327–34.

EXTRAHEPATIC PHARMACOGENETICS

EXTRAHEPATIC—CARDIOVASCULAR SYSTEM

Angiotensin-Converting Enzyme (ACE) Deletion Allele Polymorphism/Congestive Heart Failure Left Ventricular Ejection Fraction 0.24 ± 0.08/ Transplant-Free Survival Prediction
Adrenergic β-Blocker Therapy Response

Cellular/Molecular Mechanism of Action: Angiotensin-converting enzyme inhibitors disrupt the biochemical activity of angiotensin-converting enzyme in a manner that prevents the production of renal angiotensin II from angiotensin I and decreases the destruction of bradykinin.
Background Information: Activation of the renin-angiotensin and sympathetic nervous systems adversely affects the progression of heart failure. The ACE deletion allele (ACE D) is associated with increased renin-angiotensin activation; however, its influence on patient outcomes remains uncertain, and pharmacogenetic interactions with β-blocker therapy have not been thoroughly evaluated to date.
Application: Estimation of the transplant-free survival time in cases of cardiac disease as a function of ACE genotype expression related to β-blocker pharmaceuticals.
Implementation: Evaluation of the influence of the ACE-D allele on transplant-free survival (medial follow-up 21 months) in cases with systolic dysfunction (e.g., n = 328; age, 56.1 ± 11.9 years) that display abnormalities in left ventricular ejection fraction measurements (e.g., 0.24 ± 0.08).
Interpretation and Analysis: Transplant-free survival is significantly poorer for cases with the D allele (1-year percent survival II/ID/DD = 94/77/75; 2-year = 78/65/60; ordered log-rank test, p = 0.044). In cases not treated with β-blockers (e.g., n = 208), the adverse impact of the ACE D allele is dramatically increased (1-year percent survival II/ID/DD = 95/75/67; 2-year = 81/61/48; p = 0.005). Conversely, the ACE genotype has no influence on transplant-free survival when β-blocker therapy is administered (1-year percent survival II/ID/DD = 91/80/86; 2-year = 70/71/77; p = 0.73). Therefore, in cases suffering from systolic dysfunction, the ACE D allele is associated with a significantly poorer transplant-free survival. This effect is most evident in the absence of β-blockers administration and is not a pattern observed with β-blocker therapeutic regimens. Such observations suggest that a potential pharmacogenetic interaction exists between the ACE D/I polymorphism and therapy with β-blockers in the determination of heart failure survival.

Pharmacogenetic interactions between β-blocker therapy and the angiotensin-converting enzyme deletion polymorphism in patients with congestive heart failure. McNamara DM, Holubkov R, Janosko K, Palmer A, Wang JJ, MacGowan GA, Murali S, Rosenblum WD, London B, Feldman AM. Circulation 2001 Mar 27; 103 (12), pp. 1644–8.

Angiotensin-Converting Enzyme (ACE) Deletion Allele Polymorphism (ACE D/I)/Renin-Angiotensin/ Left Ventricular (LV) Modeling Post–Myocardial Infarction (MI)
Ramipril

Cellular/Molecular Mechanism of Action: Angiotensin-converting enzyme proteolytically generates angiotensin from a biologically inactive precursor. Angiotensin-converting enzyme inhibitors disrupt the biochemical activity of ACE in a manner that prevents the production of renal angiotensin II from angiotensin I and decreases bradykinin distruction.
Background Information: The D/I (deletion, D, insertion, I) polymorphism of the ACE gene has been extensively studied for its association with a number of different cardiovascular conditions and other disease states. However, its potential association with the differential clinical efficacy of ACE inhibitors (ACE-I) administered to patients who have suffered a myocardial infarction (i.e., the prevention of LV remodeling) has so far not been specifically studied.
Application: Detection and evaluation of ventricular modeling post–myocardial infarction.
Considerations: Evidence does not currently exist that demonstrates an association between ACE D/I polymorphism and the risk of LV remodeling post-MI in the presence of ACE-I therapy. Therefore, there is no relationship between the differential clinical efficacy of ACE inhibitors and D/I polymorphism of the ACE gene as a genetic marker.
Implementation: Determination of the level of association between D/I polymorphism of the ACE gene with the incidence of post-MI LV remodeling. In this context, an ACE inhibitor (ACE-I, e.g., ramipril) is administered in concert with reductions in LV enlargement in cases of acute anterior wall mitral insufficiency (n = 265 subjects: Healing and Early Afterload Reducing Therapy [HEART] study). Characterization of ACE D/I polymorphism can be determined through the application of various analytical techniques including the polymerase chain reaction (PCR).
Interpretation and Analysis: Selected frequencies for the ACE D and I alleles are 0.59 and 0.41 (placebo-high dose group), 0.56 and 0.44 (low dose-low dose group), and 0.60 and 0.40 (high dose-high dose group), respectively. All observed genotype frequencies are in

Hardy-Weinberg equilibrium. An association between genotype and outcome regarding LV size or function cannot be detected, nor with the initial blood pressure response after ACE-I administration (adjusted for co-variates).

A prospective evaluation of the angiotensin-converting enzyme D/I polymorphism and left ventricular remodeling in the "Healing and Early Afterload Reducing Therapy" study. Zee RY, Solomon SD, Ajani UA, Pfeffer MA, Lindpaintner K; Heart Investigators. Clin Genet 2002 Jan; 61 (1), pp. 21–5.

Angiotensin-Converting Enzyme (ACE) Genetic Polymorphism/Variability in Efficacy
ACE Inhibitors/Imidapril

Cellular/Molecular Mechanism of Action: Angiotensin-converting enzyme inhibitors disrupt the biochemical activity of ACE in a manner that prevents production of renal angiotensin II from angiotensin I and decreases the destruction of bradykinin.
Background Information: Angiotensin-converting enzyme inhibitors (e.g., imidapril [enalapril, captopril]) are reported to prevent neointimal formation after balloon injury in animal models, but in most prospective studies in humans, ACE inhibitors fail to prevent restenosis after percutaneous transluminal coronary angioplasty (PTCA).
Application: Imidapril can be applied as an ACE inhibitor for the purpose of preventing restenosis after PTCA. However, ACE insertion/deletion (I/D) polymorphism may influence the effectiveness of ACE inhibitors in this therapeutic role.
Dosage: Imidapril (5 mg SID) for 3 to 6 months.
Implementation: The ACE genotype assigned by an I/D polymorphism is known to affect the potency of ACE inhibitors in several conditions of renal disease. To determine how ACE genotype influences the efficacy of ACE inhibitors on restenosis related to PTCA, subjects are given imidapril (5 mg imidapril SID for 3 to 6 months starting 1 day before PTCA). Efficacy of imidapril is determined through the evaluation of vessel integrity.

Human Model: Cases with the ACE genotype and subjected to PTCA procedures (e.g., n = 46 controls [65 vessels]; n = 32 imidapril test subjects [43 vessels]).
Interpretation and Analysis: The minimal lumen diameter before and after the procedure does not differ significantly among the 3 ACE genotypes (II, ID, and DD) in both the control and imidapril groups. Late luminal loss during the follow-up period is not related to ACE genotype in the control group but is significantly related in the imidapril group (II, 0.63 ± 0.19 mm; ID + DD, 1.12 ± 0.14 mm, $p < 0.05$). Furthermore, in the II genotype, imidapril significantly reduces the late loss and restenosis rate as defined by most of the frequently used definitions.

Pharmacogenetic analysis of the effect of angiotensin-converting enzyme inhibitor on restenosis after percutaneous transluminal coronary angioplasty. Okamura A, Ohishi M, Rakugi H, Katsuya T, Yanagitani Y, Takiuchi S, Taniyama Y, Moriguchi K, Ito H, Higashino Y, Fujii K, Higaki J, Ogihara T. Angiology 1999 Oct; 50 (10), pp. 811–22.

Angiotensin-Converting Enzyme (ACE) Genetic Polymorphism/Blood Pressure: Genetic Response Variation/Antihypertensive Agents: Genetic Variation
ACE Inhibitors/Lisinopril

Cellular/Molecular Mechanism of Action: Angiotensin-converting enzyme inhibitors suppress or prevent the biochemical generation of angiotensin.
Physiological Mechanism of Action: Angiotensin-converting enzyme inhibitor agents lower peripheral vascular blood pressure.
Background Information: It has been well known for 70 years that familial factors influence blood pressure levels. Modern genetics suggest that 30–40% of blood pressure variation reflects genetic factors. Interindividual variation in response to specific antihypertensive agents has been documented for more than 40 years, with little insight beyond demographics into the responsible mechanism for the variation in efficacy. Only recently has interest been evident in attempts to bring these 2 questions together. Specifically, do familial factors influence the response to specific antihypertensive therapy?
Implementation: A substantial degree of variation can be observed between individuals in their response to ACE inhibitors.
Interpretation and Analysis: Interindividual variation in the response to ACE inhibitors is considered to be a phenotype characteristic directly influenced by genetic factors. Similarly, it has been recognized that ACE inhibition reverses many of the features of nonmodulation, which is the expression of an angiotensinogen (AGT) gene polymorphism. The ESPRIT Study was the 1st to enroll hypertensive sibling pairs into a therapeutic trial, designed to assess responses to ACE inhibitor treatment with lisinopril. This pathway of investigation is likely to be very fruitful over the next several decades, to the point that "pharmacogenetics" will be an element in selecting antihypertensive therapy.

One's grandparents as the determinant of effective antihypertensive therapy. Hollenberg NK. Blood Press Monit 1999; 4 Suppl 1, pp. S15–8.

Butyrylcholinesterase (Plasma): Genetic Asp70Gly Mutations
Succinylcholine/Mivacurium

Cellular/Molecular Mechanism of Action: Butyrylcholinesterase enzymatically metabolizes mivacurium and succinylcholine.
Background Information: The enzymatic function of butyrylcholinesterase is significantly impaired with mutations at Asp70Gly.
Implementation: Application of a simple polymerase chain reaction (PCR) method for the detection of genetic mutations of butyrylcholinesterase. A relatively high percentage of cases tested after episodes of prolonged apnea are positive for the presence of an altered genetic sequence responsible for Asp70Gly mutations (e.g., n = 13/16; homozygotes = 50.0%; heterozygotes = 31.3%). Such alterations detected by PCR tests explain certain observed clinical events and an avenue for prevention in relatives.

Rapid identification of atypical variant of plasma butyrylcholinesterase by PCR. Ceppa F, Gidenne S, Benois A, Fontan E, Burnat P. Clin Chem Lab Med 2002 Aug; 40 (8), pp. 799–801.

Cardiac Arrythmias: Congenital Long Q-T1 (LQ-T1) Syndrome/Silent LQ-T1 Mutation Carriers
Epinephrine

Cellular/Molecular Mechanism of Action: Epinephrine is an endogenous neurotransmitter that functions as a positive adrenergic agonist/sympathomimetic agent at α-adrenergic and β-adrenergic postsynaptic receptor complexes.
Background Information: The LQ-T1 form of congenital LQ-T syndrome is associated with high vulnerability to sympathetic stimulation and appears with incomplete penetrance.
Application: Detection of silent LQ-T1 mutation carriers.
Route of Administration: Epinephrine (infusion).
Dosage: Epinephrine (0.1 μg/kg/min).

Implementation: Evaluation of the LQ-T1 form of congenital LQ-T syndrome using epinephrine by infusion. Electrocardiographic (12-lead) parameters are recorded before and after epinephrine infusion.

Human Model: Congenital LQ-T1 (n = 19 mutation carriers with a baseline corrected Q-T interval (Q-Tc) of > 460 ms (Group I), 15 mutation carriers with a Q-Tc of < 460 ms (Group II), 12 non-mutation carriers (Group III), and 15 controls (Group IV).

Interpretation and Analysis: The mean corrected Q-Tend (Q-Tce), Q-Tpeak (Q-Tcp), and Tpeak-end (Tcp-e) intervals among 12 leads prior to epinephrine are significantly larger in Group I than in other case groups. Epinephrine significantly increases the mean Q-Tce, Q-Tcp, Tcp-e, and the dispersion of Q-Tcp in Groups I and II, but not in Groups III and IV. Sensitivity and specificity of Q-Tce measurements to identify mutation carriers are approximately 59% (20/34) and 100% (27/27), respectively, before epinephrine, and the sensitivity is substantially improved to 91% (31/34) without sacrificing specificity (100%, 27/27) after epinephrine. The mean Q-Tce, Q-Tcp, and Tcp-e before and after epinephrine are significantly larger in symptomatic (e.g., n = 15) than in asymptomatic (n = 19) mutation carriers in case Groups I and II, and the prolongation of the mean Q-Tce with epinephrine is significantly larger in symptomatic cases. Epinephrine challenge can function as a powerful test to establish electrocardiographic diagnosis in silent LQ-T1 mutation carriers, thus allowing implementation of prophylactic measures aimed at reducing sudden cardiac death.

Epinephrine unmasks latent mutation carriers with LQ-T1 form of congenital long Q-T syndrome. Shimizu W, Noda T, Takaki H, Kurita T, Nagaya N, Satomi K, Suyama K, Aihara N, Kamakura S, Sunagawa K, Echigo S, Nakamura K, Ohe T, Towbin JA, Napolitano C, Priori SG. J Am Coll Cardiol 2003 Feb 19; 41 (4), pp. 633–42.

Cardiac Arrythmias: Long Q-T Syndrome (LQ-TS)/Genotype LQ-T1, LQ-T2, and LQ-T3
Phenylephrine/Dobutamine

Cellular/Molecular Mechanism of Action: Phenylephrine and dobutamine function as catecholamine agonists.

Application: Electropharmacology challenge tests with phenylephrine and dobutamine for identifying alterations in repolarization lability in patients with genotyped LQ-TS. Quantification of such phenomenon may assist in identifying LQ-TS patients with an increased risk of sudden cardiac death.

Route of Administration: Intravenous.

Implementation: Determination of the effects of phenylephrine and dobutamine on repolarization lability in patients with genotyped LQ-TS. Electrocardiographic stress testing involving aperiodic repolarization lability is quantified from digitized electrocardiograms recorded during catecholamine challenge with phenylephrine and dobutamine. T wave lability quantified as a root-mean-square of the differences between corresponding signal values of subsequent beats. The magnitude of aperiodic T wave lability is quantified by using a newly derived T wave lability index (TWLI).

Human Model: Cases with the genotyped LQ-TS (n = 13 LQ-T1; n = 7 LQ-T2; n = 3 LQ-T3; n = 16 reference controls).

Interpretation and Analysis: The TWLI is significantly greater in LQ-TS genotypes relative to controls (0.0945 + 0.0517 vs 0.0445 + 0.0123; $p < 0.003$). Marked T wave lability (TWLI ≥ 0.095) can be detected in all 3 LQ-TS genotypes (10/23) but not in reference controls ($p < 0.003$). There is no correlation between TWLI and baseline corrected Q-T interval. High-risk patients that have either a history of out-of-hospital cardiac arrest or syncope almost invariably have a TWLI of 0.095 or greater. Beat-to-beat non-alternating T wave lability occurs in cases with LQ-T1, LQ-T2, and LQ-T3 during catecholamine provocation and is associated with a history of prior cardiac events. Quantification of this novel phenomenon may assist in identifying LQ-TS cases with increased risk of sudden cardiac death.

Instrumentation: Electrocardiography system.

Catecholamine-induced T wave lability in congenital long Q-T syndrome: A novel phenomenon associated with syncope and cardiac arrest. Nemec J, Hejlik JB, Shen WK, Ackerman MJ. Mayo Clin Proc 2003 Jan; 78 (1), pp. 40–50.

Lipid Metabolism: Cardiovascular Disease
Hypolipemic Agents

Background Information: Pharmaceutical therapy in the management of abnormal plasma lipid concentrations in patients suffering from cardiovascular disease often results in a wide variety of individual responses.

Considerations: Cardiovascular disease is associated with non-modifiable risk factors such as age, gender, and genetic background, and with modifiable risk factors such as lipid concentrations. Lowering serum lipid levels has been demonstrated to slow the progression of, or even induce regression in atherosclerosis.

Interpretation and Analysis: Pharmacogenetics provides the experimental foundation for understanding the variability in response to drugs as a function of individual genetic makeup. Information from small clinical trials reveals that several candidate genes may hold some promise in the quest to predict individual success to hypolipemic drug treatment. However, the current clinical relevance of this knowledge is quite limited due to the small effects observed for each of the genetic markers examined. Future progress in this area will be driven by studying gene-gene and gene-treatment interactions in much larger patient populations.

Pharmacogenetics of lipid-lowering therapies. Ordovas JM, Shen H. Curr Atheroscler Rep 2002 May; 4 (3), pp. 183–92.

3-Hydroxy-3-Methylglutaryl Coenzyme A (HMG-CoA)/HMG-CoA Genetic Polymorphism/Coronary Heart Disease (CHD)
Statins: HMG-CoA Inhibitors/Cholesterol-Lowering Agents

Background Information: Cholesterol-lowering therapy is the central approach in the primary and secondary prevention of cardiovascular disease, the leading cause of death in industrialized countries. 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) are currently the most potent and widely used cholesterol-lowering drugs. Large-scale clinical trials unequivocally demonstrate the efficacy of statin treatment in reducing the risk of cardiovascular events.

Interpretation and Analysis: In general, HMG-CoA reductase inhibitors are well tolerated, although in a minority of patients severe adverse effects like myopathy or rhabdomyolysis may develop. The incidence of these potentially life-threatening side effects increases with co-adminstration of pharmaceuticals that are metabolized via the same biochemical pathways or at high-dose statin therapy. The recent focus on the pleiotropic effects of statins that are more frequently observed at higher doses and the conclusion drawn from the large statin trials that low-density lipoprotein (LDL) cholesterol is "the lower the better" may need careful consideration in individuals at risk of adverse drug reactions. On the other hand, not all patients respond to statin therapy with a reduction in CHD risk. It is therefore of interest to develop diagnostic test systems that allow the identification of cases at increased risk of developing adverse drug reactions

or scenarios where a lack of therapeutic effect can be anticipated. Besides exogenous factors, genetic variability determines the response of an individual to drug therapy, and the analysis of genetic variants affecting pharmacokinetic or pharmacodynamic aspects of drug therapy is the subject of pharmacogenomics.

Pharmacogenomics and pharmacogenetics of cholesterol-lowering therapy. Schmitz G, Drobnik W. Clin Chem Lab Med 2003 Apr; 41 (4), pp. 581–9.

EXTRAHEPATIC—CENTRAL NERVOUS SYSTEM

Alcohol Sensitivity
Ethanol/Calcium Chloride/A23187 (Calcium Ionophor)

Physiological Mechanism of Action: Ethanol functions as a central nervous system depressant.
Application: Characterization of differences in central nervous system ethanol metabolism.
Route of Administration: Ethanol is administered orally (per os).
Implementation: Sensitivity to ethanol is measured as a function of the blood ethanol concentration at the point where the righting reflex is lost.
Interpretation and Analysis: Sensitivity to ethanol is increased significantly in SS but not LS mice following intracerebroventricular (ICV) administration of calcium chloride or A23187, a calcium ionophore. Magnesium chloride or lanthanum chloride, ICV, does not alter sensitivity to ethanol in either SS or LS mice, further indicating a specificity for calcium cation. Calcium does not affect sensitivity to halothane narcosis in LS or SS mice. Endogenous brain calcium content is similar in these mouse lines, and ethanol administration either *in vivo* or *in vitro* does not alter brain calcium concentration. Differences in brain sensitivity to ethanol are therefore mediated, in part, by genetic differences in calcium-related processes and support the hypothesis that ethanol-induced narcosis may be due to alterations in calcium metabolism in the CNS.

Calcium influence on neuronal sensitivity to ethanol in selectively bred mouse lines. Morrow EL, Erwin VG. Pharmacol Biochem Behav 1986 Apr; 24 (4), pp. 949–54.

Alcoholism: Genetic-Associated Senstivity/Central Nervous System Electroencephalogram (EEG) Patterns
Ethanol/EEG Analysis/(Electropharmacogenetics)

Physiological Mechanism of Action: Ethanol functions as a central nervous system depressant.
Background Information: Electroencephalogram analysis offers several advantages for performing pharmacogentic related studies because it simultaneously evaluates both genetic traits and challenge characteristics.
Application: Delineation of alterations in EEG tracings induced by ethanol that are associated with specific genetic characteristics that may be related to a predisposition for alcoholism.
Route of Administration: Ethanol is administered orally to effect.
Implementation: Pharmacogenetic studies employing EEG analysis have been applied to characterize the influence of acute ethanol dosing on brain function. Performance of such investigations in twin individuals has facilitated the discovery of certain ethanol-induced alterations in brain wave profiles that are directly related to genetic-related characteristics.
Interpretation and Analysis: Pharmacogenetic studies employing EEG analysis combined with acute ethanol dosing in twin individuals reveals that certain alterations in EEG tracings profiles induced by this depressant are genetic regulated in nature. Among the various genetically determined variants of EEG tracings, the poorly synchronized αEEG exhibits the most pronounced response. Examination of a series of alcoholics of both sexes as well as their 1st-degree relatives reveals that in females the poorly synchronized αEEG may be an indicator that reflects a predisposition for alcoholism. Such observations support the perception that a disposition toward alcoholism is under genetic influence not only at the metabolic but also at the pharmacodynamic level.

Pharmacogenetics of alcohol's CNS effect: Implications for the etiology of alcoholism. Propping P. Pharmacol Biochem Behav 1983; 18 Suppl 1, pp. 549–53.

Alcoholism: Genetic-Associated Sensitivity/ Cholinergic Function
Ethanol/[^{3}H]-Quinuclidinyl Benzilate

Physiological Mechanism of Action: Ethanol functions as a central nervous system depressant.
Application: Characterization of pharmacogentic properties associated with vulnerability or resistance to the development of alcoholism pertaining to different aspects of cholinergic function within the autonomic nervous system.
Route of Administration: Ethanol is traditionally administered orally (per os).
Dosage: Ethanol (4.6 g/kg).
Implementation: Evaluation of alterations in choline acetyltransferase (ChAT), acetylcholinesterase (AChE) activity, and [^{3}H]-quinuclidinyl benzilate (QNB) binding within different regions of the brain following ethanol administration (4.6 g/kg BW).
Animal Model: Murine (inbred strains).
Interpretation and Analysis: Choline acetyltransferase in striatum and septum of mouse strain C57BL/6 exhibit greater sensitivity to ethanol compared to BALB/c mice. The limbic system and related structures of BALB/c mice show greater sensitivity to ethanol relative to C57BL/6 mouse strains. Acute ethanol administration in C57BL/6 mice increases striatal ChAT activity (up to 22% with 60-minute latency). Ethanol-induced augmentation of ChAT activity in this manner is associated with non-synchronous decreases in kinetic characteristics of stratum QNB binding. In contrast, no such changes are seen in BALB/c striatum, except the observation of an increase in Kd up to 90 minutes after acute ethanol treatment. Similar significant increases in ChAT activity can also be observed in C57BL/6 septum 165 minutes after ethanol administration. However, the septum in BALB/c mice does not exhibit comparable changes. Ethanol increases ChAT activity in several brain areas of both C57BL/6 and BALB/c strains of mice. Regions of the CNS that are affected include the hippocampus, temporal limbic cortex, and piriform cortex or paleocortex. Interestingly, the latencies to increased ChAT activity in these areas are much shorter in BALB/c than in C57 mice. The kinetic characteristics of QNB binding sites (Bmax and Kd) and AChE activity are unchanged in all regions of the brain and do not differ between strains (except where previously indicated). Genetic differences in ethanol preference and sensitivity in these strains therefore are accompanied by differential sensitivity of ChAT to acute ethanol. Genotypic variations in dopaminergiccholinergic interactions in striatum, hippocampus, septum, temporal limbic, and piriform cortex could contribute to genetic differences in cholinergic sensitivity to ethanol. In addition, different blood-brain barrier and membrane properties may also contribute to genetic differences in the sensitivity of cholinergic function to ethanol. The differential

effects on ChAT activity could possibly be responsible for genetic differences in memory disorders (limbic system and related structures) and motor incoordination (basal ganglia) following high dose alcohol administration.

Genetically-determined responses of central cholinergic markers: The effects of ethanol on inbred strains of mice. Hashemzadeh-Gargari H, Mandel P. Neurotoxicology 1989 Fall; 10 (3), pp. 555–68.

Attention Deficit Hyperactivity/Dopamine Transporter Gene/Homozygote vs Heterozygote
Methylphenidate (MPH)

Background Information: The homozygosity of the 10-repeat allele at dopamine transporter gene (DAT1) seems to be associated with a poor response to MPH in children with attention deficit hyperactivity disorder (ADHD).
Implementation: Simultaneously assessment of polymorphisms at DAT1, response to MPH, and neuroimaging of cerebral blood flow in cases of childhood ADHD that display at least a moderate response to MPH.
Interpretation and Analysis: Significantly higher regional cerebral blood flows assessed by single photon emission computed tomography (SPECT) can be detected in medial frontal and left basal ganglia areas in children with homozygosity for the 10-repeat allele at DAT1 gene ($n = 4$) compared to children without this genotype ($n = 4$; $p < 0.05$). Such findings provide a preliminary connection between pharmacogenetics and neurobiological investigations on stimulant treatment of ADHD.

Dopamine transporter gene, response to methylphenidate and cerebral blood flow in attention-deficit/hyperactivity disorder: A pilot study. Rohde LA, Roman T, Szobot C, Cunha RD, Hutz MH, Biederman J. Synapse 2003 May; 48 (2), pp. 87–9.

Neurolepsy: Genetic-Associated Variable Effect
Haloperidol

Background Information: Several factors may account for the variability recognized for individual responses to neuroleptic agents. Examples include variables such as length of illness before treatment, age at onset, and the presence of negative symptoms, and pharmacologic factors such as rates of drug metabolism. However, in the general population, notable pharmacodynamic differences in therapeutic response to haloperidol have been observed between Chinese and Caucasian patients. Response rates may also reflect clinical heterogeneity, although familiality, history of obstetric trauma, or ventricular enlargement have been shown to not be significant factors. It is also possible that genetic differences in receptors that are targets for these drugs may influence response.
Considerations: Factors that influence response to neuroleptic drugs are poorly understood.

Functional neuroimaging and pharmacogenetic studies of clozapine's action at dopamine receptors. Kerwin RW, Pilowsky L, Munro J, Shaikh S, Gill M, Collier D. J Clin Psychiatry 1994 Sep; 55 Suppl B, pp. 57–62.

Nicotine Sensitivity: Genetic-Associated Sensitivity/ Induced Alterations in Neurological Function
Nicotine

Cellular/Molecular Mechanism of Action: Nictotine binds to and stimulates cholinergic nicotinic receptor sites for the neurotranmitter acetylcholine within the parasympathetic segment of the autonomic nervous system.
Physiological Mechanism of Action: Parasympathomimetic activity.
Application: Delineation of genetic-associated alterations in neurologic function related to nicotine responsiveness, nicotinic receptor properties, and nicotine metabolisms/elimination.
Implementation: Male mice from 19 inbred strains serve as an investigative platform of determining their existing genetic difference in the response to nicotine exposure. Physiological parameters measured include the responses of respiratory rate, acoustic startle, Y-maze crosses, Y-maze rears, heart rate, and body temperature. Dose-response curves are then constructed for each strain on each test in a multitest battery.
Interpretation and Analysis: Physiological responses to nicotine are strongly influenced by animal genotype. Comparison of the results from 6 evaluation parameters measured in addition to observations reported for nicotine-induced seizures in the same strain of mice indicate that responses can be grouped into 2 major classes: a set characterized by Y-maze crosses, Y-maze rears, and body temperature, and a set characterized by seizure sensitivity and seizure latency. Responses observed for respiratory rate and startle response share characteristics with both of these sets, while the effects of nicotine on heart rate are fairly unique. The results have identified strains of mice that are differentially sensitive to the effects of nicotine.

Genetic influences on nicotine responses. Marks MJ, Stitzel JA, Collins AC. Pharmacol Biochem Behav 1989 Jul; 33 (3), pp. 667–78.

Pain Sensation/Threshold
Antinoceceptive Morphine Sensitivity

Background Information: Chromosomal loci containing genes affecting antinociceptive sensitivity to morphine have been identified, but virtually nothing is known about the genetic mediation of sensitivity to over-the-counter analgesics. Such knowledge would be of great clinical interest, as prodigious interindividual variability has been noted in the efficacy of these ubiquitously used drugs.
Application: Characterization of genetic variations in pain perceptions/response and evaluation of genetic difference in analgestic properties associated with non-steroidal anti-inflammatory drugs (NSAIDs).
Implementation: Assessed heritability and genetic correlations among 3 over-the-counter analgesics.

The heritability and genetic correlations among 3 over-the-counter analgesics have been assessed against noxious stimuli (e.g., 12 inbred murine strains utilizing the 0.9% acetic acid [i.p.] writhing test). Candidate analgesics have included the centrally acting analgesic acetaminophen (150 mg/kg, s.c.), the NSAIDs indomethacin (40 mg/kg, s.c.), and lysine-acetylsalicylic acid (800 mg/kg, s.c.).
Interpretation and Analysis: Evaluation of the response to the centrally acting analgesic, acetaminophen and the NSAIDs indomethacin and lysine-acetylsalicylic acid reveals significant differences between individual strains (genetically distinct populations) of mice. Narrow-sense heritability estimates range from 23 to 45%. Similar strains are sensitive and resistant, respectively, to the 2 NSAIDs ($r^s = 0.64$). In contrast, a completely different pattern of sensitivities is observed for acetaminophen, implying a genetic dissociation ($r^s = 0.29$ and 0.02) compared with the NSAID preparations. Related investigations performed on the 2 murine strains, C57BL/6 and DBA/2, show that they display extreme sensitivities to acetaminophen. Interestingly, the plasma acetaminophen levels in these strains of mice are not significantly different during the time of

antinociception assessment, suggesting the existence of genetic factors affecting acetaminophen pharmacodynamics rather than variations in pharmacokinetic parameters.

The heritability of antinociception II: Pharmacogenetic mediation of 3 over-the-counter analgesics in mice. Wilson SG, Bryant CD, Lariviere WR, Olsen MS, Giles BE, Chesler EJ, Mogil JS. J Pharmacol Exp Ther 2003 May; 305 (2), pp. 755–64.

Pain Sensation/Threshold/Genetic-Based Variability in Analgesia/(Non-Pharmacokinetic Related)
Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)/Acetaminophen/Indomethacin/Lysine-Acetylsalicylic Acid

Background Information: Chromosomal loci containing genes affecting antinociceptive sensitivity to morphine have been identified, but virtually nothing is known about the genetic mediation of sensitivity to over-the-counter analgesics. Such knowledge would be of great clinical interest, as prodigious interindividual variability has been noted in the efficacy of these ubiquitously used drugs.
Route of Administration: Acetaminophen (s.c. in mice), indomethacin (s.c. in mice), and lysine-acetylsalicylic acid (s.c. in mice).
Dosage: Acetaminophen (150 mg/kg in mice), and the NSAIDs indomethacin (40 mg/kg in mice) and lysine-acetylsalicylic acid (800 mg/kg in mice).
Implementation: Assessment of the heritability and genetic correlations among 3 over-the-counter analgesics on the 0.9% acetic acid (i.p.) writhing test (e.g., mice of 12 inbred mouse strains: C57BL/6 and DBA/2). Analgesics evaluated can include the centrally acting analgesic acetaminophen (150 mg/kg, s.c.), the NSAIDs indomethacin (40 mg/kg, s.c.), and lysine-acetylsalicylic acid (800 mg/kg, s.c.).
Interpretation and Analysis: Significant strain differences exist in sensitivity to centrally acting acetaminophen, in addition to the NSAIDS indomethacin and lysine-acetylsalicylic acid, with narrow-sense heritability estimates ranging from 23 to 45%. Similar strains of mice are sensitive and resistant, respectively, to indomethacin and lysine-acetylsalicylic acid ($r^s = 0.64$). In contrast, a completely different pattern of sensitivities is observed for acetaminophen, implying genetic dissociation ($r^s = 0.29$ and 0.02) compared with indomethacin and lysine-acetylsalicylic acid. Investgations performed in murine strains C57BL/6 and DBA/2 reveal extreme sensitivities to acetaminophen. Plasma acetaminophen levels in these strains are not significantly different during the time of antinociception assessment, suggesting the existence of genetic factors affecting acetaminophen pharmacodynamics rather than pharmacokinetics.

The heritability of antinociception II: Pharmacogenetic mediation of 3 over-the-counter analgesics in mice. Wilson SG, Bryant CD, Lariviere WR, Olsen MS, Giles BE, Chesler EJ, Mogil JS. J Pharmacol Exp Ther 2003 May; 305 (2), pp. 755–64.

Psychosis: Dopamine D2 Receptor Gene Taq I Polymorphism
Haloperidol

Background Information: Pharmacogenetic influences on therapeutic response to neuroleptic treatment are poorly understood.
Implementation: Treatment of patients with acute psychosis with haloperidol for 28 days (e.g., n = 57). Improvement and response are measured using a Positive and Negative Syndrome Scale. Genetic profiling for the DRD2 gene Taq I polymorphism is then performed (e.g., n = 41/57 vs n = 16/57 heterozygous).
Interpretation and Analysis: On all treatment days, heterozygous patients show a greater improvement in positive, but not in negative, symptoms compared to cases that are homozygous for allele 2. Differences in improvement of positive symptoms reach statistical significance on days 14, 21, and 28. On treatment day 14, approximately 62.5% (e.g., n = 10/16) of heterozygous patients have at least a 50% improvement of positive symptoms, compared with approximately 28.9% homozygous patients (e.g., n = 11/38). Genetic variations in the DRD2 gene may therefore influence response to antipsychotics.

Association of short-term response to haloperidol treatment with a polymorphism in the dopamine D(2) receptor gene. Schafer M, Rujescu D, Giegling I, Guntermann A, Erfurth A, Bondy B, Moller HJ. Am J Psychiatry 2001 May; 158 (5), pp. 802–4.

Schizophrenia: Familial Clusters of Disease
Lithium

Background Information: Selecting a drug according to the treatment response in a family relative has been widely accepted advice in the management of mood disorders. However, this recommendation has not been adequately substantiated in the literature. Minimal information is known as to what degree response to long-term lithium treatment is a familial trait.
Implementation: Comparison of the response to long-term lithium treatment in bipolar relatives of bipolar lithium responders and bipolar controls. Validation of a diagnosis for bipolar disorder can be determined using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) and Research Diagnostic Criteria (RDC). Lithium response is evaluated on a rating scale reflecting the quality and quantity of available data.
Interpretation and Analysis: Comparison of 24 relatives with bipolar disorder, identified in families of 106 cases with lithium-responsive bipolar disorder, to 40 lithium-treated bipolar cases serving as a comparison control reveals a prevalence of unequivocal response among relatives of 67%, compared to a response rate of 35% in a comparison group ($chi^2 = 6.04$, df = 1, p = 0.014). The highly significant difference in observed responses between relatives and reference control groups supports the perspective that lithium prophylaxis clusters exist within families and the general population.

Is response to prophylactic lithium a familial trait? Grof P, Duffy A, Cavazzoni P, Grof E, Garnham J, MacDougall M, O'Donovan C, Alda M. J Clin Psychiatry 2002 Oct; 63 (10), pp. 942–7.

Schizophrenia/Serotonin (2A) Receptor: 102-T/C Genetic Polymorphism/Risperidone Efficacy Variability
Risperidone

Application: Delineation of variations in risperidone efficacy as a function of 102-T/C polymorphism in the 5-HT(2A) receptor gene.
Implementation: Administration of rispiridone in patients suffering from acute exacerbated schizophrenia (e.g., n = 100 for 42 days) performed in combination with genotype analysis for 5-HT(2A) polymorphisms. Psychopathology is measured biweekly with the Positive and Negative Syndrome Scale during the time frame of risperidone administration. Generalized estimating equation methods are used to analyze the effects of treatment duration, T/C genotypes, and other prognostic factors on Positive and Negative Syndrome Scale performance.

Interpretation and Analysis: Patients with the C/C genotype have lower total scores, negative subscale scores, and general psychopathology scores but not positive subscale scores on the Positive and Negative Syndrome Scale than patients with the 102-T/C genotype. Patients with the T/C and T/T genotypes have comparable total and subscale scores. The number of previous hospitalizations and the dose of risperidone also affects Positive and Negative Syndrome Scale total scores.

Association of risperidone treatment response with a polymorphism in the 5-HT(2A) receptor gene. Lane HY, Chang YC, Chiu CC, Chen ML, Hsieh MH, Chang WH. Am J Psychiatry 2002 Sep; 159 (9), pp. 1593–5.

Schizophrenia: Serotonin (2A) Receptor: 102-T/C Genetic Polymorphism/Risperidone: Optimal Dosing Strategies (Acute Schizophrenia)
Risperidone

Background Information: The optimal risperidone dosing strategy for acute schizophrenia requires elucidation through the conduction of clinical research investigations. Plasma levels of risperidone and its active metabolite (9-hydroxyrisperidone) at a given dose vary greatly among different individuals. For patients who metabolize risperidone slowly, a medium dose results in excessively high plasma levels, which might be related to adverse events and perhaps poor response.
Application: Detection of pharmacogenetic variations related to therapeutic response and induced side effects associated with risperodone administration.
Dosage: Risperidone (6.0 mg/day × days if tolerated).
Implementation: Evaluate the potential for reductions in dose to diminish adverse reactions associated with ordinary risperidone administration, but still maintain efficacy for acutely exacerbated schizophrenia. Toward this objective cases of acutely exacerbated schizophrenia (DSM-IV) have been managed with titrated doses of risperidone over 3 days (6 mg/day if tolerated) with administration levels lowered if undesirable side effects become apparent. Clinical efficacy and side effects are then assessed on days 0, 4, 14, 28, and 42. End point steady state plasma levels of risperidone and 9-hydroxyrisperidone are subsequently measured by high-performance liquid chromatography analysis.
Human Model: Cases suffering from acutely exacerbated schizophrenia.
Interpretation and Analysis: Approximately 50% of cases (e.g., n = 17/31) can tolerate the 6-mg target dose without significant side effects, while as little as < 30% of individuals (e.g., n = 13/31) require lowering of dosage levels (mean ± SD = 3.6 ± 0.9 mg, p = 0.0001) in order to curtail treatment-induced side effects. At end point, 92.3% of low-dose individuals respond to treatment (20% or more reduction in total Positive and Negative Syndrome Scale score), compared with 52.9% of high-dose subjects (p < 0.05). No significant between-group differences exist in other minor efficacy measures. Of note, end point plasma levels of the active moiety (risperidone + 9-hydroxyrisperidone) are similar between the low- and high-dose groups (40.4 ± 31.1 ng/mL vs 49.7 ± 13.4 ng/mL, NS).

Risperidone appears to be efficacious in the treatment of acute exacerbation of schizophrenia up to a dosage level of 6 mg/day. Approximately 60% of such cases can tolerate the administration of risperidone at this dosage level. Alternatively, approximately 40% of cases require the use of substantially lower doses of risperidone (e.g., mean = 3.6 ± 0.9 mg) in order to reverse observed side effects despite achievement of appreciable efficacy. The 2 dose groups are comparable in the end point steady state plasma drug concentrations.
Instrumentation: High-performance liquid chromatography analysis utilizing an ultraviolet-based detector modality.

Risperidone in acutely exacerbated schizophrenia: Dosing strategies and plasma levels. Lane HY, Chiu WC, Chou JC, Wu ST, Su MH, Chang WH. J Clin Psychiatry 2000 Mar; 61 (3), pp. 209–14.

EXTRAHEPATIC—ENDOCRINE/REPRODUCTIVE

Estrogen Receptor-Alpha (ERα) Polymorphism
Estrogen

Cellular/Molecular Mechanism of Action: Estrogen receptors bind estrogen, which functions as a female sex steroid.
Physiological Mechanism of Action: Functions as a critical hormone that regulates a multitude of female physiological proceses and the viability of certain cancer cell types including human mammary carcinoma.
Background Information: Recent clinical trials of hormone replacement therapy for cardiovascular disease have yielded surprisingly negative results, shifting clinical opinions from a position of presumed cardiovascular benefit to 1 of confirmed harm. Understanding why hormone replacement therapy has beneficial effects on intermediate risk markers for cardiovascular disease, but produces an increase in cardiovascular events, is an important public health question with the potential to elucidate fundamentally important aspects on atherogenesis, cardiovascular disease, and the biology of estrogen action. One question concerning the cardiovascular effects of hormone replacement therapy is whether genetic factors can substantially modify individual responses to estrogen treatment. New clinical trial evidence is emerging that links the presence of particular variants in the estrogen receptor to the response of high-density lipoprotein and other intermediate end points to hormone replacement therapy.
Application: Characterization of ERα polymorphism and correlation of these findings to observations related to response to estrogen therapy and the risk of clinical cardiovascular events.
Interpretation and Analysis: One or more common variants in ERα are associated with a differential response to hormone replacement therapy in several domains of estrogen action. However, the effect of these variants on the risk of clinical cardiovascular events in the setting of hormone replacement therapy is not yet known. Additional research focusing on the clinical impact of common variants in ERα, ERβ, and the progesterone receptor promise to improve clinical decision making concerning the use of hormone replacement therapy and other novel estrogen agonists.

Role of estrogen receptor-alpha in pharmacogenetics of estrogen action. Herrington DM. Curr Opin Lipidol 2003 Apr; 14 (2), pp. 145–50.

Hypogonadal States: Androgen Receptor Gene [AR $(CAG)_n$ Polymorphism]/Secondary Prostate Enlargement
Testosterone/T-Enanthate/T-Undecanoate/Human Chorionic Gonadotropin

Background Information: Testosterone (T) substitution in hypogonadal men results in growth of the prostate gland. The effects of testosterone are mediated via the AR. The length of the $(CAG)_n$ polymorphism of the AR gene is negatively associated with transcriptional activity and might account for variations in prostate growth during substitution therapy.
Application: Characterization of AR gene $(CAG)_n$ polymorphism to detect increased susceptibility to secondary enlargement/

growth of the prostate in hypogonadal males receiving androgen substitution therapy.

Implementation: Assessment of the influence of various testosterone preparations on enlargement of the prostate as a function of AR $(CAG)_n$, sex hormone levels, and anthropometric measures. Studies directed at achieving this objective have evaluated hypogonadal males (e.g., n = 131, 18–69 yrs) by (i) assessing prostate volume longitudinally (transrectal ultrasonography), in concert with the evaluation of (ii) AR $(CAG)_n$, (iii) sex hormone levels, and (iv) anthropometric measures. Substitution therapy with IM injections of T enanthate (n = 81), transdermal T preparations (n = 19), SQ injections of human chorionic gonadotropin (n = 17), or oral T undecanoate (n = 14) is then initiated over a prolonged period of administration (e.g., n = 69 males with primary and n = 62 males with secondary hypogonadism for 2.4 ± 0.8 years).

Interpretation and Analysis: Average prostate size increases from approximately 15.8 ± 6.1 ml to 23.0 ± 6.8 ml. ANOVA including covariates reveals initial prostate size to be dependent upon age ($p < 0.001$) and baseline T levels ($p = 0.01$) but not on the number of $(CAG)_n$ (ranging from 13 to 30; mean, 21.4 ± 3.5). Prostate growth per year and absolute prostate size under substituted T levels (6.1 ± 3.3 to 21.6 ± 10.3 nmol/liter) are strongly dependent on $(CAG)_n$, with lower treatment effects in longer repeats (both $p < 0.001$). Other significant predictors include initial prostate size (negative for growth rate and positive for absolute size) and age (positive for both growth rate and absolute size). The odds ratio for men with $(CAG)_n < 20$, compared with those with $(CAG)_n$ of 20 or more, to develop a prostate size of at least 30 ml under testosteroine substitution is 8.7 (95% confidence interval, 3.1–24.3; $p < 0.001$). Such observations demonstrate a strong age-related dependence with a more pronounced odds ratio in males older than 40 years of age.

Prostate volume and growth in testosterone-substituted hypogonadal men are dependent on the CAG repeat polymorphism of the androgen receptor gene: A longitudinal pharmacogenetic study. Zitzmann M, Depenbusch M, Gromoll J, Nieschlag E. J Clin Endocrinol Metab 2003 May; 88 (5), pp. 2049–54.

Progesterone/Testosterone Nuclear Receptor: Constitutive Active Receptor (CAR)
Testosterone/Progesterone

Background Information: Steroid hormones modulate activity of the nuclear receptor CAR (or constitutive androstane receptor) in mouse liver. Progesterone and testosterone repress the constitutive activity of mouse CAR (mCAR) in cell-mediated transfection assays, whereas estrogens activate the repressed receptor. Similar repression and activation phenomenon are not observed with CAR expressed in human cell types.

Application: Utilization of various chimeric and mutated receptor complexes to determine their responsiveness to steroid hormones in a manner that can delineate the structural basis that confers hormone responsiveness to murine CAR (mCAR).

Interpretation and Analysis: Hormone responsiveness resides near or within the AF-2 domain of mCAR. Moreover, a single mutation of threonine at position 350 to the corresponding methionine in the human counterpart abolishes the repression of mCAR by steroid hormones. Co-activation by steroid receptor co-activator 1 (SRC-1) of mCAR does not depend on the threonine 350 amino acid residue. However, overexpression of SRC-1 counteracts progesterone to repress mCAR activity. Thus, threonine at amino acid residue 350 seems to regulate hormone responsiveness of mCAR (indirect interference) and interaction of the receptor with a coactivator.

Residue threonine 350 confers steroid hormone responsiveness to the mouse nuclear orphan receptor CAR. Ueda A, Kakizaki S, Negishi M, Sueyoshi T. Mol Pharmacol 2002 Jun; 61 (6), pp. 1284–8.

EXTRAHEPATIC—RENAL SYSTEM

Cortisol Metabolism/$^{11}\beta$-HSD_2 Biochemical Activity (Renal)
Deuterium-Labeled Cortisol

Cellular/Molecular Mechanism of Action: Deuterium-labeled cortisol is a "tracer" analog of endogenous cortisol.

Application: Deuterium-labeled cortisol can be used as a labeled marker molecule to access *in vivo* alterations in endogenous cortisol metabolism mediated by $^{11}\beta$-HSD_2 activities based on urinary excretion of cortisol, cortisone, and their A-ring reduced metabolites.

Route of Administration: Deuterium-labeled cortisol (oral).

Implementation: Evaluation of cortisol metabolism through the measurement of deuterium-labeled cortisol and its deuterium-labeled metabolites. Sample evaluation involves the application of GC/MS analyses performed simultaneously with the detection of endogenous cortisol, cortisone, and their A-ring reduced metabolites.

Human Model: Normal cases without evidence of disease.

Interpretation and Analysis: The stable isotope approach offers unique advantages in assessing the appropriateness of measuring unconjugated and total (unconjugated + conjugated) cortisol, cortisone, and their A-ring reduced metabolites in urine as indices of renal $^{11}\beta$-HSD_2 activity. Measurement of urinary unconjugated cortisol and cortisone has many advantages for assessing $^{11}\beta$-HSD_2 activity.

Instrumentation: Integrated gas chromatography/mass spectrometry system.

The use of deuterium-labeled cortisol for *in vivo* evaluation of renal $^{11}\beta$-HSD_2 activity in man: Urinary excretion of cortisol, cortisone and their A-ring reduced metabolites. Kasuya Y, Shibasaki H, Furuta T. Steroids 2000 Feb; 65 (2), pp. 89–97.

Excretion Profiling: Renal Tubular Function/Tubular Transporter Mechanisms
[^{3}H]-Indoxyl Sulfate/Para-Aminohippuric Acid (PAH)/Benzylpenicillin/Cimetidine/3-Carboxy-4-Methyl-5-Propyl-2-Furanpropanoic Acid/Hippuric Acid

Cellular/Molecular Mechanism of Action: Indoxyl sulfate is a uremic toxin and radiolabeled analogs can function as tracer molecules.

Background Information: Indoxyl sulfate is a uremic toxin that accumulates in the body because of the patient's inability to excrete it, and it induces a number of uremic symptoms and leads to chronic renal failure. The functional failure of the excretion system for indoxyl sulfate causes its accumulation in blood.

Application: Uremic toxins (indoxyl sulfate; 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid hippuric acid); weak acids (PAH; benzylpenicillin); weak bases; (cimetidine); and other substances (salicylic acid, indomethacin, 3,5,3′-triiodo-l-thyronine, and indole acetic acid [IA]) can be applied in various fashions to evaluate renal excretory function.

Implementation: Evaluate and characterize transport mechanisms for the renal excretion of [^{3}H]indoxyl sulfate using an *in vivo* tissue-sampling single-injection technique (kidney uptake index [KUI] method).

Animal Model: Xenopus laevis oocyte expressing rat organic anion transporter 3 (rOAT3).
Interpretation and Analysis: Probenecid shows a concentration-dependent inhibitory effect on the uptake of [^{3}H]indoxyl sulfate using the KUI method, and uptake is inhibited by organic anions such as PAH and benzylpenicillin, by weak bases such as cimetidine, and by uremic toxins, such as 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) and hippuric acid (HA). However, salicylic acid, indomethacin, 3,5,3′-triiodo-l-thyronine, and indole acetic acid (IA) have no effect on the uptake. rOAT3-expressing oocytes exhibit uptake of [^{3}H]indoxyl sulfate by rOAT3 (Km = 158 μmol/L). Moreover, a number of uremic toxins inhibit the uptake of [^{3}H]indoxyl sulfate by rOAT3. Uptake of indoxyl sulfate appears, therefore, to be mediated by rOAT3 activity. Uremic toxins share the transport mechanism for indoxyl sulfate. Mutual inhibition of these uremic toxins via OAT3 may accelerate their accumulation in the body and, thereby, the progression of nephrotoxicity in uremia.

Major role of organic anion transporter 3 in the transport of indoxyl sulfate in the kidney. Deguchi T, Ohtsuki S, Otagiri M, Takanaga H, Asaba H, Mori S, Terasaki T. Kidney Int 2002 May; 61 (5), pp. 1760–8.

Hydroxylation Capacity: Renal Metabolizing Capacity
Phenylalanine

Cellular/Molecular Mechanism of Action: Phenylalanine is metabolized in the kidney to a hydroxylated analog.
Background Information: Although phenylalanine hydroxylase activity is detectable in *in vitro* renal tissue preparations, no data on *in vivo* phenylalanine hydroxylation in the human kidney, or its possible contribution to whole-body hydroxylation, currently exist.
Application: Evaluation of the hydroxylation capacity of the kidney.
Implementation: Evaluate measurements of whole-body, renal, and splanchnic phenylalanine hydroxylation to tyrosine, as well as phenylalanine and tyrosine rates of appearance (Ra) and disposal (Rd), in postabsorptive states by means of renal and splanchnic arteriovenous catheterization combined with phenylalanine and tyrosine isotope infusions.
Interpretation and Analysis: In the kidney, relevant phenylalanine hydroxylation activity can be detected (3.51 ± 0.97 μmol/min × 1.73 m^2 of body surface), whereas it is 2.48 + 1.35 μmol/min × 1.73 m^2 across the splanchnic area. These 2 sites together account for virtually the entire whole-body phenylalanine hydroxylation capacity. Renal production of tyrosine from phenylalanine hydroxylation accounts for approximately 13% of whole-body tyrosine Ra, whereas renal total tyrosine Ra account for approximately 34% of whole-body tyrosine Ra. In the splanchnic area, these figures are approximately 9% and 40%, respectively. Hydroxylation accounts for approximately 70% of phenylalanine Rd in the kidney, as opposed to approximately 8% in the splanchnic area.

Phenylalanine hydroxylation across the kidney in humans rapid communication. Tessari P, Deferrari G, Robaudo C, Vettore M, Pastorino N, De Biasi L, Garibotto G. Kidney Int 1999 Dec; 56 (6), pp. 2168–72.

EXTRAHEPATIC—RESPIRATORY SYSTEM

Ventilatory Responses: Regulation
S-Nitrosylation

Background Information: New genomic and proteomic techniques offer remarkable promise as tools to address long-standing questions regarding molecular mechanisms involved in the control of breathing.
Interpretation and Analysis: Application of genomic and proteomic techniques and related molecular methodologies are providing valuable observations and insight into the role of S-nitrosylation signaling reactions in regulating ventilatory responses.

Functional genomics and proteomics in control of breathing. Gaston B. Respir Physiol Neurobiol 2003 May 30; 135 (2–3), pp. 231–8.

EXTRAHEPATIC—MUSCULOSKELETAL SYSTEM

Malignant Hyperthermia (MH)
Caffeine/Diffusible CO_2

Background Information: Susceptibility to MH can be diagnosed by performing an *in vitro* contracture test, which requires acquiring an open muscle biopsy sample.
Route of Administration: Caffeine (intramuscular).
Dosage: Caffeine (500 ml of an 80 mmol/L formulation).
Implementation: Evaluate the influence of intramuscular caffeine injection on local carbon dioxide pressure (pCO_2) in conditions of MH compared to normal negative reference controls. Measurements for pCO_2 are obtained from the rectus femoris muscle during local stimulation with injected caffeine.
Human Model: Cases susceptible to MH (n = 12), cases classified as non-susceptible (n = 8), and 7 healthy/normal controls.
Interpretation and Analysis: In susceptible individuals, pCO_2 temporarily increases to a maximum of 63 mm Hg (IQR 61–69) compared with 44 mm Hg (43–47) for non-susceptible participants (p = 0.003) and 42 mm Hg (41–46) in controls (p = 0.003). Systemic side effects usually cannot be appreciated. Metabolic monitoring of the highly diffusible CO_2 stimulated by caffeine might allow a minimally invasive test for susceptibility to MH.

Diagnosis of susceptibility to malignant hyperthermia by use of a metabolic test. Anetseder M, Hager M, Müller CR, Roewer N. Lancet 2002 May 4; 359 (9,317), pp. 1579–80.

Malignant Hyperthermia (MH): Genetic Predisposition/(*Ex Vivo* Preaparation of Skeletal Muscle Biopsies)
Caffeine/Caffeine and Halothane

Application: Caffeine and caffeine + halothane can be applied as investigative probes to characterize certain intrinsic properties of skeletal muscle tissues. In this context, caffeine and caffeine + halothane can serve as indicator agents for the detection of individuals with a genetic predisposition to the development of MH (potentially fatal complication of general anesthesia).
Implementation: Skeletal muscle in *ex vivo* preparations is exposed to caffeine or caffeine + halothane and the subsequent induction of contracture responses is then measured for comparison with negative reference controls.
Interpretation and Analysis: In confirmed or suspected cases of MH and their genetic relatives, the responsiveness of muscle specimens can often vary over 2 orders of magnitude. Frequency distribution curves suggest that variations do not represent a continuum but rather are represented as 3 or more clusters of functional variants. Muscle specimens from males are on average more responsive to caffeine than is skeletal muscle collected from female subjects. Correlations within father-son and brother-brother pairs indicate complete heritability of responsiveness. Interestingly, there appears to be a lack of correlation between induced muscle contraction of muscle

biopsies collected from mother-daughter pairs. There is an intermediate correlation in father-daughter pairs. The sex difference in heritability could be due to gender-related modifying genes or due to secondary modification of the muscle response in females by sex-related, perhaps hormonal factors. Among the effects of age appeared to be poor development in early childhood of the potentiation of the caffeine contracture by halothane.

Pharmacogenetics of caffeine and caffeine-halothane contractures in biopsies of human skeletal muscle. Kalow W, Sharer S, Britt B. Pharmacogenetics 1991 Dec; 1 (3), pp. 126–35.

Malignant Hyperthermia (MH): Genetic Variation (Arg614→Cys: RYR1 C1840T)/Genomic Analyses and Provocative Testing Correlation
Caffeine/Halothane

Physiological Mechanism of Action: In conditions of MH, exposure of *ex vivo* muscle tissues to caffeine/halothane promotes an exaggerated contractile response.
Background Information: Malignant hyperthermia is an important cause of anesthesia-induced death. The caffeine/halothane contracture test (CHCT) test is highly invasive, expensive, and lacks 100% specificity. Genetic and biochemical evidence provide strong support for the view that substitution of cysteine for arginine 614 (Arg614Cys) in the human ryanodine receptor gene is 1 of several mutations that are likely to cause human MH.
Application: Detection and characterization of MH conditions.
Route of Administration: Traditionally applied by topical exposure for *ex vivo* tissue-based challenge studies.
Implementation: Delineation of a possible genetic basis for a predisposition to MH pertaining to the presence of Arg614Cys (RYR1 C1840T) mutations detected through a combination of polymerase chain reaction and restriction endonuclease digestion. Blood samples are utilized as a source of biological material for DNA analyses. Alternatively, detection of MH susceptibility can be established using the *in vitro* CHCT that employs fresh muscle biopsy specimens. Results from CHCT and DNA-based diagnostic parameters are then compared to determine their potential value in predicting MH susceptibility in large MH families that possess Arg614Cys mutations.

Human Model: Cases selected from a large Mennonite MH kindred identified by an index patient who died at age 45 years of an MH crisis after general anesthesia. Case population: n = 68 family members, n = 19/68 muscle biopsies, n = 1/681 documented crisis without biopsy. Family members can be classified as MH-susceptible or MH-normal on the basis of the CHCT.
Interpretation and Analysis: Previous investigations that have analyzed family clusters of MH have identified approximately 33% of relatives to be heterozygous for the Arg614Cys mutation (n = 22/68) and slightly less than one-fourth may have prior positive CHCT results (e.g., n = 5/22), while a small percentage may have an MH crisis without the benefit of a previous biopsy screening procedure. Utilizing DNA testing, it is possible to detect a relatively large number of cases within a related kindred that are normal for the allele (e.g., n = 44). Of these, slightly less than 25% can be classified as MH-normal while some may be determined to be MH-susceptible (e.g., n = 5) on the basis of CHCT evaluations. Based on the interpretation of the data obtained in earlier CHCT diagnoses, the condition of the muscle is poor, with no twitch, for approximately 60% of cases homozygous for the normal allele but originally classified as MH-susceptible (e.g., n = 3/5) and for a small percentage that are homozygous for the normal allele and originally classified as MH-normal. Observations from CHCT results performed for such individuals (e.g., n = 4) can often be considered invalid. The twitch response is good for a small number of cases (e.g., n = 2 remaining) that are homozygous for the normal allele but classified as MH-susceptible if contracture can be observed with appropriately low levels of both caffeine and halothane.

An absolute correlation between DNA test results and CHCT assignment can often not be established. Possible explanations for discordance are that the Arg614Cys mutation is not linked to MH, that a 2nd MH mutation is segregating in the family, or that there are errors in the CHCT. Because there is strong evidence supporting the causal nature of the Arg614Cys mutation, the discordant cases are not closely related within the pedigree as they would be if a 2nd MH mutation were segregating, and the CHCT is not 100% accurate. It has therefore been proposed that observed discordance between DNA test results and CHCT assignment in example kindred analyses can be attributed to false-positive diagnoses associated with the CHCT (e.g., n = 2 tests).

Comparison of the segregation of the RYR1 C1840T mutation with segregation of the caffeine/halothane contracture test results for malignant hyperthermia susceptibility in a large Manitoba Mennonite family. Serfas KD, Bose D, Patel L, Wrogemann K, Phillips MS, MacLennan DH, Greenberg CR. Anesthesiology 1996 Feb; 84 (2), pp. 322–9.

Malignant Hyperthermia (MH): Ryanodine Receptor (RYR1) Gene
Halothane

Background Information: Malignant hyperthermia is a potentially lethal disease triggered by volatile anesthetics and succinylcholine in genetically predisposed individuals. Because of the heterogenetic nature of MH, a simple genetic-based diagnostic test is not feasible and diagnosis requires an invasive open muscle biopsy followed by performing an *in vitro* contracture test (IVCT).
Implementation: Determine if measurements of halothane-induced increases in intracellular calcium ion concentration $[Ca^{2+}]_i$ in cultured human skeletal muscle cells can be used to phenotype MH susceptibility and if different mutations in the RYR1 gene affect halothane-induced increases in $[Ca^{2+}]_i$.
Primary cultures of human skeletal muscle cells are acquired from cases previously determined to suffer from MH. Cell lines are then individually screened for the presence of the most common mutations in the RYR1 gene. $[Ca^{2+}]_i$ is measured in 10 cells from each case that have been subjected to 5 different halothane concentrations.

Human Model: Cases of MH (n = 54) diagnosed by the IVCT according to the protocol of the European MH Group and classified as MH-susceptible (n = 22), MH-negative (n = 18), or MH-equivocal (n = 14).
Interpretation and Analysis: Halothane-induced increases in $[Ca^{2+}]_i$ vary significantly between different classification groups. Interestingly, different mutations of the RYR1 gene do not have a specific impact on halothane-induced increases in $[Ca^{2+}]_i$. Therefore, measurements of $[Ca^{2+}]_i$ in human skeletal muscle cells can be used to phenotype MH susceptibility; however, mutation in the RYR1 gene do not closely correlate with specific effects related to halothane-induced increases in $[Ca^{2+}]_i$.
Instrumentation: Fluorescent digital microscopy using fura-2/AM can be applied for the measurement of calcium ion concentrations contained within biological samples.

Phenotyping malignant hyperthermia susceptibility by measuring halothane-induced changes in myoplasmic calcium concentration in cultured human skeletal muscle cells. Girard T, Treves S, Censier K, Mueller CR, Zorzato F, Urwyler A. Br J Anaesth 2002 Oct; 89 (4), pp. 571–9.

Malignant Hyperthermia (MH)
Caffeine/Halothane/Calcium-Induced Calcium Release

Application: Detection and characterization of MH conditions.
Route of Administration: Topical application (emersion) of *ex vivo* muscle biopsy specimens.
Implementation: Comparison of results of the *in vitro* caffeine-halothane contracture test (CHCT) according to the protocols of the North American Malignant Hyperthermia Group (NAMHG) and the European Malignant Hyperthermia Group (EMHG) with the Ca^{++}-induced Ca^{++} release (CICR) rate test. Muscle biopsies are usually obtained from the musculus vastus lateralis. Diagnostic cutoff points and procedures for CHCT protocols are as described in the original and renewal versions of NAMHG and EMHG. The CICR rate test is performed according to the protocol reported by Endo et al.

Human Model: Cases of suspected MH (n = 16; and n = 5 normal negative reference controls).
Interpretation and Analysis: Normal controls and cases with abortive MH (e.g., n = 2), postoperative hyperthermia (e.g., n = 2), and high serum creatinine kinase levels (e.g., n = 3) may all display normal results in diagnostic screening tests. According to NAMHG criteria, patients with MH reactions (e.g., n = 3) or with a history of masseter spasms (e.g., n = 1) may be classified as MH-positive, while the EMHG guidelines would classify such cases as MH-susceptible and MH-equivocal, respectively. Some patients may display discordant findings based on a comparison of results from CHCT and CICR rate tests (e.g., n = 5 cases). It has therefore been proposed that muscle biopsy for the diagnosis of MH susceptibility should combine the CHCT with the CICR rate test, which may identify the defective site of Ca^{++} release channels.

Comparison of the *in vitro* caffeine-halothane contracture test with the Ca-induced Ca release rate test in patients suspected of having malignant hyperthermia susceptibility. Oku S, Mukaida K, Nosaka S, Sai Y, Maehara Y, Yuge O. J Anesth 2000 Jan 20; 14 (1), pp. 6–13.

Malignant Hyperthermia (MH)
4-Chloro-m-Cresol (4-CmC)/Caffeine/Halothane

Background Information: *In vitro* contracture test (IVCT) for diagnosis of MH has been reported to have a sensitivity of 100% and a specificity of 93%. The results are equivocal in 10 to 15%, and supplementary tests may thus be required.
Application: The agents 4-CmC, caffeine, and halothane can be applied to detect and characterize conditions of MH.
Implementation: Evaluation of the potential for 4-CmC to function as a supplementary test for MH. One investigative approach involves the collection of muscle tissue in the form of biopsy specimens for utilization in an IVCT. In this context, muscle biopsies are topically exposed *ex vivo* to increasing concentrations of 4-CmC (25, 50, 75, 100, 150, and 200 μmol/l) and the corresponding force profiles recorded. Diagnosis of MH susceptibility is established with standard halothane and caffeine tests and classified as MHS (MH-susceptible), MHN (MH-negative), or MHE (equivoval result).

Human Model: Cases subjected to skeletal muscle biopsy (n = 41 from n = 7 families).
Interpretation and Analysis: At all concentrations of 4-CmC, the increase in baseline force is significantly greater in the MHS group compared to the MHN group ($p < 0.05$). Muscle from MH-susceptible cases (e.g., n = 15) responds to 4-CmC with increasing force at a threshold concentration of 75 μmol/l or less, whereas muscle tissues from cases classified as MH-non-susceptible (e.g., n = 23) have thresholds of 100 μmol/l or more. The accuracy of the chlorocresol test is approximately 100% (95% confidence limits 90.75–100%) at a threshold of 75 μmol/l. Amplitude of contractures at 2 mmol/l caffeine is not different from contractures at 75 μmol/l of 4-CmC in either the MHS or the MHN group ($p > 0.05$). *In vivo* concentrations of chlorocresol from clinical use of insulin and somatropin are estimated to be 20 times less than the threshold concentration and thus these drugs seem safe in MH cases. Therefore, 4-CmC may be a suitable aid to clarify puzzling results of standard testing of MH susceptibility.

4-Chloro-m-cresol test—A possible supplementary test for diagnosis of malignant hyperthermia susceptibility. Ording H, Glahn K, Gardi T, Fagerlund T, Bendixen D. Acta Anaesthesiol Scand 1997 Sep; 41 (8), pp. 967–72.

Rhabdomyolysis: Recurrent/Exertional (Equine)/Myoplasmic Calcium Concentration Estimation/Heritable Myopathy
Caffeine/4-Chloro-m-Cresol (4-CmC)

Physiological Mechanism of Action: Caffeine promotes contraction of skeletal muscle tissue in *ex vivo* preparations.
Route of Administration: Topical (*ex vivo)* bathing of muscle tissue biopsy specimens.
Implementation: Delineate if alterations in myoplasmic calcium regulation can be identified in muscle cell cultures (myotubes) and intact muscle fiber bundles derived from Thoroughbreds affected by recurrent exertional rhabdomyolysis (RER). Laboratory procedures that can be employed to address such an objective may utilize myotube cell cultures grown from satellite cells obtained from muscle biopsy specimens from horses affected by RER. Fura-2 fluorescence can be used to measure resting myoplasmic calcium concentration as well as caffeine- and 4-CmC-induced increases in myoplasmic calcium. In addition, intact intercostal muscle fiber bundles are prepared from both types of horses, and their sensitivities to caffeine and 4-CmC-induced contractures determined.

Animal Model: Equine (n = 6 related Thoroughbreds with RER; n = 8 clinically normal control Thoroughbred or crossbred horses).
Interpretation and Analysis: Myotubes of RER-affected and control horses have identical resting myoplasmic calcium concentrations. Myotubes from RER-affected horses have significantly higher myoplasmic calcium concentrations than myotubes from control horses following the addition of ≥ 2mM caffeine; however, there is no difference in their response to 4-CmC (≥ 1 mM). Caffeine contracture thresholds for RER and control intact muscle cell bundles (2 vs 10mM, respectively) are significantly different, but 4-CmC contracture thresholds of muscle bundles from RER-affected and control horses (500 μM) do not differ. An increase in caffeine sensitivity for muscle cells derived from a family of related RER-affected horses has been detected *in vitro* by use of cell culture with calcium imaging and by use of fiber bundle contractility techniques. An alteration in muscle cell calcium regulation is a primary factor in the cause of this heritable myopathy.

Myoplasmic calcium regulation in myotubes from horses with recurrent exertional rhabdomyolysis. Lentz LR, Valberg SJ, Herold LV, Onan GW, Mickelson JR, Gallant EM. Am J Vet Res 2002 Dec; 63 (12), pp. 1724–31.

PHARMACOGENETICS OF PROGNOSIS AND THERAPEUTIC RESPONSE

The number of citations described in this section represents only a small segment of the extensive amount of research and clinical knowledge developed to date related to this particular subject.

Chemotherapeutic Resistance Potential/ Radioresistance: Mammary Carcinoma
Insulin-Like Growth Factor-I Receptor mRNA/Antisense Insulin-Like Growth Factor-I Receptor (IGF-IR)

Cellular/Molecular Mechanism of Action: The IGF-IR plays a critical role in breast cancer cell growth regulation and transformation. Antisense IGF-IR nucleotides inhibit the transcriptin of IGF-IR mRNA gene sequences.
Application: Expression of insulin like growth factor I receptor mRNA can serve as a prognostic markers pertaining to the potential of antisense IGF-IR to reverse resistance to radiation and chemotherapy in neoplastic cell populations (e.g., mammary carcinoma). Strategies designed to disrupt the biological expression of IGF-IR may be a fruitful avenue for achieving enhanced resolution of neoplastic disease states.
Route of Administration: Antisense IGF-IR nucleotide (intravenous).
Dosage: Antisense IGF-IR nucleotide.
Implementation: Evaluaton of the radiosensitivity of NIH 3T3 fibroblasts overexpressing either wild-type or mutant IGF-IR complex, complemented by immunohistochemical analysis of primary mammary carcinoma neoplastic lesions for the expression of IGF-IR complexes.
Interpretation and Analysis: High levels of wild-type IGF-IR confer radioresistance, and mutational analysis has revealed that this effect correlates with the transforming capacity but not the mitogenic activity of the receptor. The radioresistant phenotype is reversed when the cells are incubated with antisense oligonucleotides targeted to IGF-IR mRNA, demonstrating that IGF-IR directly influences radioresistance. Immunohistochemical analysis of primary breast tumors reveals that high levels of IGF-IR in tumor samples highly correlates with ipsilateral breast tumor recurrence (IBTR) following lumpectomy and radiation therapy ($p = 0.001$). Subgroup analysis reveals that, for early breast tumor relapses (within 4 years of initial breast tumor diagnosis), a strong association exists between elevated IGF-IR levels and ipsilateral breast tumor recurrence (IBTR, $p = 0.004$). However, IGF-IR expression appears to have minimal prognostic value for IBTR from breast cancer patients with late relapses ($p = ns$).

Insulin-like growth factor-I receptor overexpression mediates cellular radioresistance and local breast cancer recurrence after lumpectomy and radiation. Turner BC, Haffty BG, Narayanan L, Yuan J, Havre PA, Gumbs AA, Kaplan L, Burgaud JL, Carter D, Baserga R, Glazer PM. Cancer Res 1997 Aug 1; 57 (15), pp. 3079–83.

Colorectal Cancer: Metastatic Potential
Tegafur-Uracil/CD44 Expression/Thymidylate Synthase Expression

Cellular/Molecular Mechanism of Action: Tegafur-uracil may induce apoptosis of tumor cells by inhibition of thymidylate synthase. The cell adhesion molecule CD44 may contribute to the pathogenesis of metastatic processes.
Background Information: Tegafur-uracil is commonly used to treat digestive cancers. However, the inhibition of metastasis by this agent has not been fully characterized.
Application: Expression of CD44 could potentially be used as a possible independent predictor of survival and represents an independent prognostic factor. Tegafur-uracil, via the inhibition of CD44 expression, may promote an inhibition of distant metastasis. Analysis of CD44 expression and apoptosis phenomenon may serve as predictive variables pertaining to potential efficacy of tegafur-uracil.
Route of Administration: Tegafur-uracil (oral/per os).
Dosage: Tegafur-uracil—preoperatively (600 mg SID $\times$ 3–7 days); postoperatively (400 mg SID $\times$ 2 years).
Implementation: Characterization of CD44 and apoptosis phenomenon in neoplastic tissue harvested from cases subjected to curative resection of colorectal cancer (e.g., $n = 66$). Postoperative therapy includes tegafur-uracil (600 mg) administered orally every day for 3 to 7 days before surgery, and tegafur-uracil (400 mg) administered orally every day for 2 years after surgery.

Detection of CD44 and apoptosis is determined in neoplastic tissue samples applying immunohistochemical analysis and by the TUNNEL method, respectively. The thymidylate synthase inhibition rate is calculated from the total thymidylate synthase level, and free thymidylate synthase levels by a modified Spears' method using fresh tumor tissue specimens.
Interpretation and Analysis: The thymidylate synthase inhibition rate of non-recurrent cases is significantly higher than that of recurrent cases ($p < 0.05$). The 5-year survival rate in CD44-low grade positive/negative patients (81.6%) is significantly higher than that in CD44-high grade positive patients (46.4%) ($p < 0.005$). The 5-year survival rate in apoptosis-high grade positive patients (89.7%) is significantly higher than that in apoptosis-low grade positive/negative patients (46.4%) ($p < 0.001$). With respect to the relationship between CD44 and apoptosis, the proportion of apoptosis-high grade positive patients among CD44-low grade positive/negative patients (55.3%) is significantly higher than that among CD44-high grade positive patients (28.6%) ($p < 0.05$). Multivariate analysis suggests that CD44 expression may represent an independent prognostic factor.

Do the expression of CD44, apoptosis, and thymidylate synthase inhibition rate correlate with the efficacy of chemotherapy in colorectal cancer? Tachikawa D, Arima S, Futami K, Iwashita A. Anticancer Res 2000 Jan–Feb; 20 (1C), pp. 595–600.

Diamminedichloroplatinum (II) Resistance/ Squamous Cell Carcinoma: Head and Neck (HNSCC)/Membrane Protein Fraction Markers: Glycoprotein Hormone Marker

Cellular/Molecular Mechanism of Action: The chemotherapeutic agent *cis*-diamminedichloroplatinum (II) (cDDP) exerts antineoplastic properties by promoting intrastrand and interstrand DNA cross-linking.
Background Information: Mechanisms involved in the acquired resistance of cancer cells to to *cis*-diamminedichloroplatinum (II) (cDDP) have not been thoroughly characterized.
Application: Discovery of gene sequences and protein fractions expressed by head and neck squamous cell carcinoma that correlate with resistance and vulnerability to the antineoplastic properties of *cis*-diamminedichloroplatinum (II).
Implementation: Gene expression profiles can be established for HNSCC cell populations that are both sensitive and resistant to the antineoplastic properties of *cis*-diamminedichloroplatinum (II). Both a fluorescent differential display and cDNA microarray analyses can be applied to distinguish gene profiles expressed between KB, a human HNSCC line, and its cDDP-resistant variant (KB/cDDP). Laboratory findings can subsequently be validated by Northern blot analysis.
Interpretation and Analysis: One upregulated gene, glycoprotein hormone alpha-subunit, and 2 downregulated genes coding membrane proteins, human folate receptor and tumor-associated

antigen L6, have been identified in KB/cDDP cell lines. Based on these findings, it is believed that development of cDDP-resistant phenotypes is accompanied by alternations of gene expression including a glycoprotein hormone and membrane proteins. Such gene products could represent new molecular markers for identifying cDDP resistance.

Differentially expressed genes associated with CIS-diamminedichloroplatinum (II) resistance in head and neck cancer using differential display and CDNA microarray. Higuchi E, Oridate N, Furuta Y, Suzuki S, Hatakeyama H, Sawa H, Sunayashiki-Kusuzaki K, Yamazaki K, Inuyama Y, Fukuda S. Head Neck 2003 Mar; 25 (3), pp. 187–93.

Leukemia and Lymphoma/Dihydropteridine Reductase/Efficacy of Chemotherapeutic Intervention
Neopterin: [Urine]

Application: Neopterin levels in the urine and dihydropteridine reductase concentrations have been applied as sensitive and reliable early prognostic marker systems pertaining to the severity of malignancy and long-term survival in conditions of leukemia and lymphoma ($p < 0.00001$). Alterations in concentrations of these prognostic marker systems in response to chemotherapy can provide prognostic information pertaining to therapeutic response and long-term survival.

Implementation: Evaluation of urinary neopterin levels and blood dihydropteridine reductase activity as well as other frequently used clinical parameters in cases suffering from various types of lymphomas and leukemias (e.g., n = 110).

Interpretation and Analysis: All patients with active disease have significantly raised urinary neopterin levels compared to those in remission and healthy controls. Of cases with active disease approximately 96% are above the upper limit seen in healthy subjects (e.g., n = 66/69). In addition, the highest neopterin excretion is found in patients with active chronic myeloid leukemia (1,469 ± 479 μmol/mol creatinine n = 16). In contrast, only approximately 2.4% of stable responsive disease and remission states have increases in urinary neopterin levels above the upper limit (e.g., n = 1/41). Dihydropteridine reductase (DHPR) activities can also be detected in all patients and control groups. In active disease slightly reduced (DHPR) activities are evident (3.42 ± 0.37 for controls, 2.92 ± 0.39 in active disease, and 3.28 ± 0.42 nmol red cytochrome C/min/5 mm diameter disc in remission patients). However, in patients under medication this pattern of association is strengthened. Therefore, dihydropteridine reductase activity may be affected by chemotherapeutic intervention. Reductions in dihydropteridine reductase activities may also be an underlying cause for the neurological disorders that are commonly seen in patients with hematological malignancies.

Dihydropteridine reductase activity and neopterin levels in leukemias and lymphomas: Is there any correlation between these 2 parameters? Altindag ZZ, Sahin G, Isimer A, Akpek G, Kansu E. Leuk Lymphoma 1999 Oct; 35 (3–4), pp. 367–74.

Mammary Carcinoma/Reversion-Inducing Cysteine-Rich Protein/Kazal/Response to Therapeutic Intervention/Type 1 Matrix Metalloproteinase

Cellular/Molecular Mechanism of Action: Reversion-inducing cysteine-rich protein with Kazal motifs (RECK) inhibits membrane Type 1 matrix metalloproteinase (MMP-14), MMP-2, and MMP-9 secretion and enzymatic activity. Its expression is essential for normal vasculogenesis, RECK being an important tumor-suppressor gene.

Application: Detection of downregulated levels of RECK has been implicated in tumor angiogenesis and progression of neoplastic disease states. Therefore, possibility exists of (i) applying RECK as a pharmaceutical mimetic; (ii) drugs activating endogenous RECK expression as therapeutic or preventive agents; (iii) the reality of prognostic factor for predicting response to therapeutic intervention pertaining to breast carcinoma.

Implementation: Assessment of the prognostic value of RECK expression in human mammary carcinoma tissue specimens (e.g., n = 278 cases; median follow-up = 75 months; range, 2–169 months). Quantitative measurement of RECK mRNA sequences is performed by real-time quantitative reverse transcriptase-polymerase chain reaction.

Interpretation and Analysis: In some cases, the expression levels of RECK are lower in tumor tissue specimens than in adjacent normal breast tissue (e.g., n = 10; p = 0.028). No relevant associations of RECK with established clinicopathologic factors or treatment regimens can be appreciated. Expression of RECK can serve as an indicator for predicting a longer recurrence-free survival time (RFS; p = 0.037) at the optimal cutoff value (hazard ratio, 0.66; 95% confidence interval, 0.44–0.98). A large number of cases with neoplastic lesions that exhibit low levels of RECK have a mean RFS time of 80.4 months and a 61.8% 5-year RFS rate (e.g., n = 100 cases), in contrast to cases with tumors with high RECK expression, which have a mean RFS time of 91.2 months and a 73.0% 5-year RFS rate (e.g., n = 178 cases). Based on multivariate Cox regression analysis, RECK expression provides significant independent prognostic value for RFS time (p = 0.047).

Matrix metalloproteinase inhibitor reversion-inducing cysteine-rich protein with Kazal motifs: A prognostic marker for good clinical outcome in human breast carcinoma. Span PN, Sweep CG, Manders P, Beex LV, Leppert D, Lindberg RL. Cancer 2003 Jun 1; 97 (11), pp. 2710–5.

Rheumatoid Arthritis: Severe vs Mild-Moderate (Prediction of Disease Severity)/5,10-Methylenetetrahydrofolate Reductase Gene (C677) Polymorphism/G Allele (TT vs TG/GG Genotypes)/Tumor Necrosis Factor-Alpha (TNF-α) Receptor Type II (TNFRII): Exon 6 Polymorphism
Methotrexate/Anti-TNF-α Response

Application: Characterizing severe vs mild-moderate conditions of rheumatoid arthritis as a function of exon 6 TNFRII gene polymorphism. The G allele appears to have utility in predicting the degree of responsiveness to anti-TNF-α treatment while the GG genotype for the exon 6 TNFRII polymorphism may be usefull in predicting more aggressive forms of rheumatoid arthritis.

Implementation: Characterization of exon 6 TNFRII gene polymorphism in cases of (i) mild-moderate synovitis classified as responders to methotrexate therapy (e.g., n = 97) for comparison with; (ii) "non-responder" cases receiving anti-α-TNF treatment due to severe and aggressive disease (e.g., n = 78); and (iii) cases with severe rheumatoid arthritis monitored for 6 months during anti-TNF-α therapy. Results are evaluated pertaining to response according to genotype and compared to matched age, sex and ethnic reference controls (e.g., n = 84).

Interpretation and Analysis: A distinct trend exists towards increases in the frequency of the GG genotype in patients with severe rheumatoid arthritis (6.4%) compared to cases of mild-moderate disease (3.1%) and reference controls (1.2%). Evalaution of the response to anti-TNF-α therapy after 12 weeks of treatment reveals that 37.8% of the TT genotype pass from high to medium-low disease activity. Such observations are in contrast to the 10.7% of the

TG/GG genotype that pass from high to medium-low disease activity ($p = 0.03$).

Collective evaluation of laboratory results therefore suggest that in cases of rheumatoid arthritis classified according to response to the conventional therapy and by disease severity, there is a decernable trend towards a higher prevalence of the GG genotype for the exon 6 TNFRII polymorphism in less responsive cases displaying a more aggressive form of disease. In addition, the G allele tends to promote a lower degree of response to anti-TNF-α treatments.

Tumor necrosis factor-alpha receptor II polymorphism in patients from southern Europe with mild-moderate and severe rheumatoid arthritis. Fabris M, Tolusso B, Di Poi E, Assaloni R, Sinigaglia L, Ferraccioli G. J Rheumatol. 2002 Sep;29(9):1847–50.

Sampling Methods for Nucleic Acids (DNA)
Buccal Mouthwash

Application: Collection of cellular populations suitable for the extraction of DNA to be utilized in pharmacogenetic-based methodologies. The mouthwash method for collection of genomic DNA is a simple, inexpensive, and non-invasive method that poses less risk than venipuncture and may be used in a variety of settings. Genomic DNA in mouthwash is stable for prolonged periods at room temperature, and the quantity of DNA recovered from this method is more than sufficient for pharmacogenetic studies. Such an approach should be valuable to pharmacogenetic researchers and others who are conducting genetic research.

Implementation: Collection of biological material in the form of buccal cell populations suitable of application in molecular biology-oriented pharmacogenetic methodologies. Buccal cells are harvested from collected mouthwash samples. Stability of genomic DNA is determined by measuring DNA yield from mouthwash samples. Different volumes of mouthwash are used to identify optimization of the cell collection technique (e.g., 5-ml vs 10-ml mouthwash). Quality of genomic DNA is assessed by determining the success rate of DNA amplification with polymerase chain reaction (PCR) testing and by genotyping.

Interpretation and Analysis: In stability studies, the quantity of genomic DNA decreases over time with storage at room temperature (overall $p < 0.01$), with the largest declines occurring at 60 and 90 days. Median DNA recovery at 30 and 90 days is 59% and 28% of that at baseline, respectively. Mean ± standard deviation, median, and range for recovery of genomic DNA (e.g., $n = 201$ samples) is 45.2 ± 55 μg, 25.2 μg, and 1–330 μg, respectively. Median recoveries of DNA from the 1-rinse and 3-rinse methods are not statistically significantly different (9.1 vs 10.5 μg). Essentially all samples can be amplified successfully by PCR for the purpose of genotyping. The quality of the DNA samples for such purposes therefore is sufficient.

Collection of genomic DNA by the non-invasive mouthwash method for use in pharmacogenetic studies. Andrisin TE, Humma LM, Johnson JA. Pharmacotherapy 2002 Aug; 22 (8), pp. 954–60.

7 Integumentary System

Anhydrosis
Epinephrine/Ephedrine

Diagnostic Application: Diagnosis of equine anhydrosis based upon a lack of response at the site of intradermal injection.
Route of Administration and Dosage: Intradermal injection at dilutions of 1:1,000, 1:10,000, 1:100,000, 1:1,000,000. A positive diagnosis of anhydrosis is based on a lack of an observed dermal response at epinephrine injection sites using preparations formulated at concentrations of ≥ 1:1,000 (e.g., identical or highly similar to the negative reference control injection site).
Considerations: Epinephrine occasionally produces localized leukotrichia at the site of intradermal injection.
Interpretation and Analysis: Presence of cutaneous sweating at the site of intradermal injection is considered a positive (normal) response that would be expected to be observed in negative reference controls unaffected by anhydrosis.

Eczema: Atopic/Efficacy Evaluation of Therapeutic Intervention
Aeroallergen Challenge/Atopy Patch Test (APT)

Background Information: Pharmacologic studies in atopic eczema (AE) are difficult to standardize. Patients with AE differ in the stage of their skin disease (acute, subacute, chronic).
Application: Aeroallergen challenge (APT) can be applied to evaluate the effect of topical anti-inflammatory agents (topical glucocortico-steroids; tar) on allergic inflammatory responses in conditions of AE.
Route of Administration: Aeroallergen challenge (APT: topical/intradermal injection).
Implementation: Efficacy evaluation of candidate therapeutics for AE by performing topical tests on non-lesional skin regions of the back of cases. Segments of skin are treated for approximately 3 weeks at different sites (e.g., n = 3) with agents such as triamcinolonacetonide (1%) in cetamacrogol ointment (GCSs), pix liquida (10%) in cetamacrogol ointment (tar), and cetamacrogol ointment (vehicle). Atopy patch tests are then performed, and biopsy specimens collected from each test site (time = 0 and 24 hours) for immunohistochemical analysis.

Human Model: Cases of AE (n = 6).

Interpretation and Analysis: Treatment with both GCSs and tar reduces the severity of responses detected on macroscopic examination of APT reactions. Furthermore, both treatment modalities have an almost equal inhibitory effect on the influx of T cells, eosinophils, $CD1^+$, $RFD1^+$, interferon-gamma$^+$, and interleukin-4^+ cell populations, , as well as on the percentage of vessels expressing adhesion molecules, E-selectin, and vascular cell adhesion molecule 1 (VCAM-1) in response to epicutaneous aeroallergen challenge. Although both treatments significantly reduce the various cellular constituents of allergic inflammation, all cell types remain present. In this context, aeroallergen challenge (e.g., ATP) can be used to evaluate the effect of topical anti-inflammatory treatments on allergic inflammation in patients with AE.

Modulation of the atopy patch test reaction by topical corticosteroids and tar. Langeveld-Wildschut EG, Riedl H, Thepen T, Bihari IC, Bruijnzeel PL, Bruijnzeel-Koomen CA. J Allergy Clin Immunol 2000 Oct; 106 (4), pp. 737–43.

Neoplasia: Melanoma/Non-Invasive Transcutaneous Detection
Fluorescein-Isothiocyanate-Labeled Antimelanoma IgG/Texas Red-Labeled Dextran

Refer to the neoplastic disease chapter (chapter 12) in Section II.

In vivo detection of small subsurface melanomas in athymic mice using non-invasive fiber optic confocal imaging. Anikijenko P, Vo LT, Murr ER, Carrasco J, McLaren WJ, Chen Q, Thomas SG, Delaney PM, King RG. J Invest Dermatol 2001 Dec; 117 (6), pp. 1442–8.

Neoplasia Differentiation/Basal Cell Carcinoma/ Squamous Cell Carcinoma/ Precancerous Lesions/ Benign Lesions
Nitrogen/Dye Laser (410 nm)

Refer to the neoplastic disease chapter (chapter 12) in Section II.

Laser-induced fluorescence spectroscopy for *in vivo* diagnosis of non-melanoma skin cancers. Panjehpour M, Julius CE, Phan MN, Vo-Dinh T, Overholt S. Lasers Surg Med 2002; 31 (5), pp. 367–73.

Scleroderma/Systemic Sclerosis (SSc)/Follicle-Stimulating Hormone (FSH)/Lutenizing Hormone (LH)/Prolactin/Skin Sclerosis/Prolactin/Peripheral Vascular Involvement/Prolactin/Pulmonary Involvement
Gonadotropin-Releasing Hormone (GnRH)/ Thyrotropin-Releasing Hormone (TRH)

Background Information: Scleroderma is a chronic hardening and shrinking of the connective tissues on any part of the body, including the skin, heart, esophagus, kidney, and lung. Skin may appear thickened, hard, and rigid with pigmented patches. The condition can be generalized or limited to distal parts of the extremities, face, or digits (oval or linear in shape).
Application: To evaluate basal and dynamic levels of pituitary gonadotropin release in female cases of SSc that are of child-bearing age in addition to postmenopausal SSc women.
Implementation: Evaluation of responses to GnRH and TRH stimulation tests during the early follicular phase. Serum concentrations of FSH, LH, and prolactin are then measured. Blood samples may also be collected after performing TRH stimulations in postmenopausal women with SSc. In the latter situation, only serum prolactin concentrations are determined because elevated basal concentrations of FSH and LH can be expected to be observed. Hormone

concentrations are estimated by radioimmunoassay. Comparisons are made with healthy control women matched for age and reproductive status.

Human Model: Female cases in their child-bearing years with systemic sclerosis (n = 12 SSc with mean age [S.E.M] 34.8 + 2.4 years). In addition, postmenopausal female cases (n = 6) with systemic sclerosis (mean age 46.8 [2.4] years).

Interpretation and Analysis: In cases with systemic sclerosis of child-bearing age, basal FSH, LH, and oestradiol (E_2) levels are not significantly different from reference controls, whereas basal prolactin concentrations are significantly higher than reference controls ($p = 0.0001$). After a stimulation test, the peak concentrations of FSH ($p = 0.0001$) and prolactin ($p < 0.0001$) are significantly higher than controls. The net integrated response curves (net area under the curve [AUC]) for FSH and LH do not differ significantly between systemic sclerosis cases and controls. On the contrary, the net AUC for prolactin in response to TRH stimulation is significantly higher than reference controls ($p = 0.001$). In postmenopausal patients, basal estrogen E_2, FSH, LH, and prolactin levels are not significantly different between women with systemic sclerosis and controls. Similarly, after TRH stimulation, peak levels and net AUC for prolactin are not significantly higher in cases than those in reference controls. No significant correlations can be appreciated between basal and stimulated FSH, LH, and prolactin levels and the severity of involvement of various organ systems. However, multiple regression analysis reveals that basal and stimulated prolactin concentrations are associated with skin sclerosis and peripheral vascular and lung involvement. Results suggest that subclinical primary hypogonadism can occur in SSc patients. Some studies have established that alterations exist in the mechanism for prolactin secretion and release, which may not only contribute to further disturbance of the reproductive axis but may also have an influence on the pathogenesis of disease.

Assessment of pituitary gonadotropin release to gonadotropin releasing hormone/thyroid-stimulating hormone stimulation in women with systemic sclerosis. La Montagna G, Baruffo A, Pasquali D, Bellastella A, Tirri G, Sinisi AA. Rheumatology (Oxford) 2001 Mar; 40 (3), pp. 310–4.

Ulcers: Dermal Microvascular Disease (Diabetes-Associated Foot Lesions)
Fluorescein

Background Information: Fluorescein dermofluorometry can be used to relate the skin uptake of fluorescein to regional vascular blood flow.

Application: The dye wash-in time constant parameters are inversely proportional to local dermal blood flow.

Implementation: Evaluation of fluorescein dermofluorometry for the detection and characterization of dermal ulcers. Following intravenous injection of sodium fluorescein, the change in the fluorescein signal with time is continuously measured at the plantar surface of the foot. Both the initial slope of the signal and the wash-in time constant are then calculated. The outcomes of the patients with foot ulcers are then documented 4–14 months after diagnostic evaluation.

Human Model: Cases of cutaneous diabetic foot ulcers (n = 4 groups: non-diabetic control patients, diabetic control patients, diabetic patients with chronic foot ulcers, and diabetic patients with acute foot ulcers).

Interpretation and Analysis: Significant differences in the wash-in time constant can be appreciated between diabetic and non-diabetic cases and between diabetic subjects with and without foot ulcers. In cases with foot ulcers, a relatively large percent may not display an early washout in the dermofluorometer signal and later require amputation (e.g., n = 2/8 or 25%). The fluorescein wash-in time constant demonstrates better correlation with the presence of diabetic microvascular disease than does the initial slope of the signal. Differences in the wash-in time constants of non-diabetic and diabetic subjects support the hemodynamic hypothesis for the development of microvascular disease. The indication of early washout of the fluorescein signal may also be useful in the prediction of ulcer healing.

Fluorescein dermofluorometry for the assessment of diabetic microvascular disease. Oh DK, Jones RN, Marshall W, Magin RL. Skin Res Technol 2001 May; 7 (2), pp. 105–11.

Urticaria/Idiopathic-Acquired Cold Urticaria
Tiaramide

Application: Characterization of conditions of idiopathic-acquired cold urticaria.

Route of Administration: Tiaramide (oral).

Dosage: Multiple doses of 250 mg, q.i.d., for 1 week administered after a single oral dose of 500 mg.

Considerations: Provocative test is associated with no adverse effects with the exception of the possible development of idiosyncratic reactions.

Implementation: The skin of the forearm of each subject is exposed to cold stimuli for 1, 2, 3, 4, and 5 minutes by placing 5 ice cubes on the ventral surface at 1-minute intervals followed by their simultaneous removal 5 minutes after contact with the 1st cube.

Interpretation and Analysis: Challenge sites are observed for 10 minutes and the wheal surface area, intensity of edema, and time of contact necessary to induce the skin response recorded. The results of the provocative test following the single and multiple dosage administration of tiaramide are compared to baseline skin responses. After 1 week of tiaramide treatment at 250 mg, q.i.d., there is a marked attenuation of skin responses to cold-challenge.

Preliminary report on the effects of tiaramide on the ice cube test in patients with idiopathic cold urticaria. Petillo JJ, Natbony SF, Zisblatt M, Vukovich RA, Neiss ES, Kaplan AP. Ann Allergy 1983 Nov; 51 (5), pp. 511–4.

Vasculature Perfusion: Microvasculature/Ulcers: Dermal Vasomotor Responses in Diabetes-Associated Foot Lesions
Sodium Fluorescein

Background Information: A loss of sympathetic function could lead to changes in capillary fluid filtration in diabetic patients.

Application: Delineation of an association between abnormal capillary leakage with decreases in sympathetically mediated dermal vasomotion in diabetic conditions.

Implementation: Evaluation of sensory and motor nerve function of the distal extremities assessed by standard neurography and expressed as a sensory-motor nerve function score. Sympathetic vasomotion of the skin microcirculation is assessed by determining the power of blood flow variability in the low-frequency (0.02–0.14 Hz) band by spectral analysis of laser Doppler flowmetry at the median ankle. Skin capillary leakage is evaluated by sodium fluorescein videodensitometry at the same site of the foot.

Human Model: Cases of diabetic cutaneous foot ulcers (n = 3 matched groups: n = 18 diabetic patients with documented peripheral neuropathy [DN]; n = 18 diabetic patients without peripheral neuropathy [D]; n = 18 healthy control subjects [C]).

Interpretation and Analysis: Sympathetically mediated vasomotion of the microcirculation in the skin of the foot is lower in diabetic patients with documented peripheral neuropathy compared with diabetic patients without peripheral neuropathy and control subjects ($p < 0.001$). Capillary sodium fluorescein leakage is greater in diabetic patients with documented peripheral neuropathy ($n = 18$) than in diabetic patients without peripheral neuropathy ($p < 0.02$) and C ($p < 0.005$). Multiple regression analysis reveals that a reduced sympathetically mediated vasomotion, together with a lower sensory-motor nerve function score, independently contributes to variance in sodium fluorescein leakage, for 30% ($p < 0.001$) and 17% ($p < 0.01$), respectively. A loss of sympathetic tone, apart from sensory-motor nerve dysfunction, seems to be a major determinant for increased capillary permeability in diabetic patients with neuropathy.

Sympathetic mediated vasomotion and skin capillary permeability in diabetic patients with peripheral neuropathy. Lefrandt JD, Bosma E, Oomen PH, Hoeven JH, Roon AM, Smit AJ. Diabetologia 2003 Jan; 46 (1), pp. 40–7.

8 Musculoskeletal System

BONE AND SYNOVIAL STRUCTURES

Bone/Growth and Physiology/Aluminum ($Al(OH)_3$) Body Stores/Overload/Toxicity/Hemodialysis/ Hematopoietin/Iron Absorption: Iron Deficiency
Tetracycline/Desferrioxamine/Deferoxamine/[^{59}Fe]

Cellular/Molecular Mechanism of Action: Tetracycline is incorporated into bone hydroxyapetite deposits. Desferrioxamine and deferoxamine are metallic ion chelating agents. Desferrioxamine can induce elevations in basal plasma aluminum concentrations.
Application: Desferrioxamine can be applied to characterize body aluminum stores while tetracycline can be used as a marker for profiling bone physiology and the rate or extent of new bone deposition.
Implementation: Evaluation of the influence of hemofiltration (HF) and hemodialysis (HD) in connection with the risk of aluminum overload/intoxication and renal osteodystrophy by analysis of double bone biopsies after double tetracycline labeling and use of the desferrioxamine test.

Human Model: Cases managed by hemofiltration (n = 12 HF) and hemodialysis (n = 15 HD). No cases are included that have received 1 alpha-OH-D.
Interpretation and Analysis: Aluminum concentrations are low (less than 0.6 μmol/l) in both the dialysate and the substitution fluid. The duration of treatment (about 2 years) and the cumulative doses of $Al(OH)_3$ and $CaCO_3$ are comparable in the 2 groups. The aluminum balance during an HF run ranges from −22 to +1.8 μmol/l, the balance being positive only when the plasma aluminum is less than 0.5 μmol/l. Basal plasma aluminum and its increase induced by desferrioxamine are comparable in the 2 groups. Bone aluminum content is also comparable but is about 10 times higher than in 7 non-uremic controls. Bone aluminum content and plasma aluminum increase after desferrioxamine can be correlated to $Al(OH)_3$ cumulative dose. No cases have florid osteomalacia with osteoid thickness, and few have traces of stainable aluminum (e.g. n = 1/27). The mineralization front is decreased in most cases of hemofiltration (e.g., n = 8/12) and hemodialysis cases (n = 9/14) to the extent that differences cannot be appreciated between each management scheme. The predominant histological bone picture of the patients is osteitis fibrosa, which is present in a large percent of hemofiltration and hemodialysis cases (e.g., n = 10/12 HF and n = 13/15 HD). Mean osteoclast count and active resorption surface are comparable in the 2 groups but are increased (5–10 times the mean of the controls).

Comparative evaluation of bone aluminum content and bone histology in patients on chronic hemodialysis and hemofiltration. Sebert JL, Fournier A, Leflon P, Fohrer P, de Frémont JF, Morinière P, Galy C, Marie A, Demontis R, Boudailliez B, et al. Nephron 1986; 42 (1), pp. 34–40.

Bone Growth Marker
Tetracycline

Cellular/Molecular Mechanism of Action: Tetracycline becomes associated with and incorporated into hydroxyapetite crystals within the osseous bony matrix.
Diagnostic Application: Detection and measurement of new osseous bone growth. Recently, tetracyclines have also been reported to be beneficial in correcting certain equine angular limb deformities if administered in the early days of life shortly after parturition.
Route of Administration and Dosage: Oral or intravenous.
Considerations: Can cause severe diarrhea in equine patients due to disruption of normal gastrointestinal bacterial flora that can occasionally lead or predispose to development of life-threatening Salmonella enteritis.
Implementation: Following administration, a portion of the dose is incorporated into bone in a manner that can function as a physiological marker for assessment of the rate and extent of bone growth.
Interpretation and Analysis: Measurement of the osseous segment deposited following tetracycline administration is an indication of the extent of new bone growth.

Bioassayable growth hormone activity in blood from healthy individuals and acromegalic patients. Thorngren KG, Hallengren B. Acta Endocrinol (Copenh) 1986 Jan; 111 (1), pp. 3–9.

Effects of unilateral arterial infusion of GH and IGF-I on tibial longitudinal bone growth in hypophysectomized rats. Nilsson A, Isgaard J, Lindahl A, Peterson L, Isaksson O. Calcif Tissue Int 1987 Feb; 40 (2), pp. 91–6.

Osteopenia and Osteoporosis/Tubular Acidosis Type I (Incomplete)/Distal Renal Tubular Acidification Capacity
Ammonium Chloride Loading/Frusemide

Cellular/Molecular Mechanism of Action: Chloride ion is transported by the renal epithelium into the urinary filtrate.
Background Information: Chronic metabolic acidosis may increase alkali mobilization from the bone and thus promote the development of osteoporosis.
Application: Detection and characterization of incomplete renal tubular acidosis type I (RTA I).
Route of Administration: Frusemide (oral ingestion); ammonium chloride loading (oral).
Dosage: Frusemide (40 mg); ammonium chloride loading (0.1 g/kg body weight).
Implementation: Evaluation and comparison of renal function in cases of osteopenia and osteoporosis (reduced mineral content) relative to negative reference control subjects with normal bone

density. Distal tubular acidification can be characterized applying an oral ammonium chloride loading test and the oral frusemide test.

Human Model: Cases of osteopenia or osteoporosis devoid of overt metabolic acidosis, derangement of potassium homeostasis, or renal insufficiency (n = 46; n = 41 females; n = 5 males). Negative age- and sex-matched (normal) reference controls (n = 20; n = 17/20 females, n = 3/20 males).

Interpretation and Analysis: The urinary pH in controls is <5.5 following frusemide ingestion. In cases with reduced bone mineral density, the oral ammonium chloride loading test can aid in establishing a diagnosis of incomplete RTA I (n = 10/46 or 22%). Disorders possibly related to RTA I can also be detected (e.g., n = 8/10). A large percentage of cases will display normal urinary pH responses following oral ammonium chloride loading (e.g., n = 36/46). Oral frusemide fails to lower urinary pH < 5.5 in a little more than one-third of cases (e.g., n = 16/46 or 35%) and encompasses cases with incomplete RTA I (e.g., n = 10) or with a normal oral ammonium chloride loading test (n = 6). An abnormal frusemide test may be appreciated in 35% of cases with reduced bone mass but is a feature rarely if ever detected in normal controls ($chi^2 = 7.39$; < 0.01). With the ammonium chloride test functioning as a gold standard for the diagnosis of distal RTA, the frusemide test shows a sensitivity of 1.0 (95% CI, 0.69–1.0) and a specificity of 0.89 (95% CI, 0.78–0.96) for the diagnosis of distal RTA. Patients with incomplete RTA I tend to be younger in age than those without incomplete RTA I (42 ± 16 vs 54 ± 14 years; p = 0.025; mean ± SD). Basal serum bicarbonate concentrations and capillary pH do not differ between evaluation groups.

Therefore, incomplete RTA I may be prevalent in a significant proportion of cases suffering from osteopenia or osteoporosis. The outcome of the frusemide test suggests either a defect of the H^+ATPase in the cortical collecting tubule (CCT) or a defective Na^+ reabsorption in the CCT. Prospective studies are needed to further elucidate the impact of incomplete RTA I on the development of reduced bone mineral content.

Prevalence and characterization of renal tubular acidosis in patients with osteopenia and osteoporosis and in non-porotic controls. Weger W, Kotanko P, Weger M, Deutschmann H, Skrabal F. Nephrol Dial Transplant 2000 Jul; 15 (7), pp. 975–80.

Osteosarcoma: Hydroxyapatite (HA) Deposition/ Osteoblastic Activity/Atherosclerosis
Near-Infrared (NIR) Light Emitting Probes/Fluorescent Bisphosphonate Derivatives

Background Information: In vertebrates, the development and integrity of the skeleton requires HA deposition by osteoblasts. Sites of osteoblastic activity have most commonly been imaged *in vivo* using gamma-emitting radioisotopes. The scan times required are long, and the resultant radioscintigraphic images suffer from relatively low resolution.

Application: Hydroxyapatite deposition can function as an indicator of, or participant in, processes as diverse as cancer and atherosclerosis.

Semi-Synthetic Methods: Production of an NIR fluorescent bisphosphonate derivative.

Implementation: Evaluation of the ability for NIR fluorescent bisphosphonate derivatives to physically bind to hydroxyapatite deposits.

Interpretation and Analysis: Near-infrared light-based probes like fluorescent bisphosphonate derivatives possess an ability to bind to hydroxyapatite under *in vitro* conditions, in addition to recognizing osteoblastic activity in living animals. Such biotechnology can be applied to study skeletal development, osteoblastic metastasis, coronary atherosclerosis, and other disease states.

In vivo near-infrared fluorescence imaging of osteoblastic activity. Zaheer A, Lenkinski RE, Mahmood A, Jones AG, Cantley LC, Frangioni JV. Nat Biotechnol 2001 Dec; 19 (12), pp. 1148–54.

Pain and Lameness Disorders/Musculoskeletal vs Neurologic Disorders
Lidocaine/Mepivicaine/Bupivicaine

Cellular/Molecular Mechanism of Action: Inhibits axonal membrane-associated Na^+/K^+ pump activity, thereby diminishing the rate or completely preventing transmission of neurologic impulses.

Diagnostic Application: Localize sites of musculoskeletal-associated pain. Local peripheral nerve blocks can also be applied to differentiate musculoskeletal pain from neurologic deficits.

Route of Administration: Intra-articular or selective injection in a juxtaposition in the immediate proximity of peripheral nerves.

Considerations: Lidocaine has an onset of action of approximately 5 minutes with a duration of action approaching 30–45 minutes. Mepivicaine (Carbocaine) has a slightly longer onset of action and a duration of activity lasting approximately 45–60 minutes. Bupivicaine (Marcaine) has the longest onset of action and the longest duration of activity that on average can last between 3 and 4 hours.

Onset of action and duration of activity are inversely related to and are dependent upon the individual diffusion characteristics of each local anesthetic agent in order across axonal membrane structures. Duration of activity can be enhanced with preparations containing epinephrine (promotion of localized vasoconstriction), thereby inhibiting diffusion away from the site of local injection. Dextrose has also been included in preparations of local anesthetic agents in order to increase their osmotic characteristics, thereby promoting a net accumulation of tissue fluid at the site of injection and a decreased net diffusion away from the site of local injection.

Interpretation and Analysis: Alleviation of pain or signs of abnormal locomotion or gait usually suggest that clinical signs are due to a musculoskeletal condition instead of a neurological disease state.

Pain/Lameness: Lower Extremity (Coxalgic vs Neuropathic)
Bupivicaine

Application: Characterization of lower-extremity pain below the knee in cases of spinal and hip osteoarthritis (OA) to determine whether the leg symptoms are coxalgic or neuropathic.

Route of Administration: Intra-articular or selective injection in a juxtaposition in close proximity to peripheral nerves.

Interpretation and Analysis: The test procedure facilitates accurate identification of the anatomical location of painful foci with a sensitivity of 87%, a specificity of 100%, and an efficiency of 88%. This office test also provides significant savings in terms of diagnostic tests and patient discomfort.

The value of bupivicaine hip injection in the differentiation of coxarthrosis from lower extremity neuropathy. Kleiner JB, Thorne RP, Curd JG. J Rheumatol 1991 Mar; (3), pp. 422–7.

Rheumatoid Arthritis/Matrix Metalloprotease Protein 3 (MMP-3)
Corticosteroids

Cellular/Molecular Mechanism of Action: Corticosteroids inhibit phospholipase A2 biochemical activity.

Physiological Mechanism of Action: Corticosteroids exert anti-inflammatory and immunosuppressive properties, presumably due to their ability to generally suppress protein synthesis and stabilize cellular membrane structures.
Application: Delineation of potential relationships between rheumatoid arthritis, expression profiles for interleukins (IL) and metalloproteases (MMP), and corticosteroid production.
Implementation: Evaluate expression profiles for IL-1β, tumor necrosis factor-alpha (TNF-α), IL-6, IL-4, IL-10, MMP-1, MMP-3, and MMP-13 in freshly isolated peripheral blood (PBMC) and synovial fluid mononuclear cells (SFMC) in early rheumatoid arthritis (never-treated [ENT-RA] vs non-acute, treated rheumatoid arthritis [NAT-RA]). Characterization of expression profiles entails the application of ELISA and quantitative and semi-quantitative RT-PCR/analyses.

Human Model: Cases suffering from early rheumatoid arthritis (n = 14 RA; n = 7/14 early RA [< 1 year of evolution] never treated with corticosteroids or disease-modifying antirheumatic drugs, and n = 7/14 non-acute RA [> 2 years of evolution] treated with disease-modifying antirheumatic drugs). Reference controls: A group of 14 healthy subjects matched for sex and age.
Interpretation and Analysis: Statistically significant differences in the protein or transcript levels for the cytokines of interest cannot be appreciated between ENT-RA and NAT-RA case groups. The cytokine mRNA expression by freshly isolated PBMC and SFMC in both case types is IL-1β > TNF-α > IL-10 > IL-6, with no mRNA IL-4 expression. In contrast, cytokine serum levels in ENT-RA and NAT-RA patients exist in an inverse order as follows: IL-6 > IL-10, while IL-1 β, TNF-α, and IL-4 are undetectable. Metalloprotease protein 3 mRNA expression by the PBMC of NAT-RA patients is statistically different to that in ENT-RA patients. Similar levels of mRNA expression of MMP-1, MMP-3 and MMP-13 by the PBMC and SFMC in both RA groups can be appreciated. A close equilibrium between MMP and pro-/anti-inflammatory cytokine production is observed in ENT-RA and NAT-RA patients. This balance is apparently not influenced by the length of the disease. Highly sensitive methods such as quantitative RT-PCR and ELISA, and even studying freshly isolated MC, show sustained cytokine secretion at the local level (synovial fluid/SFMC) and scarce translation at the peripheral level (serum/PBMC). Expression of MMP mRNA needs to be further evaluated in order to know whether its peripheral expression reflects local activity in RA patients.

Expression of interleukin-1β, tumor necrosis factor-alpha, interleukins-6, -10 and -4, and metalloproteases by freshly isolated mononuclear cells from early never-treated and non-acute treated rheumatoid arthritis patients. Vázquez-Del Mercado M, Delgado-Rizo V, Muñoz-Valle JF, Orozco-Alcalá J, Volk HD, Armendáriz-Borunda J. Clin Exp Rheumatol 1999 Sep–Oct; 17 (5), pp. 575–83.

Rheumatoid Arthritis: Severe vs Mild-Moderate/ Prediction of Disease Severity: G Allele (TT vs TG/GG Genotypes)/Tumor Necrosis Factor-Alpha (TNF-α) Receptor Type II (TNFRII): Exon 6 Polymorphism
Anti-TNF-α Response/Methotrexate

Application: Characterizing severe vs mild-moderate conditions of rheumatoid arthritis as a function of exon 6 TNFRII gene polymorphism. The G allele appears to have utility in predicting the degree of responsiveness to anti-TNF-α treatment while the GG genotype for the exon 6 TNFRII polymorphism may be useful in predicting more aggressive forms of rheumatoid arthritis.
Implementation: Characterization of exon 6 TNFRII gene polymorphism in cases of (i) mild-moderate synovitis classified as responders to methotrexate therapy (e.g., n = 97) for comparison with (ii) "non-responder" cases receiving anti-TNF-α treatment due to severe and aggressive disease (e.g., n = 78); and (iii) cases with severe rheumatoid arthritis monitored for 6 months during anti-TNF-α therapy. Results are evaluated pertaining to response according to genotype and compared to matched age, sex, and ethnic reference controls (e.g., n = 84).
Interpretation and Analysis: A distinct trend exists toward increases in the frequency of the GG genotype in patients with severe rheumatoid arthritis (6.4%) compared to cases of mild-moderate disease (3.1%) and reference controls (1.2%). Evaluation of the response to anti-TNF-α therapy after 12 weeks of treatment reveals that 37.8% of the TT genotype passes from high to medium-low disease activity. Such observations are in contrast to the 10.7% of the TG/GG genotype that passes from high to medium-low disease activity (p = 0.03).

Collective evaluation of laboratory results therefore suggests that in cases of rheumatoid arthritis classified according to response to the conventional therapy and by disease severity, there is a discernable trend toward a higher prevalence of the GG genotype for the exon 6 TNFRII polymorphism in less-responsive cases displaying a more aggressive form of disease. In addition, the G allele tends to promote a lower degree of response to anti-TNF-α treatments.

Tumor necrosis factor-alpha receptor II polymorphism in patients from southern Europe with mild-moderate and severe rheumatoid arthritis. Fabris M, Tolusso B, Di Poi E, Assaloni R, Sinigaglia L, Ferraccioli G. J Rheumatol 2002 Sep; 29 (9), pp. 1847–50.

SKELETAL MUSCLE

Devitalized Tissues
Organic Iodine (e.g., Betadine)

Cellular/Molecular Mechanism of Action: Exclusionary vitality stain. Damaged or devitalized tissues (musculoskeletal lacerations) assimilate a greater amount of organic iodine relative to normal tissues.
Diagnostic Application: Identification of devitalized musculoskeletal tissues such as those found in lacerated wounds.
Route of Administration and Dosage: Topical application onto regions of suspected tissue devitalization.
Considerations: Septic wounds with extensive neutrophil infiltration will convert organic iodine solution from a rich brown color to an almost colorless iodine preparation. This occurs due to the activity of halogen-myeloperoxidase system within neutrophil populations.
Interpretation: Devitalized tissues will appear a different color than non-affected (normal) tissues.

Hyperkalemic Periodic Paralysis (HYPP): Equine
Potassium Chloride (KCl)

Cellular/Molecular Mechanism of Action: Stabilizes propagation of electrolyte-mediated electrical impulses across (along) cell membranes. Normally, potassium (K^+) is maintained at a relatively higher concentration intracellularly in contrast to the extracellular environment. Additionally, sodium (Na^+) is usually in relatively higher concentration in the extracellular environment than in the intracellular milieu. During membrane depolarization, Na^+ conductance intracellularly increases, which is followed by increased extracellular K^+ conductance during the repolarization process. In this

context, administration of large amounts of K^+ serves to inhibit the processes of membrane depolarization/repolarization.

Diagnostic Application: Stabilization of excitable membrane structures for the diagnosis of conditions including cases suffering from HYPP syndrome. Positive diagnosis is recognized by a progressive increase in muscle weakness that can advance to a state of collapse.

Route of Administration: Orally by nasogastric tube.

Dosage: KCl (88–160 gm/kg).

Considerations: False negative results can be observed when the lower dose range is administered.

Implementation: Administration should begin at the lower dose of approximately 40 g of KCl in water (6 L). Blood samples are collected for measurement of KCl concentrations. Some patients with periodic hyperkalemic syndrome will not respond to the potassium chloride challenge test. In such instances, these cases can be diagnosed by increasing the dosage of potassium chloride in increments of 20 grams. Only 1 challenge test should be performed within any given 24-hour period and horses should not be left unattended following KCl administration (2- to 4-hour period following dosage).

Interpretation and Analysis: Normal horses have a modest increase in plasma potassium concentrations but do not develop abnormal clinical signs even at the 180 mg/kg KCl dose level. Reversal of clinical signs of HYPP following induction by potassium chloride administration can be achieved with the subsequent administration of calcium chloride or calcium gluconate. Calcium gluconate can be administered at a dose of 0.2 to 0.4 ml/kg diluted to 1 to 2 liters in 5% dextrose and given intravenously. Response rate following calcium gluconate is rapid. Alternatively dextrose 5% (D5W) at 4.4 to 6.6 ml/kg or $NaHCO_3$ at 1 mEq/kg can be administered rapidly by intravenous infusion.

Laboratory Instrumentation: Electron capture instrumentation is applied to measure concentrations of potassium cation.

Hyperkalemic Periodic Paralysis (HYPP)
Potassium/Acetazolamide/Calcium Glucuronate/ Biocarbinate/Bicarbonate/Glucose (+/− Insulin)

Cellular/Molecular Mechanism of Action: Acetazolamide inhibits carbonic anhydrase activity. Pharmaceuticals within the thiazide class act directly on the kidney and increase the excretion of sodium chloride, potassium chloride, and water. At sufficient doses, pharmaceuticals within this class such as acetazolamide also promote bicarbonate excretion. Potassium excretion is achieved through a combination of enhanced excretion at the distal tubule, accented by drug-induced microvascular hyperperfusion at the level of the distal renal tubule.

Diagnostic Application: Hyperkalemic periodic paralysis in the acute stages can be reversed by calcium gluconate, bicarbonate, glucose, and/or insulin. Hyperkalemic periodic paralysis can be induced or initiated by the administration of potassium electrolyte.

Background Information: Hyperkalemic periodic paralysis is characterized by sporadic attacks of muscle tremors, weakness, and/or collapse, lasting for variable periods of time. Diagnosis is based on physical findings in association with hyperkalemia. In horses with HYPP, the regulation of ion transport through myocyte sodium channels occasionally fails, causing uncontrollable muscle twitching. Further investigations into molecular genetic profiles reveal a mutation in the gene responsible for sodium and potassium regulation. The identification of this gene mutation is the basis for the blood test used to diagnose HYPP. Hyperkalemic periodic paralysis is inherited as an autosomal dominant trait.

Dosage: Reports have described the use of acetazolamide at 2.2 mg/kg body weight in the horse. Other pharmacokinetic-based studies have characterized serum acetazolamide concentration profiles following the administration of intravenous (4 mg/kg IV) and oral(8 mg/kg) doses.

Route of Administration: Acetazolamide (IV or oral).

Considerations: Administration can promote a hypokalemia. Acetazolamide can also promote the development of hyperglycemia, in addition to elevations in triglycerides and cholesterol.

Pharmacokinetics: The pharmacokinetics of acetazolamide (IV or per os) can be established in horses (e.g., $n = 6$) by the administration of 2 doses (4 mg/kg of body weight, IV; 8 mg/kg, per os) and blood samples collected at regular intervals before and after administration. Assay for acetazolamide concentrations can be determined by high-performance liquid chromatography. Following IV administration of acetazolamide, data analysis reveals a median mean residence time of 1.71 ± 0.90 hours and median total body clearance of 263 ± 38 ml/kg/h. Median steady state volume of distribution is 433 ± 218 ml/kg. After oral administration, mean peak plasma concentration is 1.90 ± 1.09 μg/ml. Mean time to peak plasma concentration is 1.61 ± 1.24 hours. Median oral bioavailability is $25 \pm 6\%$. Oral pharmacokinetic disposition of acetazolamide in horses is characterized by rapid absorption, low bioavailability, and slower elimination than observed initially after IV administration. Pharmacokinetic data can therefore facilitate estimation of appropriate dosages for acetazolamide use in horses with hyperkalemic periodic paralysis.

Interpretation and Analysis: During HYPP attacks, the intravenous administration of calcium gluconate, bicarbonate, and glucose results in a rapid reversal of clinical signs. Administration of potassium as an electrolyte solution can precipitate the condition in cases with a genetic predisposition.

HYPP—Hyperkalemic periodic paralysis in horses. Zeilmann M. Tierarztl Prax 1993 Dec; 21 (6), pp. 524–7.

Pharmacokinetics of acetazolimide after intravenous and oral administration in horses. Alberts MK, Clarke CR, MacAllister CG, Homer LM. Am J Vet Res 2000 Aug; 61 (8), pp. 965–8.

Malignant Hyperthermia (MH)
Midazolam/Caffeine/Halothane

Cellular/Molecular Mechanism of Action: Midazolam (water-soluble benzodiazepine).

Application: Premedicant for induction of anesthesia, but can additionally be applied to screen for patients susceptible to the development of MH. Midazolam is not an effective diagnostic pharmaceutical for the screening of patients susceptible to the development of MH.

Dosage: Midazolam (ca. 0.5 μg/ml).

Considerations: Midazolam is applied to determine its influence on the resting tension of directly stimulated muscle biopsies. Interactions between midazolam and the 2 most commonly used MH diagnostic agents (halothane and caffeine) have also been examined.

Interpretation and Analysis: Midazolam, at maximum therapeutic concentrations (ca. 0.5 μg/ml), has no detectable effects on muscle contraction in control and MH preparations. However, midazolam does elicit contracture responses in control and MH-positive preparations when used within a concentration range between 160 and 1,280 μg/ml. There are no significant differences between control and MH-positive patients at minimum concentration of midazolam causing contracture or the strength of contracture at the respec-

tive eliciting concentration. There appears to be no interaction between midazolam and either halothane or caffeine on the resting tension of directly stimulated muscle twitch preparation.

Effects of midazolam on directly stimulated muscle biopsies from control and malignant hyperthermia positive patients. Fletcher JE, Rosenberg H, Hilf M. Can Anaesth Soc J 1984 Jul; 31 (4), pp. 377–81.

Malignant Hyperthermia (MH)
Caffeine/Halothane/Haloperidol/Succinylcholine/Caffeine

Cellular/Molecular Mechanism of Action: Succinylcholine is an analog of the neurotransmitter acetylcholine.
Physiological Mechanism of Action: Droperidol and haloperidol are butyrophenone neuroleptic agents that induce a state of quiescence and reduced motor activity. Ketamine is a dissociative anesthetic agent.
Application: Utilized in the *in vitro* diagnostic screening of muscle strips from human patients being diagnostically screened for MH and muscle strips from rat diaphragm preparations are exposed to droperidol, haloperidol, or ketamine prior to challenge with halothane, succinylcholine, or caffeine (used to screen the MH potential of droperidol, haloperidol, ketamine).
Route of Administration: Halothane (inhalation); succinylcholine (intravenous).
Considerations: If any agent augments the contracture response to the MH triggering or diagnostic agents, then the agent is considered unsafe for use in MH-susceptible patient cases.
Interpretation and Analysis: Droperidol (10 μmol/l) and ketamine (100 μmol/l) do not induce contractures in human or rat skeletal muscle when added alone, nor do they augment halothane, succinylcholine, or caffeine contractures. Therefore, these agents appear to be safe for use in patients susceptible to MH. In contrast, haloperidol (10 μmol/l) augments responses to succinylcholine about 1.5-fold and may be contraindicated in MH-susceptible cases.

Effects of droperidol, haloperidol, and ketamine on halothane, succinylcholine, and caffeine contractures: Implications for malignant hyperthermia. Fletcher JE, Rosenberg H, Lizzo FH. Acta Anaesthesiol Scand 1989 Apr; 33 (3), pp. 187–92.

Malignant Hyperthermia (MH)
Caffeine/Diffusible CO_2

Background Information: Susceptibility to MH can be diagnosed by implementation of a contracture test, which requires obtaining an open muscle biopsy sample.
Route of Administration: Caffeine (intramuscular).
Dosage: Caffeine (500 μl of an 80 mmol/L formulation).
Implementation: Evaluate the influence of intramuscular caffeine injection on local carbon dioxide pressure (pCO_2) in conditions of MH compared to normal negative reference controls. Measurements for pCO_2 are obtained from the rectus femoris muscle during local stimulation with injected caffeine.

Human Model: Cases susceptible to MH (n = 12); cases classified as non-susceptible (n = 8); and 7 healthy/normal controls.
Interpretation and Analysis: In susceptible individuals, pCO_2 temporarily increases to a maximum of 63 mm Hg (IQR 61–69) compared with 44 mm Hg (43–47) for non-susceptible participants ($p = 0.003$) and 42 mm Hg (41–46) in controls ($p = 0.003$). Systemic side effects usually cannot be appreciated. Metabolic monitoring of highly diffusible CO_2 stimulated by caffeine could possibly serve as a minimally invasive test for susceptibility to MH.

Diagnosis of susceptibility to malignant hyperthermia by use of a metabolic test. Anetseder M, Hager M, Müller CR, Roewer N. Lancet 2002 May 4; 359 (9317), pp. 1579–80.

Malignant Hyperthermia (MH)
Caffeine/Halothane/Calcium-Induced Calcium Release

Application: Detection and characterization of MH conditions.
Route of Administration: Topical application (emersion) of muscle biopsy specimens.
Implementation: Comparison of results of the *in vitro* caffeine/halothane contracture test (CHCT) according to the protocols of the North American Malignant Hyperthermia Group (NAMHG) and the European Malignant Hyperthermia Group (EMHG) with the Ca^{2+}-induced Ca^{2+} release (CICR) rate test. Muscle biopsies are usually obtained from the musculus vastus lateralis. Diagnostic cut-off points and procedures for CHCT protocols are as described in the original and renewal versions of NAMHG and EMHGs. The CICR rate test is performed according to the protocol reported by Endo et al.

Human Model: Cases of suspected MH (n = 16; n = 5 normal negative reference controls).
Interpretation and Analysis: Essentially a very large proportion of normal subjects (e.g., n = 5/5 or 100%); some abortive cases of MH (e.g., n = 2); some cases of postoperative hyperthermia (e.g., n = 2); and some cases with high serum creatinine kinase (e.g., n = 3) will have normal results from all 3 tests. Some cases with MH reactions (e.g., n = 3) or that have a history of masseter spasm (e.g., n = 1) may be classified as MH-positive according to NAMHG criteria and MH-susceptible and MH-equivocal according to EMHG criteria. Some cases will have discordant results between the CHCT and CICR rate tests (e.g., n = 5 patients). It has therefore been proposed that muscle biopsy for diagnosis of MH susceptibility should combine the CHCT with the CICR rate test, which may identify the defective site of Ca^{2+} release channels.

Comparison of the *in vitro* caffeine-halothane contracture test with the Ca-induced Ca release rate test in patients suspected of having malignant hyperthermia susceptibility. Oku S, Mukaida K, Nosaka S, Sai Y, Maehara Y, Yuge O. J Anesth 2000 Jan 20; 14 (1), pp. 6–13.

Malignant Hyperthermia (MH)
4-Chloro-m-Cresol (4-CmC)/Caffeine/Halothane

Background Information: *In vitro* contracture test (IVCT) for diagnosis of MH has been reported to have a sensitivity level of 100% and a specificity of 93%. The results are equivocal in 10 to 15% of cases, and supplementary tests may thus be required
Application: The agents 4-CmC, caffeine, and halothane can be applied to detect and characterize conditions of MH.
Implementation: Evaluation of muscle tissue collected as a biopsy specimen applying an IVCT performed by topical exposure to increasing concentrations of 4-CmC (25, 50, 75, 100, 150, and 200 μmol/l). Corresponding real-time force profiles are then recorded. Diagnosis of MH susceptibility is established with standard halothane and caffeine tests and test subjects classified as MHS (MH-susceptible), MHN (MH0negative), or MHE (equivocal result).

Human Model: Cases subjected to skeletal muscle biopsy (n = 41 from n = 7 families).
Interpretation and Analysis: At all concentrations of 4-CmC, the increase in baseline force is significantly greater in the MHS group compared to the MHN group ($p < 0.05$). Muscle from MH-susceptible cases (e.g., n = 15 MHS) responds to 4-CmC with increasing force at a threshold concentration of 75 μmol/l or less,

whereas muscle tissues from cases classified as MH-non-susceptible (e.g., n = 23 MHN) have thresholds of 100 μmol/l or more. The accuracy of the chlorocresol test is approximately 100% (95% confidence limits, 90.75–100%) at a threshold of 75 μmol/l. Amplitude of contractures at 2 μmol/l caffeine is not different from contractures at 75 μmol/l of 4-CmC in either the MHS or the MHN group ($p > 0.05$). *In vivo* concentrations of chlorocresol from clinical use of insulin and somatropin are estimated to be 20 times less than the threshold concentration and thus these drugs seem safe in MH cases. Therefore, 4-CmC may be a suitable aid to clarify puzzling results of standard testing of MH susceptibility.

4-Chloro-m-cresol test—A possible supplementary test for diagnosis of malignant hyperthermia susceptibility. Ording H, Glahn K, Gardi T, Fagerlund T, Bendixen D. Acta Anaesthesiol Scand 1997 Sep; 41 (8), pp. 967–72.

Malignant Hyperthermia (MH)/Genomic Analyses and Provocative Testing Correlation/Mutation: Arginine-614 → Cysteine (Arg614Cys: RYR1 C1840T)

Caffeine/Halothane

Physiological Mechanism of Action: In conditions of MH, exposure of muscle tissue to caffeine/halothane promotes an exaggerated contractile response.

Background Information: Malignant hyperthermia is an important cause of anesthesia-induced death. The caffeine/halothane contracture test (CHCT) is highly invasive, expensive, and lacks 100% specificity. Genetic and biochemical evidence provide strong support for the view that the substitution of cysteine for arginine 614 (Arg614Cys) in the human ryanodine receptor gene is 1 of several mutations that are likely to be responsible for MH in humans.

Application: Detection and characterization of MH conditions.

Route of Administration: Traditionally applied topically during exposure of biopsy tissue specimens in *ex vivo* based challenge studies.

Implementation: Delineation of a possible genetic basis for a predisposition to MH pertaining to the presence of Arg614Cys (RYR1 C1840T) mutations detected through a combination of polymerase chain reaction and restriction endonuclease digestion. Blood samples are utilized as a source of biological material for DNA analyses. Alternatively, detection of MH susceptibility can be established using the *in vitro* CHCT that employs fresh muscle biopsy specimens. Results in the form of CHCT and DNA-based diagnostic parameters are then compared to determine their potential value in predicting MH susceptibility in large MH families that possess Arg614Cys mutations.

Human Model: Cases selected from a large Mennonite MH kindred identified by an index patient that died at age 45 years of an MH crisis after general anesthesia. Case population: N = 68 family members, n = 19/68 muscle biopsies, n = 1/68 documented crisis without biopsy.

Interpretation and Analysis: Family members can be classified as MH-susceptible or MH-normal on the basis of results from CHCT procedures. Approximately 33% of cases can be found to be heterozygous for the Arg614Cys mutation (n = 22/68) and slightly less than one-fourth have prior positive CHCT results (e.g., n = 5/22), while a small percentage have an MH crisis without a biopsy procedure. On DNA testing, it is possible to detect a relatively large number of cases within a related kindred that are normal for the allele (e.g., n = 44). Of these, slightly less than 25% can be classified as MH-normal, while some may be determined to be MH-susceptible (e.g., n = 5) on the basis of CHCT evaluations. Based on the interpretation of the data obtained in earlier CHCT diagnoses, the condition of the muscle is poor, with no twitch, for approximately 60% of cases of individuals homozygous for the normal allele but originally classified as MH-susceptible (e.g., n = 3/5) and for a small percentage that are homozygous for the normal allele and originally classified as MH-normal. Observations from caffeine/halothane contraction test results performed for such individuals (e.g., n = 4) can often be considered invalid. The twitch response is good for a small number of cases (e.g., n = 2 remaining) that are homozygous for the normal allele but classified as MH-susceptible if contractures can be observed with appropriately low levels of both caffeine and halothane.

An absolute correlation between DNA test results and CHCT assignment can often not be established. Possible explanations for discordance are that the Arg614Cys mutation is not linked to MH, that a 2nd MH mutation is segregating in the family, or that there are errors in the CHCT. Because there is strong evidence supporting the causal nature of the Arg614Cys mutation, the discordant cases are not closely related within the pedigree as they would be if a 2nd MH mutation were segregating, the CHCT is not 100% accurate, and it has been proposed that the observed discordance between DNA test results and CHCT assignment in this kindred results from 2 false-positive diagnoses by the CHCT.

Comparison of the segregation of the RYR1 C1840T mutation with segregation of the caffeine/halothane contracture test results for malignant hyperthermia susceptibility in a large Manitoba Mennonite family. Serfas KD, Bose D, Patel L, Wrogemann K, Phillips MS, MacLennan DH, Greenberg CR. Anesthesiology 1996 Feb; 84 (2), pp. 322–9.

Malignant Hyperthermia (MH)/Ryanodine Receptor (RYR1) Gene

Halothane

Background Information: Malignant hyperthermia is a potentially lethal disease triggered by volatile anesthetics and succinylcholine in genetically predisposed individuals. Because of the heterogenetic nature of MH, a simple genetic-based diagnostic test is not feasible and diagnosis requires an invasive open muscle biopsy followed by the *in vitro* contracture test (IVCT).

Implementation: Establish if measurements of halothane-induced increases in intracellular calcium ion concentration $[Ca^{2+}](i)$ in cultured human skeletal muscle cells can be used to phenotype MH susceptibility and if different mutations in the RYR1 gene affect halothane-induced increases in $[Ca^{2+}](i)$.

Primary cultures of human skeletal muscle cells can be acquired from cases previously determined to suffer from MH. Cell lines are then individually screened for the presence of the most common mutations in the RYR1 gene. $[Ca^{2+}](i)$ is measured in 10 cells from each case that has been subjected to 5 different halothane concentrations.

Human Model: Cases of MH (n = 54) diagnosed by the IVCT according to the protocol of the European Malignant Hyperthermia Group can be classified as MH-susceptible (n = 22), MH-negative (n = 18), or MH-equivocal (n = 14).

Interpretation and Analysis: Halothane-induced increases in $[Ca^{2+}](i)$ differ significantly between different classification groups. Interestingly, different mutations of the RYR1 gene do not have a specific impact on halothane-induced increases in $[Ca^{2+}](i)$. Therefore, measurements of $[Ca^{2+}](i)$ in human skeletal muscle cells can be used to phenotype MH susceptibility; however, mutation in the RYR1 gene does not closely correlate with a specific effect related to halothane-induced increases in $[Ca^{2+}](i)$.

Instrumentation: Fluorescent digital microscopy using fura-2/AM can be applied for the measurement of calcium ion concentrations contained within biological samples.

Phenotyping malignant hyperthermia susceptibility by measuring halothane-induced changes in myoplasmic calcium concentration in cultured human skeletal muscle cells. Girard T, Treves S, Censier K, Mueller CR, Zorzato F, Urwyler A. Br J Anaesth 2002 Oct; 89 (4), pp. 571–9.

Malignant Hyperthermia (MH): Genetic Predisposition/(*Ex Vivo* Preparation of Skeletal Muscle Biopsies)
Caffeine/Caffeine and Halothane

Application: Caffeine and caffeine + halothane can be applied as investigative probes to characterize certain intrinsic properties of skeletal muscle tissues. In this context, caffeine and caffeine + halothane can serve as indicator agents for the detection of individuals with a genetic predisposition to the development of MH (potentially fatal complication of general anesthesia).
Implementation: Skeletal muscle *ex vivo* preparations are exposed to caffeine or caffeine + halothane and the subsequent induction of contracture responses is measured for comparison with negative reference controls.
Interpretation and Analysis: In confirmed or suspect cases of MH and their genetic relatives, the responsiveness of the muscle specimens often varies over 2 orders of magnitude. Frequency distribution curves suggest that the variation does not represent a continuum but rather represents 3 or more clusters of functional variants. Muscle specimens from males are on average more responsive to caffeine than is skeletal muscle collected from female subjects. Correlations within father-son and brother-brother pairs indicate complete heritability of responsiveness. Interestingly, there appears to be a lack of correlation between induced contraction of muscle biopsies collected from mother-daughter pairs. There is an intermediate correlation in father-daughter pairs. The sex difference in heritability could be due to gender-related modifying genes or due to secondary modification of the muscle response in females by sex-related characteristics such as hormonal factors. Among the effects of different variables as a function of age, it appears that poor development in early childhood corresponds to or correlates with the potentiation of the caffeine contracture by halothane.

Pharmacogenetics of caffeine and caffeine-halothane contractures in biopsies of human skeletal muscle. Kalow W, Sharer S, Britt B. Pharmacogenetics 1991 Dec; 1 (3), pp. 126–35.

Myasthenia Gravis (MG)
4-Aminopyridine (4-AP)

Cellular/Molecular Mechanism of Action: 4-aminopyridine is a general blocker of voltage-dependent K^+ channels.
Background Information: This pyridine derivative has also been shown to inhibit T cell proliferation, to modulate immune responses, and to alleviate some of the symptoms associated with neurological disorders such as multiple sclerosis, MG, and Alzheimer's disease. 4-aminopyridine triggers a Ca^{2+} response in lymphocytes, astrocytes, neurons, and muscle cells, but little is known about the regulation of the 4-AP response in these cells.
Implementation: Evaluation of suspect conditions of MG.
Interpretation and Analysis: Alleviation of clinical signs suggests a diagnosis of MG.

Myasthenia Gravis (MG)
Thyrotropin-Releasing Hormone (TRH)

Cellular Mechanism of Action: Hypothalamic TRH promotes the synthesis/release of thyroid-stimulating hormone (TSH) from the pituitary.
Implementation: Administration of TRH to measure TSH responses in cases of MG.
Interpretation and Analysis: Some cases of MG display an elevated response to TRH administration reflected by abnormally high increases in TSH produced following administration (e.g., n = 6/13 MG cases). Findings are usually accompanied by normal free thyroxine indices and the absence of antithyroid antibodies. This latter observation is relevant when the association of MG with hyperthyroidism, thyroiditis, and hypothyroidism is considered.

Gonadotropin, prolactin, and TSH secretion in patients with myasthenia gravis. Shapiro MS, Weiss E, Kott E, Taragan R, Shenkman L. J Endocrinol Invest 1984 Dec; 7 (6), pp. 579–83.

Myasthenia Gravis (MG)/Complications/Side Effects/Sequellae
Edrophonium (Tensilon)/Sleep Test/Ice Test

Background Information: The incidence of life-threatening complications from edrophonium chloride (Tensilon) testing for suspected MG is thought to be extremely low.
Implementation: Survey of physician members of the North American Neuro-ophthalmology Society (n = 357) regarding (i) number of years clinician had practiced neuro-ophthalmology; (ii) the estimated number of edrophonium tests performed since completion of training; (iii) number and nature of major complications from edrophonium; and (iv) preference for the sleep test or ice test to edrophonium testing.
Interpretation and Analysis: Of a group of 199 respondents, 105 (53%) may have practiced neuro-ophthalmology for at least 10 years. Estimates of the number of edrophonium tests performed for diagnostic purposes can be very high (e.g., n = 21,111), of which approximately 0.16% (e.g., n = 37) may be associated with a serious complication, mostly attributed to bradyarrythmias and syncope. Respiratory failure, seizure, severe vomiting, and transient ischemic attack may also be recognized. Approximately one-sixth (e.g., n = 31) of respondents (16%) prefer the sleep test or ice test to the edrophonium test and one-third of this group may have observed serious complication with edrophonium. The rate of significant complications of edrophonium testing is low, but the complications can be potentially life threatening.

The complication rate of edrophonium testing for suspected myasthenia gravis. Ing EB, Ing SY, Ing T, Ramocki JA. Can J Ophthalmol 2000 Apr; 35 (3), pp. 141–4; discussion 145.

Myasthenia Gravis (MG)/(Safety Evaluation)
Edrophonium Chloride (Tensilon Test)

Cellular/Molecular Mechanism of Action: Edrophonium chloride is a potent inhibitor of cholinesterase biochemical activity. Inhibition of this enzyme promotes elevated accumulation of acetylcholine (cholinergic neurotransmitter) in the vicinity of presynaptic membranes.
Physiological Mechanism of Action: Edrophonium chloride enhances cholinergic activity (parasympathomimetic properties).
Background Information: Cholinesterase inhibitors (CEIs) have been known to improve strength in patients with MG since the 1930s and have been used as a diagnostic test since the early 1950s.
Application: To evaluate the safety of edrophonium chloride in the course of the Tensilon test by measurement of hemodynamic and ECG parameters and the observation of adverse events.
Implementation: Evaluation of the influence of Tensilon in conditions of suspected MG. Blood pressure, heart rate, continuous

ECG, and adverse events are recorded 10 minutes following intravenous application of Tensilon.

Human Model: Cases of suspected MG (n = 25: open, prospective study).

Interpretation and Analysis: Blood pressure and heart rate do not change significantly during the observation period. Some patients on β-blockers may develop grade I AV block. Self-limiting adverse events of short duration have also been observed (e.g., n = 11 cases). Serious adverse events such as syncope or hemodynamic deterioration usually do not occur. The Tensilon test therefore appears to be a safe procedure. A detailed clinical cardiac history, the history of medication, and 12 lead ECG recordings should be documented in all patients undergoing the Tensilon test. Patients with a history of dysrhythmia receiving digitalis, β-blocking agents, or Ca antagonist therapy should be managed with special care, as Tensilon enhances vagal effects.

Edrophonium chloride (Tensilon) test: A safe method in diagnosing myasthenia gravis. Domanovits H, Wenger S, Schillinger M, Mayr N, Holzer M, Laggner AN, Zeitlhofer J. Wien Klin Wochenschr 2000 Jul 7; 112 (13), pp. 592–5.

The edrophonium test. Pascuzzi RM. Semin Neurol 2003 Mar; 23 (1), pp. 83–8.

Myasthenia Gravis (MG): Dysphagia
Edrophonium Chloride (EC)/Esophogeal Function

Cellular/Molecular Mechanism of Action: Edrophonium chloride is a short-acting acetylcholinesterase inhibitor.

Background Information: Myasthenia gravis is an autoimmune disease in which impairment of neuromuscular transmission results in a pathological fatigability of striated muscles. Dysphagia is a common symptom in MG. It is caused by a weakness of the striated muscles in the pharynx and esophagus.

Application: Detection and characterization of esophageal dysfunction in conditions of MG.

Dosage: Edrophonium chloride (10 mg).

Implementation: Evaluation of esophageal transit facilitated by the application of scintigraphy to assess responses to a multiple swallow test protocol. Cases are studied (i) under baseline conditions, and (ii) immediately after pharmacological stimulation with EC.

Human Model: Cases of clinically proven MG (n = 15; n = 10/15 with a history of dysphagia; n = 6/15 oculopharyngeal manifestation; n = 9/15 generalized weakness).

Interpretation and Analysis: Under baseline conditions, the vast majority of cases (e.g., n = 15/15) show impaired esophageal function (emptying [%]= 58% ± 21; normal range > 85%). In some individuals (e.g., n = 14/15), esophageal transit improves after EC administration (emptying [%]= 75% ± 18; $p < 0.01$), reaching the normal range in slightly less than 30% of cases (e.g., n = 6/15). Esophageal transit, therefore is often compromised in MG. Functional abnormalities may be also present in patients without a history of dysphagia. Inhibition of cholinesterase positively affects striated muscles in the pharynx and upper esophagus, thus improving esophageal transit. Esophageal scintigraphy may be considered a simple, non-invasive method for diagnosing impairment of esophageal function in MG and for monitoring the changes under pharmacological stimulation.

Assessment of esophageal function in patients with myasthenia gravis. Linke R, Witt TN, Tatsch K. J Neurol 2003 May; 250 (5), pp. 601–6.

Protein (Muscle) Breakdown Rate
L-[ring-$^{13}C_6$]Phenylalanine/Leucine Kinetics/ Phenylalanine Kinetics

Cellular/Molecular Mechanism of Action: L-[ring-$^{13}C_6$] phenylalanine is a radioactive analog of the amino acid phenylalanine and therefore closely mimics its metabolism, catabolism, and synthetic incorporation into biological proteins (translation).

Application: L-[ring-$^{13}C_6$]phenylalanine can serve a non-invasive marker molecular for detecting alterations in phenylalanine metabolism, phenylalanine catabolism, protein synthesis, and protein catabolism.

Dosage: L-[ring-$^{13}C_6$] phenylalanine (4–6 mg/kg).

Implementation: Determination of the fractional synthesis rate (FSR) and breakdown rate (FBR) of muscle protein employing a method that utilizes a pulse tracer injection and measurement of enrichment in the arterial blood and muscle at 3 time points. The calculations of FSR and FBR are based on the precursor-product principle. One agent that can be applied for such purposes includes L-[ring-$^{13}C_6$] phenylalanine (4–6 mg/kg).

Animal Model: Rabbit (n = 5 normal/healthy).

Interpretation and Analysis: Measured FBR values (0.233 ± 0.060%/h) are almost identical ($p = 0.35$) to that (0.217 ± 0.078%/h) estimated with a leg arteriovenous balance model (Biolo G, Chinkes D, Zhang X-J, Wolfe RR. J Parenter Enteral Nutr 1992; 16, pp. 305–15). The measured FSR value tends to be lower than that estimated from a leg model (0.125 ± 0.036 vs 0.185 ± 0.086%/h; $p = 0.14$), possibly because the new method measures only muscle FSR, whereas the leg balance model also includes the determination of skin and bone contributions. The pulse tracer injection does not affect muscle protein kinetics as measured by leucine and phenylalanine kinetics in the leg. In related studies (n = 5 rabbits), it has been demonstrated that sampling can be reduced to either 1 or 2 muscle biopsies when multiple pulse injections are used. This method can be completed within a period of 1 hour with 1 muscle biopsy and has technical advantages over currently used methods.

Measurement of muscle protein fractional synthesis and breakdown rates from a pulse tracer injection. Zhang XJ, Chinkes DL, Wolfe RR. Am J Physiol Endocrinol Metab 2002 Oct; 283 (4), pp. E753–64.

Rhabdomyolysis: Recurrent/Exertional (Equine)/Myoplasmic Calcium Concentration Estimation/Heritable Myopathy
Caffeine/4-Chloro-m-Cresol (4-CmC)

Physiological Mechanism of Action: Caffeine promotes contraction of skeletal muscle tissue in *in vitro* preparations.

Route of Administration: Topical (*in vitro*) bathing of muscle tissue biopsy specimens.

Implementation: Delineate if alterations in myoplasmic calcium regulation can be identified in muscle cell cultures (myotubes) and intact muscle fiber bundles derived from Thoroughbreds affected with recurrent exertional rhabdomyolysis (RER). Laboratory procedures that can be employed to address such an objective may employ myotube cell cultures grown from satellite cells obtained from muscle biopsy specimens collected from horses affected by RER. Fura-2 fluorescence can be used to measure resting myoplasmic calcium concentration as well as caffeine- and 4-CmC-induced increases in myoplasmic calcium. In addition, intact intercostal muscle fiber bun-

dles can be prepared from both types of horses, and their sensitivities to caffeine and 4-CmC-induced contractures determined.

Animal Model: Equine (n = 6 related Thoroughbreds with RER; n = 8 clinically normal control Thoroughbred or crossbred horses).

Interpretation and Analysis: Myotubes of RER-affected and control horses have identical resting myoplasmic calcium concentrations. Myotubes from RER-affected horses have significantly higher myoplasmic calcium concentrations than myotubes from control horses following the addition of ≥ 2mM caffeine; however, there is no difference in their response to 4-CmC (≥ 1 mM). Caffeine contracture thresholds for RER and control intact muscle cell bundles (2 vs 10 mM, respectively) are significantly different, but 4-CmC contracture thresholds of muscle bundles from RER-affected and control horses (500 μM) do not differ. An increase in caffeine sensitivity of muscle cells derived from a family of related RER-affected horses has been detected *in vitro* by use of cell culture with calcium imaging and by use of fiber bundle contractility techniques. An alteration in muscle cell calcium regulation is a primary factor in the cause of this heritable myopathy.

Myoplasmic calcium regulation in myotubes from horses with recurrent exertional rhabdomyolysis. Lentz LR, Valberg SJ, Herold LV, Onan GW, Mickelson JR, Gallant EM. Am J Vet Res 2002 Dec; 63 (12), pp. 1724–31.

9 Reproductive System

Amniotic Fluid Volume Estimation
Aminohippurate Sodium

Cellular/Molecular Mechanism of Action: Aminohippurate sodium can be applied as a tracer molecule to monitor physiological processes and estimate fluid compartment volumes.
Application: Utilization of aminohippurate sodium as a reagent in dye-dilution techniques suitable for accurately estimating amniotic fluid volume.
Route of Administration: Amniocentesis.
Implementation: Evaluation of the fluid volume for amniotic fluid obtained from normal singleton pregnancies with intact membranes (Cesarean deliveries).

Human Model: Women (n = 15; cases with amniocentesis at delivery n = 6; Cesarean delivery n = 9 with samples collected 4–24 hours prior to surgery).
Interpretation and Analysis: Good concordance between the dye-determined and direct measurement of amniotic fluid volume can be appreciated in women undergoing amniocentesis (e.g., n = 6/6 cases; r = 0.99, p = < 0.001). Among women with varying times from amniocentesis to direct assay measurements (e.g., n = 9 cases), the correlation is not significant (e.g., n = 9 cases; r = 0.36, p = 0.08). The percent difference between dye-determined and directly measured amniotic fluid volume is significantly smaller in women undergoing amniocentesis just prior to delivery (7%) compared to the women with varying times from amniocentesis to delivery (37%, $p < 0.001$). Dye-determined amniotic fluid volume accurately reflects actual amniotic fluid volume but the dye-determined concentrations, *in vivo*, may undergo rapid changes.

Dye-dilution techniques using aminohippurate sodium: Do they accurately reflect amniotic fluid volume? Magann EF, Whitworth NS, Files JC, Terrone DA, Chauhan SP, Morrison JC. J Matern Fetal Neonatal Med 2002 Mar; 11 (3), pp. 167–70.

Cervical Cell Type Differentiation/Normal vs Abnormal/Cell Nuclei Visualization
Acetic Acid

Cellular/Molecular Mechanism of Action: Acetic acid chemically modifies the physical properties of different cellular constituents.
Application: The use of high-resolution *in vivo* confocal imaging may offer a clinically useful adjunct to standard methods for the diagnosis and screening of epithelial precancers.
Implementation: Assess the feasibility of real-time confocal reflectance imaging of cervical tissue and the use of acetic acid as a contrast agent to increase visualization of cell nuclei.

***Ex Vivo* Model:** Confocal microscopy image evaluation of cervical cells and colposcopically normal and abnormal cervical biopsy specimens. Images are obtained before and after the application of 6% acetic acid.
Interpretation and Analysis: Confocal microscopy imaging can resolve subcellular detail throughout the entire epithelial thickness. Normal and abnormal tissues are clearly able to be differentiated. Addition of acetic acid enhances nuclear signal in all acquired images. High-contrast reflected light images of cervical tissue are attainable in near real time. Acetic acid significantly increases light scattering from cell nuclei, which may partially explain why acetowhitening occurs.

Laser scanning confocal microscopy of cervical tissue before and after application of acetic acid. Drezek RA, Collier T, Brookner CK, Malpica A, Lotan R, Richards-Kortum RR, Follen M. Am J Obstet Gynecol 2000 May; 182 (5), pp. 1135–9.

Endometriosis/Prolactin Response: Lack of Discrimination
Thyrotropin-Releasing Hormone (TRH)/Insulin Challenge

Application: Detection and characterization of endometriosis conditions.
Implementation: Evaluation of prolactin secretion applying a TRH test and an insulin challenge test in conditions of female endometriosis.

Human Model: Female cases of endometriosis (n = 10) treated with danazol at a dose of 200 mg 3 times daily for 6 months (evaluated after the reappearance of 2nd menses). Reference controls include normal females (n = 10 in follicular phase; test performed only once).
Interpretation and Analysis: The absence of a significant difference in basal prolactin levels or responses to the TRH and insulin challenge tests between controls and patients with endometriosis, before and after danazol treatment, supports the view that hyperprolactinemia should not be considered as a cause of infertility in endometriotic women. Furthermore, significant reductions in prolactin response to provocative tests during danazol treatment may be associated with the low estrogen levels caused by the medication. Finally, inadequate prolactin responses to insulin-induced hypoglycemia in cases with endometriosis and in normal women shows that this provocative test is not effective for hypothalamic stimulation of prolactin.

Provocative tests of prolactin before, during and after long-term danazol treatment in patients with endometriosis. Panidis D, Vavilis D, Rousso D, Panidou E, Kalogeropoulos A. Gynecol Endocrinol 1992 Mar; 6 (1), pp. 19–24.

Endometriosis: Infertility (Hyperprolactenemia)/ Ovulatory Dysfunction and Infertility
Thyrotropin-Releasing Hormone (TRH)/ [Metoclopramide]

Cellular/Molecular Mechanism of Action: Metoclopramide functions as a dopaminergic type 2 (DA2) receptor competitive antagonist.
Background Information: The origin of infertility in patients with endometriosis without tubal occlusion has not yet been clearly

defined. Several reports show an abnormal pituitary-ovarian axis in this group of patients. Moreover, prolactin (PRL) and growth hormone (GH) secretion is closely related to reproductive status.

Implementation: Evaluation of minimal/mild endometriosis conditions as a function of PRL and GH responses following the infusion of metoclopramide and TRH infusion. Metoclopramide or TRH is administered randomly in 2 sequential menstrual cycles (cycle days 3–5). Serum PRL and GH secretion before and after dopaminergic type 2 (DA2) receptor blockade and TRH are compared.

Human Model: Cases of infertility due to minimal/mild endometriosis (n = 64 females; composed of n = 33 fertile without endometriosis; n = 10 fertile with minimal/mild endometriosis; n = 21 infertile with mild/moderate endometriosis)

Interpretation and Analysis: Higher serum PRL levels are observed in cases with endometriosis at baseline and after 15 and 30 minutes of TRH administration. Also, infertile cases with endometriosis have lower serum estradiol levels than fertile patients. Moreover, the dopaminergic blockade does not result in abnormal PRL or GH secretion. Decreases in serum estradiol levels and altered PRL secretion after TRH administration in infertile cases with minimal/mild endometriosis are related to ovulatory dysfunction and infertility in this group of patients without tubal occlusion.

Prolactin and growth hormone secretion after thyrotrophin-releasing hormone infusion and dopaminergic (DA2) blockade in infertile patients with minimal/mild endometriosis. Cunha-Filho JS, Gross JL, Lemos NA, Dias EC, Vettori D, Souza CA, Passos EP. Hum Reprod 2002 Apr; 17 (4), pp. 960–5.

Endometriosis Stages I, II, III, and IV
Thyrotropin-Releasing Hormone (TRH)

Background Information: Hyperprolactinemia has been described in previous reports to be associated with infertility and endometriosis.

Application: Characterize serum prolactin concentrations in cases with infertility and endometriosis.

Implementation: Evaluation of basal serum prolactin levels and prolactin levels after TRH administration.

Human Model: Cases of infertility and laparoscopically proven endometriosis (n = 40).

Interpretation and Analysis: The mean basal serum prolactin concentrations are 12.5, 16.5, 19.5, and 26.5 ng/ml compared to post-TRH administration levels of 88.3, 114.2, 125.3, and 138.8 ng/ml in cases with stages I, II, III, and IV endometriosis, respectively. A statistically significant relationship exists between basal serum prolactin levels as well as those after TRH injection and the stage of the endometriosis. In cases that either do not receive treatment or have been initially treated with gonadotropin-releasing hormone (GnRH) analogues (24 weeks) and other therapeutic schemes to improve fecundity, the fertility rate is not noticeably different. Cases that do not become pregnant, however, have higher serum prolactin concentrations after TRH administration as compared to those who conceive. Occult hyperprolactinemia may therefore be a cause of infertility in cases with endometriosis.

Evaluation of serum prolactin levels in patients with endometriosis and infertility. Gregoriou G, Bakas P, Vitoratos N, Papadias K, Goumas K, Chryssicopoulos A, Creatsas G. Gynecol Obstet Invest 1999; 48 (1), pp. 48–51.

Impotency: Male
Papaverine

Route of Administration: Intracavernous (IC) applying autoinjection techniques.

Considerations: Vasoactive drugs can be described as being inducers (drugs that induce a rigid erection, even in the presence of the doctor), facilitating drugs (pharmaceuticals that produce a rigid erection only if sexual stimulation is also present), and inhibitors (agents that stop an erection). The former group (principally papaverine) produces a significant number of side effects, not least of these being priapism; there is a risk of lasting iatrogenic impotence that is not negligible.

Sequelae are reduced considerably when one uses facilitating drugs that, although less powerful, suffice in treating a large proportion of cases of impotence.

Interpretation and Analysis: A substantial number of impotent cases (25%) improve after papaverine, while 50% of cases suffering from impotence of psychological origin also note an improvement. Intracavernous treatment now seems justified in most cases that have not responded to traditional therapeutic strategies, and this includes cases of psychological origin. Papaverine cannot be replaced as a diagnostic drug but facilitating drugs should be used 1st in therapy and inducers should be used only if facilitating drugs have failed.

The role of adrenomedullin in varicocele and impotence. Ozbek E, Yurekli M, Soylu A, Davarci M, Balbay MD. BJU Int 2000 Oct; 86 (6), pp. 694–8.

Infertility/Female Fertility/Thyroid-Stimulating Hormone (TSH)/Prolactin (PRL) Responses
Metoclopramide/Thyrotropin-Releasing Hormone (TRH)

Cellular/Molecular Mechanism of Action: Metoclopramide functions as a dopaminergic DA2 receptor competitive antagonist.

Application: Characterization of female fertility and infertility conditions.

Implementation: Evaluation of TSH determinations before and after TRH stimulation. During the same period, PRL may be determined after TRH stimulation in the early follicular phase and after metoclopramide stimulation in the luteal phase.

Human Model: Cases of female infertility (n = 834 women subjected to a TRH stimulation test between 1982 and 1985 in Germany; and n = 759 females subjected to the metoclopramide stimulation test).

Study Design: Cross-sectional study.

Interpretation and Analysis: Thyroid function disturbances can be appreciated in 20% of women evaluated, which is in accordance with the prevalence in South Germany. Postcoital tests are significantly poorer in females with subclinical hypothyroidism than in euthyroid patients. Spontaneous conception is more frequent in euthyroid (16%) than in hypothyroid (6%) conditions. The pregnancy rate is not improved by the administration of dopamine agonists in women with an exaggerated response to TRH or metoclopramide.

Such findings suggest that subclinical hypothyroidism as well as disorders of PRL secretion may play a role in female infertility. The TRH test has been proposed as an approach for ruling out thyroid dysfunction. Neither the TRH nor the metoclopramide test is useful for the prognostic differentiation of PRL secretion disorders.

Thyrotropin-releasing hormone (TRH) and metoclopramide testing in infertile women. Gerhard I, Eggert-Kruse W, Merzoug K, Klinga K, Runnebaum B. Gynecol Endocrinol 1991 Mar; 5 (1), pp. 15–32.

Infertility/Female Fertility/Growth Hormone (GH)/Estradiol/Insulin-Like Growth Factor (IGF)/Prolactin (PRL)/Thyroid-Stimulating Hormone (TSH)
Metoclopramide/Thyrotropin-Releasing Hormone (TRH)

Cellular/Molecular Mechanism of Action: Metoclopramide functions as a dopaminergic DA2 receptor competitive antagonist.
Background Information: The origin of infertility in patients with endometriosis without tubal occlusion has been shown in several reports to be due to abnormal pituitary-ovarian axis in this group of patients. Moreover, PRL and GH secretion is closely related to reproductive status.
Application: Characterization of infertility in women with endometriosis without tubal occlusion.
Implementation: Evaluation of PRL and GH secretion during the early follicular phase. Metoclopramide is administered and blood samples collected at −15, 0, 15, 30, 45, and 60 minutes for the measurement of PRL, GH, estradiol, IGF-I, TSH, and glucose concentrations (insulin measured at −15 and 0 minutes). Existence of a correlation between GH and PRL secretion can then be determined.

Human Model: Cases of normal fertility (n = 22 females) presented with normal serum levels of estradiol, PRL, insulin, fasting glucose, and IGF-I.

Study Design: Cross-sectional study.
Interpretation and Analysis: Serum GH levels are not changed after metoclopramide infusion ($p = 0.302$), but there is a significant alteration in serum PRL ($p = 0.0001$) with the highest levels after 30 (mean: 237.20 ng/ml ± 95.86) and 45 (mean: 211.80 ng/ml ± 83.24) minutes. Serum GH levels do not correlate with serum PRL levels after the dopaminergic DA2 blockade. Therefore, GH secretion is not modulated by a direct effect of Type 2 dopamine receptor.

Growth hormone and prolactin secretion after metoclopramide administration (DA2 receptor blockade) in fertile women. Cunha-Filho JS, Gross JL, Vettori D, Dias EC, Passos EP. Horm Metab Res 2001 Sep; 33 (9), pp. 536–9.

Infertility: Female vs Male/Oocyte Maturity
Follicle-Stimulating Hormone: Purified (pFSH)/Menopausal Gonadotropin

Physiological Mechanism of Action: Promotes maturation of ovarian follicles and subsequent ovulation.
Application: Both pFSH and human menopausal gonadotropin (hMG) can be used in concert with intraplasmic sperm injection to improve the fertilization potential in females experiencing persistent infertility through improved follicle/oocyte maturation.
Route of Administration: Follicle-stimulating hormone (purified).
Dosage: Follicle-stimulating hormone (purified).
Background Information: Intracytoplasmic sperm injection (ICSI) is widely employed today in cases of severe male factor infertility. The technique requires denuding the oocytes from the surrounding granulosa cells prior to sperm injection. One can thus assess oocyte maturity more accurately and can study the effects of various ovarian stimulation protocols on egg maturation and the rest of the parameters of the outcome of ICSI.
Implementation: Evaluation of the influence of ovarian stimulation using hMG compared to that achieved with pFSH.
Interpretation and Analysis: Significantly higher fertilization rate can be appreciated with the administration of pFSH preparations. The parameters of percentage of mature oocytes and pregnancy rate are similar for both pFSH and hMG preparations. Pregnancy rate does not seem to be affected by the gonadotropin preparation employed for ovarian stimulation. Recognition of this consideration has economic implications due to the financial cost of certain gonadotropin preparations and quantities of such products are frequently limited.

A comparative study of the effect of ovarian stimulation protocols with different gonadotropin preparations on the biological and clinical parameters of the outcome of intracytoplasmic sperm injection. Drakakis P, Loutradis D, Kallianidis K, Bletsa R, Milingos S, Dionyssiou-Asteriou A, Michalas S. Clin Exp Obstet Gynecol 2002; 29 (4), pp. 286–9.

Infertility: Male/Spermatozoa Fertilization Potential
Hemizona Assay

Application: Prediction of the fertilization potential of domestic animal and human spermatozoa. Theoretically, the analysis assay would also be of value to exotic animal breeding programs in zoo wildlife refuge impoundments.
Validation Investigations: Evaluation of spermatozoa fertilization potential (e.g., equine) can be established by obtaining equine ovarian oocytes collected immediately postmortem or by ultrasound-guided follicle aspiration. Oocytes are then denuded from cumulus cells and stored in salt solution at 4°C prior to use. Oocytes are then bisected to provide 2 equal matching hemizonae from each oocyte. Semen samples from cases with known fertility data are then used to assess the number of spermatozoa bound to the outer surface of the hemizona after *in vitro* incubation. Sperm binding to matching hemizonae of a particular individual (e.g., stallion) has been found to be similar and corresponds to results observed with the hemizona assay. In horses, spermatozoa hemizona binding capacity of 10 pairs of stallions has been compared by incubating 1 hemizona with the semen of a stallion and the matching hemizona with the semen of another stallion from the same stud farm. Applying 5 matching pairs of hemizonae for each pair of stallions, it can be observed that there is a significant relationship between the mean number of spermatozoa bound to matching hemizonae and stallion fertility indices from each stud farm ($p < 0.0001$).
Interpretation and Analysis: The hemizona assay can be used as a valuable diagnostic tool in analyzing the quality and function of spermatozoa.

Testicular Function/Cryptorchidism (Unilateral or Bilateral) vs Unilateral or Bilateral Castration/Aggressive Cryptorchid Behavior
Human Chorionic Gonadotropin (hCG)

Cellular/Molecular Mechanism of Action: Human chorionic gonadotropin induces synthesis and release of testosterone from testicular Leydig cells.
Application: Detection of conditions of cryptorchidism (unilateral or bilateral) when it is uncertain if cases may actually be normal but have previously been subjected to bilaterial or unilateral castration.
Background Information: Horses with cryptorchidism tend to be ill-tempered. Sometimes insufficient history is available to know if a horse has or has not been previously castrated (e.g., non-castrated cryptorchid vs unilateral castration).
Considerations: Horses less than 3 years of age and all donkeys cannot be evaluated using a single measurement of (resting) blood

esterone sulfate for the purpose of detecting conditions of cryptorchidism.

Route of Administration: Human chorionic gonadotropin (intravenous injection).

Dosage: Intravenous injection of hCG at a dosage of 6,000 IU per 450 kg with blood sample collected prior to and 30 to 120 minutes after injection.

Implementation: Detection of conditions of unilateral or bilateral cryptorchidism by measuring increases in serum testosterone levels induced by hCG administration compared to preinjection concentrations.

Interpretation and Analysis: Cryptorchid patients have basal systemic esterone sulfate concentrations in excess of 400 pg/ml. Alternatively, in situations where the esterone sulfate assay is not an appropriate diagnostic approach, the administration of hCG will induce increases in blood testosterone levels. Prior to hCG administration, cryptorchid cases will have resting testosterone levels in excess of 100 pg/ml, whereas horses not affected by cryptorchidism will have resting testosterone concentrations below 40 pg/ml that will not change following hCG administration.

Laboratory Instrumentation: Assay of either growth hormone (GH) concentrations by either radioimmunoassay (RIA) or ELISA.

Cryptorchidism. Cox JE (pp. 571–3). Current Therapy in Equine Medicine 2, ed Robinson NE. WB Saunders, Philadelphia (Copyright 1987).

Is human chorionic gonadotropin useful for identifying and treating nonpalpable testis? Bukowski TP, Sedberry S, Richardson B. J Urol 2001 Jan; 165 (1), pp. 221–3.

10 Renal and Urinary Systems

Anuria/Oligouria
Dopamine

Cellular/Molecular Mechanism of Action: Promotes direct dilation of the renal artery, thereby increasing renal perfusion.
Diagnostic Application: Detection of acute oliguria or anuria associated with hypoxia resulting from decreased renal perfusion as occurs with hypotensive (e.g., endotoxic) shock. Initially, patients suspected of being oliguric or anuric are administered polyionic intravenous fluids. If urine production is not appreciated, the 2nd phase of diagnostic evaluation entails the administration of 1 to 2 doses of furosemide. Failure to produce urine after furosemide administration implies the necessity to evaluate the potential for renal urine production with dopamine in stage 3 of the diagnostic procedure.
Route of Administration and Dosage: Administered as a slow drip diluted in 5% sterile dextrose. In potentially oliguric and anuric patients, dopamine is administered intravenously at a rate of between 1 to 5 μg/kg/min, thereby directly promoting vasodilation of the renal artery. Administration of dopamine at dose rates greater than 5 μg/kg/min results in renal artery vasoconstriction. Heart rate and abdominal discomfort should be monitored closely.
Considerations: Due to the adrenergic (sympathetic) properties of dopamine, gastrointestinal ileus and possibly physical (feed-related) obstruction of the gastrointestinal tract may be experienced. Dietary management can often assist in reducing the severity and risk of such sequela (e.g., equine).
Interpretation and Analysis: Absence of urine production following administration suggests the presence of oliguria or anuria condition that may be temporary or permanent in duration.
Laboratory Instrumentation: Not applicable.

Anuria/Oligouria/Severity in Acute Renal Failure: Reversible/Non-Reversible
Furosemide (Lasix)/Bumetanide

Cellular/Molecular Mechanism of Action: Furosemide is a classic potassium-wasting "loop diuretic" that inhibits electrolyte reabsorption primarily in the "thick" ascending region of the loop of Henle. Perfusion of blood within the kidney is increased but the filtration rate remains essentially unchanged. This latter phenomenon is most prominent when furosemide is administered intravenously. Furosemide also weakly inhibits carbonic anhydrase and promotes vasodilation in part through induction of prostacyclin (PGI_2) synthesis.
Physiological Mechanism of Action: Bumetanide is a potent, rapid-acting loop diuretic (potency 40 > furosemide) applied to determine the severity of acute renal failure.
Diagnostic Application: Detection of acute oliguria or anuria associated with hypoxia resulting from decreased renal perfusion as occurs with hypotensive (e.g., endotoxic) shock or other forms of acute renal failure. Initially, patients suspected of being oliguric or anuric are administered intravenous polyionic fluids. If urine production is not appreciated, the 2nd phase of diagnostic evaluation entails the administration of 1 to 2 doses of furosemide. Failure to produce urine after furosemide administration implies the necessity to evaluate the potential for renal urine production with dopamine in stage 3 of the diagnostic procedure.
Route of Administration and Dosage: Intravenous and/or intramuscular injection of standard dose normally applied to induce diuresis.
Considerations: Lasix is a potassium-wasting diuretic.
Interpretation and Analysis: Urinary output should occur within 30 minutes of intravenous injection. Absence of urine production during this time period implies the presence of profound renal dysfunction.
Laboratory Instrumentation: Not applicable.

Biochemical Metabolism: Cortisol/11β-HSD2 Biochemical Activity
Deuterium-Labeled Cortisol

Cellular/Molecular Mechanism of Action: Deuterium-labeled cortisol is an analog of endogenous cortosol.
Application: Deuterium-labeled cortisol can be used as a labeled marker molecule for assessing *in vivo* alterations in endogenous cortisol metabolism mediated by 11β-HSD2 activities based on urinary excretion of cortisol, cortisone, and their A-ring reduced metabolites.
Route of Administration: Deuterium-labeled cortisol (oral).
Implementation: Evaluation of cortisol metabolism determined through the measurement of deuterium-labeled cortisol and its deuterium-labeled metabolites. Sample evaluation involves the application of GC/MS analyses simultaneously with the detection of endogenous cortisol, cortisone, and their A-ring reduced metabolites.
Human Model: Normal cases without evidence of disease.
Interpretation and Analysis: The stable isotope approach offers unique advantages in assessing the appropriateness of measuring unconjugated and total (unconjugated + conjugated) cortisol, cortisone, and their A-ring reduced metabolites in urine as indices of renal 11β-HSD2 activity. Measurement of urinary unconjugated cortisol and cortisone has many advantages for assessing 11β-HSD2 activity.
Instrumentation: Integrated gas chromatography/mass spectrometry system.

The use of deuterium-labeled cortisol for *in vivo* evaluation of renal 11β-HSD activity in man: Urinary excretion of cortisol, cortisone and their A-ring reduced metabolites. Kasuya Y, Shibasaki H, Furuta T. Steroids 2000 Feb; 65 (2), pp. 89–97.

Biochemical Metabolism: Hydroxylation Capacity
Phenylalanine

Cellular/Molecular Mechanism of Action: Phenylalanine is metabolized in the kidney to a hydroxylated analog.
Background Information: Although phenylalanine hydroxylase activity is detectable in *in vitro* renal tissue preparations, minimal

knowledge exists regarding *in vivo* phenylalanine hydroxylation across the human kidney or the possible contribution of the kidney to whole-body hydroxylation.

Application: Evaluation of renal hydroxylation capacity.

Implementation: Evaluate measurements of whole-body, renal, and splanchnic hydroxylation of phenylalanine to tyrosine, as well as phenylalanine and tyrosine rates of appearance (Ra) and disposal (Rd), in postabsorptive states by means of renal and splanchnic arteriovenous catheterization combined with phenylalanine and tyrosine isotope infusions.

Interpretation and Analysis: In the kidney, relevant phenylalanine hydroxylation activity can be detected (3.51 ± 0.97 μmol/min $\times$ 1.73 m^2 of body surface), whereas it is 2.48 ± 1.35 μmol/min $\times$ 1.73 m^2 across the splanchnic area. These 2 sites together account for virtually the entire whole-body phenylalanine hydroxylation capacity. Renal production of tyrosine from phenylalanine hydroxylation accounts for approximately 13% of whole-body tyrosine Ra, whereas renal total tyrosine Ra accounts for approximately 34% of whole-body tyrosine Ra. In the splanchnic area, these figures are approximately 9% and 40%, respectively. Hydroxylation accounts for approximately 70% of phenylalanine Rd in the kidney, as opposed to approximately 8% in the splanchnic area.

Phenylalanine hydroxylation across the kidney in humans rapid communication. Tessari P, Deferrari G, Robaudo C, Vettore M, Pastorino N, De Biasi L, Garibotto G. Kidney Int 1999 Dec; 56 (6), pp. 2168–72.

Detrusor Contractions: Spontaneous/Urinary Frequency Secondary to Benign Prostatic Obstruction

Adenosine Triphosphate (ATP)-Sensitive Potassium Channel Openers (KCOs)/A-278637 (Dihydropyridine Analog)/ZD6169 WAY-133537

Cellular/Molecular Mechanism of Action: Adenosine triphosphate–sensitive potassium channel openers.

Physiological Mechanism of Action: Relaxation of involuntary detrusor contractions.

Application: Characterization of male urinary frequency/urgency.

Route of Administration: Oral (rat).

Dosage: ZD6169 (10 μmol/kg) and A-278637 (3 μmol/kg).

Considerations: Symptoms of urinary frequency and urgency secondary to benign prostatic obstruction are common in elderly men. In many patients, these symptoms correspond to the urodynamic finding of involuntary detrusor contractions during filling cystometry (i.e., detrusor instability). Spontaneous non-voiding contractions during filling can be modeled in animals by subchronic, partial urethral obstruction. However, many investigators remove the obstructive ligature a few days prior to cystometrical evaluation (which may not be an ideal representation of the clinical situation where obstruction is still present), and all perform cystometry within 3 days post-bladder catheterization surgery (i.e., while considerable wound healing is present).

Implementation: Evaluation of structurally diverse ATP-sensitive KCOs ($n = 3$) for their ability to influence spontaneous detrusor contractions secondary to obstruction with an intact obstructive ligature at the time of testing and 2 weeks post–bladder catheterization.

Animal Model: Rats.

Interpretation and Analysis: The agents ZD6169, WAY-133537, and the novel dihydropyridine KCO, A-278637, all significantly decrease spontaneous bladder contractions at 30 minutes post-dosing (p.o.). However, only ZD6169 (10 μmol/kg) and A-278637 (3 μmol/kg) attenuate such bladder contractions at doses that concurrently do not significantly affect mean arterial blood pressure and heart rate. These data confirm the efficacy of KCOs to inhibit unstable contractions in obstructed rats, and they further demonstrate the positive effect of the bladder-selective KCO, A-278637, in an animal model with potentially less artifact than observed in previous animal models.

K-ATP opener-mediated attenuation of spontaneous bladder contractions in ligature-intact, partial bladder outlet obstructed rats. Lynch JJ 3rd, Brune ME, Lubbers NL, Coghlan MJ, Cox BF, Polakowski JS, King LL, Sullivan JP, Brioni JD. Life Sci 2003 Mar 14; 72 (17), pp. 1931–41.

Effective Renal Plasma Flow (ERPF)

Paraaminohippuric Acid/[^{131}I]-Iodohippurate

Cellular/Molecular Mechanism of Action: Hippuric acid perfuses through the glomerular apparatus, thereby becoming a constituent within the plasma filtrate. Within the renal tubule, hippuric acid is reabsorbed from the glomerular filtrate.

Diagnostic Application: Determination of the relative level of effective renal plasma flow (ERPF) in disease conditions. The agent does not per se selectively evaluate renal tubular function. Various forms of hippuric acid can be used to evaluate the potentially renal toxic properties associated with new candidate pharmaceutical agents or injury precipitated during the course of disease.

Route of Administration: Intravenous administration only.

Implementation: Evaluation of renal glomerular function by measuring plasma clearance of the radiolabeled marker molecules.

Interpretation and Analysis: Estimates of ERPF using [^{131}I]-iodohippurate are slightly higher than values obtained using [^{14}C]-inulin and [^{3}H]-tetraethylammonium. Estimates of ERPF in cats are lower than those observed in dogs. [^{125}I]-iothalamate has also been used to measure estimated glomerular filtration rate (GFR). Note: Filtration fraction = GF/ERPF.

Concentration measured as a function of spectrophotometric absorbance [^{123}I]-ortho-iodohippuric acid. Prévot A, Mosig D, Martini S, Guignard JP. Pediatr Res 2004 Feb; 55 (2), pp. 254–60.

Evaluation of renal function in transplant patients on tacrolimus therapy. Agarwala S, Culligan E, Jain A, McCauley J, Shapiro R, Chakrabarti P, Burckart G, Venkataramanan R. J Clin Pharmacol 2002 Jul; 42 (7), pp. 798–805.

Effective Renal Plasma Flow (ERPF)

Hippurate/Benzoate Metabolite/[^{14}C]Hippurate/[^{3}H]Hippurate/[^{14}C]Benzoate

Cellular/Molecular Mechanism of Action: Hippurate functions as a passive tracer/marker molecular that is compatible with *in vivo* living systems.

Application: Assessment of effective renal plasma flow.

Implementation: Evaluation of the relative extent of excretion for [^{14}C]hippurate and [^{3}H]hippurate utilizing a single-pass flow through the kidney.

***Ex Vivo* Model:** Rat (isolated kidney preparation perfused at 8 ml/min containing tracer concentrations of [^{14}C]benzoate and [3H]hippurate).

Interpretation and Analysis: The steady state renal extraction ratio of [^{14}C]benzoate is 0.26 ± 0.04 and is associated with a renal clearance of 1.13 ± 0.17 ml/min/g. The urinary clearance of [^{14}C]benzoic acid is low (0.011 ± 0.01 ml/min/g), yielding a low fractional excretion (unbound urinary clearance/glomerular filtration rate [GFR}) value of 0.27 ± 0.19 and suggesting that glycination of [^{14}C]benzoate to [^{14}C]hippurate accounts almost completely for the

total renal clearance. Fractional excretion for preformed [^{3}H]hippurate is 8 times that of GFR, but the steady state renal extraction ratio of preformed [^{3}H]hippurate, E_{pmi} (0.24 ± 0.05) is much lower than the apparent extraction ratio of the renally formed [^{14}C]hippuric acid [E_{mi} = 0.39 ± 0.09] ($p < 0.05$). The theoretical basis for the discrepancy can be explored with mathematical formulations developed from a physiologically based model of the kidney. It has been found that parent drug kinetic parameters (transport and metabolic intrinsic clearance of benzoate) are unimportant for E_{mi} or E_{pmi}. Rather, the value of EK_{mi} exceeds EK_{pmi} because the ratio of efflux clearances at the basolateral and luminal membranes for hippurate (corrected) is less than 26.089, a value determined by the GFR, plasma renal flow, and the unbound fraction of hippurate of the system that would render E_{mi} to equal E_{pmi} in the system. The influx clearance for hippurate to enter from plasma to cell at the basolateral membrane and the reabsorption clearance of hippurate to enter from tubular urine to cell at the luminal membrane fails to alter the ratio of EK_{pmi}/EK_{mi}. Note: Filtration fraction = GF/ERPF.

Differences in excretion of hippurate, as a metabolite of benzoate and as an administered species, in the single-pass isolated perfused rat kidney explained. Geng W, Pang KS. J Pharmacol Exp Ther] 1999 Feb; 288 (2), pp. 597–606.

Glomerular Filtration
Creatinine Clearance

Cellular/Molecular Mechanism of Action: Creatinine is a substance that is endogenously synthesized by muscle tissue (myocytes). Creatinine in the intravascular compartment is excreted by glomerular filtration.

Diagnostic Application: The rate at which creatinine is cleared from the intravascular compartment can be utilized to estimate the rate of glomerular filtration (GFR). It is often used to diagnose renal disease in patients with normal blood urea nitrogen (BUN) and serum creatinine concentrations. Inulin can also be used to evaluate GFR in addition to [^{51}Cr] ethylene-diamine-tetra-acetic acid (EDTA) and [^{99m}Tc]-diethylenetriamine pentaacetic acid (DTPA).

Route of Administration: Subcutaneous.

Considerations: Tubular secretion of creatinine (in dogs) can lead to an overestimation of GFR of ≤ 25%. The influence of tubular creatinine secretion on U_{cr} is compensated for by non-creatinine chromagens on P_{cr} that makes this measurement an effective diagnostic approach.

Interpretation and Analysis: Endogenous creatinine clearence in the dog and cat is approximately 2–5 ml/min/kg. Results often approximate those recognized for inulin clearance tests.

Glomerular Filtration
Inulin Excretion Test/Iohexol

Cellular/Molecular Mechanism of Action: Inulin and iohexol per se do not exert any significant molecular or cellular mechanism of action.

Application: Iohexol and inulin can be used simultaneously to calculate estimates of glomerular filtration rate (GFR) as can inulin, [^{51}Cr] ethylene-diamine-tetra-acetic acid (EDTA), and [^{99m}Tc]-diethylenetriamine pentaacetic acid (DTPA).

Route of Administration: Inulin and iohexol can be applied as inert molecular tracer molecules for the purpose of estimating GFR.

Implementation: Measurement and estimation of GFR using a single injection inulin methodology. The total clearance can be calculated from the decay of serum inulin concentrations after injection of 3,000 mg/m^2 body surface area using a 2-compartment model.

Animal Model: Feline (n = 53 young healthy adults; n = 23 > 10 years or near abnormal serum creatinine).

Interpretation and Analysis: Concomitant inulin and iohexol clearance can potentially show excellent correlation with 1 another (e.g., n = 9 cats). Calculated normal values for GFR are 35.9–58.5 (median 46.0) ml/min/m^2 or 2.07–3.69 (median 2.72) ml/min/kg (e.g., n = 30/50 cats). Some cases with normal creatinine or blood urea nitrogen levels can have reduced GFR and therefore may be in a state of early renal dysfunction. Studies indicate that single-injection inulin clearance is a valuable tool for routine GFR measurements in cats. An "inulin excretion test" using only 1 blood sample 3 hours after the administration of 3,000 mg/m^2 body surface area can be a viable alternative for assessing renal function in daily practice.

Single-injection inulin clearance for routine measurement of glomerular filtration rate in cats. Haller M, Rohner K, Müller W, Reutter F, Binder H, Estelberger W, Arnold P. J Feline Med Surg 2003 Jun; 5 (3), pp. 175–81.

Glomerular Filtration
Iohexol/Exogenous Creatinine Clearance

Application: Analysis of the pharmacokinetic profile for iohexol to estimate glomerular filtration rate (GFR). Inulin can also be used to evaluate GFR, in addition to [^{51}Cr] ethylene-diamine-tetra-acetic acid (EDTA) and [^{99m}Tc]-diethylenetriamine pentaacetic acid (DTPA).

Route of Administration: Iohexol (intravenous). If creatinine is administered in the same animal it can be given 1 hour following iohexol (s.c. in 3 approximately equal amounts on the side under the skin near the axilla, pectoral area, and caudolateral to the withers followed 25 minutes later by the administration of the remaining 35% injected at the same sites on the contralateral side of the animal).

Dosage: Iohexol (150 mg/kg/30 seconds). Creatinine is administered as a solution (1 g/12 ml sterile LRS; final concentration = 80 mg/ml) at a dose of 60 mg/kg.

Implementation: Delineation of the pharmacokinetic profile of iohexol following a single injection and measurement of concentrations in blood samples collected at 5, 20, 40, 60, 120, 180, 240, and 360 minutes. High-pressure liquid chromatography is then used as an analysis technique for the measurement of serum iohexol concentrations (Shihabi et al and Finco et al). Exogenous creatinine can be administered as a reference control for the measurement of creatinine clearance parameters.

The bladder is evacuated and urine samples collected serially for analysis in a manner that chronologically parallels the collection of serum samples.

Animal Model: Equine (foals n = 10).

Interpretation and Analysis: Serum iohexol concentrations best display a 3-compartment open model. Comparison of calculated values from the model can then be made with creatinine clearance measurements. Separate limited-sample models can be created with various combinations of sample times from the terminal slope of the plasma vs time profile for iohexol. Mean exogenous creatinine clearance is 2.17 mL/min/kg, while the mean value for iohexol is 2.15 mL/min/kg, which is not significantly different from mean creatinine clearance. Iohexol can therefore be applied to predict exogenous creatinine clearance and can be determined from 2 blood samples taken after a single intravenous injection. Appropriate correction factors for adult horses and horses with abnormal glomerular filtration rate need to be determined.

Evaluation of iohexol clearance used to estimate glomerular filtration rate in clinically normal foals. Gonda KC, Wilcke JR, Crisman MV, Ward DL, Robertson JL, Finco DR, Braselton E. Am J Vet Res 64; 12, pp. 1486–90.

Iohexol determination by direct injection of serum on the HPLC column. Shihabi ZK, Thompson EN, Constantinescu MS. J Liquid Chromatogr 1993; 16, pp. 1289–96.

Relationship between plasma iohexol clearance and urinary exogenous creatinine clearance in dogs. J Vet Intern Med 2001; 15, pp. 368–73.

Glomerular Filtration/Neonatal Renal Function
β-2-Microglobulin

Cellular/Molecular Mechanism of Action: Excreted by glomerular filtration.

Application: Assessment of glomerular filtration rate (GFR) as an alternative or complementary test to radioisotopic filtration and creatinine/blood urea nitrogen (BUN) measurements.

Considerations: The protein β-2-microglobulin can be labeled/radiolabeled with an appropriate marker system for application as a radiobiopharmaceutical agent designed to assess elimination/clearance by glomerular filtration.

Route of Administration: Intravenous.

Implementation: Evaluation of renal function utilizing a panel of analysis procedures including standard tests for estimating BUN, serum creatinine, blood pH, serum bicarbonate, serum and urinary electrolytes, urine output, and urinalysis. Urinary β-2-microglobulin levels are estimated from urine collected on day 1 and day 3 (e.g., all neonates) and on day 7 (n = 18 neonates) applying radioimmunoassay methodologies.

Human Model: Term "sick" neonates (n = 46 cases) transferred for neonatal care compared to healthy term neonates (n = 40 reference control cases).

Interpretation and Analysis: Statistically significant elevations of mean urinary β-2-microglobulin concentrations can be appreciated in sick neonates compared to normal controls irrespective of primary disease, indicating tubular dysfunction (41/46 = 90%). Approximately 15% (e.g., n = 7/46) may have abnormalities indicative of renal involvement when judged by standard tests. Very high levels of urinary β-2-microglobulin can be detected in conditions of birth asphyxia (e.g., n = 9), sepsis (e.g., n = 8), and renal disease (e.g., n = 7). Transient elevation of urinary β-2-microglobulin can be noted in meconium aspiration syndrome (e.g., n = 4).

Some surgical cases with non-renal congenital malformations (e.g., n = 10) may show high urinary β-2-microglobulin, while a relatively large percent of cases (e.g., n = 12/18 or 67%) tested on day 7 have persistently high urinary β-2-microglobulin due to multiple factors.

Elevated urinary β-2-microglobulin may be detected in approximately 90% of sick neonates with apparently normal renal parameters (e.g., n = 34/41), which indicates the presence of subclinical proximal tubular dysfunction, especially in neonates with asphyxia, sepsis, and congenital malformations. Persistent elevations of urinary β-2-microglobulin appears to be a sensitive diagnostic indicator for defining a group of neonates with subtle renal tubular dysfunction. Serum β-2-microglobulin is a simple, economical, specific, sensitive, and accurate test for assessing glomerular filtration. It is superior to radioisotopic GFR, serum creatinine, and BUN.

Renal dysfunction detected by β-2 microglobulinuria in sick neonates. Mehta KP, Ali US, Shankar L, Tirthani D, Ambadekar M. Indian Pediatr 1997 Feb; 34 (2), pp. 107–11.

Glomerular Filtration
Creatinine/Cystatin C/Sodium Thiosulfate

Cellular/Molecular Mechanism of Action: Creatinine enters the urinary filtrate at the level of the renal glomerulus. Once creatinine enters the plasma/urinary filtrate, its concentration is not affected by renal tubular transport mechanisms. Passive filtration of creatinine into the plasma/urinary filtrate is the major route and rate-limiting step by which the compound is eliminated from the intravascular compartment.

Application: Creatinine, cystatin C, and sodium thiosulfate can be utilized to evaluate glomerular filtration parameters.

Implementation: Evaluate the reliability and efficacy of serum cystatin C measurements for the estimation of glomerular filtration rate (GFR). Complementary analysis can include the evaluation of 24-hour creatinine clearance (24-hour C(Cr)) and performance of a standard sodium thiosulfate clearance test (C(Thio)) within a week of blood sample collection.

Human Model: Cases of renal disease (n = 212).

Interpretation and Analysis: Serum cystatin C concentrations determined by a particle-enhanced immunonephelometry method. C(Thio) and 1/cystatin C, 24-hour C(Cr), 1/β-2-microglobulin, and 1/creatinine are well correlated. The correlation coefficients for C(Thio) obtained by 24-hour C(Cr) and 1/cystatin C are comparable to each other (0.701 vs 0.679). Receiver-operated characteristic (ROC) analysis reveals that 24-hour C(Cr) shows the highest area under the curve when C(Thio) = 60 ml/min or C(Thio) = 100 ml/min are applied as the discrimination point. However, the ROC value obtained by cystatin C is slightly greater than 24 hours C(Cr) when C(Thio) = 80 ml/min is used as the discrimination point. Patient age, gender, glucose tolerance, presence of proteinuria, systemic inflammation, lupus, or systemic use of steroids do not interfere in the relationship between C(Thio) and 1/cystatin C. Serum cystatin C measurement is an excellent diagnostic test for detecting conditions of subclinical renal dysfunction.

Serum cystatin C reliably detects renal dysfunction in patients with various renal diseases. Kazama JJ, Kutsuwada K, Ataka K, Maruyama H, Gejyo F. Nephron 2002 May; 91 (1), pp. 13–20.

Glomerular Filtration Rate (GFR): Chronic Renal Failure
Creatinine Clearance/[^{99m}Tc]-Diethylenetriamine Pentaacetic Acid (DTPA)

Cellular/Molecular Mechanism of Action: Creatinine and DTPA when applied to measure GFR do not per se exert a molecular or cellular mechanisms of action. Instead, they function as molecular tracers that are passively excreted into the urinary plasma filtrate.

Application: Creatinine and DTPA in the form of [^{99m}Tc]-DTPA can both be applied to estimate renal GFR. In this context, [^{99m}Tc]-DTPA can be detected externally by scintigraphy methods, while progressive declines in intravascular compartment creatinine concentrations can be measured directly within plasma samples.

Estimation of changes in glomerular filtration can be evaluated in the assessment of conditions like chronic renal failure where parameters are compared to reference controls. Additionally, methodologies can enhance the accuracy of the captorpril test. Accuracy of the original method is improved by replacing the Tonnesen formula that estimates renal depth with direct measurement from lateral views to calculate tissue attenuation correction.

Route of Administration: Intravascular.

Implementation: Evaluation of GFR estimated through the implementation of a computerized version of the Gates' method for calculating radionuclide glomerular filtration rate (RGFR). In this context, the creatinine clearance test (CCT) and dynamic [^{99m}Tc]-DTPA renal scintigraphy (DRS) can be used to estimate renal GFR. Renal GFR is quantified from the attenuation-corrected absolute DTPA uptake for kidneys on DRS between 120 and 180 seconds after injection. Attenuation correction is estimated using lateral views of the kidneys taking into account the distance from the computed geometric center of the kidneys to the posterior body surface along a line vertical to the collimator surface. Creatinine clearance and GFR estimates from DRS are then compared by linear regression.

Human Model: Conditions of chronic renal failure (n = 38).

Interpretation and Analysis: Radionuclide GFR estimates agree well with CCT, yielding a correlation coefficient of 0.92 (e.g., n = 38 cases) and 0.90 (e.g., n = 11, case subgroup suffering from chronic renal failure). Modifications can be made to improve the accuracy of RGFR estimates to the precision range of blood sample–based methods. This modified method requires little additional work and no extra cost in patients undergoing DRS. Calculation of RGFR may be advantageous in cases when 24-hour urine collection for CCT cannot be obtained, and it should improve the accuracy of the captopril test.

Renal depth estimates to improve the accuracy of glomerular filtration rate. Steinmetz AP, Zwas ST, Macadziob S, Rotemberg G, Shrem Y. J Nucl Med 1998 Oct; 39 (10), pp. 1822–29.

Glomerular Filtration Rate (GFR) *Creatinine (Exogenous)/[^{14}C]-Inulin/Iothalamate*

Cellular/Molecular Mechanism of Action: Creatinine, iothalamate, and [^{14}C]-inulin are passively excreted into the urine/ plasma filtrate. In this context, plasma creatinine enters the urinary filtrate after passing across membranes of the renal glomerulus. Once creatinine enters the plasma/urinary filtrate, its concentration is not affected by renal tubular transport mechanisms. Passive filtration of creatinine into the plasma/urinary filtrate is the major route and rate-limiting step by which the compound is eliminated from the intravascular compartment.

Physiological Mechanism of Action: Estimation of renal GFR.

Application: Assessment of GFR in a manner similar to the applications of [^{51}Cr] ethylene-diamine-tetra-acetic acid (EDTA) and [^{99m}Tc]-diethylenetriamine pentaacetic acid (DTPA).

Route of Administration: Creatinine (intravenous); iothalamate (intravenous); [^{14}C]-inulin (intravenous).

Dosage: Creatinine (exogenous at 40, 80, and 160 mg/kg).

Implementation: Evaluate the capacity of creatinine plasma clearance to accurately assess GFR in dogs. Experimental Protocol #1: Determintion of 24-hour urine clearance of endogenous creatinine, plasma, and urine clearances of exogenous creatinine administered in a crossover design (linearity study), plasma iothalamate clearance, and plasma and urine clearances of [^{14}C]-inulin. Experimental Protocol #2: Plasma creatinine and iothalamate clearances are compared, and a linearity study performed analogous to that applied for Protocol #1 with surgically induced renal impairment. Experimental Protocol #3: compare plasma creatinine clearance with plasma iothalamate clearance before and 3 weeks after induction of moderate renal impairment. Plasma creatinine clearances are calculated by both non-compartmental and compartmental analyses.

Animal Model: Canine (n = 6 healthy dogs/research protocol).

Interpretation and Analysis: In Protocol #1, plasma inulin clearance is higher ($p < 0.001$) than other clearance values. Plasma creatinine clearances at the 3 dose rates do not differ from urine inulin clearance and each other. In Protocol #2, plasma creatinine clearances are about 14% lower than plasma iothalamate clearance ($p < 0.05$). In Protocol #3, decreases in GFR assessed by plasma clearances of iothalamate and creatinine are similar. Renal failure decreases the daily endogenous input rate of creatinine by 25%. Limiting sampling strategies for optimizing GFR calculation have been designed in a manner that allows an error lower than 6.5% with the collection of only 4 blood samples. Determination of plasma creatinine clearance by a non-compartmental approach offers a reliable, inexpensive, rapid, and convenient means of estimating GFR in routine practice.

Plasma exogenous creatinine clearance test in dogs: Comparison with other methods and proposed limited sampling strategy. Watson AD, Lefebvre HP, Concordet D, Laroute V, Ferré JP, Braun JP, Conchou F, Toutain PL. J Vet Intern Med 2002 Jan–Feb; 16 (1), pp. 22–33.

Glomerular Filtration Rate (GFR) *[^{131}I]-Iothalamate/Serum Creatinine [Cockcroft-Gault Formula]*

Cellular/Molecular Mechanism of Action: [^{131}I]-Iothalamate in the plasma enters the urinary filtrate after passing across membranes of the renal glomerulus. Once it enters the plasma/urinary filtrate, its concentrations are not affected by renal tubular transport mechanisms. Passive filtration of [^{131}I]-iothalamate is the rate-limiting step in the elimination of this compound from the intravascular compartment.

Application: Evaluation and characterization of renal excretory function.

Route of Administration: [^{131}I]-iothalamate (intravascular).

Implementation: Assessment of the degree of association between renal function and frequent nighttime urination in older adults. One approach to identifying any such correlation can involve utilization of a 3-day examination period employing 3 GFR measurements. Relevant examples include an estimation of serum creatinine using the Cockcroft-Gault formula, a calculation using 24-hour urine volume, and a determination by [^{131}I]-iothalamate clearance.

Human Model: Cases of advanced age (n = 48; ≥ 65 years old; n = 45/48 completed a 7-day voiding record and were restricted to eating a research diet for 4 days).

Interpretation and Analysis: Cases with frequent nighttime voiding, defined as a mean of 2 or more voiding episodes from 11 P.M. to 7 A.M., have slightly lower but statistically similar GFR measurements than those with a mean of less than 2 nighttime voids. The glomerular filtration rate does not correlate with the number of nighttime voids (Pearson correlation coefficient −0.006, p = 0.971). Mean 24-hour urine output is similar in the 2 groups and the correlation of the number of nighttime voids with 24-hour urine output is insignificant (Pearson correlation coefficient −0.06, p = 0.683). A greater number of nighttime voids is associated with producing more urine at night and smaller mean voided volume. As determined by a gold standard GFR measurement, renal function does not appear to be associated with the number of nighttime voids. Total 24-hour urine output is also not associated with the number of nighttime voids. Such findings confirm the positive association of a greater number of nighttime voids with greater nighttime urine production and lower bladder voided volume.

A prospective evaluation of the glomerular filtration rate in older adults with frequent nighttime urination. Johnson TM 2nd, Sands JM, Ouslander JG. J Urol 2002 Jan; 167 (1), pp. 146–50.

Glomerular Filtration Rate (GFR)/Hepatic Substrate Metabolism/Hepatic Substrate Clearance
Toborinone Pharmacokinetics/Iothalamate/ Caffeine/Indocyanine Green (ICG)

Cellular/Molecular Mechanism of Action: Caffeine is a substrate for hepatic cytochrome P450 1A2 (CYP1A2) and the biochemical activity of this enzyme system yields a metabolite that can be measured or quantified. Indocyanine green is not metabolized by the liver but instead is removed from the intravascular compartment by hepatocytes and eliminated in the bile. Iothalamate is simply eliminated from the intravascular compartment by passive mechanisms of glomerular filtration and excreted in the urinary filtrate.
Application: Plasma clearance of iothalamate can be used to evaluate glomerular filtration. Caffeine metabolism is useful as a molecular probe for evaluating hepatic metabolic capacity, while ICG can serve as a marker molecule for characterizing hepatic clearance parameters.
Implementation: Evaluation of the GRF using iothalamate clearance. Hepatic function is assessed by utilizing the caffeine metabolism test and determining ICG clearance.

Human Model: Delineation of the pharmacokinetic profile of toborinone in cases of congestive heart failure (CHF) complicated by concomitant renal and/or hepatic disease. Cases are grouped based on estimated creatinine clearance and serum bilirubin concentrations.
Interpretation and Analysis: No significant differences can be observed for mean toborinone pharmacokinetic parameters among the 4 study groups. Positive correlations can be appreciated between toborinone clearance and measured indices for renal and hepatic function: creatinine clearance, iothalamate renal clearance, paraxanthine/caffeine ratio, and ICG clearance. Toborinone clearance decreases with decreasing creatinine clearance, decreasing GFR, decreasing demethylation metabolic activity, and decreasing hepatic blood flow, although no significant differences can be observed in any mean toborinone pharmacokinetic parameters evaluated among the 4 study groups.

The pharmacokinetics of toborinone in subjects with congestive heart failure and concomitant renal impairment and/or concomitant hepatic impairment. Tammara B, Trang JM, Kitani M, Miyamoto G, Bramer SL. J Clin Pharmacol 2002 Dec; 42 (12), pp. 1318–25.

Glomerular Filtration Rate (GFR) ($<$ 20 ml/min)/ [Plasma/Renal Clearance]
Iohexol

Cellular/Molecular Mechanism of Action: Iohexol is an iodinated organic molecule that when used to estimate glomerular filtration rate does not per se exert a cellular or molecular mechanism of action. Instead, it functions as a passive marker or tracer molecule.
Application: Iohexol can be applied as a passive marker/tracer compound for measuring or estimating glomerular filtration, plasma clearance, and renal clearance.
Route of Administration: Iohexol (intravenous).
Dosage: Iohexol (10 ml; 300 mg iodine/ml).
Implementation: Evaluation of iohexol (non-ionic low-osmolar contrast medium) as a molecular marker for measuring GFR. Simultaneous determinations of renal clearance and plasma clearance, both as slope clearance and single sample clearance, are performed after intravenous injection of iohexol. Plasma samples are collected early (around 3 hours) and late (up to 24 hours) after injection. Urine is collected during 4 40-minute periods (Case Group I) and during 1 3-hour period (Case Group II along with estimation of residual urine by ultrasound). Plasma and urine iodine concentrations are analyzed by x-ray fluorescence. Tubular function test and S-creatinine are monitored to detect signs of nephrotoxicity. Iohexol is injected to assess extrarenal clearance in anuric cases (e.g., n = 6 Case Group III).

Human Model: Cases with stable renal function (n = 53 total; Group I: n = 32, Group II: n = 21) with clearance values $\leq$ 30 ml/min/1.73 m^2 body surface (40 patients $<$ 20 ml/min/1.73 m^2 body surface). Anuric cases (n = 6).
Interpretation and Analysis: In Case Groups I and II the slope clearance closely correlates with the single sample clearance (r = 0.99) when a late plasma sample is used in both techniques. In Case Group II, where residual urine is estimated by ultrasound, renal clearance correlates better with slope clearance than in Case Group I (r = 0.94 vs r = 0.89). There are no signs of nephrotoxicity in the parameters noted. In Case Group III, extrarenal plasma clearance of iohexol does not exceed 2 ml/min/1.73 m^2. Glomerular filtration rates $<$ 20 ml/min can accurately and safely be determined as renal clearance or plasma clearance of iohexol after an intravenous dose of 10 ml 300 mg I/ml. Plasma clearance techniques that have the practical clinical advantage of no urine sampling do at low GFR require collection of a late plasma sample, for instance, 24 hours after injection of iohexol, irrespective of whether slope technique or single sample technique or 1-compartment or poly-compartment models are used.
Instrumentation: Reanalyzer PRX90, Provalid AB, Sweden.

The use of iohexol clearance to determine GFR in patients with severe chronic renal failure—A comparison between different clearance techniques. Frennby B, Sterner G, Almén T, Hagstam KE, Hultberg B, Jacobsson L. Clin Nephrol 1995 Jan; 43 (1), pp. 35–46.

Glomerular Function/Albuminuric States/Albumin Clearance
[^{3}H]-Albumin

Cellular/Molecular Mechanism of Action: Albumin *in vivo* primarily maintains plasma oncotic pressure and can serve as a reserve protein fraction during episodes of starvation. The inorganic compound NH_4Cl is an inhibitor of renal protein uptake.
Background Information: Recent studies have demonstrated that the normal glomerular capillary wall (GCW) is not charge selective to albumin. This means that albumin flux across the GCW is high. This has been confirmed in studies where albumin uptake by the tubules has been inhibited. Therefore, there must be a high-capacity postglomerular retrieval pathway in normal kidneys that returns filtered albumin back into the intravascular compartment.
Application: Evaluation of the albumin-filtering capacity of the kidney.
Implementation: Identify the presence of glomerular filtered albumin in the renal vein from the analysis of the decrease of radioactivity in the venous effluent after the injection of a pulse of [^{3}H]-labeled albumin into the renal artery *in vivo* and in the isolated perfused kidney (IPK).

Animal Model: Rat.
Interpretation and Analysis: Glomerular filtered albumin is returned to the blood supply by a high-capacity pathway that transports labeled albumin at a rate of 1,830 $\pm$ 292 μg/min rat kidney (n = 14) (mean $\pm$ SEM). This pathway has been identified under physiological conditions *in vivo* and in the IPK. The pathway is specific for albumin, as it does not occur for horseradish peroxidase (HRP).

The pathway is inhibited in a non-filtering kidney. The pathway is also inhibited by NH_4Cl, an inhibitor of protein uptake. The high-capacity retrieval pathway for albumin is most likely associated with transtubular cell transport. It is also apparent that most albuminuric states could be accounted for by the malfunctioning of this pathway without resorting to any change in glomerular permselectivity.

The return of glomerular filtered albumin to the rat renal vein—The albumin retrieval pathway. Eppel GA, Osicka TM, Pratt LM, Jablonski P, Howden B, Glasgow EF, Comper WD. Ren Fail 2001 May–Jul; 23 (3–4), pp. 347–63.

Glomerular Hydrostatic Pressure/(Calcium Antagonist)
Efonidipine/Lisinopril

Cellular/Molecular Mechanism of Action: Calcium antagonist.
Physiological Mechanism of Action: Dilation of afferent and efferent arterioles in a manner that reduces glomerular hydrostatic pressure.
Laboratory Methodology: Four-week-old spontaneously hypertensive rats (SHRs) given efonidipine (25 mg/kg/day) or lisinopril (3 mg/kg/day) until the age of 22 weeks.
Dosage: Efonidipine (25 mg/kg/day) and lisinopril (3 mg/kg/day) in rats.
Implementation: Evaluation of glomerular hydrostatic pressure utilizing efonidipine or lisinopril.

Animal Model: Rat (4-week-old SHRs) given efonidipine or lisinopril until the age of 22 weeks.
Interpretation and Analysis: Fixed, maximal dilation can be observed at 22 weeks after receiving either efonidipine (25 mg/kg/day) or lisinopril (3 mg/kg/day). Morphometrical measurements show that efonidipine and lisinopril treatments cause structural alteration of the vasculature, resulting in significantly greater efferent arteriolar diameters than in untreated reference controls. In addition, lisinopril-treated rats have wider afferent lumina. The renoprotective effect of efonidipine and lisinopril might be partly due to the structurally larger efferent arteriolar lumen.

Effects of efonidipine hydrochloride on renal arteriolar diameters in spontaneously hypertensive rats. Nakamura M, Notoya M, Kohda Y, Yamashita J, Takashita Y, Gemba M. Hypertens Res 2002 Sep; 25 (5), pp. 751–5.

Glomerulonephritis (Chronic)/Renal Function/ Prognostic Factors: Markovian Analysis
Phenolsulfonphthalein (PSP)

Cellular/Molecular Mechanism of Action: Phenolsulfonphthalein is secreted by the proximal renal tubular epithelium.
Application: Estimation of prognosis for conditions of chronic glomerulonephritis.
Implementation: Delineation of the incidence of hypertension in chronic glomerulonephritis and its influence on the clinical course of the disease applying statistical techniques (especially Markovian process).
Interpretation and Analysis: In cases of chronic glomerulonephritis, the incidence of hypertension is approximately 15.2% (e.g., n = 283/1,862) but if renal function is approximately one-half, then the incidence increases to 50% (e.g., n = 930/1,862).

The average blood pressure in such disease states tends to become elevated with decreases in 15-minute values for PSP excretion tests and with increasing serum creatinine levels (Note: Variations are large). After a fall of renal function to approximately one-third of normal, progression rates for declines in the clinical course of the disease become markedly more rapid. The prognosis for the progressive type of chronic glomerulonephritis is obviously shortened by the presence of hypertension. According to the results obtained by the Markovian approach, survival years of the patients with a wide range of renal functions can be predicted with relative accuracy.

Prognosis of chronic glomerulonephritis with special reference to hypertension and renal function. Urakabe S, Shirai D, Orita Y, Ando A. Jpn Circ J 1975 Jul; 39 (7), pp. 815–21.

Low-Density Lipoprotein (LDL) Assimilation: Hemodialysis and Diabetes/Atherosclerotic Lesion Pathogenesis: LDL Accumulation
[^{125}I]-LDL

Cellular/Molecular Mechanism of Action: [^{125}I]-labeled LDLs are radiolabeled analogs of LDLs. Low-density lipoproteins can function as a transport platform in the plasma and contain a relatively high amount of triglycerides and cholesterol compared to high-density lipoproteins.
Background Information: In conditions requiring chronic hemodialysis treatment there is an elevated atherogenic risk, and dyslipidemia appears to be 1 of the major risk factors. However, most affected cases exhibit elevated serum triglycerides, whereas serum cholesterol and LDL cholesterol levels are in the normal range.
Application: Characterization of *in vitro* and *in vivo* LDL metabolism.
Route of Administration: In animal (*in vivo*) models, [^{125}I]-labeled LDLs are usually given intravenously.
Implementation: Evaluate the influence of hypertriglyceridemia under the condition of hemodialysis and diabetes mellitus on LDL metabolism.

Low-density lipoproteins are isolated from whole blood and separated into 6 subfractions by density gradient ultracentrifugation and then characterized pertaining to lipid/protein composition, degree of glycation, and oxidation. Uptake of [^{125}I]-labeled LDL is examined via LDL receptors of HepG2 cells and scavenger receptors of mouse peritoneal macrophages.

Human Model: Cases of hypertriglyceridemic diabetes, non-diabetic hemodialysis cases, and healthy reference controls (n = 30, n = 10/case group).
Interpretation and Analysis: Hemodialysis procedures promote elevations in serum triglycerides but cholesterol levels are within normal ranges. Triglyceride enrichment occurs in the very low-density lipoprotein (VLDL) class and LDL class, and an accumulation of a highly atherogenic small dense LDL subfraction can be detected predominantly in cases of non-insulin-dependent diabetes mellitus. Low-density lipoprotein concentrations in response to hemodialysis also contain elevated levels of lipid peroxidation products, which are at even higher levels in diabetic conditions. Alterations in composition, size, and configuration of LDL in response to hemodialysis in diabetic and non-diabetic conditions impair LDL receptor-mediated degradation and enhance uptake of such modified LDL particles via non-saturable scavenger receptors. Diminished LDL receptor-mediated uptake of modified, triglyceride-rich, small dense LDL most likely leads to accumulation of these lipoproteins *in vivo*, favoring development of atherosclerotic lesions. Future clinical studies must be conducted to demonstrate whether patients benefit from reducing atherogenic particles by lipid-lowering intervention.

Abnormalities in lipoprotein metabolism in hemodialysis patients. Köniğer M, Quaschning T, Wanner C, Schollmeyer P, Krämer-Guth A. Kidney Int Suppl 1999 Jul; 71, pp. S248–50.

Nephropathy: Chronic Renal Failure (Reflux Type)/ Effective Renal Plasma Flow (ERPF)/Glomerular Filtration Rate (GFR)
Inulin/Para-Hippurate/Amino Acid Formulations

Application: Inulin-based molecules can be applied to evaluate GFR, while *p*-hippurate can be used to measure (effective) renal plasma flow.

Considerations: Reflux nephropathy is an important cause of chronic renal failure in children. After the parenchymal scar, the progression is thought to be mediated by glomerular hypertension in remnant nephrons, resulting in modifications in permselectivity to macromolecules. Proteinuria correlates with a progressive course.

Implemenation: Evaluation of glomerular permselectivity to macromolecules in basal conditions and after acute hemodynamic stress. Bilateral renal scarring (0–8 scale for both kidneys) is 4.3 ± 1.6. Albuminuria (UAE) is evaluated in basal and under acute hyperfiltration conditions induced by infusion of amino acid formulations. After isotonic saline at 310 ml/hour/1.73 m^2, 6 mg/kg/min of amino acid formulations are infused for 2 hours.

Human Model: Cases with bilateral vesico-ureteric reflux (VUR; n = 28) that have previously been surgically corrected (meanly 5.6 years before) accompanied by normal creatinine clearance (CrCl).

Interpretation and Analysis: Urine albumin is significantly higher than controls in basal conditions ($p < 0.01$) and further increases after amino acid formulation infusion ($p < 0.02$). Microalbuminuria is detectable in 53.5% of children in basal conditions and in 64.3% after infusion of amino acid formulations. Also urinary β-2-microglobulin significantly increases at the end of the test ($p < 0.001$). Creatinine clearance significantly increases at the 1st hour ($p < 0.05$). Children with severe renal parenchymal scarring have greater urine albumin ($p < 0.01$) and β-2M ($p < 0.02$) values after provocative tests than those with mild renal damage. In children, GFR and ERPF can be measured by means of inulin and *p*-hippurate clearance, respectively. The variations in UAE during the infusion of amino acid formulations significantly correlate with GFR dynamics ($p < 0.05$), while they are not influenced by ERPF modifications. Note: Filtration fraction = GF/ERPF.

Concentration measured as a function of spectrophotometric absorbance [^{123}I]-ortho-iodohippuric acid. Prévot A, Mosig D, Martini S, Guignard JP. Pediatr Res 2004 Feb; 55 (2), pp. 254–60.

Evaluation of renal function in transplant patients on tacrolimus therapy. Agarwala S, Culligan E, Jain A, McCauley J, Shapiro R, Chakrabarti P, Burckart G, Venkataramanan R. J Clin Pharmacol 2002 Jul; 42 (7), pp. 798–805.

Perfusion/Effective Renal Plasma Flow (ERPF)/ Glomerular Filtration Rate (GFR)
Sodium Sulfanilic (SS) Acid/Phenolsulfonphthalein (PSP)/Hippuric Acid/Inulin

Cellular/Molecular Mechanism of Action: Removal from the systemic intravascular compartment primarily occurs through proximal renal tubular excretion. Inulin is eliminated from the intravascular compartment by passive diffusion into the plasma filtrate at the level of the glomerulus.

Diagnostic Application: Selective assessment of renal tubular function.

Dosage: Injected intravenously as an aqueous solution (20%) at a dose rate of 10 mg/kg. Dosage in dogs is 20 mg/kg and 11 mg/kg for cats.

Considerations: The test should not be performed in animals that have received systemic sulfonamide therapy because they cross react with sulfanilite. The advantage of this test is that collection of urine is not required, but the disadvantage is that a numerical value for GFR is not obtained.

Implementation: Evaluation of the excretion of creatinine, SS, and PSP. Heparinized blood samples are collected at 10, 15, 30, 60, and 90 minutes following administration.

Animal Model: Companion animals and domestic livestock.

Reference Example: Caprine (healthy goats).

Interpretation and Analysis: Renal clearance of SS acid after intravenous injection displays a $T_{1/2}$ = 39.5 ± 4.4 minutes. In dogs normal values have been reported to be 50–80 minutes and 42–82 minutes. The normal sodium sulfanilic acid clearance in cats is 37–57 minutes. Plasma $T_{1/2}$ half-life ranges between 30 to 90 minutes in normal horses with a mean value of 39.5 ± 4.4 minutes.

In conscious goats, mean (± SEM) inulin clearance is 2.26 0.08 ml/min/kg of body weight. Endogenous creatinine clearance, 1.97 ± 0.09 ml/min/kg, underestimates inulin clearance ($p < 0.01$), probably because of the presence of non-creatinine chromogens in caprine plasma. The estimated renal clearance of PSP is 6.88 ± 0.39 ml/min/kg, whereas the estimated renal clearance of SS is 3.71 ± 0.39 ml/min/kg. Both exceed inulin clearance ($p < 0.01$), confirming renal tubular secretion of both compounds. In anesthetized goats (e.g., n = 6), exogenous creatinine clearance and SS clearance exceed inulin clearance ($p < 0.05$). Results of stop-flow experiments document secretion of creatinine and SS by the proximal portion of the caprine nephron. Plasma half-life of PSP in uninephrectomized goats exceeds that in intact goats (20.2 ± 1.5 minutes vs 11.9 ± 0.7 minutes; $p < 0.01$). Similarly, plasma half-life of SS is greater in goats after uninephrectomy (58.2 ± 6.2 minutes vs 30.4 ± 1.2 minutes; $p < 0.01$). Note: Filtration fraction = GF/ERPF.

Determination of excretion of inulin, creatinine, sodium sulfanilate, and phenolsulfonphthalein to assess renal function in goats. Brown SA, Groves C, Barsanti JA, Finco DR. Am J Vet Res 1990 Apr; 51 (4), pp. 581–6.

Perfusion (Renal): Microperfusion and Free-Flow Micropuncture/Fluid Reabsorption in Microperfused Nephron Segments/Glomerular Filtration Rate (GFR)
Iothalamate/[^{3}H]-Methoxy-Inulin

Background Information: Inulin remains the gold standard for measurements of fluid reabsorption (Jv) and single nephron glomerular filtration rate (SNGFR) in micropuncture experiments. However, the method used to measure cold inulin in nanoliter samples is time-consuming, while the use of radiolabeled inulin is disadvantaged by possible radioactive contamination, disposal of radioactive material, and cost of the isotope. It has been reported that non-radiolabeled iothalamate may be a suitable alternative for estimation of whole kidney GFR.

Application: Iothalamate and inulin can both be applied to assess glomerular filtration. Iothalamate has been used to measure fluid reabsorption (Jv) in microperfusion and free-flow micropuncture experiments and therefore can function as a standard reference method for the detection of fluid reabsorption in microperfused nephron segments.

Route of Administration: Iothalamate (intravenous).

Dosage: Iothalamate IV (18.3 mg/h).

Implementation: Evaluate the potential for iothalamate to be used to measure Jv in microperfusion and free-flow micropuncture experiments. Superficial loops of Henle (LOH) are perfused from late proximal to early distal tubules with an end-like proximal solution. In the 1st set of experiments, the perfusate contains both iothalamate (1.9 mmol/L) and [^{3}H]-methoxy-inulin (50 μCi/mL). To test if iothalamate is able to detect changes in Jv, 2 additional sets of experiments can be performed: (i) mannitol (61 mmol/L) added to the perfusate to partially replace NaCl, a condition known to inhibit Jv; and (ii) LOH of remnant kidneys perfused (shown in previous investigations to have a higher Jv). Lastly, free-flow micropuncture experiments are performed by infusing iothalamate IV at 18.3 mg/h. Iothalamate analysis in nanoliter samples of renal tubular fluid obtained *in vivo* is performed by capillary electrophoresis (CE).

Animal Model: Rat.

Interpretation and Analysis: In the 1st set of experiments, liquid scintillation counting of [^{3}H]-methoxy-inulin vs iothalamate analysis with CE results in almost identical calculations of perfusion rates (20.4 ± 0.6 vs 20.6 ± 0.7 nL./min, n = 20) and tubular fluid/perfusate ratios (TF/P; 1.35 ± 0.04 vs 1.36 ± 0.04) and thus also fluid reabsorption (Jv 5.17 ± 0.50 vs 5.38 ± 0.59 nL/min). In mannitol investigations, iothalamate measurements show that the addition of mannitol significantly reduces fluid reabsorption (Jv) from 4.98 ± 0.40 (n = 19) to 0.72 ± 0.58 nL/min (n = 33; $p < 0.0001$). Iothalamate determinations by CE are able to detect a significant increase in fluid reabsorption (Jv) in LOH of remnant rats perfused at 40 nL/min (from 8.40 ± 0.73 [n = 20] in sham-operated to 17.8 ± 2.9 nL/min [n = 6] in remnant animals; $p < 0.0001$]. In free-flow micropuncture experiments the ratio of tubular fluid to plasma iothalamate (TF/P) along the proximal tubule is 1.62 ± 0.10 (n = 15). Therefore, iothalamate can replace inulin to measure Jv in microperfusion and free-flow micropuncture experiments. Since iothalamate analysis by CE technique is a fast, easy, and highly reproducible technique, it may become the gold standard method for the detection of fluid reabsorption in microperfused nephron segments.

Instrumentation: Capillary electrophoresis.

Iothalamate measured by capillary electrophoresis is a suitable alternative to radiolabeled inulin in renal micropuncture. Capasso G, Unwin RJ, Pica A, Quagliuolo L, Giovane A. Kidney Int 2002 Sep; 62 (3), pp. 1068–74.

Polyuria
Antidiuretic Hormone (ADH, Vasopressin)

Diagnostic Application: Utilized to evaluate renal function in debilitated patients in which a water deprivation test would be considered hazardous.

Route of Administration and Dosage: Intravenous administration of ADH at a dose rate of 11 mU/kg over a period of 60 minutes. The bladder is then emptied of urine. Solute of the urine is measured at time zero, and then at 30-minute intervals for 3 hours. After each measurement, the bladder is emptied to improve the accuracy of the test.

Considerations: Values are usually lower than those observed with the standard water deprivation test, which is the preferred initial test procedure.

Implementation: The repositol vasopressin test entails IM administration of 3 to 5 units of ADH tannate in oil. Evacuation of the bladder is done within 6 hours of administration.

Interpretation and Analysis: Maximal response to aqueous ADH is observed at 60 minutes (S.G. 1.009–1.033; urine osmolality 429–1437 mOsm/kg; urine/plasma osmolality 1.5–5.1).

In the repositol vasopresson test, urine solute concentrations are measured at 6-hour intervals over a period of 24 hours. Maximal response occurs at 8 to 12 hours (USG 1.024–1.060; urine osmolalities 1.033–2.001; urine/plasma 3.8–7.4).

Polyuria
Hypertonic Saline (Hickey-Hare Test)

Diagnostic Application: Utilized to distinguish polyuric states complicated by renale medulary washout. Administration of hypertonic saline evaluates the ability of the pituitary-renal axis (antidiuretic hormone [ADH]: renal tubule) to reduce urine production following induction of plasma hyperosmolality.

Route of Administration and Dosage: Hypertonic saline (2.5%) is administered to a water-"loaded" patient (e.g., dog).

Considerations: The water deprivation test is safer, and easier to perform in most patients suffering from polyuria and polydypsia conditions.

Interpretation and Analysis: Administration of hypertonic saline normally should simultaneously stimulate pituitary ADH secretion and replenish sodium chloride in situations of renal "washout." Normal patients (e.g., canine) display a ≥ 25% reduction in urine production (flow rate). Patients suffering from central (pituitary) diabetes insipidus or nephrogenic diabetes insipidus fail to reduce urine production following adminstration of hypertonic saline. In contrast, psychognic polydypsia patients reduce urine production following administration of hypertonic saline.

Renal Failure: Acute/Plasma Protein Binding Changes with Phenolsulfonphthalein (PSP) in Acute Renal Failure (ARF)
PSP

Cellular/Molecular Mechanism of Action: Phenolsulfonphthalein is secreted by the proximal renal tubular epithelium.

Application: Phenolsulfonphthalein can be applied to detect alterations in renal tubular function such as those associated with acute and chronic renal failure.

Route of Administration: Phenolsulfonphthalein (intravenous).

Implementation: Determine the influence of ARF on the disposition of PSP.

Animal Model: Rat (acute renal failure induced by subcutaneous injection of uranyl nitrate). In order to clarify the regulatory mechanisms of PSP excretion between liver and kidney in ARF, a simultaneous perfusion system of rat liver and kidney can be utilized that controls the flow rate and the constituents of the perfusate.

Interpretation and Analysis: Renal excretion of PSP decreases significantly in ARF compared to that in normal controls. In conditions of ARF, increases in biliary PSP excretion can be detected and reflects a process that compensates for reductions in renal excretion. Consequently no significant change can be appreciated in total body clearance of PSP between control and ARF. Binding investigations (*in vitro*) reveal that the bound fraction of PSP to plasma proteins in conditions of ARF is significantly lower than observed in reference control plasma.

Applying the simultaneous perfusion system, neither biliary excretion nor the protein binding of PSP differs significantly between control and ARF, though its renal excretion decreases in ARF in a similar manner as observed *in vivo*. These results suggest

that alterations in plasma protein binding as well as renal excretory function are determinants of PSP disposition in ARF.

Influence of acute renal failure on pharmacokinetics of phenolsulfonphthalein in rats: A comparative study *in vivo* and in the simultaneous perfusion system of liver and kidney. Yasuhara M, Katayama H, Fujiwara J, Okumura K, Hori R. J Pharmacobiodyn 1985 May; 8 (5), pp. 377–84.

Renal Plasma Flow: Effective Renal Plasma Flow (ERPF)
[^{123}I]-Orthoiodohippurate (OIH)

Cellular/Molecular Mechanism of Action: [^{123}I]-orthoiodohippurate is a radioiodinated analog of OIH that functions as a passive tracer/marker molecule and does not per se exert any true biological effect.
Background Information: The methods of measuring ERPF based on the 2-compartment analysis of plasma clearance of [^{123}I]-OIH disregard the distribution of [^{123}I]-OIH into erythrocytes, resulting in an overestimation of ERPF.
Application: Passive molecular marker for measuring ERPF.
Route of Administration: [^{123}I]-orthoiodohippurate (intravascular).
Implementation: Measurement of ERPF using a modified methodology in order to obtain more accurate estimates. Intercompartmental clearance rates among plasma, erythrocytes, and extracellular fluid are determined through *in vitro* and *in vivo* experiments to facilitate performing 3-compartment analysis of plasma clearance curves for [^{123}I]-OIH using multiple blood samplings in comparison with 2-compartment analysis. Multiplying renal blood flow derived from 2-compartment analysis of the whole blood clearance curve by (1-hematocrit/100) is a new modified approach for measuring ERPF resulting in reduced complexity of 3-compartment analysis methods and can serve to characterize the distribution of [^{123}I]-OIH to erythrocytes.
Interpretation and Analysis: Comparing ERPF derived from the modified 2-compartment analysis technique with that from 3-compartment analysis technique demonstrates good agreement ($r = 0.883$, $p = 0.001$) between the 2 methods. Effective renal plasma flow derived by this new method is also compared with the clearance of paraaminohippurate (PAH), resulting in a regression line that is much closer to unity and produces a better correlation between them ($r = 0.926$) than between PAH clearance and ERPF ($r = 0.909$) derived from conventional 2-compartment analysis. Note: Filtration fraction = GF/ERPF.

Modification of measurement of effective renal plasma flow from blood pool clearance curve of [^{123}I]-orthoiodohippurate using single blood sampling. Akutsu T, Komatani A, Yamaguchi K, Takahashi K, Konta T. Radiat Med 1998 Jul–Aug; 16 (4), pp. 245–50.

Renal Tubular Absorption Mechanisms/Organic Anion Transporter 1 and 3
Phenolsulfonphthalein (PSP)/Paraaminohippuric Acid/[Cimetadine]

Cellular/Molecular Mechanism of Action: Removed from the systemic intravascular compartment predominantely by excretion through the proximal renal tubule (94%). A minor component (6%) is eliminated through the glomerulus.
Background Information: Paraaminohippuric acid, a substrate of rat organic anion transporter1 (rOat1), and cimetidine, a substrate of rOat3, both reduce the urinary excretion of PSP.
Diagnostic Application: Selective assessment of renal tubular function. Formulated at 1% solution in sodium bicarbonate (0.1 N) and administered systemically by intravenous injection (pH adjusted to 7.4). Collection of blood is performed at 2, 5, 10, 15, 20, 30, 35, 45, and 60 minutes following injection. Plasma $T_{1/2}$ = 6 minutes (range = 5.6–9.3 minutes).
Route of Administration and Dosage: Intravenous injection at a dose of 1 mg/kg.
Interpretation and Analysis: The uptake of PSP by kidney slices is found to consist of 2 components. The IC50 values of rOat1 substrates are higher than those of rOat3 substrates. In the presence of cimetidine, the Eadie-Hofstee plot is a single straight line. The profile of PSP uptake component in the presence of cimetidine is similar to that of the low-affinity component in the absence of cimetidine. Therefore, rOat1 and rOat3 are involved in the renal uptake of PSP, and PSP is a high-affinity substrate for rOat3 but is a relatively low-affinity substrate for rOat1.

Major role of organic anion transporters in the uptake of phenolsulfonphthalein in the kidney. Itagaki S, Sugawara M, Kobayashi M, Nishimura S, Fujimoto M, Miyazaki K, Iseki K. Eur J Pharmacol 2003 Aug 15; 475 (1–3), pp. 85–92.

Renal Tubular Acidosis
Ammonium Chloride (NH_4Cl)

Diagnostic Application: Diagnostic detection of renal tubular acidosis, which can be either a primary or secondary condition.
Background Information: Renal tubular acidosis Type I–Distal renal tubular acidosis due to inability to excrete sufficient H^+ ion that prevents the kidney from being able to optimally acidify the urine. Type II–Proximal renal tubules are unable to appropriately absorb urinary bicarbonate. Type IV–Distal renal tubular acidosis results in an inability to secrete both potassium and hydrogen ions.
Route of Administration: Preparations of NH_4Cl are given orally.
Dosage: Preparations of NH_4Cl are given at a dose of 0.1 gm/kg.
Considerations: Administration of NH_4Cl should be avoided in conditions of metabolic and respiratory acidosis (academia). In dogs, the test is discontinued if the urine pH falls below 6.0, which occurs in normal dogs between 2 and 8 hours following NH_4Cl administration.
Interpretation and Analysis: Following administration of NH_4Cl, and removal of urine from the bladder by catheterization, the urine pH is measured at 6-hour intervals. In dogs, the NH_4Cl test is performed after a fast and hourly urine specimens are collected with the 1st sample obtained at 2 hours postinjection. Normal (equine) patients display a significant decrease in urine pH to approximately 6.5 by 4 hours postadministration. The degree of acidification in herbivores is less than in humans and dogs that usually display urine with a pH of approximately 5.5.
Laboratory Instrumentation: Measurement of renal pH using conventional clinical pathology instrumentation and methodologies.

Renal Tubular Acidosis/Tubular Acidosis Type I (Incomplete)/Distal Renal Tubular Acidification Capacity/Osteopenia and Osteoporosis
Ammonium Chloride Loading/Frusemide

Cellular/Molecular Mechanism of Action: Chloride ion is transported by the renal epithelium into the urinary filtrate.
Background Information: Chronic metabolic acidosis may increase alkali mobilization from the bone and thus promote the development of osteoporosis.

Application: Detection and characterization of incomplete renal tubular acidosis Type I (RTA I).
Route of Administration: Frusemide (oral ingestion); ammonium chloride loading (oral).
Dosage: Frusemide (40 mg); ammonium chloride loading (0.1 g/kg body weight).
Implementation: Evaluation of renal function and urinary acidification in cases of osteopenia and osteoporosis. Distal tubular acidification can be characterized by applying an oral ammonium chloride loading test and the oral frusemide test.

Human Model: Cases of osteopenia or osteoporosis devoid of overt metabolic acidosis, derangement of potassium homeostasis, or renal insufficiency (n = 46; n = 41 females, n = 5 males). Negative age- and sex-matched (normal) reference controls (n = 20; n = 17/20 females, n = 3/20 males).
Interpretation and Analysis: The urinary pH in controls is < 5.5 following frusemide ingestion. In contrast, in cases with reduced bone mineral density, the oral ammonium chloride loading test can aid in establishing a diagnosis of incomplete RTA I (n = 10/46 or 22%). Disorders possibly related to RTA I can also be detected (e.g., n = 8/10). A large percentage of cases will display normal urinary pH responses following oral ammonium chloride loading (e.g., n = 36/46). Oral frusemide fails to lower urinary pH < 5.5 in a little more than one-third of cases (e.g., n = 16/46 or 35%) and may include cases with incomplete RTA I (e.g., n = 10) or with a normal oral ammonium chloride loading test (n = 6). An abnormal frusemide test may be appreciated in 35% of cases with reduced bone mass but is a feature rarely if ever detected in normal controls ($chi^2 = 7.39$; $p < 0.01$). With the ammonium chloride test functioning as a gold standard for the diagnosis of distal RTA, the frusemide test shows a sensitivity of 1.0 (95% CI, 0.69–1.0) and a specificity of 0.89 (95% CI, 0.78–0.96) for the diagnosis of distal RTA. Patients with incomplete RTA I tend to be younger in age than those without incomplete RTA I (42 ± 16 vs 54 ± 14 years; p = 0.025; mean ± SD). Basal serum bicarbonate concentrations and capillary pH do not differ between evaluation groups.

Therefore, incomplete RTA I may be prevalent in a significant proportion of cases suffering from osteopenia or osteoporosis. The outcome of the frusemide test suggests either a defect of the H^+ATPase in the cortical collecting tubule (CCT) or a defective Na^+ reabsorption in the CCT. Prospective studies are needed to further elucidate the impact of incomplete RTA I on the development of reduced bone mineral content.

Prevalence and characterization of renal tubular acidosis in patients with osteopenia and osteoporosis and in non-porotic controls. Weger W, Kotanko P, Weger M, Deutschmann H, Skrabal F. Nephrol Dial Transplant 2000 Jul; 15 (7), pp. 975–80.

Renal Tubular Acidosis: Distal Tubular Acidosis/ Reduced Distal Nephron Proton Secretion
Bicarbonate Loading

Background Information: Recent classifications of the several pathophysiologic types of distal renal tubular acidosis (secretory, voltage dependent, and gradient) have been based on the response of acidification parameters to a series of provocative *in vivo* and *in vitro* maneuvers. A reduction in the difference in urine and blood CO_2 tension during bicarbonate loading (U-B pCO_2 gradient), a widely applied parameter, has been employed as an index of reduced distal nephron proton secretion.

Application: Detection and characterization of reductions in distal nephron proton secretion associated with conditions of distal renal tubule acidosis.
Route of Administration: Sodium bicarbonate (Na_2CO_3; oral).
Implementation: Evaluation of reductions in distal nephron proton secretion by measuring reductions in the difference in urine and blood CO_2 tension during bicarbonate loading (U-B pCO_2 gradient). Such parameters can be employed for the detection and evaluation of distal renal tubular acidosis conditions. Example: Evaluation of the validity of U-B pCO_2 gradients in a variety of experimental models of distal renal tubular acidosis by measuring and comparing disequilibrium pH (a direct technique to detect H^+ secretion *in situ*) with *in vivo* pCO_2 in the papillary collecting duct during bicarbonate loading. Chronic amiloride, lithium chloride, and amphotericin-B administration and the postobstructed kidney models can all potentially be employed.

Animal Model: Rat (papillary collecting duct).
Interpretation and Analysis: Amiloride results in an acidification defect that does not respond to sulfate infusion (urine pH = 6.15 ± 0.08) and is associated with an obliteration of the acid disequilibrium pH (−0.26 ± 0.05 – −0.08 ± 0.03) and reduction in papillary pCO_2 (116.9 ± 3.2 – 66.9 ± 2.5 mm Hg). Defects induced by lithium administration respond to Na_2SO_4 (urine pH = 5.21 ± 0.06) but are similar to amiloride with respect to observed reductions in disequilibrium pH (−0.04 ± 0.02) and pCO_2 (90.3 ± 3.0 mm Hg). The postobstructed kidney model is characterized by an abnormally alkaline urine pH that is unresponsive to sulfate (6.59 ± 0.06) and a reduction in disequilibrium pH (+0.02 ± 0.06) and pCO_2 (77.6 ± 3.6 mm Hg). Amphotericin-B results in a gradient defect characterized by excretion of an acid urine after infusion of sodium sulfate (5.13 ± 0.06). Unlike other models, however, amphotericin-B is associated with a significant acid disequilibrium pH (−0.11 ± 0.05) and an appropriately elevated urine pCO_2 (119.8 ± 6.4 mm Hg) that does not differ from the corresponding control values. Thus, these findings support the use of the U-B pCO_2 as a reliable means of demonstrating impaired distal nephron proton secretion in secretory and voltage-dependent forms of distal renal tubular acidosis.

Validation of the difference in urine and blood carbon dioxide tension during bicarbonate loading as an index of distal nephron acidification in experimental models of distal renal tubular acidosis. DuBose TD Jr, Caflisch CR. J Clin Invest 1985 Apr; 75 (4), pp. 1116–23.

Renal Tubular Concentrating Capacity
1-Desamino-8-Arginine Vasopressin (DDAVP)/ (Pitressin Tannate in Oil Alternative)

Cellular/Molecular Mechanism of Action: 1-desamino-8-arginine vasopressin promotes the production of concentrated urine in normal individuals.
Application: Assessment of renal urine concentrating capability.
Route of Administration: Intranasal.
Dosage: A reappraisal of a previous opinion and subsequent comparative work leads to the conclusion that 40 μg DDAVP administered instransally is a preferable dose to 20 μg to use as a urinary concentrating agent in the diagnostic context, and that it is quite comparable to the standard agent, pitressin tannate (5 IU) in oil.
Considerations: 1-desamino-8-arginine vasopressin is superior to the use of pitressin tannate in oil as a diagnostic agent.
Interpretation and Analysis: Consistently results in an increased production of urine with an osmolality of 800 mosmol/kg or more, with no untoward effect (evaluation performed over a period of approximately 8 and a half hours).

Renal Tubular Dysfunction: Distal Tubule Injury
Vasopressin (Inhalation)/Ammonium Chloride (Short Urinary Acidification Test)

Cellular/Molecular Mechanism of Action: Desamino-*d*-arginine vasopressin, also known as antidiuretic hormone (ADH), interacts with receptor complexes expressed on the membrane surface of renal epithelial cells lining the distal renal tubules.
Physiological Mechanism of Action: One function of desamino-*d*-arginine vasopressin is the promotion of increased urinary filtrate concentration.
Application: Desamino-*d*-arginine vasopressin inhalation.
Route of Administration: Desamino-*d*-arginine vasopressin (intranasal inhalation).
Implementation: Evaluation of glomerular filtration rate (GFR) and plasma electrolyte concentrations. In addition, renal distal tubular function is assessed by measurement of overnight urinary concentration after intranasal desamino-*d*-arginine vasopressin inhalation and by a standard short urinary acidification test following the administration of oral ammonium chloride.

Human Model: Cases treated with a serial course of cisplatinum combination chemotherapy (n = 22).
Interpretation and Analysis: Cumulative and dose-related hypomagnesemia can be observed in the vast majority of cases (e.g., n = 22/22), but can be unassociated with clinical symptoms. No significant fall in GFR is seen as a whole but approximately 30% of cases evaluated in a comprehensive manner develop impairment of urinary concentrating and acidifying abilities, independent of any change in GFR (e.g., n = 4/12). Such profiles indicate that cisplatinum exerts a selective effect at the level of the renal distal tubule.

Cis-platinum and distal renal tubule toxicity. Swainson CP, Colls BM, Fitzharris BM. N Z Med J 1985 May 22; 98 (779), pp. 375–8.

Renal Tubular Excretion/Renal Plasma Flow/Urine Flow/Protein Binding: Non-Linear/Glomerular Filtration/Tubular Secretion/Tubular Accumulation
Phenolsulfonphthalein (PSP)

Cellular/Molecular Mechanism of Action: Phenolsulfonphthalein is secreted by the proximal renal tubular epithelium.
Route of Administration: Phenolsulfonphthalein (intravenous).
Dosage: Phenolsulfonphthalein (1.0 g).
Implementation: Evaluation of renal tubular function. Plasma kinetics and renal excretion of intravenous PSP (1.0 g) can be evaluated with and without concomitant administration of probenecid or salicyluric acid (SUA). A physiologically based kidney model has been developed that incorporates the functional characteristics of the kidney and is capable of determining (predicting) the excretion of PSP taking into account the variables of renal plasma flow, urine flow, non-linear protein binding, glomerular filtration, tubular secretion, and tubular accumulation.

Animal Model: Canine (beagle dog).
Interpretation and Analysis: Pharmacokinetic analysis reveals that tubular secretion is the predominant route of excretion, and that secretion is inhibited by both SUA and probenecid. The model enables an accurate description and analysis of the measured plasma levels and renal excretion rates. The interaction with probenecid and SUA can be adequately described as a inhibition of the carrier-mediated uptake of PSP into the proximal tubular cells. However, both compounds clearly differ in their inhibitory action. Whereas probenecid shows simple competitive inhibition, for SUA a considerably more complex interaction (2-site competitive system) has to be taken into consideration. Especially in the interaction experiments, only satisfactory fits to the model are obtained when secretion is assumed to be dependent on unbound PSP concentrations. Model calculations show that in controlled experiments tubular secretion is accompanied by a pronounced accumulation of PSP within the proximal tubular cells, which is clearly diminished in the presence of SUA or probenecid. The predicted accumulation ratios are in good agreement with previous studies.

Physiologically based pharmacokinetic model for the renal clearance of phenolsulfonphthalein and the interaction with probenecid and salicyluric acid in the dog. Russel FG, Wouterse AC, van Ginneken CA. J Pharmacokinet Biopharm 1987 Aug; 15 (4), pp. 349–68.

Renal Tubular Excretion/Renal Glomerular Filtration
Phenolsulfonphthalein (PSP) Excretion/Inulin Clearance/Paraaminohippurate (PAH) Clearance

Cellular/Molecular Mechanism of Action: Phenolsulfonphthalein is excreted into the urine by the proximal renal tubular epithelium. Alternatively, inulin and PAH are removed passively from the plasma and pass into the urinary filtrate at the level of the renal glomerulus.
Application: Detection of alterations in renal tubule excretory capacity (PSP), effective renal plasma flow (ERPF) (PAH), and glomerular filtration rate (GFR) (inulin).
Implementation: Evaluation of alterations in renal function induced by exposure to a toxic agent. Assessment of renal function can be determined by measuring (i) exogenous PSP excretion; (ii) inulin clearance; (iii) PAH clearance (monkeys only); (iv) endogenous serum creatinine; and (v) blood urea nitrogen (BUN) at multiple time points during the study.

Toxicity Model: Toxic challenge can be induced by exposure to triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) in order to determine its influence on renal function following oral administration (e.g., beagle dog and rhesus monkey). Male rhesus monkeys are orally administered triclopyr by gavage at a dose of 5 mg/kg/day, 7 days/week for 28 days, after which the dosage is increased to 20 mg/kg/day for 102 consecutive days. Groups of male dogs can be administered either a single oral dose of 5 mg/kg triclopyr or are fed a diet spiked with triclopyr at a dose of 5 mg/kg/day for 47 consecutive days.

Animal Model: Canine (beagle dog).
Animal Model: Primate (male rhesus monkey).
Interpretation and Analysis: Creatinine, BUN, and inulin clearance are within the normal range from both species following triclopyr administration and indicate that repeated administration of triclopyr in the dog and monkey has no effect on GFR. In monkeys, the percentage excretion of PSP and PAH appears to increase following triclopyr administration (20 mg/kg/day), suggesting that these weak organic acids may be competing for the same plasma protein-binding site enhancing their clearance. More importantly, triclopyr does not appear to be competing with PSP or PAH for the active secretory site within the monkey kidney proximal tubules. In contrast, PSP clearance studies in dogs reveal that triclopyr administration (5 mg/kg) can significantly decrease the percentage of PSP excreted even following a single-dose administration. The decrease in percentage PSP is reversible and inversely related to the plasma triclopyr concentration. Therefore, triclopyr appears to effectively compete with PSP for the active secretory site within the dog kidney proximal tubules. In contrast, monkeys are insensitive to the effects of triclopyr on the active secretory process even at doses 4-fold higher

(20 mg/kg/day) than the effective dose in the dog (5 mg/kg/day). These findings suggest that the effect observed on PSP and PAH excretion in the dog represent a physiological competition for excretion and not toxicity. Note: Filtration fraction = GF/ERPF.

Evaluation of renal function in rhesus monkeys and comparison to beagle dogs following oral administration of the organic acid triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid). Timchalk C, Finco DR, Quast JF. Fundam Appl Toxicol 1997 Mar; 36 (1), pp. 47–53.

Renal Tubular Excretion: Purines (Allantoin)/ Ruminant Allantoin Plasma Clearance *[^{14}C]-Allantoin*

Application: Evaluation of purine derivative plasma clearance by renal mechanisms. The agent 4,5-[^{14}C]allantoin can be applied to measure the proportion of plasma allantoin excreted in the urine of sheep. Using this method it may be feasible to validate purine derivative excretion models in ruminant livestock.
Route of Administration: 4,5-[^{14}C]allantoin (intravenous).
Implementation: Evaluate the recovery of [^{14}C]allantoin in urine following intravenous injection and degradation of allantoin by rumen microorganisms. The urine [^{14}C]-allantoin is then recovered for measurement.

Animal Model: Sheep (n = 8 normal/healthy animals).
Interpretation and Analysis: Individual values of [^{14}C]allantoin recovery vary from 66 to 95% (mean value 83 [SE 1.6%]). The recovery of [^{14}C]allantoin shows no relation to the level of feed intake. There is some evidence that glomerular filtration rate is an important factor affecting the amount of urinary allantoin recovered. Incomplete recovery of plasma [^{14}C]allantoin in the urine indicates losses of plasma [^{14}C]allantoin via non-renal routes. Such observations occur in concert with the disappearance of [^{14}C] from rumen contents incubated *in vitro* with [^{14}C]allantoin for 48 hours (88%) and the presence of [^{14}C] in saliva *in vivo* from sheep sampled after dosing with [^{14}C]allantoin. However, the amount of [^{14}C] activity in the saliva is very low (equivalent to only 1.5% of the total dose in sheep producing saliva at a rate of 15 liters/day). The proportion of renal and non-renal excretion of purine derivatives is unpredictable both between and within individual animals. The factors responsible for this variability need to be identified, and existing models of excretion of purine derivatives may need to be modified accordingly to improve their accuracy of prediction. A single intravenous injection of 4,5-[^{14}C]allantoin provides a simple alternative to infusion methods used to measure the proportion of plasma allantoin excreted in the urine of sheep. Using this method it may be feasible to validate PD excretion models in other ruminant livestock.

Variation between sheep in renal excretion of [^{14}C]allantoin. Prasitkusol P, Ørskov ER, Chen XB, Hovell FD, Kyle DJ. Br J Nutr 2002 Jun; 87 (6), pp. 561–8.

Renal Tubular Excretion Profiling/Tubular Transporter Mechanisms *[^{3}H]-Indoxyl Sulfate/Paraaminohippuric Acid (PAH)/ Benzylpenicillin/Cimetidine/3-Carboxy-4-Methyl-5-Propyl-2-Furanpropanoic Acid/Hippuric Acid*

Cellular/Molecular Mechanism of Action: Indoxyl sulfate is a uremic toxin and radiolabeled analogs function as tracer molecules.
Background Information: Indoxyl sulfate is a uremic toxin that accumulates in the body because of the patient's inability to excrete it, thereby inducing a number of uremic symptoms that can lead to chronic renal failure. The functional failure of the excretion system for indoxyl sulfate causes its accumulation in the intravascular compartment.
Application: Uremic toxins (indoxyl sulfate, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid hippuric acid); weak acids (paraaminohippuric acid, benzylpenicillin); weak bases (cimetidine); and other substances (salicylic acid, indomethacin, 3,5,3′-triiodo-l-thyronine, and indole acetic acid [IA]) can be applied in various fashions to evaluate renal excretory function.
Implementation: Evaluate and characterize [^{3}H]indoxyl sulfate renal transport mechanisms involved in excretion using an *in vivo* tissue-sampling single-injection technique (kidney uptake index [KUI] method).

***Ex Vivo* Model:** Xenopus laevis oocyte expressing rat organic anion transporter 3 (rOAT3).
Interpretation and Analysis: Probenecid shows a concentration-dependent inhibitory effect on the uptake of [^{3}H]indoxyl sulfate using the KUI method, and uptake is inhibited by organic anions such as PAH and benzylpenicillin, by weak bases such as cimetidine, and by uremic toxins, such as 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) and hippuric acid (HA). However, salicylic acid, indomethacin, 3,5,3′-triiodo-l-thyronine, and indole acetic acid (IA) have no effect on the uptake. rOAT3-expressing oocytes exhibit uptake of [^{3}H]indoxyl sulfate by rOAT3 (Km = 158 μmol/L). Moreover, a number of uremic toxins inhibit uptake of [^{3}H]indoxyl sulfate by rOAT3. Uptake of indoxyl sulfate appears therefore to be mediated by rOAT3 activity. Uremic toxins share the transport mechanism for indoxyl sulfate. Mutual inhibition of these uremic toxins via OAT3 may accelerate their accumulation in the body and, thereby, the progression of nephrotoxicity in uremia.

Major role of organic anion transporter 3 in the transport of indoxyl sulfate in the kidney. Deguchi T, Ohtsuki S, Otagiri M, Takanaga H, Asaba H, Mori S, Terasaki T. Kidney Int 2002 May; 61 (5), pp. 1760–8.

Renal Tubular Reabsorptive Mechanisms *Myo-Inositol/[^{14}C]-Inulin*

Background Information: Filtered myo-inositol, an important renal intracellular organic osmolyte, is almost completely reabsorbed.
Implementation: Evaluation of tubule reabsorption sites and specificity for myo-inositol, and thus identification of possible mechanisms involved in this process. Investigations involve microinfusion of myo-[^{3}H]inositol or D-[^{3}H]glucose into early proximal (EP), late proximal (LP), or early distal tubule sections of superficial nephrons and into long loops of Henle (LLH) of juxtamedullary nephrons and papillary vasa recta in rats *in vivo* et *situ* for the determination of urinary fractional recovery of the [^{3}H] label compared with co-microinfused [^{14}C]-inulin. To determine the extent to which the proximal convoluted tubule (PCT) alone contributes to myo-inositol reabsorption, tubular segments can be microperfused between EP and LP puncture sites. Specificity of reabsorptive carrier(s) can be examined by adding high concentrations of other polyols and monosaccharides to the infusate.

Animal Model: Rat EP, LP, early distal tubule sections of superficial nephrons; LLH of juxtamedullary nephrons; papillary vasa recta.
Interpretation and Analysis: Greater than 60% of the physiological glomerular load of myo-inositol can be reabsorbed in the PCT and > 90% in the short loop of Henle (SLH) by a saturable, phloridzin-sensitive process. Myo-inositol can also be reabsorbed in

the ascending limb of LLH and can move from the papillary vasa recta blood into ipsilateral tubular structures. Essentially no reabsorption occurs in nephron segments beyond the SLH or in collecting ducts. Specificity studies indicate that reabsorption probably occurs via a luminal [Na^+]-myo-inositol co-transporter.

Tubular reabsorption of myo-inositol vs that of D-glucose in rat kidney *in vivo* et *situ*. Silbernagl S, Völker K, Dantzler WH. Am J Physiol Renal Physiol 2003 Jun; 284 (6), pp. F1181–9.

Renal Tubular Resorption/Differentiation between Competitive vs Non-Competitive Tubular Absorption
Phenolsulfonphthalein (PSP)

Cellular/Molecular Mechanism of Action: Phenolsulfonphthalein excreted by the proximal renal tubular epithelium.
Application: Evaluation of renal tubular function pertaining to excretory mechanisms.
Implementation: Evaluation of the plasma kinetics and renal excretion of salicyluric acid (SUA; 0.8 g), administered intravenously with and without concomitant PSP administration.
Animal Model: Canine (beagle dogs).
Interpretation and Analysis: Pharmacokinetic analysis reveals that tubular secretion is the predominant route of elimination and that secretion is inhibited by PSP. A physiologically based kidney model has been developed that comprises all the functional characteristics of the kidney that determine the excretion of salicyluric acid (renal plasma flow, urine flow, non-linear protein binding, glomerular filtration, tubular secretion, and tubular accumulation). The model facilitates an accurate description and analysis of measured plasma levels and renal excretion rates. The interaction with PSP can be adequately described with the model by non-competitive inhibition of a carrier-mediated uptake of SUA into the tubular cells. Furthermore, a small but significant reduction in non-renal SUA clearance can be observed. Model calculations show that, in controlled investigations, tubular secretion is accompanied by a pronounced accumulation of SUA within cells, which is clearly diminished in the presence of phenolsulfonophthalein. The predicted accumulation ratios are in good agreement with previously reported *in vitro* values.

Physiologically based pharmacokinetic model for the renal clearance of salicyluric acid and the interaction with phenolsulfonphthalein in the dog. Russel FG, Wouterse AC, van Ginneken CA. Drug Metab Dispos 1987 Sep–Oct; 15 (5), pp. 695–701.

Renal Tubule Acid Transport/Prolongation of Pharmaceutical Clearance
Probenecid

Cellular/Molecular Mechanism of Action: Competes with other acidic pharmaceuticals that are excreted by mechanisms dependent upon renal tubular acid transport systems.
Physiological Mechanism of Action: Competitively decreases the renal excretion of acidic pharmaceutical agents circulating within the intravascular compartment in a manner that prolongs their pharmacokinetic profile.
Application: Prolong the exposure of acidic diagnostic pharmaceutical agents to desired organ/tissue/lesion target sites.
Route of Administration: Probenecid (oral).
Dosage: Probenecid (100 mg/kg).
Implementation: In rabbits receiving pranofen (5 mg/kg IV), levels of (−)-(R)- and (+)-(S)-pranoprofen and their glucuronide (after hydrolysis with sodium hydroxide) can be measured in plasma, urine, and several tissues to established pharmacokinetic parmeters. The plasma concentration of the (+)-(S)-isomer is higher than that of the (−)-(R)-form. Oral co-administered probenecid (100 mg/kg) results in increased plasma concentrations of both enantiomers. Probenecid reduces the apparent total clearance and excretion of pranoprofen enantiomers in urine. Probenecid (100 mg/kg) has a slight effect on the tissue distribution of pranoprofen but significantly reduces the formation of glucuronide for both enantiomers to the same extent in kidney microsomes. The differences caused by probenecid are significant with respect to its ability to inhibit glucuronidation in the kidney and subsequent excretion into urine, but enantioselective effects are negligible.

Probenecid-induced changes in the clearance of pranoprofen enantiomers. Imai T, Nomura T, Otagiri M. Chirality 2003 May 5; 15 (4), pp. 318–23.

Renal Tubule Concentrating Capacity/Renal Failure
Desamino Arginine Vasopressin (DDAVP)/[Antidiuretic Hormone (ADH)]

Physiological Mechanism of Action: Promotes constriction of smooth muscle tissues of capillaries and arterioles resulting in an elevation in blood pressure. Secondary affects include stimulation of intestinal smooth muscle and increased peristaltic activity. One of the most significant properties of vasopressin is its effect on epithelial cells of the distal renal tubule in a manner the promotes water resorption independent of solids, resulting in elevations in urine concentration.
Background Information: Vasopressin is 1 of the hormones formed by the neuronal cells of the hypothalamic nuclei and stored in the posterior lobe of the pituitary gland.
Application: Comprehensive 1-day renal function test.
Route of Administration: Desamino arginine vasopressin (intranasal).
Dosage: Desamino arginine vasopressin (20 μg).
Implementation: Evaluation of the influence of DDAVP on urine osmolality over a period of 8 and a half hours.
Human Model: Normal "young" cases (n = 33).
Interpretation and Analysis: A 20-μg intranasal dose of DDAVP consistently results in the production of a urinary osmolality of 800 mosmol/kg or more, with no detectable untoward effects. There are several advantages associated with DDAVP that make it a viable alternative to pitressin tannate in oil as a diagnostic agent.

Desamino arginine vasopressin (DDAVP) as a diagnostic agent. Somerfield SD, Hocken AG. N Z Med J 1977 Nov 23; 86 (600), pp. 472–3.

Renal Tubule Excretion of Phosphorous/Renal Dopamine Metabolism/Renal Brush Border Membranes
Gamma-L-Glutamyl-L-dopa (Gludopa)/Dopamine (DA)/L-Dopamine (L-dopa)

Cellular/Molecular Mechanism of Action: Synthetic dipeptide gludopa can serve as an inactive precursor (pro-prodrug) of dopamine (DA).
Background Information: For treatment of phosphate (Pi) overload in various pathophysiological states, an agent that selectively increases renal Pi excretion would be of major value. Previ-

ously, it has been shown that DA inhibits Na^+-Pi co-transport in renal epithelia. However, the administration of DA or its immediate precursor L-dopa increases DA in multiple tissues. Synthetic dipeptide gludopa can serve as an inactive precursor (pro-prodrug) of DA.
Application: Delineation of renal phosphorous physiology.
Implementation: Evaluation of the hypothesis that gludopa may elicit phosphaturia by action of DA generated within the kidney due to the unique co-localization of gamma-glutamyltransferase (gamma-GT), aromatic amino acid decarboxylase, Na^+-Pi co-transporter, and Na^+-L-dopa co-transporter in brush border membrane (BBM) of proximal tubular cells.

Investigation Design: Evaluation of gludopa, or gludopa in combination with acivicin (gamma-GT inhibitor). Urinary excretion of Pi, Ca^{2+}, Na^+, K^+, DA, cAMP, and cGMP can then be determined along with Na^+-Pi co-transport measured in renal BBMs prepared from rats at the end of the investigation period.

Animal Model: Rat (thyroparathyrectomized).
Interpretation and Analysis: Administration of gludopa results in (i) an inhibition of Na^+-Pi co-transport, but not co-transport of Na^+-proline and Na^+-alanine in BBM; (ii) an increase (+300%) of fractional excretion (FE) of Pi and declines (−35%) in plasma Pi, whereas plasma levels and FEs of Ca^{2+}, Na^+, and K^+ remain unchanged; (iii) an increase in urinary excretion of cAMP, but not cGMP; (iv) a 1,000-fold increase of urinary excretion of DA, without a change in excretion of norepinephrine; and (v) incubation of gludopa with BBM *in vitro,* which causes a release of L-dopa, and the *in vivo* administration of acivicin, which blocks actions of gludopa to inhibit Na^+-Pi co-transport and increases urinary Pi and DA excretion. Co-localization of enzymes of biotransformation, BBM transporters, and the autocrine/paracrine DA system in cells of proximal tubules constitutes a cellular basis for the potent and specific phosphaturic action of gludopa.

Gamma-L-glutamyl-L-DOPA inhibits Na(+)-phosphate co-transport across renal brush border membranes and increases renal excretion of phosphate. de Toledo FG, Thompson MA, Bolliger C, Tyce GM, Dousa TP. Kidney Int 1999 May; 55 (5), pp. 1832–42.

Renal Tubule Fractional Clearance Rates/Charge Selectivity
Negatively Charged Ficoll/Uncharged Ficoll

Cellular/Molecular Mechanism of Action: Passive marker system.
Background Information: Recent studies using low-temperature perfusion of rat kidneys have claimed the existence of renal charge selectivity simply on the basis of the differential excretion rates of uncharged Ficoll and charged proteins.
Application: Evaluation of renal tubule charge selectivity and fractional clearance rates.
Route of Administration: Intravenous.
Implementation: Determination of the existence of *in vivo* charge selectivity evaluated by measuring the clearance of negatively charged Ficoll compared with uncharged Ficoll. A short-term approach to steady state is used to study renal tubule fractional clearance rates. Relative clearances can also be examined using an osmotic pump technique where the tracers reach a steady state value after a period of 7 days.

Animal Model: Rat (n = 5 conscious).
Interpretation and Analysis: Carboxymethyl Ficoll is stable during filtration and renal passage, is not taken up by the kidneys, and does not bind to plasma proteins. There is no significant difference in the fractional clearance of molecules with a radius of 36 Å for Ficoll (fractional clearance = 0.048 ± 0.038) and negatively charged carboxymethyl Ficoll (fractional clearance = 0.028 ± 0.019). For molecules with radii greater than 36 Å, carboxymethyl Ficoll has facilitated clearance with respect to uncharged Ficoll (example: radius of 60 Å fractional clearance for Ficoll = 0.0012 ± 0.0005, compared to carboxymethyl Ficoll = 0.015 ± 0.005). Renal function is not compromised by carboxymethyl Ficoll as uncharged Ficoll in urine exhibits similar hydrodynamic size profiles when studied in the presence of excess unlabeled carboxymethyl Ficoll. The facilitated clearance of negatively charged Ficoll with respect to uncharged Ficoll reveals a property of the capillary wall, which has been previously observed with other non-proteinaceous polyanions. The glomerular capillary wall is not charge selective in the form of excluding negatively charged Ficoll. However, the charge properties of the capillary wall may influence the facilitated transport of charged Ficoll compared with uncharged Ficoll.

Anomalous fractional clearance of negatively charged Ficoll relative to uncharged Ficoll. Guimaraes MA, Nikolovski J, Pratt LM, Greive K, Comper WD. Am J Physiol Renal Physiol 2003 Dec; 285 (6), pp. F1118–24.

Renal Tubule Resorption Capacity
Phenol Red/Bromophenol Blue/Bromothymol Blue

Cellular/Molecular Mechanism of Action: Phenol red, bromophenol blue, and bromothymol blue appear to be excreted by renal tubule acid transport mechanisms and are reabsorbed in a manner that is proportional to glomerular load.
Application: Evaluation of renal tubule excretory function.
Route of Administration: Phenol red, bromophenol blue, and bromothymol blue (intravenous).
Implementation: Potential application for evaluating renal tubule acid transport mechanisms.

Animal Model: Rabbit.
Interpretation and Analysis: Net tubular excretion of phenol red reaches a maximal value of 8 μmol/min at a plasma concentration of ultrafilterable dye of about 0.1 mM and is decreased at higher plasma concentrations. Decreases in net tubular excretion at high plasma concentrations are also obtained for bromophenol blue and bromothymol blue, suggesting tubular reabsorption in addition to tubular secretion of the dye. Conclusive evidence for reabsorption is provided by administration of probenecid, which causes a fall in the excretion of the dyes below that filtered by the glomeruli. Tubular reabsorption of phenol red during probenecid administration appears to be proportional to the glomerular load and is increased under experimental conditions leading to decreases in urinary pH. Experiments involving efflux of phenol red from liposomes provides no evidence of a significant role of trans-membrane passage by non-ionic diffusion. The dependence of the reabsorptive process is the result of preferential reabsorption of the acid as compared to the basic form of the indicator dye across a hydrophilic pathway in the transporting membranes. The clearance ratio of phenol red to that of *p*-aminohippurate at low plasma concentrations is about 0.3. The low degree of extraction of phenol red from renal plasma is attributed both to tubular reabsorption and binding of the dye by plasma proteins. Note: Filtration fraction = GF/ERPF.

Renal handling of phenol red. III. Bidirectional transport. Gerdes U, Kristensen J, Møller JV, Sheikh MI. J Physiol 1978 Apr; 277, pp. 115–29.

URINARY BLADDER

Contractions/(Potassium Adenosine Triphosphate [KATP] Channel Opener) *[^{125}I]A-312110*

Cellular/Molecular Mechanism of Action: [^{125}I]A-312110 or [(9R)-9-(4-fluoro-3-^{125}iodophenyl)-2,3,5,9-tetrahydro-4H-pyrano[3,4-b]thieno[2,3-e]pyridin-8(7H)-one-1,1-dioxide] functions as a KATP channel opener in cardiac tissues.
Physiological Mechanism of Action: The *in vivo* application of [^{125}I]A-312110 or [(9R)-9-(4-fluoro-3-^{125}iodophenyl)-2,3,5,9-tetrahydro-4H-pyrano[3,4-b]thieno[2,3-e]pyridin-8(7H)-one-1,1-dioxide] can suppress action potential duration (APD) in Purkinje fibers and relax electrical field-stimulated bladder contractions.
Application: [^{125}I]A-312110 or [(9R)-9-(4-fluoro-3-^{125}iodophenyl)-2,3,5,9-tetrahydro-4H-pyrano[3,4-b]thieno[2,3-e] pyridin-8(7H)-one-1,1-dioxide] can be applied to detect and characterize KATP channels. In this context, [^{125}I]A-312110 can be used to delineate the molecular and functional properties of the KATP channel complex and the identification of new channel blockers and openers that interact with cardiac/smooth muscle-type K^+ATP channels.
Route of Administration: [^{125}I]A-312110 (intravenous).
Dosage: [^{125}I]A-312110 or [(9R)-9-(4-fluoro-3-^{125}iodophenyl)-2,3,5,9-tetrahydro-4H-pyrano[3,4-b]thieno[2,3-e]pyridin-8(7H)-one-1,1-dioxide].
Considerations: Although ATP-sensitive K^+ channels continue to be explored for their therapeutic potential, developments in high-affinity radioligands to investigate native and recombinant KATP channels have been less forthcoming.
Interpretation and Analysis: The agent [^{125}I]A-312110 binds with high affinity to a single class of binding sites in guinea pig cardiac (KD = 5.8 nM) and urinary bladder (KD = 4.9 nM) membranes in a manner that is saturable. Displacement of [^{125}I]A-312110 by structurally diverse potassium channel openers (K^+COs) indicates a similar rank order of potency in both guinea pig cardiac and bladder membranes (Ki, heart): A-312110 (4.3 nM) > N-cyano-N′-(1,1-dimethylpropyl)-N′′-3-pyridylguanidine (P1075) > (−)-N-(2-ethoxyphenyl)-N′-(1,2,3-trimethylpropyl)-2-nitroethene-1,1-diamine (Bay X 9228) > pinacidil > (−)-cromakalim > N-(4-benzoyl phenyl)-3,3,3-trifluro-2-hydroxy-2-methylpropionamine (ZD6169) > 9-(3-cyanophenyl)-3,4,6,7,9,10-hexahydro-1,8-(2H,5H)-acridinedione (ZM244085) >> diazoxide (16.7 μM). Displacement by KATP channel blockers, the sulfonylurea glyburide, and the cyanoguanidine N-[1-(3-chlorophenyl)cyclobutyl]-N′-cyano-N′′-3-pyridinyl-guanidine (PNU-99963) are biphasic in the heart but monophasic in bladder with about a 100- to 500-fold difference in Ki values between high- and low-affinity sites. Good correlations exist between cardiac or bladder-binding affinities of potassium channel openers and functional activation as assessed by their respective potencies to either suppress APD in Purkinje fibers or to relax electrical field-stimulated bladder contractions. Collectively, these results demonstrate that [^{125}I]A-312110 binds with high affinity and has an improved activity profile compared with other radiolabeled potassium channel opener agents. [^{125}I]A-312110 is a useful tool for investigating the molecular and functional properties of the K^+ATP channel complex and for the identification, in a high throughput manner, of both novel channel blockers and openers that interact with cardiac/smooth muscle-type KATP channels.

[^{125}I]A-312110, a novel high-affinity 1,4-dihydropyridine ATP-sensitive K^+ channel opener: Characterization and pharmacology of binding. Davis-Taber R, Molinari EJ, Altenbach RJ, Whiteaker KL, Shieh CC, Rotert G, Buckner SA, Malysz J, Milicic I, McDermott JS, Gintant GA, Coghlan MJ, Carroll WA, Scott VE, Gopalakrishnan M. Mol Pharmacol 2003 Jul; 64 (1), pp. 143–53.

Detrusor Muscle Overactivity/Lower Motor Neuron vs Upper Motor Neuron Hyperactivity *Tramadol*

Physiological Mechanism of Action: Tramadol can function as an effective analgesic agent.
Background Information: Cerebrovascular disease, such as stroke, frequently results in incontinence by reducing suprapontine micturition control. Intraluminal occlusion of the middle cerebral artery (MCA), which produces detrusor overactivity, has been introduced as a useful model of stroke-induced lower urinary tract dysfunction. Recently, tramadol has been found to possess inhibitory actions on normal rat micturition.
Application: Potential differentiation of upper motor neuron disease from lower motor neuron disease pertaining to dysfunction related to micturition processes.
Route of Administration: Tramadol (intravenous).
Dosage: Tramadol (5 mg/kg).
Implementation: Evaluation of detrusor muscle overactivity due to cerebral infarction applying cystometry analysis in combination with tramadol administration. At 72 hours posturinary catheterization, continuous cystometry is performed in awake animals and the effects of tramadol determined.
Animal Model: Rat (female Sprague-Dawley strain with cerebral ischemia induced by occlusion of the MCA). Additional features/requirements involved in the application of the animal model include the placement of urinary bladder catheters.
Interpretation and Analysis: In cerebral infarcted rats, bladder capacity is lower (48 ± 9%) and micturition pressure higher (76 ± 21%) than in control rats. Tramadol increases bladder capacity (59 ± 29%) and threshold pressure (47 ± 32%) to values similar to those detected in reference controls. However, micturition pressure is not significantly altered. Tramadol induces diuresis in some, but not all, conditions of cerebral infarction (rat). Tramadol normalized detrusor overactivity in MCA-occluded rats. Tramadol may therefore have applicability in cases with detrusor overactivity following vascular accidents like cerebral stroke.

Effects of tramadol on rat detrusor overactivity induced by experimental cerebral infarction. Pehrson R, Stenman E, Andersson KE. Eur Urol 2003 Oct; 44 (4), pp. 495–9.

Neoplastic Disease: Urinary Bladder Cancer *5-Aminolevulinic Acid (ALA)/5-Aminolevulinic Acid Methyl Ester (AME)*

Refer to the neoplastic disease chapter (chapter 12) in Section II.

A study of 5-aminolevulinic acid and its methyl ester used in *in vitro* and *in vivo* systems of human bladder cancer. Manivasager V, Heng PW, Hao J, Zheng W, Soo KC, Olivo M. Int J Oncol 2003 Feb; 22 (2), pp. 313–8.

Overactive Bladder: Urgency/Detrusor Pressure/ Increase Precision of Urodynamic Procedures *Lidocaine/Provocative Water Cystometry*

Cellular/Molecular Mechanism of Action: Lidocaine combines with a specific receptor site within sodium channel complexes associated with cell membrane structures, resulting in the inhibition of trans-membrane Na^+ transport.

Physiological Mechanism of Action: Inhibition of depolarization events normally propagated along cell membrane structures including those associated with neurons. In this manner, normal nerve transmission becomes compromised. The effect is most prominent in small, unmyelinated neurons.
Application: Increase the precision of the urodynamic diagnosis of bladder overactivity/urgency.
Route of Administration: Lidocaine (intravesicular delivery).
Dosage: Lidocaine (3 g for 20 minutes).
Implementation: Evaluation of the effect of intravesical lidocaine on urinary bladder control. Repeat cystometry is performed after the bladder had been bathed in lidocaine (3 g for 20 minutes).

Human Model: Cases with symptoms of urinary urgency with or without incontinence.
Interpretation and Analysis: Provocative water cystometry is not able to demonstrate signs of unstable detrusor function. However, all cases evaluated display a continuous, steep rise of detrusor pressure at the end phase of urinary bladder filling. Repeat cystometry performed after intravesicular lidocaine administration (3 g; 20 minutes) results in no detectable change in the configuration of the cystometrogram profiles in most affected cases. Certain groups of cases display decreases in detrusor pressure, and there is also a detectable influence on phasic bladder contractions. In some cases (e.g., n = 3), phasic detrusor contractions develop after lidocaine administration. Thus, intravesical lidocaine seems to be a useful tool to increase the precision of the urodynamic diagnosis in bladder overactivity.

Lidocaine cystometry in the diagnosis of bladder overactivity. Edlund C, Peeker R, Fall M. Neurourol Urodyn 2001; 20 (2), pp. 147–55.

Overactive Bladder vs Sensory Urgency
Edrophonium Chloride/Provocative Filling Cystometry

Cellular/Molecular Mechanism of Action: Edrophonium chloride is a potent inhibitor of cholinesterase biochemical activity. Inhibition of this enzyme promotes elevated accumulation of acetylcholine (cholinergic neurotransmitter) in the vicinity of presynaptic membranes.
Physiological Mechanism of Action: Edrophonium chloride enhances cholinergic activity (parasympathomimetic properties). Bethanecol stimulates bladder activity.
Background Information: Overactive bladder, a highly prevalent disorder, is suspected of having a low detection rate due to the lack of sensitive diagnostic tools. Recent studies imply the involvement of the cholinergic system in the pathophysiological mechanism underlying overactive bladder.
Application: Edrophonium chloride can significantly increase the sensitivity of provocative filling cystometry procedures, thereby facilitating the identification of case subsets with overactive bladder that have previously been classified with sensory urgency.
Route of Administration: Edrophonium chloride (intravenous).
Dosage: Edrophonium chloride (10 mg).
Implementation: Determination of the potential for *in vivo* pharmaceutical enhancement of cholinergic activity to be applied as a means of detecting and evaluating subtle conditions of urinary bladder urgency. Bladder function in this context can be established using multichannel video urodynamic methodologies to evaluate filling cystometry procedures following edrophonium chloride administration. Response to edrophonium is defined as a significant change in sensation and decreased bladder capacity, the induction or amplification of involuntary detrusor contractions, or significant decreases in detrusor compliance. Findings are then compared in responders and non-responders.

Human Model: Cases of bladder hyperactivity (n = 27).
Interpretation and Analysis: Slightly more than 30% of cases evaluated can be classified as responders (e.g., n = 11/27; compared to n = 16 non-responders). In addition, a response can be noted in 78% of the cases with the symptomatology of overactive bladder. A response cannot be appreciated in the vast majority of cases with no specific complaints suggestive of bladder overactivity (e.g., approximately 100%). In approximately two-thirds of responders, values for baseline cystometry can be interpreted as being normal (e.g., n = 7/12 or 64%). In cases classified as responders, uninhibited urinary leakage can be observed in response to edrophonium (e.g., n = 6/11 or 54%). Therefore, preliminary findings to date imply that edrophonium may serve as a novel, practical, and safe drug for provocative cystometry. By significantly increasing cystometry sensitivity the drug would facilitate the identification of case subsets with overactive bladder that have previously been classified with sensory urgency. Note: Serious adverse reactions to the drug are rarely observed.

The effect of cholinergic enhancement during filling cystometry: Can edrophonium chloride be used as a provocative test for overactive bladder? Yossepowitch O, Gillon G, Baniel J, Engelstein D, Livne PM. J Urol 2001 May; 165 (5), pp. 1441–5.

Overactive Conditions of the Urinary Bladder/ Serotonin (5-HT) Production in the Central Nervous System (CNS)
Clomipramine CL)/Fluoxetine

Cellular/Molecular Mechanism of Action: Clomipramine modifies (reduces) concentrations of serotonin within the CNS. Fluoxetine is a selective serotonin uptake inhibitor within the CNS.
Physiological Mechanism of Action: Clomipramine promotes endogenous CNS depression. In contrast, fluoxetine can reverse these CNS properties induced by clomipramine.
Background Information: Serotonin in the CNS may inhibit bladder activity. Based on this consideration, it has been postulated that CNS depression associated with altered 5-HT production may be associated with, or the cause of, conditions such as overactive bladder (OAB).
Application: Detection and evaluation of physiological functions or conditions (e.g., OAB) that may be associated with alterations in 5-HT production within the CNS.
Dosage: Clomipramine hydrochloride (22.5 mg/kg body weight) compared to an equal volume of sterile physiological saline administered by injection to negative reference controls. Fluoxetine (20 mg/kg daily).
Implementation: Evaluation of the influence of CL hydrochloride on urinary voiding frequency at 10 and 15 weeks. Behavioral correlates of depression are assessed using a forced-swim challenge. At age 15 weeks cystometry (CMG) analysis is performed. Fluoxetine is then co-administrated with clomipramine or saline followed by the measurement of void frequency (VF) and reevaluation of CMG analyses.

Animal Model: Rat (Wistar pups, postnatal days 8 to 21), endogenous model for depression promoted by lowering serotonin (5-HT) concentrations.
Interpretation and Analysis: Clomipramine increases immobility when combined with forced-swim challenge testing (indication of depression). Clomipramine promotes more frequent voiding compared to negative saline controls at 10 and 15 weeks. Interestingly, the difference is significant only in females. Cystometry of

CL/females reveals decreased bladder capacity, micturition volume, and intermicturition contractions compared with negative saline reference controls. Fluoxetine reverses these changes (comparable to saline negative reference controls). Such observations support the hypothesis that OAB may be associated with altered 5-HT function and may explain previous descriptions of an association between depression and OAB.

Alterations in voiding frequency and cystometry in the clomipramine induced model of endogenous depression and reversal with fluoxetine. Lee KS, Na YG, Dean-McKinney T, Klausner AP, Tuttle JB, Steers WD. J Urol 2003 Nov; 170 (5), pp. 2067–71.

Traumatic Rupture
Fluorescein Dye

Cellular/Molecular Mechanism of Action: Functions as a detectable dye marker in biological systems. Reacts with ultraviolet light to produce a fluorescent green color.
Diagnostic Application: Detection of traumatic rupture of the urinary bladder. Fluorescein dye is an appropriate visible *in vivo* marker for a variety of purposes.
Route of Administration and Dosage: Traumatic rupture of the urinary bladder is detected by direct infusion into the urinary bladder utilizing a urethral catheter.
Considerations: Fluorescein dyes can on occasion diffuse rather rapidly across intact barriers such as the urinary bladder. For this reason, the interval between infusion and collection must be of a relatively short duration. Alternatively, fluorescein dye can be conjugated to large molecular weight substances, such as a polypeptide protein or dextran molecule, that do not readily diffuse across intact anatomical structures such as the bladder or diaphragm.
Interpretation and Analysis: Detection of fluorescein dye in abdominal fluid following direct infusion into the urinary bladder utilizing a urethral catheter system implies the presence of a physical defect within the bladder wall.
Laboratory Instrumentation: In a clinical environment, presence of fluorescein dye localized within devitalized segments of devitalized intestinal tract, or within abdominal fluid samples, is usually accomplished by direct visual observation.

Traumatic Rupture
Glucose

Cellular/Molecular Mechanism of Action: Detectable molecular marker molecule.
Diagnostic Application: Detection of a ruptured urinary bladder.
Route of Administration: Direct infusion into the urinary bladder by catheter following the removal of any residual urine.
Dosage: Infusion of a 50% formulation of dextrose sufficient to moderately distend the urinary bladder of a normal individual.
Considerations: Dextrose may immediately drain into the abdomen of a patient with a ruptured urinary bladder. Obvious care must be taken to avoid puncture of the urethra and urinary bladder with the catheter, or rupture of an already distended bladder. Peritoneal fluid samples should be collected within a few minutes of bladder infusion of dextrose due to the diffusible nature of the molecule.
Interpretation and Analysis: Traumatic rents in the urinary bladder can be diagnosed by measuring the concentration of glucose within the peritoneal fluid depending on the size, shape, and location of the full-thickness defect.
Laboratory Instrumentation: Measurement of glucose concentrations applying conventional clinical pathology techniques and instrumentation (spectrophotometer).

Urolithiasis: Feline Struvite Crystal Formation
Walpole's Buffer Solution pH 4.5

Application: Diagnosis of struvite urolithiasis.
Implementation: Sterile Walpole's solution is formulated at a pH of 4.5 and can be used to detect and treat struvite urolithiasis.
Animal Model: Feline.

The use of sterile Walpole's buffer solution pH 4.5 as a diagnostic agent and a treatment of struvite urolithiasis in the cat. Jackson OF. Vet Rec 1970 Nov 21; 87 (21), pp. 667–8.

IMAGING ENHANCEMENT

Renal Artery Stenosis (RAS) with Hypertension
Captopril/[^{99m}Tc]-Mercaptoacetyltriglycine-3 (MAG-3) Validation

Cellular/Molecular Mechanism of Action: Captopril (oral) functions as an angiotensin-converting enzyme (ACE) inhibitor. [^{99m}Tc]-MAG3 is eliminated from the body through mechanisms of renal glomerular filtration.
Application: Detection of RAS. Captopril can enhance [^{99m}Tc]-MAG3 renography for assessment of renal plasma flow.
Route of Administration: Captopril (oral).
Dosage: Captopril (50 mg) premedication 1 hour before performing [^{99m}Tc]-MAG3 renal scintigraphy.
Considerations: In the reference trial, baseline studies are obtained only for those patients showing abnormal findings during provocative diagnostic tests.
Interpretation and Analysis: Sensitivity and specificity for the detection of RAS with greater than 50% occlusion are 89% and 91%, respectively. After captopril administration, tracer parenchymal transit time increases significantly in ischemic kidneys (334 $\pm$ 93 seconds in baseline conditions vs 468 $\pm$ 96 seconds after captopril, $p < 0.001$) but not in kidneys without RAS or RAS $<$ 50% (243 $\pm$ 46 seconds vs 271 $\pm$ 95 seconds, $p =$ ns). False-positive responses are mostly bilateral and associated with marked decreases in blood pressure. Therefore, [^{99m}Tc]-MAG3 is an effective compound for detecting RAS $\geq$ to 50% with captopril renal scintigraphy. Performing the provocative test as a 1st step considerably reduces the number of scintigraphic studies required.
Instrumentation: Computer-integrated scintigraphic camera.

Use of technetium-99m-MAG3 for renal scintigraphy after angiotensin-converting enzyme inhibition. Dondi M, Monetti N, Fanti S, Marchetta F, Corbelli C, Zagni P, De Fabritis A, Losinno F, Levorato M, Zuccalá A. J Nucl Med 1991 Mar; 32 (3), pp. 424–8.

Renal Artery Stenosis (RAS) with Hypertension
Captopril/[^{99m}Tc]-Mercaptoacetyltriglycine ([^{99m}Tc]-MAG3)

Application: Detection of RAS in cases of hypertension.
Route of Administration: Captopril (oral) [^{99m}Tc]-MAG3 (intravascular).
Dosage: Captopril (50 mg) followed in 60 minutes by [^{99m}Tc]-MAG3 (120 mBq).
Implementation: Evaluation of the renogram shape, relative tracer uptake, time to peak counts, and parenchymal transit time uti-

lizing [^{99m}Tc]-MAG3 for scintigraphic detection of RAS. In cases with a normal outcome, no further scintigraphic test are performed. In all other cases a baseline study is performed within 2 to 3 days. Where an apparent improvement is found in baseline conditions compared to the provocative test (upslope of the renogram, increased tracer uptake, and reduction of parenchymal transit time), the study is considered positive for significant RAS.

Human Model: Renal artery stenosis in 102 hypertensive patients who underwent renal angiography within 4 weeks of scintigraphic assessment.

Interpretation and Analysis: At renal angiography, a number of arteries with RAS can be detected (e.g., n = 48 with occlusion > 50%; n = 27 RAS with < 50% occlusion). Sensitivity and specificity for detection of RAS > 50% occlusion is approximately 89.6% and 91.6%, respectively. In kidneys affected by RAS > 50%, captopril administration typically induces prolongation in parenchymal transit time (324 ± 90 seconds, baseline conditions, vs 408 ± 122 seconds, postcaptopril, $p < 0.01$) compared to 255 ± 70 vs 251 ± 64, p = ns, in kidneys supplied by patent arteries or with RAS < 50% occlusion.

Captopril renal scintigraphy with [^{99m}Tc]-mercaptoacetyltriglycine (^{99m}Tc-MAG3) for detecting renal artery stenosis. Dondi M. Am J Hypertens 1991 Dec; 4 (12 Pt 2), pp. 737S–40S.

Renal Obstruction: Chronic/Partial
Captopril/[^{99m}Tc]-Mercaptoacetyltriglycine-3 (MAG-3) Validation/Furosemide

Cellular/Molecular Mechanism of Action: Captopril is an angiotensin-converting enzyme (ACE) inhibitor.

Background Information: A growing body of evidence suggests that the renin-angiotensin system is a key factor in the onset and progression of renal damage in chronic partial obstruction, and therefore often represents a complex diagnostic challenge.

Application: Characterization and evaluation of chronic partial renal obstruction.

Dosage: Captopril (0.75 mg/kg).

Considerations: Angiotensin-converting enzyme inhibitors may worsen the excretory phase of diuretic renography for obstructive hydronephrosis.

Implementation: Evaluation of the efficacy of captopril/[^{99m}Tc]-mercaptoacetyltryglycerine-3 or [^{99m}Tc]-MAG-3 renography as a diagnostic approach for the detection of early obstruction. Basal and captopril-enhanced diuretic renography with [^{99m}Tc]-MAG-3 is performed within 24 hours using the same hydration and diuretic stimulus (furosemide 0.75 mg/kg), and captopril is administered orally 60–90 minutes before scintigraphy.

Human Model: Postsurgical cases (n = 12 pyeloplasty including n = 10 males, 2–72 months old; median age 7) with unilateral hydronephrosis, normal renal function, and normal blood pressure.

Interpretation and Analysis: Captopril enhances diuretic renography with [^{99m}Tc]-MAG-3. No adverse effects or modifications of blood pressure can be observed after captopril administration. The diuretic response is markedly worsened by ACE inhibition in each hydronephrotic kidney even when the basal study is only slightly abnormal (15-minute washout basal −27 ± 16%, after captopril −9 ± 13, $p < 0.005$). After surgical correction the diuretic washout during angiotensin inhibition appears normal in the vast majority of cases (e.g., n = 12/12; 15-minute washout −56 ± 14%). Separate renal function and parenchymal transit of MAG-3 are not modified by ACE inhibition, preoperatively or postoperatively.

Data confirm the influence of angiotensin on the kidney excretory system in human hydronephrosis and suggest a role for captopril-enhanced diuretic renography in the early diagnosis of pyeloureteral obstruction. Further work is needed to evaluate ACE inhibition as a protective agent in obstructive nephropathy.

Angiotensin-converting enzyme inhibition worsens the excretory phase of diuretic renography for obstructive hydronephrosis. Zucchetta P, Carasi C, Marzola MC, Murer L, Passerini-Glazel G, Rigamonti W, Zacchello G, Bui F, Montini G. J Urol 2001 Jun; 165 (6 Pt 2), pp. 2296–9.

11 Respiratory System

RESPIRATORY PHYSIOLOGY

Bronchial Constriction Responses/Sites of Action
Methacholine (MCh)/Helium/Oxygen (HeO_2)

Cellular/Molecular Mechanism of Action: Methacholine is a parasympathomimetic/cholinergic agonist that recognizes, binds to, and activates parasympathetic receptor complexes.
Background Information: Bronchodilator agents are an important class of drug for patients with chronic obstructive pulmonary disease. Methacholine is used as a popular bronchial provocative agent. Although the major site of action for bronchodilator agents is known, the site of action for MCh is still in debate.
Application: Methacholine can be applied as a reactive agent in bronchial provocation tests.
Route of Administration: HeO_2 is administered by inhalation.
Implementation: Determination of the site of action for MCh. One approach to achieving this objective involves the evaluation of upper respiratory tract infection applying spirometry, MCh bronchial provocation, and 5 minutes of inhalation of an HeO_2 mixture over the course of a single 24-hour period. Spirometry and the bronchodilator test, in concert with 5-minute inhalations of HeO_2 and expiratory flow-volume (F-V) curve, are subsequently performed on a 2nd (separate) day. Changes in pre- and post-HeO_2 VE_{Max50} are calculated as delta VE_{Max50}. The pre- and postbronchodilator VE_{Max50} and delta VE_{Max50} differences are then counted to determine the acting site for a given bronchodilator. After a bronchial provocative test with MCh, the volume of isoflow (VisoV) can be estimated from pre- and post-HeO_2 F-V curve to establish the acting site for MCh.

Human Model: Cases with upper respiratory tract infection (n = 30).
Interpretation and Analysis: Small airways appear to be the major acting sites of bronchodilators, in contrast to large airways that are the reaction target sites for MCh. Similarly, upper respiratory tract infections (URIs) affect primarily the larger airways. Although airway hyperresponsiveness is more severe in cases with positive MCh responses, the recovery of spirometry values is not significantly different between MCh-positive and MCh-negative groups. Therefore, it appears that the major sites of action for bronchodilator, MCh, and URI are the small and large bronchioles, and large airways, respectively. Bronchial hyperresponsiveness is not a cause of quick restoration of spirometry values in subjects with positive MCh response.

The acting site of bronchodilator, methacholine and upper respiratory tract infection on airways. Ting YM, Liang L, Lu JY. Zhonghua Yi Xue Za Zhi (Taipei) 1997 Dec; 60 (6), pp. 283–9.

Bronchial Constriction Responses/Bronchial Provocation/Cardiac Effects/Provocative Test Safety
Methacholine (MCh)

Cellular/Molecular Mechanism of Action: Methacholine is a cholinergic receptor agonist (parasympathomimetic agent).
Physiological Mechanism of Action: Promote parasympathetic activity.
Application: During a bronchial provocation test (BPT), the performance of maximal inspiratory-expiratory maneuvers, causing abrupt and marked shifts in intrathoracic pressure, may increase the risk of cardiac arrhythmias. Moreover, the inhalation of MCh, a cholinergic agonist agent, could favor the development of unwelcome cardiovascular events, namely, cardiac arrhythmias.
Implementation: Evaluation of the severity of cardiac arrhythmias by ECG-Holter monitoring before, during, and after BPT with MCh challenge. A routine pulmonary function test (PFT) is performed, followed by BPT, during ECG-Holter monitoring. Complementary tests include measurement of serum potassium concentrations, baseline arterial blood gas analysis, and monitoring of oxyhemoglobin saturation.

Human Model: Cases with clinical indications for BPT without pre-existing cardiovascular diseases and not receiving arrhythmogenic drugs (n = 46 cases; n = 28 males; n = 18 female).
Interpretation and Analysis: No significant increase in the number of supraventricular and ventricular arrhythmias can be detected during the performance of PFTs and of BPTs with MCh, either with or without bronchial hyperresponsiveness (BHR). However, during the performance of BPTs, a significant reduction in mean heart rate can be detected. Such findings indicate that the performance of PFTs and BPTs with MCh does not increase the risk of cardiac arrhythmias in conditions without evidence of cardiovascular diseases, as well as during BHR evaluations suggesting that these tests are safe to perform in most subjects.

Cardiac arrhythmia monitoring during bronchial provocation test with methacholine. Malerba M, Radaeli A, Politi A, Ceriani L, Zulli R, Grassi V. Chest 2003 Sep; 124 (3), pp. 813–8.

Bronchial Constriction Responses: Provocative Test Frequency/Incidence of Side Effects/Doubling Dose
Histamine/Methacholine (MCh)/Forced Expiratory Volume in 1 Second (FEV1)

Cellular/Molecular Mechanism of Action: Methacholine is a parasympathomemetic/cholinergic agonist that recognizes, binds to, and activates parasympathetic receptor complexes.
Dosage: Increasing doses reaching a maximum of 4 μM (histamine) and 12 μM (MCh) according to the method of Yan et al corresponding to the dose of agonist causing a 20% fall in FEV1 referred to as PD20.
Considerations: Measurement of bronchial reactivity is widely used in epidemiological surveys. Side effects are mild with both histamine and MCh, but histamine more frequently causes voice change (21% vs 11%).
Implementation: Evaluation of bronchial reactivity in conditions of respiratory wheeze signs.

Human Model: Case subjects (n = 108 subjects in addition to n = 95 subjects selected because of wheeze in the last 12 months).

Interpretation and Analysis: More subjects have a measurable PD20 with MCh than with histamine (e.g., n = 25/108 vs 11/108, $p < 0.01$; and wheeze subjects n = 67/108 vs 48/95). A random sample of repeatability of tests in subjects with signs of wheezing within the last year reveals that the 95% range for a single estimation of PD20 in cases with a measured PD20 on at least 1 occasion is ± 2.5 doubling doses for histamine (n = 25) and ± 2.1 doubling doses for MCh (n = 33). Thus MCh has advantages over histamine for community studies of bronchial reactivity, as it is possible to use doses that produce more PD20 measurements with fewer side effects.

Comparison of histamine and methacholine for use in bronchial challenge tests in community studies. Higgins BG, Britton JR, Chinn S, Jones TD, Vathenen AS, Burney PG, Tattersfield AE. Thorax 1988 Aug; 43 (8), pp. 605–10.

Bronchial Contriction Responses: Reflex Airway/ Asthma Safety
Lidocaine/Bupivacaine/Procaine/Histamine/Serotonin/ Methacholine (MCh)

Application: Intravenous lidocaine is commonly used to treat ventricular arrhythmias and to attenuate reflex airway constriction and intracranial pressure elevation during airway manipulation in intensive care units. Much controversy exists as to the actions of lidocaine on the airway.
Route of Administration: Intravenous local anesthetics (lidocaine, bupivacaine, procaine).
Dosage: Lidocaine (0–10 mg/kg)/, bupivacaine (0–2.5 mg/kg), procaine (0–20 mg/kg), histamine (10 μg/kg + 500 μg/kg/hr; serotonin (10 μg/kg + 500 μg/kg/hr; and MCh (0.5 μg/kg + 300 μg/kg/hr).
Implementation: Compare, in detail, the actions of lidocaine with those of bupivacaine and procaine on airway caliber and the associated changes in plasma catecholamine concentrations in the dog. Such an objective can be addressed through the evaluation of the effects of (i) local anesthetics (lidocaine n = 7; bupivacaine n = 7; procaine n = 7) on basal airway tone; (ii) lidocaine on agonist-induced bronchoconstriction mediated by histamine (n = 6; MCh n = 7); (iii) lidocaine on vagal nerve stimulation (n = 7). Bronchial cross-sectional area at the 3rd bronchial bifurcation is subsequently monitored continuously through the use of a fiberoptic bronchoscope.
Animal Model: Canine (mongrel dogs). Design: Prospective, randomized, controlled experimental *in vivo* and *in vitro* study.
Interpretation and Analysis: Local anesthetics produce a dose-dependent decrease in basal bronchial cross-sectional area. Similarly, lidocaine significantly potentiates histamine and serotonin-induced bronchoconstriction. Lidocaine antagonized MCh- and vagal nerve stimulation-induced bronchoconstriction. Lidocaine may produce direct bronchoconstriction and worsen some agonist-induced bronchoconstriction, but it prevents reflex airway constriction. Therefore, it has been by suggested by some clinicians that this agent be used with caution in asthmatics.

Bronchoconstrictive and relaxant effects of lidocaine on the airway in dogs. Hirota K, Hashimoto Y, Sato T, Yoshioka H, Kudo T, Matsuki A, Lambert DG. Crit Care Med 2001 May; 29 (5), pp. 1040–4.

Bronchial Constriction Responses: Standardized Inhalation Test Procedure
Histamine/Methacholine (MCh)

Cellular/Molecular Mechanism of Action: Histamine binds to and activates histamine H_1 and histamine H_2 receptor complexes. The H_2 receptor complexes are recognized for their influence on gastric acid secretion. Alternatively, MCh binds to and activates muscarinic acetylcholine receptor complexes.
Physiological Mechanism of Action: Histamine promotes inflammatory responses (e.g., increased vascular permeability, leukocyte activation, bronchoconstriction) while MCh functions as a parasympathomimetic agent, thereby promoting bronchoconstrictive responses along the network of the bronchial tree.
Implementation: Evaluation of bronchial responses to standardized inhalation test procedures. In standardized inhalation tests, histamine and MCh have been compared to determine their degree of reproducibility and the relative extent to which each agent can induce a response. Each set of duplicate tests is carried out on a separate day within a given 1-week period. Results are expressed as the concentration of histamine or MCh that causes a 20% fall in forced expiratory volume in 1 second (PC20).
Interpretation and Analysis: Responses to histamine and MCh are highly reproducible (coefficients of determination [r^2] = 0.994 and 0.990, respectively). Responsiveness to histamine correlates closely with responsiveness to MCh ($r^2 = 0.85$). There is a small but significant cumulative dose effect with MCh ($p < 0.01$) but not with histamine. Side effects of throat irritation, flushing, and headache are more frequent with histamine than MCh and are dose-related. The high level of reproducibility indicates the efficiency of the test procedure. The similar severity of effects by the 2 agents with different mechanisms of action suggests that the primary cause of non-specific bronchial hyperreactivity lies at the level of bronchial smooth muscle.

Reproducibility and comparison of responses to inhaled histamine and methacholine. Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE. Thorax 1978 Dec; 33 (6), pp. 705–10.

Bronchial Response Test: Allergen Sensitivity-Induced Constriction
Salbutamol/Beclomethazone Diproprionate/ [Methacholine (MCh) Hyperresponsive Test]

Cellular/Molecular Mechanism of Action: Methacholine functions as a cholinergic/parasympathomimetic agent. Salbutamol functions as a β_2-adrenoceptor agonist.
Physiological Mechanism of Action: Methacholine promotes bronchoconstriction. Corticosteroids like beclomethazone inhibit phospholipase A2 (inhibit prostaglandin/leukotriene production) and stabilize cell membrane structures (inhibit leukocyte degranulation). In addition, like adrenergic agonists, and clenbuterol, corticosteroids promote bronchodilation by promoting increases in intracellular adenylcyclase activity (increased cAMP) within bronchial smooth muscle.
Application: Determination of bronchial dilation capacity in conditions of allergen-induced asthma.
Route of Administration: Salbutamol can be delivered by inhalation methods.
Dosage: Salbutamol (0.3 mg, t.i.d.); beclomethazone diproprionate (0.2 mg, t.i.d.).
Implementation: Determine the effect of a 1-week treatment schedule with inhaled salbutamol plus placebo (S + P) vs salbutamol combined with beclomethasone dipropionate (S + BDP) on early and late asthmatic responses to inhaled allergen. Affected cases are subjected to 2 different weeklong exposure periods, with a 15-day interval between them: (i) salbutamol (t.i.d.) + placebo, and (ii) salbutamol + BDP (t.i.d.); at the end of each treatment period, a specific bronchial provocative test (sBPT) is performed and the last treatments are given 1.5–2 hours before and 3–4 hours after allergen

challenge. As an adjuct form of evaluation, non-specific bronchial hyperresponsiveness to MCh can be induced before each treatment period, after 6 days of treatment before sBPT, and the day after sBPT at the end of the treatment period.

Human Model: Cases of atopy (e.g., n = 10) employed in a randomized, double blind, crossover study. All cases have previous evidence of a dual-type response to an sBPT.

Interpretation and Analysis: Salbutamol + beclomethasone dipropionate completely prevents both early and late responses to allergen, while S + P reduces but does not completely inhibit early and late responses. The difference between the 2 exposure schemes is significant for early and late asthmatic responses. Following MCh challenge, there is only a mild increase in PD15FEV1 MCh after 6 days of treatment with S + BDP in comparison with S + P treatment. These results suggest that salbutamol + beclomethasone effectively prevents the occurrence of early and late asthmatic reactions.

Salbutamol plus beclomethasone dipropionate, but not salbutamol alone, completely prevent early and late asthmatic responses to allergen. Paggiaro PL, Dente FL, Vagaggini B, Bacci E, Talini D, Testi R, Mapp CE, Fabbri LM, Giuntini C. Respir Med 1991 Sep; 85 (5), pp. 401–6.

Bronchodilator Response (BDR)/Bronchoconstrictor Response/Childhood Asthma/ *Salbutamol (Albuterol)/Methacholine (MCh) Influence on BDR*

Cellular/Molecular Mechanism of Action: Methacholine is a parasympathomimetic agent that recognizes, binds to, and activates cholinergic receptor complexes. Salbutamol belongs to the class of selective β_2-adrenergic agonists that recognizes, binds to, and activates sympathetic, adrenergic β_2 receptor complexes.

Physiological Mechanism of Action: Methacholine can promote bronchoconstriction of the major airways. Salbutamol promotes bronchodilation.

Background Information: Airway responses to a BDR and to MCh are frequently measured in the assessment of childhood asthma and in pulmonary research.

Route of Administration: Salbutamol (inhaled).

Dosage: Salbutamol (800 μg).

Implementation: Evaluation of the BDR measured twice in random order on consecutive days in conditions of childhood asthma in concert with assessment of MCh hyperresponsiveness. In this manner, it can be determined if it is possible to obtain a reliable BDR immediately following completion of an MCh challenge test. Bronchodilator response can be calculated as a function of the change in forced expiratory volume in 1 second (FEV1) before and 20 minutes after salbutamol inhalation (metered dose inhaler with a spacer), expressed as a percentage of the predicted value (deltaFEV1%pred). On 1 day, BDR is measured immediately after completing an MCh challenge, on another separate day without any preceding challenge.

Human Model: Cases of asthmatic children (n = 24).

Interpretation and Analysis: Mean (SD) baseline FEV1%pred is not significantly different between test days (98.6 ± 14.2% and 98.1 ± 13.8%, respectively, p = 0.53). The geometric mean provocative dose of MCh producing a 20% fall in FEV1 (PD20) is 56.2 μg (range 10.3–306.2 μg). The mean (SEM) BDR without preceding MCh challenge is approximately 10.8 ± 1.4%, while after a preceding MCh challenge it is 5.2 ± 1.5% (mean difference 5.6%, 95% CI 3.0–8.1%, p < 0.001). Mean (SEM) postbronchodilator FEV1%pred is 109.4 ± 3.0% without and 103.4 ± 2.7% with preceding MCh challenge (mean difference 6.0%, 95% CI 3.5–8.6%, p < 0.001). Therefore, a preceding MCh challenge significantly reduces BDR in asthmatic children. And therefore, a BDR, measured immediately after completing an MCh challenge, cannot be used as a substitute for a separate bronchodilator test.

Bronchodilator response in asthmatic children is significantly reduced when measured immediately after methacholine challenge. Kamps AW, Brand PL, Vermeer K, Roorda RJ. J Asthma 2002 Dec; 39 (8), pp. 737–41.

Bronchodilator Response Test: Allergen Sensitivity *Phleum Pratensis/Methacholine (MCh) Inhalation Test*

Cellular/Molecular Mechanism of Action: Methacholine activates muscarinic acetylcholine receptor complexes.

Physiological Mechanism of Action: Methacholine promotes bronchoconstriction.

Application: Detection and characterization of patterns for airway response to a specific bronchial provocative test (sBPT)

Route of Administration: Inhalation.

Dosage: Phleum pratensis extracts (0.01–2.5 standardized biological units [BU]).

Implementation: Evaluation of seasonal asthma conditions by characterizing airway response patterns to sBPT utilizing Phleum pratensis extracts in addition to MCh inhalation challenge tests and assessement of allergologic parameters. Respiratory function is determined by monitoring FEV1 to assess early (EAR) and late (LAR) airway responses.

Human Model: Cases of seasonal asthma due to grass pollen (n = 27) tested during non-pollen seasons with n = 10/27 cases also tested during the peak pollen seasons.

Interpretation and Analysis: Outside the pollen season sBPT elicites an isolated EAR in about 60% of cases (n = 16/27) and a dual asthmatic response (DAR) in about 41% of cases (n = 11/27). Significant differences do not exist between EAR and DAR cases with regard to the provocative dose causing a 15% fall in FEV1 (PD15FEV1) MCh, EAR (expressed by PD15FEV1 allergen), or allergologic data. There is a significant relationship between PD15FEV1 allergen and PD15FEV1 MCh (r = 0.43, p < 0.05). Assessment of sBPT outside the pollen season reveals correlation coefficients between the 2 tests are r = 0.91, p < 0.001 for the total delivered dose of allergen during sBPT; r = 0.79, p < 0.01 for PD15FEV1 allergen; and r = 0.95, p < 0.001 for the maximum percent fall in FEV1 during LAR. In situations when the evaluation tests are performed during grass pollen season, PD15FEV1 MCh and baseline FEV1 decrease significantly but PD15FEV1 allergen does not change significantly during the season.

Instrumentation: A DeVilbiss 646 nebulizer can be used to deliver increasing doses (0.01n2.5 BU) of the allergen solution.

Natural exposure to pollen reduces the threshold but does not change the pattern of response to the allergen in allergic subjects. Dente FL, Bacci E, Di Franco A, Giannini D, Vagaggini B, Paggiaro PL. Respir Med 2000 Nov; 94 (11), pp. 1073–8.

Bronchodilatory Capacity Postbronchoconstriction/ Asthma: Mild/Bronchodilator Efficacy *Methacholine (MCh)/(Isoflurane)*

Cellular/Molecular Mechanism of Action: Methacholine activates acetylcholine receptor complexes (cholinergic/parasympathomimetic agonist).

Physiological Mechanism of Action: Methacholine promotes bronchoconstrictive responses within the pulmonary airways.

Background Information: Volatile anesthetics used in high concentrations are potent bronchodilators. The effect of lower

subanesthetic concentrations has been not completely documented, particularly in humans with provoked bronchial obstruction.

Application: Methacholine can be applied as a bronchoconstrictive agent for evaluation of the efficacy of candidate bronchodilation agents.

Implemenation: Evaluation of the potential of either 100% oxygen or 0.75% isoflurane in oxygen (8 minutes via a face mask) to reverse MCh-induced bronchconstriction. The MCh provocative test is 1st performed to produce a decrease in forced expiratory volume in 1 second (FEV1) of at least 20%. Either 100% oxygen or 0.75% isoflurane in oxygen is then inhaled for 8 minutes using a face mask.

Human Model: Cases with mild asthma (n = 7) during asymptomatic periods.

Interpretation and Analysis: During isoflurane inhalation, subjects lose consciousness but recover quickly. There is no significant improvement in FEV1 with isoflurane inhalation and maximum expiratory flow at 50% vital capacity (MEF50) recorded 3, 6, 9, 14, and 19 minutes after the end of isoflurane inhalation compare with time-control values. Therefore, lung function in mild asthmatic subjects challenged with MCh is not improved after administration of a low subanesthetic concentration of isoflurane. This may be important during recovery from general anesthesia.

Lack of bronchodilator effect after administration of subanaesthetic concentration of isoflurane in mild asthmatic subjects challenged with methacholine. Mercier FJ, Benhamou D, Denjean A. Br J Anaesth 1995 Mar; 74 (3), pp. 301–5.

Bronchodilatory Response
Atropine

Cellular/Molecular Mechanism of Action: Atropine inhibits parasympathomimetic activity through blockage of muscarinic cholinergic neuromuscular receptor sites. At the level of the smooth muscle, atropine inhibits phosphodiesterase, thereby reducing degradation of cAMP and promoting bronchodilation.

Physiological Mechanism of Action: Atropine promotes bronchodilation during conditions of excessive parasympathetic (cholinergic) tone.

Diagnostic Application: Evaluate dilatory capacity of respiratory bronchial tree.

Dosage: Subcutaneous or intramuscular injection.

Considerations: Atropine can produce undesirable sequela associated with inhibition of the parasympathetic autonomic nervous system. A viable example is the profound ability of atropine to inhibit gastrointestinal motility in equine patients.

Interpretation and Analysis: Atropine can promote bronchodilation by suppressing parasympathetic tone at the level of the bronchial smooth muscle.

Bronchodilatory Response/Capacity
Isoproterenol

Cellular/Molecular Mechanism of Action: Isoproterenol is a selective β_1/β_2-adrenergic agonist and has essentially no effect as an α-adrenergic receptor complex. At the level of the smooth muscle, isoproterenol and other adrenergic agonists (e.g., isoproterenol, clenbuterol) enhance adenylcyclase activity, thereby increasing cAMP synthesis.

Physiological Mechanism of Action: Increases in intracellular cAMP created by the influence of adrenergic agonists (e.g., isoproterenol, clenbuterol) on adenylcyclase activity promote bronchodilatory responses.

Diagnostic Application: Evaluate the dilatory capacity of the respiratory bronchial tree in disease conditions of compromised function (equine chronic obstructive lung disease [COPD], heaves). Parameters evaluated during isoproterenol challenge include alterations in respiratory rate, blood gas profile, expired vs inspired gas composition profile, and upper respiratory tract luminal pressure profile.

Route of Administration: Isoproterenol (intravenous; given by slow continuous infusion in horses).

Dosage: Isoproterenol (0.1 mg added to 250 ml of lactated Ringer's solution in horses).

Considerations: Diagnostically, isoproterenol is superior to atropine in horses because it imposes significantly fewer alterations in gastrointestinal motility. Isoproterenol administration should be terminated when the heart rate doubles compared to preadministration values.

Implementation: Evaluation of the capacity for isoproterenol to reverse bronchoconstrictive processes.

Animal Model: Equine.

Interpretation and Analysis: Evidence of induced bronchodilation is a positive respose to isoproterenol and suggests that the bronchocontrictive process is reversible (lack of permanent fibrous tissue replacement).

Laboratory Instrumentation: Measurement of arterial blood oxygen levels, mass spectroscopy for gas composition determinations, and micro-pressure sensor device for airway measurements.

Bronchodilatory Response Postbronchoconstriction
Clenbuterol (β_2-Adrenergic Bronchodilator)

Cellular/Molecular Mechanism of Action: Clenbuterol is a selective β_2-adrenergic agonist. Adrenergic agonists (e.g., isoproterenol, clenbuterol) and corticosteroids promote increases in intracellular adenylcyclase activity (increases cAMP) within bronchial smooth muscle.

Physiological Mechanism of Action: Increases in intracellular cAMP created by the influence of adrenergic agonists (e.g., isoproterenol, clenbuterol) on adenylcyclase activity promote bronchodilation responses.

Application: Bronchodilator agent (differentiation between responsive and non-responsive bronchoconstriction due to elevated production of inflammatory mediators).

Interpretation and Analysis: Improved respiratory function due to bronchodilation following administration suggests that chronic fibrosis of the respiratory tree has not occurred and that the pulmonary system is responsive to bronchodilatory agents.

Carbon Monoxide Diffusing Capacity
Pulmonary Function Test

The carbon monoxide diffusing capacity test [D(LCO)] is a commonly performed pulmonary function test that requires technical expertise and attention to detail to get acceptable results. With the advent of automated devices and powerful computer programs, D(LCO) measurement has rapidly gained wide clinical acceptance. But there are many subtle aspects to performing the test that can diminish its accuracy and repeatability. During its application, it must be ensured that D(LCO) instrumentation is correctly calibrated; that inhalation is at least 90% of the largest previously measured vital capacity; that quick, smooth inhalations are executed within 2 seconds; that the breath-hold is 9–11 seconds; that the breath-hold is without straining (devoid of Valsalva or Müller maneuvers); that exhalation is quick and smooth; that a representative gas sample is obtained from the correct portion of the exhalation; and that at least 5 minutes elapse between D(LCO) tests. At least 2 but no more than 5 D(LCO) tests should be conducted, and testing is complete when 2

tests are within 10% or 3 D(LCO) units (mL CO/min/mm Hg) of each other. The reported D(LCO) value is the average of the 1st 2 tests that meet the reproducibility criteria, but if 5 tests are performed and no 2 meet the reproducibility criteria, the reported value is the average of the 2 tests with the highest inspiratory volumes. These quality controls will help laboratories achieve consistent high D(LCO) accuracy.

Diffusing capacity: How to get it right. Jensen RL, Crapo RO. Respir Care 2003 Aug; 48 (8), pp. 777–82.

Carboxylic Acid Metabolism: Hereditary Disorders/ Nitrogen Metabolism Disorders (Acquired)/ Gastrectomy, Hepatic Failure, Renal Failure, Myotonic Dystrophy/Valine Metabolism
1-[^{14}C]-Alpha-Ketoisovaleric Acid (KIV)/[$^{14}CO_2$ Breath Excretion Test]

Cellular/Molecular Mechanism of Action: 1-[^{14}C]-alpha-ketoisovaleric acid is a keto analogue of valine and can function as a biochemical substrate for mechanisms of carboxylic acid metabolism.
Background Information: The keto analogues of essential amino acids represent a promising therapeutic modality in hereditary and acquired disorders of nitrogen metabolism. The utilization of these substances in humans has been primarily for nitrogen balance studies.
Application: Endogenous biochemical metabolism of KIV can be measured using the $^{14}CO_2$ breath excretion test. In this context, the agent can be applied as a tool for evaluation of certain diet formulations.
Route of Administration: Oral.
Implementation: Assessement of the *in vivo* decarboxylation of KIV through measurement of $^{14}CO_2$ using a simple and accurate breath excretion test. Protein diets (5 g) supplemented with essential amino acids and KIV (in place of valine) are then evaluated. Diets of 120 g of protein/day are then reevaluated in normal volunteers and gastrectomy cases.
Human Model: Normal volunteers and diseased cases (n = 6 diseased; gastrectomy, hepatic failure, renal failure, and myotonic dystrophy).
Interpretation and Analysis: In situations of low protein intake, 13–32% of ingested KIV undergoes rapid decarboxylation, and this proportion appears to correlate inversely with damage to organ systems containing the branched-chain keto acid dehydrogenase. With high protein intake, the proportion of KIV decarboxylated increases to 44% to 53%. Such findings confirm that the decarboxylation of KIV in man varies under different conditions of dietary intake and metabolic disease. The $^{14}CO_2$ breath excretion test is applicable to other related analyses of carboxylic acid metabolism in human subjects.

Decarboxylation of alpha-ketoisovaleric acid after oral administration in man. Epstein CM, Chawla RK, Wadsworth A, Rudman D. Am J Clin Nutr 1980 Sep; 33 (9), pp. 1968–74.

Epithelium Integrity and Viability/Pulmomary Pathophysiology/Asthma Bronchiale
L-[Guanidino$^{15}N_2$]-Arginine Monohydrochloride/ [^{15}NO] Exhalation/[Breath Test]

Cellular/Molecular Mechanism of Action: The amino acid L-[guanidino$^{15}N_2$]-arginine monohydrochloride is resorbed within the lung and is partially metabolized to [^{15}NO].
Background Information: Conventional diagnostic evaluation of the pulmonary tract uses physical methods such as spirometry and oscillometry. However, the inhalation of a chemical diagnostic agent ought to provide novel ways of more specific diagnosis, for instance of inflammatory states of the bronchial and lung mucosa. The stable isotope technique using a [^{15}N]-labeled substrate appears to be a suitable tool for this application.
Application: Inhalation breath test for evaluating pulmonary integrity/function. The amount of [^{15}NO] yielded after the inhalation of [^{15}N]-labeled arginine could be a potential marker for demonstrating pathophysiological changes in the lung epithelium.
Route of Administration: L-[guanidino$^{15}N_2$]-arginine monohydrochloride (inhaled).
Dosage: L-[guanidino$^{15}N_2$]-arginine monohydrochloride (700 mg of aqueous solution, 20 atom % [^{15}N]).
Implementation: Evaluation of conditions of asthma bronchiale with defined amounts of inhaled amino acid L-[guanidino$^{15}N_2$]-arginine monohydrochloride. The exhaled air is collected under defined conditions in 10-L breath bags and analyzed for NO using chemiluminescence.
Human Model: Cases suffering from asthma bronchiale (n = 7) and healthy/normal volunteers (n = 6).
Interpretation and Analysis: Under standardized test conditions, healthy cases exhale 0.97 ± 0.08 μmol NO/m^3 and asthmatics 1.17 ± 0.14 μmol NO/m^3. A better distinction can be expected when making comparisons using measurements of [^{15}NO] exhalation. Between 30 and 80 minutes after inhalation of [^{15}N]arginine, a maximum (plateau of the [^{15}NO] abundance) is found in exhaled air at which time healthy and asthmatic cases exhibit clear differences in amounts of [^{15}NO] exhaled. Under standardized test conditions, healthy cases exhale 102.3 ± 6.7 nmol [^{15}NO/m^3], whereas asthmatic cases exhale only 76.1 ± 10.9 nmol [^{15}NO/m^3].
Instrumentation: Cryotrap gas chromatography-mass spectrometry system for analyzing the [^{15}N] abundance of NO gas.

[$^{15}N_2$]arginine as a 1st potential inhaled diagnostic agent to characterize respiratory diseases. Krumbiegel P, Denk E, Russow R, Rolle-Kampczyk U, Metzner G, Herbarth O. Exp Lung Res 2002 Oct–Nov; 28 (7), pp. 535–42.

Epithelium Integrity and Viability/Respiratory Inhalation Test/Regional Inflammation/Localized Decompensated Cellular Function
1-[^{13}C]Hexadecanol/1-[^{13}C]Glucose

Cellular/Molecular Mechanism of Action: 1-[^{13}C]hexadecanol and 1-[^{13}C]glucose can be absorbed across the surface of the pulmonary epithelium, transported to the liver through the intravascular compartment, and metabolized by hepatocytes to CO_2 that is then transported to the lung where it can be detected in exhaled air.
Background Information: Conventional [$^{13}CO_2$] breath tests, and also [^{15}N] urine tests, always start with an oral administration of a test substrate. The test person swallows a stable isotope-labelled diagnostic agent. This technique has been used to study several pathophysiological changes in gastrointestinal organs. However, to study pathophysiological changes of the bronchial and lung epithelium, the inhalative administration of a stable isotope labeled agent appears to have some distinct advantages.
Application: Detection and evaluation of alterations in respiratory function (e.g., pulmonary inflammation of certain sections and decompensated cell functions).
Implementation: Evaluation of 1-[^{13}C]exadecanol and 1-[^{13}C] glucose as inhaled pulmonary diagnostic agents. Complementary investigations can involve assessment of the practicality and reproducibility of 1-[^{13}C]glucose as an agent in inhalation tests. Using the APS aerosol provocation system and a nebulizer, a 25% aqueous solution can be inhaled with breath samples then collected at 15-

minute intervals and analyzed for [$^{13}CO_2$] 75–120 minutes after the end of inhalation.

Human Model: Healthy individuals (n = 18).

Interpretation and Analysis: Inhaled 1-[^{13}C]hexadecanol does not yield [$^{13}CO_2$] in exhaled air in contrast to 1-[^{13}C]glucose. A highly reproducible maximum delta [^{13}C] value of 6% over baseline (DOB) is detectable (e.g., n = 12 healthy probands). The optimum inhalative dose of [^{13}C]glucose has been determined to be 205 mg under certain conditions (e.g., n = 6 self-tests). Speculation that the pulmonary resorption of [^{13}C]glucose is the rate-limiting step of elimination suggests that decompensations in the epithelium should be reflected by changes in 1-[^{13}C]glucose resorption rates and changes in [$^{13}CO_2$] output. Inhalation of suitable [^{13}C]-labeled substrates may ultimately facilitate the development of improved [$^{13}CO_2$] breath tests that can aid in characterizing specific pathophysiological changes in the pulmonary tract, such as areas of regional inflammation with decompensated cellular function.

Instrumentation: "Medic Aid" nebulizer (Erich Jaeger, Würzburg).

Toward an inhalative ^{13}C breath test method. Krumbiegel P, Rolle-Kampczyk U, Liebergeld P, Herbarth O, Körich R. Isotopes Environ Health Stud 2002 Jun; 38 (2), pp. 65–70.

Respiratory Center Depression/Brainstem Suppression vs Primary Pulmonary Dysfunction
Doxopram (Dopram)

Cellular/Molecular Mechanism of Action: Doxopram directly stimulates the central respiratory centers located in the reticular formation of the brainstem.

Diagnostic Application: Stimulate elevated respiratory function through direct stimulation of the brainstem respiratory center.

Route of Administration and Dosage: Intravenous injection.

Application: Differentiation between reversible suppression of central brainstem respiratory center function and primary respiratory function as a cause for subnormal arterial oxygen levels (e.g., blood gas analysis of premature neonates).

Interpretation and Analysis: Subnormal blood oxygen concentrations attributed to primary pulmonary dysfunction will not be corrected by Doxopram administration.

Laboratory Instrumentation: Evaluation of respiratory rate, in concert with measurement of arterial blood oxygen and carbon dioxide concentrations.

Ventilation/Perfusion Mismatch
Oxygen Inhalation

Cellular/Molecular Mechanism of Action: Oxygen diffuses from the respiratory alveoli across the pulmonary vascular endothelium. Oxygen then ultimately diffuses across the red blood cell (RBC) membrane and binds to 1 of the porphyrin Fe^{+2} sites within molecular hemoglobin. Oxygen is not as lipid soluble as CO_2 so the latter gas is able to diffuse faster and to a greater extent across cell membrane/basement membrane structures.

Diagnostic Application: Characterization of causes for observed declines in arterial oxygen concentration including detection and classification of ventilation/prefusion mismatch conditions. Related examples include conditions such as those recognized with pulmonary thromboembolism, congenital cardiac malformation, and neonatal hylan membrane disease. In this condition, there is either an anatomical malformation or physical barrier present that limits or prevents normal pulmonary-cardiovascular gas exchange and oxygenation of arterial blood.

Route of Administration and Dosage: Respiratory inhalation of oxygen gas.

Interpretation and Analysis: In instances of compromised oxygenation of RBC hemoglobin due to deficiencies in ventilation, inhalation of gas with a higher oxygen content results in improved oxygenation of the peripheral blood. Alternatively, in instances of hylan membrane disease (e.g., oxygen diffusion barrier), there is no subsequent increase in systemic arterial blood oxygen level. Similarly, in instances of congenital cardiac defects such as a right to left shunt, increased oxygen inhalation fails to result in an increase in arterial blood oxygen concentrations. In this context, abnormalities classified as a mismatch in ventilation (respiratory) and perfusion (cardiovascular) almost invariably fail to respond to inhalation of gas with an increased oxygen content.

Alveolar Gas Concentrations

Ventilation Perfusion Ratio: Normal and Abnormal:

	PO_2 (mm HG)	PCO_2 (mm HG)
Normal Ventilation/Perfusion Ratio	104	40
Ventilation Deficit (V/Q Mismatch)	40	45
Perfusion Deficit (V/Q Mismatch)	149	0

If V/Q is below normal, then ventilation function is insufficient and a portion of the blood perfusing the lung is not oxygenated (amount = physiological shunt blood).

If V/Q is abnormally large, then pulmonary perfusion is insufficient. In the table above, CO_2 more rapidly and more extensively diffuses out of the alveolus compared to O_2, which is known to have a lower capacity of ablity to diffuse across intact cell membranes.

Laboratory Instrumentation: Mass spectroscopic analysis.

RESPIRATORY DISEASE CONDITIONS

Bronchial Reactivity/(Hyperresponsiveness): Occupational Agents
Histamine

Cellular/Molecular Mechanism of Action: Histamine binds to and activates histamine H_1 and H_2 receptor complexes.

Physiological Mechanism of Action: Histamine directly promotes contraction of smooth muscle of the bronchi and intestine (H_1 receptors), while in small vascular structures it promotes relaxation of smooth muscle tissues (H_1 and H_2 receptor complexes). Depending on the receptor complex, histamine can promote profound inflammatory responses. Gastric acid secretion is mediated through H_2 receptor complexes.

Background Information: Specific bronchial reactivity to occupational agents may decline after exposure in the workplace ceases, leading to falsely negative specific inhalation challenges.

Application: The increase in non-specific bronchial hyperresponsiveness after a specific inhalation challenge can be an early and sensitive marker of bronchial response to occupational agents, especially in subjects removed from workplace exposure for a long time. Non-specific bronchial hyperresponsiveness should be systematically assessed after specific inhalation challenges in the absence of changes in airway caliber (diameter).

Route of Administration: Inhalation.

Implementation: Histamine administration can be applied to determine if increases in non-specific bronchial hyperresponsiveness

can facilitate the detection of bronchial responses to occupational agents during specific inhalation challenges.

In cases thought to possibly suffer from occupational asthma due to various causes, challenges are performed over a 2-hour period/day with the suspected agent. Cases not displaying an asthmatic reaction are then rechallenged in 24 hours over a 2- to 3-hour period. The provocative concentration of histamine that causes a 20% fall (PC20) in the forced expiratory volume in 1 second (FEV1) is then assessed at the end of the control day as well as 6 hours after each challenge that does not cause a $\geq$ 20% fall in FEV1. Cases that have significant ($\geq$ 3.1-fold) reductions in PC20 value at the end of the 2nd challenge day are requested to perform additional specific inhalation challenges.

Interpretation and Analysis: The 1st test day elicits an asthmatic reaction in less than half of suspected cases (e.g., n = 25/66). A relatively low percentage of the remaining cases (e.g., 4/45 or 12% with a CI of 95% between 4% and 26%) will exhibit a $\geq$ 3.1-fold fall in PC20 values after inhalation challenge and develop an asthmatic reaction during the 2nd (n = 3) or 3rd (n = 2) challenge exposure. Examples of offending "occupational" agents include persulphate (n = 1), wood dust (n = 2), isocyanate (n = 1), or amoxycillin (n = 1). In some instances, workers have left their job-place for a longer period (mean 21 $\pm$ 14) months) than those who react after the 1st specific inhalation challenge (mean 8 $\pm$ 11 months).

Increase in non-specific bronchial hyperresponsiveness as an early marker of bronchial response to occupational agents during specific inhalation challenges. Vandenplas O, Delwiche JP, Jamart J, Van de Weyer R. Thorax 1996 May; 51 (5), pp. 472–8.

Cell Infiltration: Inflammatory
Methacholine (MCh)/Nitric Oxide Exhalation

Cellular/Molecular Mechanism of Action: Methacholine is a parasympathomimetic agent that recognizes, binds to, and activates cholinergic receptor complexes.

Physiological Mechanism of Action: Methacholine can promote bronchoconstrictive responses.

Background Information: Monitoring of inflammation is an important factor in asthma management. The gold standard for measuring direct airway inflammation is bronchial biopsy specimens taken from proximal airways through a fiber optic bronchoscope. As a non-invasive procedure, the use of exhaled nitric oxide (FENO) for monitoring airway inflammation has been reported in many studies.

Implementation: Evaluate the degree of correlation between FENO with direct measurements of airway inflammation in biopsy specimens and pulmonary function tests (PFTs). Histopathologic features are characterized for bronchial biopsy specimens collected from cases of stable mild-moderate asthma. Parameters are also assessed for measurements acquired from PFTs, FENO levels, blood eosinophil count, and bronchoscopy with bronchial biopsies and bronchoalveolar lavage. In some cases with forced expiratory volume in 1 second (FEV1) > 80% the MCh challenge test can be performed as a complementary diagnostic procedure.

Human Model: Cases of stable mild-moderate asthma (n = 9), in addition to individuals with FEV1 > 80% (n = 5) subjected to the MCh challenge test. Ideally, cases should have had no prior anti-inflammatory therapy for asthma.

Interpretation and Analysis: There is no correlation among PFTs, blood eosinophil count, and FENO levels. There is a negative correlation between PC20 and FENO. Though there is no correlation between bronchial biopsy eosinophil, monocyte and lymphocyte counts, and FENO, a weak positive correlation exists between total inflammatory cell count in bronchial biopsies and FENO levels. A negative significant correlation exists between FENO levels and epithelial desquamation ($p < 0.05$, $r = 0.7$). These results suggest that FENO levels reflect the increased number of activated inflammatory cells in airways, while a negative correlation with epithelial desquamation reflects the role of epithelium in NO syntheses. Exhaled nitric oxide should not be interpreted as a specific inflammation marker for asthma.

Correlation of exhaled nitric oxide levels and airway inflammation markers in stable asthmatic patients. Turktas H, Oguzulgen K, Kokturk N, Memis L, Erbas D. J Asthma 2003 Jun; 40 (4), pp. 425–30.

Chronic Obstructive Pulmonary Disease (COPD)/ Autonomic Dysfunction and Breathlessness
Acetylcholine/[Dermal Sweat-Spot Test]

Cellular/Molecular Mechanism of Action: Induces parasympathomimetic activity at nicotinic and muscarinic neuromuscular receptor sites.

Background Information: Cases with hypoxic COPD have evidence of a subclinical parasympathetic autonomic neuropathy, with apparent preservation of sympathetic function. However, these findings discovered using cardiovascular-respiratory tests might have been biased by concomitant chest disease.

Diagnostic Application: Detection and assessement of pulmonary hypersensitivity to allergen exposure. The acetylcholine sweat-spot test avoids the bias of concomitant chest disease.

Route of Administration: Acetylcholine (intradermal).

Dosage: Acetylcholine (0.1 ml of 1%).

Considerations: Gastrointestinal stimulation.

Implementation: Following intradermal injection of acetylcholine into the dorsum of the feet, the number of sweatglands able to respond in a given surface area is recorded. Cardiovascular autonomic nerve function, arterial oxygen and carbon dioxide tensions, lung function, and cigarette consumption are also recorded.

Human Model: Cases with hypoxemic COPD (n = 35); and age-matched controls (n = 7).

Interpretation and Analysis: The sweat-spot test assesses sympathetic nerve function and it relies upon the fact that denervated sweat glands do not produce sweat. Excessive bronchoconstriction (pulmonary hypersensitivity) implies that environmental exposure to an allergenic substance has occurred. Positive results may suggest the need to avoid further exposure if possible and the prophylactic administration of agents that prevent activation of peroxidation reactions in an effort to prevent development of COPD.

The acetylcholine sweat-spot test is highly repeatable in COPD patients (e.g., n = 8/35 and no person with normal or frankly abnormal function being wrongly assigned). Age-matched controls have normal acetylcholine sweat-spot scores and cardiovascular autonomic tests. The acetylcholine sweat-spot test can be abnormal in a relatively large percent of suspect cases (e.g., n = 24/35), with small numbers showing borderline (e.g., n = 8/35) or normal (e.g., n = 3) responses. Case groups with abnormal sweat-spot tests have significantly worse FEV1, arterial blood gases, and autonomic function. The acetylcholine sweat-spot score correlates with the severity of arterial hypoxemia ($r = 0.78$, $p < 0.001$) and with the parasympathetic cardiovascular tests ($r = 0.80$, $p < 0.001$). Therefore, patients with hypoxemic COPD have a parasympathetic cardiovascular and a peripheral sympathetic autonomic neuropathy. The acetylcholine sweat-spot test is repeatable, easy to perform, and a sensitive indicator for autonomic dysfunction in breathless individuals with COPD.

Autonomic nerve dysfunction in COPD as assessed by the acetylcholine sweat-spot test. Stewart AG, Marsh F, Waterhouse JC, Howard P. Eur Respir J 1994 Jun; 7 (6), pp. 1090–5.

Chronic Obstructive Pulmonary Disease (COPD)/ Pulmonary Function/Forced Vital Capacity and Forced Expiratory Volume
Salbutamol/Forced Expiratory Volume in 1 Second (FEV1)

Cellular/Molecular Mechanism of Action: Salbutamol selectively recognizes, binds to, and activates β_2-sympathetic/adrenergic receptor complexes within the autonomic nervous system.
Physiological Mechanism of Action: Salbutamol (bronchodilation).
Implementation: Evaluation of the effects of an inspiratory maneuver preceding forced expiration on functional tests performed under routine conditions before and after bronchodilator inhalation (salbutamol). Forced vital capacity (FVC) maneuvers are performed either immediately after a rapid inspiration (maneuver no. 1) or after a slow inspiration with a 4- to 6-second pause (maneuver no. 2).
Human Model: Cases of COPD (n = 150).
Interpretation and Analysis: Under baseline conditions, FEV1 values are approximately 8% (% control) larger with maneuver no. 1 than no. 2. Forced expiratory volume in 1 second values increase with salbutamol administration by approximately 8% and are, on average, still 7% larger with maneuver no. 1 than no. 2. The incidence of reversibility, assessed according to American Thoracic Society criteria, is 76% when maneuver no. 2 is selected to represent baseline conditions and maneuver no. 1 is chosen to represent the effects of bronchodilator administration, whereas the lowest incidence (2%) is found when maneuver no. 1 is selected to represent baseline conditions and maneuver no. 2 is chosen to represent the effects of bronchodilator administration. The time dependence of the FVC maneuver has an important impact on the assessment of routine lung function in a clinical setting and supports the notion that the time course of the inspiration preceding the FVC maneuver should be standardized.

Bronchodilation test in COPD: Effect of inspiratory manoeuvre preceding forced expiration. Santus P, Pecchiari M, Carlucci P, Boveri B, Di Marco F, Castagna F, Centanni S. Eur Respir J 2003 Jan; 21 (1), pp. 82–5.

Chronic Obstructive Pulmonary Disease (COPD) vs Asthma/Pulmonary Function Tests: [Forced Expiratory Volume in 1 Second (FEV1), Maximal Mid-Expiratory Flow (MMEF), Airway Resistance (Raw), Specific Airway Conductance (sGaw)]/ Differentiation: Asthmas vs COPD (sGaw)
Albuterol/[Isoproterenol]

Cellular/Molecular Mechanism of Action: Albuterol is a selective sympathomimetic β_2-adrenergic receptor agonist. Proterenol is a non-selective sympathomimetic β_1/β_2-adrenergic agonist. At the level of the bronchial smooth muscle, adrenergic agonists and corticosteroids increase the activity of adenylcyclase, thereby increasing intracellular cAMP levels.
Physiological Mechanism of Action: Increases in intracellular cAMP created by adrenergic agonists promote bronchodilation responses.
Implementation: Evaluation of the relative influence of albuterol and isoproterenol as a bronchodilator in pulmonary function tests and their value as diagnostic probes in the differential diagnosis of asthma and COPD.
Human Model: Cases of asthma (n = 59) and COPD (n = 37). Patients of each group are randomly divided into 2 subgroups. The 2 adrenergic agonists are evaluated using a crossover study design on odd or even days.
Interpretation and Analysis: The changing rates of pulmonary functions induced by albuterol and isoproterenol in the 2 case group types are positively correlated ($p < 0.005$). The changing rates of FEV1, MMEF, Raw, and sGaw induced by albuterol are higher than the corresponding measurements for isoproterenol in asthma conditions ($p < 0.02$); however, the changing rates of forced vital capacity (FVC) and peak expiratory flow rate (PEF) in asthma cases and all the above parameters mentioned for COPD cases are not significantly different between the 2 drugs ($p > 0.10$). The comparison of positive case numbers between asthma and COPD cases induced by both drugs shows significant difference ($p < 0.005$), especially when the positive rate of sGaw induced by albuterol in asthma cases reaches 98%, the positive rates in COPD cases is generally much lower. Bronchodilation testing, therefore, is a valuable diagnostic method for asthma. Albuterol appears to be superior to isoproterenol. The most sensitive index in bronchodilation testing is sGaw, then, in order, FEV1(92%), MMEF, and Raw; the FVC and PEF are relatively insensitive evaluation parameters.

A comparison between the effects of albuterol and isoproterenol in bronchodilation test. Yuan Y, He T, Wang L, Zeng J, Luo Y. Hua Xi Yi Ke Da Xue Xue Bao 2001 Dec; 32 (4), pp. 551–4.

Epithelium: Inflammation vs Non-Inflamed
Arachidonic Acid/Prostaglandin E_2 (PGE_2)

Background Information: Eicosanoids such as PGE2, thromboxane A2 (TXA_2), and peptidoleukotrienes (pLT) are known to be highly biologically active lipid-derived endogenous mediators, especially in human lung epithelium. PGE2 is thought to have mostly bronchoprotective effects, whereas pLT and TXA_2 are bronchoconstrictive.
Implementation: Assessment of the release and interaction of eicosanoids in human bronchial biopsy specimens of normal and inflamed mucosa. One laboratory approach to achieving this objective involves evaluation of the *ex vivo* release of pLT, TXA_2 (measured as TXB_2), and PGE_2 in tissue biopsies.
Human Model: Cases with severe bronchial epithelial inflammatory processes (n = 7) and cases without signs of inflammation (n = 9).
***Ex Vivo* Preparation:** Inflammed bronchial epithelium harvested as a biopsy specimen and subjected to a "functional *in vitro* test" and exposure to various molecular stimuli (e.g., arachidonic acid, PGE_2).
Interpretation and Analysis: Bronchial tissue biopsies incubated with arachidonic acid release higher amounts of pLT, TXB_2, and PGE_2 than unstimulated specimens. Preincubation with PGE_2 results in a significant inhibition of arachidonic acid-induced release of pLT and TXB_2 (> 50%). The inhibitory effect is higher in normal than in inflamed epithelium. Therefore, exogenous PGE_2 has inhibitory effects on the release of pLT and TXB_2 in human bronchial biopsy specimens. This finding could explain the bronchoprotective effect of inhaled PGE_2 in normal subjects and asthmatic subjects as direct eicosanoid interactions. It also supports the concept of PGE_2 as a bronchoprotective endogenous substance. The complex effects of PGE_2 as a modulating mediator in inflammation may justify further investigation.

Effect of prostaglandin E2 on eicosanoid release by human bronchial biopsy specimens from normal and inflamed mucosa. Schäfer D, Lindenthal U, Wagner M, Bölcskei PL, Baenkler HW. Thorax 1996 Sep; 51 (9), pp. 919–23.

Immune Hyperactivity: Asthma
n-3 Polyunsaturated Fatty Acids (PUFA)/Methacholine (MCh)-Induced Respiratory Distress

Application: Detection and characterization of asthmatic conditions and efficacy evaluation of agents that can potentially be applied for therapeutic intervention.
Dosage: Sequential oral ingestion of n-3 PUFAs in ratios to n-6 PUFAs of 0.1:1 and 0.5:1 for 1 month each.
Considerations: Asthma may respond to dietary modification, thereby reducing the need for pharmacologic agents.
Implementation: Evaluation of the potential effectiveness of n-3 PUFA ingestion to ameliorate MCh-induced respiratory distress in conditions of asthma through the assessment of urinary leukotriene excretion and alterations in respiratory function parameters. Forced vital capacity (FVC), forced expiratory volume for 1 second (FEV1), peak expiratory flow (PEP), and forced expiratory flow 25–75% (FEF25–75) are measured along with weekly 24-hour urinary leukotriene concentrations.
Interpretation and Analysis: Methacholine-induced respiratory distress increases with low n-3 PUFA ingestion. Alterations in urinary 5-series leukotriene excretion can predict treatment efficacy with high n-3 PUFA ingestion. Elevated n-3 PUFA ingestion results in a positive MCh bronchoprovocation dose change in $> 40\%$ of the test subjects (responders). The provocative dose that causes a 20% reduction (PD20) in FEV1, FVC, PEF, and FEF25–75 values cannot be calculated because of a lack of significant respiratory reduction. Conversely, elevated n-3 PUFA ingestion causes some cases (non-responders) to further lose respiratory capacity. Five-series leukotriene excretion with high n-3 PUFA ingestion is significantly greater for responders than for non-responders. A urinary ratio of 4-series to 5-series leukotrienes < 1, induced by n-3 PUFA ingestion, may predict respiratory benefit.

Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. Broughton KS, Johnson CS, Pace BK, Liebman M, Kleppinger KM. Am J Clin Nutr 1997 Apr; 65 (4), pp. 1011–7.

Immune Hyperimmune Response/Rhinitis: Seasonal Allergic Reactions
Allergen Provocation/[Interleukin (IL)-4 Expression Profile]

Cellular/Molecular Mechanism of Action: Allergens stimulate the immune system and may create hypersensitivity responses.
Application: Detection and characterization of seasonal allergic rhinitis-type hyperimmune reactions. Measurement or detection of IL-4 can therefore theoretically be applied as an indicator of disease or a molecular marker for validating pharmaceutical efficacy.
Implementation: Evaluation of conditions of seasonal allergy as a function of cytokine expression analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR). The time-course study during winter includes repeated local allergen provocation with subsequent nasal biopsies as well as biopsies taken during pollen season.
Human Model: Cases of seasonal allergic rhinitis ($n = 17$; $n = 10$ healthy controls).
Interpretation and Analysis: The RT-PCR for CD44 yields positive bands in the vast majority of affected cases (e.g., $n = 65/71$ cases). Evaluation of messenger RNA (mRNA) for IL-2, IL-4, and IL-5 (PCR analyses/semi-nested) in these cases reveals that IL-4 mRNA is almost exclusively expressed by allergic patients. During provocation a significant increase in IL-4 can be detected compared to reference controls ($p = 0.043$). Similarly, during the natural pollen season, IL-4 mRNA expression is significantly higher in cases not using nasal corticosteroids compared with those who do ($p = 0.011$). No differences in IL-2 or IL-5 can be appreciated in these same case group types. Such findings, together with the observations of T-cell activation (e.g., phenotype switch toward T-helper 2 [Th2] cells; accumulation [homing] of these T cells in the nasal mucosa) indicate that T cells constitute the main source for IL-4 in the nasal mucosa. Therefore, allergic patients have an increased synthesis of IL-4 when provoked with the allergen, and during natural pollen season this synthesis can be downregulated by corticosteroids. Furthermore, this study exemplifies the versatility of molecular biology in surgical pathology and that even low-copy-number cytokine mRNA can be examined in routinely snap-frozen surgical specimens.

Nasal messenger RNA expression of interleukins 2, 4, and 5 in patients with allergic rhinitis. Karlsson MG, Davidsson A, Viale G, Graziani D, Hellquist HB. Diagn Mol Pathol 1995 Jun; 4 (2), pp. 85–92.

Immune Hypersensitivity/Allergic Asthma/Smokers: Chronic Airflow Obstruction
Citric Acid Cough Threshold/Histamine/Airway Responsiveness

Application: Characterization of respiratory function in cases of asthma and in smokers.
Route of Administration: Citric acid.
Implementation: Determination of parameters for the citric acid cough threshold and airway hyperresponsiveness in non-smokers with allergic asthma. Cough thresholds are determined on 2 occasions by administering doubling concentrations of citric acid.
Human Model: Non-smokers with allergic asthma ($n = 11$; means FEV1 94% predicted) and non-smokers with chronic airflow obstruction (e.g., $n = 25$; mean FEV1 65%).
Interpretation and Analysis: Greater than 50% of asthmatic subjects (e.g., $n = 7/11$) and smokers with chronic airflow obstruction (e.g., $n = 14/25$) have a positive cough threshold on both test days. Cough threshold measurements are reproducible in both groups (standard deviation of duplicate measurements 1.2 doubling concentrations in asthma, 1.1 doubling concentrations in chronic airflow obstruction). Citric acid provocation does not cause bronchial obstruction in most patients, though some cases (e.g., $n = 4/36$) display a fall in FEV1 of more than 20% for a short time on 1 occasion only. No significant difference in cough threshold can be appreciated between non-smokers with allergic asthma and non-smokers with chronic airflow obstruction despite appreciable differences in baseline FEV1 values. There is no significant correlation between cough threshold and the provocative concentration of histamine causing a 20% fall in FEV1 (PC20) histamine in either case group. Based on observations in previous investigations, it can be concluded that sensory nerves can be activated with a tussive agent in cases with asthma and chronic airflow obstruction without causing bronchial smooth muscle contraction.

Citric acid cough threshold and airway responsiveness in asthmatic patients and smokers with chronic airflow obstruction. Auffarth B, de Monchy JG, van der Mark TW, Postma DS, Koeter GH. Thorax 1991 Sep; 46 (9), pp. 638–42.

Immune Hypersensitivity: Airway/Allergen-Induced Stimulation/Type Th2-Dominated Allergic Airway Response
Interleukin-16 (IL-16)

Cellular/Molecular Mechanism of Action: Interleukin-16 has been described as a natural soluble CD4-ligand.
Physiological Mechanism of Action: In an *in vitro* environment, IL-16 exerts immunosuppressive effects but little is known about the *in vivo* effect of IL-16 on immune responses.
Application: Suppression of antigen-induced asthma.
Route of Administration: Interleukin-16 (intraperitoneally in a murine animal model).
Dosage: Interleukin-16.
Interpretation and Analysis: Intraperitoneal administration of IL-16 completely inhibits antigen-induced airway hyperresponsiveness and significantly decreases the number of eosinophils in bronchoalveolar lavage fluid (> 90%) and airway tissue of ovalbumin-sensitized and challenged mice. Thoracic lymph node cells isolated from *in vivo* IL-16-treated ovalbumin-challenged animals produce less IL-4 (77%) and IL-5 (85%) upon antigenic restimulation, compared to vehicle-treated mice. Preincubation of lymphocytes with IL-16 *in vitro* reduces antigen-induced proliferation (55%) and T-helper 2 (Th2)-type cytokine production (IL-4: 56%, IL-5: 77%). The presence of IL-16 during priming cultures of TCR transgenic T cells (DO11.10) reduces IL-4 (33%) and IL-5 (35%) but not IL-10 and interferon-gamma levels upon restimulation. Collectively, these findings support the concept that IL-16 has potent immunosuppressive effects on a Th2-dominated allergic airway response.

Exogenous interleukin-16 inhibits antigen-induced airway hyperreactivity, eosinophilia, and Th2-type cytokine production in mice. De Bie JJ, Jonker EH, Henricks PA, Hoevenaars J, Little FF, Cruikshank WW, Nijkamp FP, Van Oosterhout AJ. Clin Exp Allergy 2002 Nov; 32 (11), pp. 1651–8.

Immune Hypersensitivity: Asthma/Allergen-Induced: House Dust Mite (HDM)-Provoked Asthma/Aspirin-Intolerant Asthma (AIA)
Aspirin/Pranlukast/HDM Inhalation Provocation/ Luekotriene Receptor Antagonist (LTRA)

Cellular/Molecular Mechanism of Action: Panlukast is an LTRA.
Background Information: Leukotrienes (LTs) are important in asthma, and LT modifiers modulate antigen-induced asthma. Overproduction of LT by suppression of cyclooxygenase activity occurs in patients with AIA.
Application: Possible differentiation between HDM-provoked asthma (LT overproduction and Ag/Ab reactions) and AIA (LT overproduction and 5-lipoxygenase shift in the arachidonic cascade).
Route of Administration: Aspirin oral provocation.
Implementation: House dust mite–inhalation provocation tests performed in HDM-sensitive asthmatic inpatients without AIA (HDM type cases). In addition, an aspirin oral provocation test can be employed to evaluate cases of asthma that are intolerant to aspirin administration (ASA type cases). Tests are repeated using the same regimen after 7 days of treatment with an LTRA. The effects of an LTRA on changes in sputum LTC_4-LTD_4, eosinophil cationic protein (ECP), eosinophil count, urinary LTE_4/creatinine, 11-dehydrothromboxane B_2 (11-dhTXB_2)/creatinine, serum LTC_4-LTD_4, and peripheral blood eosinophil count, during immediate asthmatic reaction (IAR) and late asthmatic reaction (LAR) in the HDM cases and during immediate asthmatic reaction IAR in ASA cases for each test, are then compared.
Human Model: Cases of HDM-sensitive asthma without AIA (n = 6 HDM group type), and aspirin-insensitive asthma conditions subjected to an aspirin provocation test (n = 7 ASA group type).
Interpretation and Analysis: In cases of HDM-sensitive asthma without aspirin insensitivity, immediate (IAR) and late (LAR) asthmatic reactions can be observed. Sputum LTC_4-LTD_4 and urinary LTE_4/creatinine both increase significantly during IAR and LAR. Sputum ECP increases during IAR and further increases during LAR. Eosinophil count in the sputum does not increase during IAR but significantly increases during LAR. Leukotriene receptor antagonism (pranlukast) suppresses declines in FEV1 both during IAR and LAR (73.8% and 51.9%, respectively) and inhibits increases in sputum eosinophil count during LAR and sputum ECP during IAR and LAR. In cases of AIA subjected to an aspirin provocation test, aspirin-induced IAR is associated with a fall in urinary 11-dhTXB_2/creatinine and increases in levels of sputum LTC_4-LTD_4, ECP, and urinary LTE_4/creatinine. Leukotriene receptor antagonism (pranlukast) suppresses IAR and inhibits increases in the level of sputum ECP but fails to change aspirin-induced LT production in the sputum and urine. The levels of sputum LTC_4-LTD_4 and urinary LTE_4/creatinine in the stable phase in the ASA group are significantly greater than those in the HDM group. Therefore, it appears that HDM-provoked asthma is associated with overproduction of LT with an antigen-antibody reaction, while AIA is associated with overproduction of LT with a shift to the 5-lipoxygenase series of the arachidonate cascade. Leukotriene receptor antagonist may be useful against both types of asthma through inhibition of LT activity and eosinophilic inflammation of the airways.

Effects of pranlukast on chemical mediators in induced sputum on provocation tests in atopic and aspirin-intolerant asthmatic patients. Obase Y, Shimoda T, Tomari SY, Mitsuta K, Kawano T, Matsuse H, Kohno S. Chest 2002 Jan; 121 (1), pp. 143–50.

Immune Hypersensitivity: Asthma/Aspirin-Induced Bronchoconstriction
Lysine Acetylsalicylate (LASA)/Cromoglycate (Sodium)/ Nedocromil (Sodium)

Background Information: The protective activity of nedocromil sodium and of sodium cromoglycate against aspirin-induced asthma has never been investigated in controlled studies. Reports have described the occurrence of aspirin-induced platelet-mediated cytotoxic activity *in vitro* is inhibited after treatment *in vivo* with nedocromil but not with cromoglycate.
Application: Partial validation of bronchoconstriction induced by aspirin administration.
Route of Administration: Nedocromil sodium (inhalation), sodium cromoglycate (inhalation) or placebo.
Dosage: Nedocromil sodium (4 mg), cromoglycate (10 mg), or placebo.
Implementation: Delineation of the potential protective activity of nedocromil and cromoglycate against aspirin-induced bronchoconstriction. Three bronchial challenges with a single dose of LASA are performed that are capable of causing decreases in forced expiratory volume in 1 second (FEV1) of 25% or more in preliminary dose-response tests 30 minutes after inhalation of nedocromil sodium, sodium cromoglycate, or placebo. Forced expiratory volume in 1 second and specific airway resistance (SRaw) are recorded at intervals for 195 minutes.
Human Model: Cases of aspirin-induced asthma (n = 10).
Interpretation and Analysis: Following a placebo, LASA causes a maximal decrease in FEV1 that is 42 ± 4% of baseline. After cromoglycate and nedocromil the maximal decrease in FEV1

is reduced to 20 ± 3% and 18 ± 4%, respectively ($p < 0.01$ vs placebo for both treatments), without significant differences between the 2 treatments. Similar results can be observed for SRaw. Therefore, at recommended therapeutic doses, sodium cromoglycate and nedocromil sodium are equally effective in attenuating aspirin-induced bronchoconstriction, and it is unlikely that platelet activation participates in the pathogenesis of aspirin-induced asthma.

Attenuation of aspirin-induced bronchoconstriction by sodium cromoglycate and nedocromil sodium. Robuschi M, Gambaro G, Sestini P, Pieroni MG, Refini RM, Vaghi A, Bianco S. Am J Respir Crit Care Med 1997 Apr; 155 (4), pp. 1461–4.

Immune Hypersensitivity: Asthma/Asthma-Associated Severe Bronchial Obstruction/Aspirin-Intolerant Asthma/Rhinitis
Lysine Acetylsalicylic Acid (L-ASA)

Background Information: Nasal provocation tests with lysine-aspirin have recently been introduced for assessment of aspirin-intolerant asthma.
Application: Nasal challenge with L-ASA can be used as a diagnostic test even in asthmatic patients with severe bronchial obstruction.
Route of Administration: Lysine acetylsalicylic acid (intranasal infusion).
Dosage: Lysine acetylsalicylic acid (25 mg).
Implementation: Evaluation of the usefulness of acoustic rhinometry, a new non-invasive technique, for the diagnosis of aspirin-intolerant asthma/rhinitis. Methodologies that can be used in investigative protocols include challenge tests with L-ASA or saline (0.9% NaCl) and 25 mg L-ASA instilled into each nostril of the nose on 2 separate days. Clinical response is evaluated based on nasal symptoms (sneezes, itching, secretion, and blockage). Nasal response is measured by acoustic rhinometry. Symptoms and rhinometry curves are recorded at 10-minute intervals for 3 hours, 1 hour before challenge and 2 hours after challenge.

Human Model: Cases of aspirin-intolerant asthma/rhinitis ($n = 15$; $n = 9/15$ females age 54.7 ± 14 years; $n = 8$ aspirin-tolerant asthma/rhinitis; $n = 3/8$ women mean age = 52.6 ± 7.8 years; $n = 8$ normal healthy volunteers; $n = 2/8$ women, mean age 32.5 ± 9.7 years).
Interpretation and Analysis: L-lysine acetylsalicylic acid challenge induces a significant increase in symptoms in conditions of aspirin-intolerant asthma/rhinitis. No difference in the clinical response can be detected in cases with aspirin-tolerant asthma/rhinitis or healthy subjects. Lysine acetylsalicylic acid challenge induces a significant decrease in nasal volume measured by acoustic rhinometry in cases of aspirin intolerance. No differences can be detected between challenges in aspirin-tolerant cases. If a 25% decrease in nasal volume is taken as the cutoff point, the specificity of the test is approximately 94% with a corresponding 73% level of the sensitivity. The nasal challenge is well tolerated by all subjects. Acoustic rhinometry may be used to study the nasal response to L-ASA.
Instrumentation: Acoustic rhinometry.

Intranasal challenge with aspirin in the diagnosis of aspirin intolerant asthma: Evaluation of nasal response by acoustic rhinometry. Casadevall J, Ventura PJ, Mullol J, Picado C. Thorax 2000 Nov; 55 (11), pp. 921–4.

Immune Hypersensitivity: Asthma/Asthmatic Responses: Early vs Late/Chemotactic Properties Mediated by Mast Cell vs Mononuclear Cells
Lysine Aspirin Bronchoprovocation (L-ASA BPT)

Application: Differentiation between "early" and "dual" aspirin-sensitive asthma response based on the liberation of serum chemotactic properties by either mast cell or monocyte cell populations.
Route of Administration: Lysine aspirin (inhalation).
Implementation: Evaluation of pathogenic mechanisms involved in the early vs late asthmatic response as a function of serum neutrophil chemotactic activity (NCA) and histamine production. Following bronchoprovocation with lysine aspirin. Sera is collected before, then 30 minutes and 240 minutes after L-ASA BPT.

Human Model: Cases of aspirin-sensitive asthma ($n = 13$ ASA; $n = 7$ early responders; $n = 6$ dual responders).

Analytical: Chemotactic activity of human neutrophils can be evaluated using a Boyden chamber apparatus. Histamine can be assayed using an automated fluorometric analyzer.
Interpretation and Analysis: Serum neutrophil chemotactic activity increases significantly after 30 minutes ($p = 0.02$) and decreases significantly at 240 minutes ($p = 0.02$) in aspirin-sensitive asthma cases/early responders, while serum NCA in aspirin-sensitive asthma/dual responders increases significantly at 30 minutes ($p = 0.04$) but tends to increase further up to 240 minutes (not statistically significant). Neutrophil chemotactic activity at 240 minutes in aspirin-sensitive asthma/dual responders is significantly higher than baseline NCA ($p = 0.02$). Serum NCA values collected before and at 240 minutes are significantly higher in aspirin-sensitive asthma/dual responders than in aspirin-sensitive asthma/early responders ($p < 0.05$). There are no significant changes in serum histamine levels during lysine aspirin bronchoprovocation in either of these case group types.

Neutrophil chemotactic activity derived from mast cell may contribute to the development of early asthmatic responses induced by L-ASA inhalation. There may be possible involvement of NCA derived from mononuclear cells during late asthmatic response.
Instrumentation: Boyden chemotactic chamber and automated fluorometric analyzer (histamine assay).

Enhanced serum neutrophil chemotactic activity was noted in both early and late asthmatic responses during lysine-aspirin bronchoprovocation test in ASA-sensitive asthmatic patients. Kim SS, Park HS, Yoon HJ, Lee YM, Lee SK, Nahm DH. J Korean Med Sci 2003 Feb; 18 (1), pp. 42–7.

Immune Hypersensitivity: Asthma/β-Adrenergic Receptor Expression (Bronchial)/Function
Isoproterenol Sulfate (Isoprenaline)

Cellular/Molecular Mechanism of Action: Isoproterenol is a selective β_1/β_2-adrenergic agonist and has essentially no effect as α-adrenergic receptor complexes. At the level of the smooth muscle, isoproterenol and other adrenergic agonists (e.g., clenbuterol) enhance adenylcyclase activity, thereby increasing cAMP synthesis.
Physiological Mechanism of Action: Increases in intracellular cAMP created by the influence of adrenergic agonists (e.g., isoproterenol, clenbuterol) on adenylcyclase activity promote bronchodilation responses.
Route of Administration: Isoproterenol (intravenous).
Implementation: Evaluation of complete *in vitro* and *in vivo* dose-response curves for isoproterenol. Complete *in vivo* dose-response curves for isoproterenol are obtained to determine its effect on bronchial muscle (forced expiratory volume in 1 second), heart rate, blood pressure, and skeletal muscular tremor in conditions of chronic intrinsic asthma. In this context, an isoproterenol test is performed before and during oral treatment with a long-acting selective β-adrenergic stimulator (terbutaline sulfate, 5 mg 3 times daily). Evaluation is performed over 12 months to avoid seasonal variation in basal levels of obstruction and is concluded by adding inhaled terbutaline (2 inhalations 4 times daily) to oral therapy.

Human Model: Cases of chronic intrinsic asthma (n = 3 for *in vitro* studies; n = 8 for *in vivo* studies; n = 60 negative reference controls).

Interpretation and Analysis: Complete *in vitro* dose-response curves for isoproterenol (isoprenaline) sulfate reveal no functional defects in bronchial muscular β-adrenergic receptors in chronic intrinsic asthma, compared to negative reference controls. Complete *in vivo* dose-response curves for intravenously infused isoproterenol reveal that no patterns of "resistance" develop in bronchial β-adrenergic receptors during prolonged exposure. Inhalation therapy in addition to oral therapy improves bronchodilation without causing resistance. Even at 6 inhalations given 4 times daily (4- to 5-hour intervals), isoproterenol does not cause any bronchial resistance; however, resistance develops in skeletal muscles with decreased tremor and in cardiac β-adrenergic receptors.

Development of "resistance" in β-adrenergic receptors of asthmatic patients. Svedmyr NL, Larsson SA, Thiringer GK. Chest 1976 Apr; 69 (4), pp. 479–83.

Immune Hypersensitivity: Asthma/Bronchial Obstruction (Severe)/Aspirin-Intolerant Asthma/ Rhinitis: Leukocyte Response
Lysine Acetylsalicylic Acid (L-ASA)/[Cysteinyl-Leukotriene]

Background Information: Aspirin-induced asthma (AIA) is a clinical syndrome related to cysteinyl leukotriene overproduction in airways. The confirmation of the diagnosis requires inconvenient provocation tests with acetylsalicylic acid (ASA).

Application: Detection and characterization of AIA cannot be effectively determined through the measurement of leukocyte cysteinyl leukotrienes.

Implementation: Detection of AIA conditions with ASA evaluated through the *in vitro* measurement of cysteinyl leukotrienes (cys-LTs) release from isolated peripheral blood leukocytes. In this context, a cellular allergen stimulation test, CAST, can be adapted for the measurement of leukotriene release from leukocytes following stimulation with L-ASA, N-formyl-Met-Leu-Phe (*f*MLP), or both *f*MLP and L-ASA, in buffer containing IL-3, and results compared with the response of human leukemia cell line (Hl-60) to L-ASA. Cysteinyl leukotrienes measured in cell supernatant fluids by ELISA.

Human Model: Isolated leukocyte populations harvested from cases of aspirin-tolerant asthma (ATA; n = 32; n = 26 aspirin-intolerant asthmatics).

Interpretation and Analysis: Acetylsalicylic acid has a rather weak stimulatory effect on cys-LT release in both case group types. Contrary to some previous studies, no significant differences can be appreciated between cys-LT release by leukocytes from AIA and ATA or by differentiated Hl-60 cells. Measurement of cysteinyl-leukotriene release by peripheral blood leukocytes pretreated with aspirin has no value for diagnosis of AIA.

A moderate and unspecific release of cysteinyl leukotrienes by aspirin from peripheral blood leucocytes precludes its value for aspirin sensitivity testing in asthma. Pierzchalska M, Mastalerz L, Sanak M, Zazula M, Szczeklik A. Clin Exp Allergy 2000 Dec; 30 (12), pp. 1785–91.

Immune Hypersensitivity: Asthma/Bronchial Responsiveness
Salmeterol/Methacholine (MCh)

Cellular/Molecular Mechanism of Action: Salmeterol functions as a β_2 agonist at adrenergic receptor complexes while MCh is a cholinergic receptor agonist.

Physiological Mechanism of Action: Salmeterol promotes bronchodilation by sympathomimetic qualities, while MCh promotes bronchoconstriction through parasympathomimetic properties.

Background Information: Asthma is characterized by hyperresponsiveness of the airways to bronchoconstrictive stimuli. Long-acting β_2-adrenoceptor agonists have been introduced as a new therapeutic approach, but there is growing concern about whether control of asthma may deteriorate with the regular use of these agents.

Application: Detection and characterization of the bronchodilatory capacity of airways in asthmatic cases and their potential hyperresponsiveness to bronchoconstrictive stimuli induced by MCh.

Route of Administration: Salmeterol (inhalation).

Dosage: Salmeterol (50 μg, b.i.d.).

Implementation: Evaluation of the bronchodilatory response to salmeterol, complemented by the delineation of potential hyperresponsiveness to the bronchoconstrictive properties of MCh, can provide valuable diagnositic information in cases of respiratory asthma. In the performance of such survey studies, patients are given inhaled salmeterol (or saline palacebo) with MCh challenge being performed before, during, and after each exposure period. Methacholine responsiveness is measured as the provocative concentration (PC20) that causes a 20% decrease in the forced expiratory volume over a period of 1 second (FEV1).

Interpretation and Analysis: Significant increases in FEV1 are present 1 hour after inhalation of salmeterol ($p = 0.006$), which does not differ significantly on days 0, 28, and 56 of the exposure period (increase, 9.8, 9.4, and 8.8% of predicted FEV1, respectively; $p = 0.91$). On the 1st exposure day, salmeterol evokes significant protection against MCh-induced bronchoconstriction, as reflected by a 10-fold increase in the PC20 compared to analogous values from pretreated reference controls ($p < 0.001$). After 4 and 8 weeks of salmeterol, however, the salmeterol-induced changes in PC20 are significantly attenuated ($p < 0.001$) to only a 2-fold increase. Two and 4 days after termination of salmeterol exposure, the PC20 is not significantly different from pre-exposure reference control values ($p = 0.15$). Appreciation of these findings suggests that regular treatment of mild asthma cases with salmeterol leads to tolerance phenomenon with regard to the protective effects against bronchoconstrictor stimulus (e.g., inhaled MCh). Such a finding raises concern about the effectiveness of prolonged therapy with long-acting β_2-adrenoceptor agonists in asthma and may in concept be similar to observations of the downregulation of β receptor complexes in cardiac cases treated long-term with propanolol.

Long-term effects of a long-acting β_2-adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. N Engl J Med 1993 Mar 4; 328 (9), pp. 665–6.

Immune Hypersensitivity: Asthma/Bronchial Responsiveness
Cromolyn Sodium/Cockroach (CR) Antigen

Physiological Mechanism of Action: Cromolyn sodium inhibits histamine release mediated through basophil/mast cell degranulation events.

Application: Characterization of asthmatic responses associated with CR antigen.

Implementation: Application of the bronchial provocative test (BPT) with CR antigen in cases of asthma. Evaluation of allergen-specific asthmatic responses induced by CR utilizing a skin test protocol.

Human Model: Cases of asthma (n = 22).

Interpretation and Analysis: Skin tests utilizing CR antigen can result in postive reactions in a large percentage of subjects (e.g., n = 16/22). Coversely, immediate bronchoconstrictive responses can be appreciated following antigen inhalation (e.g., n = 14/16) in CR-positive asthmatics, while no CR-negative asthmatics show bronchospasm (n = 6). Late asthmatic responses can also be appreciated in a large number of CR-positive asthmatic cases following a BPT and CR antigen challenges (e.g., n = 13/16). The dual asthmatic reactions in CR-positive individuals can to a large degree be inhibited by the prior administration of cromolyn sodium. Threefold increases in peripheral eosinophil counts can be appreciated 24 hours following BPT with CR antigen. Speculation therefore suggests that CR-induced asthmatic responses are allergen-specific and CR plays a causative role in allergic asthma in certain populations.

Study on cockroach antigen as a probable causative agent in bronchial asthma. Kang B. J Allergy Clin Immunol 1976 Sep; 58 (3), pp. 357–65.

Immune Hypersensitivity: Asthma/Bronchial Responsiveness
Acetaldehyde/Adenosine 5′-Monophosphate (AMP)/ Methacholine (MCh)

Cellular/Molecular Mechanism of Action: Methacholine binds to and activates cholinergic receptor complexes (parasympathomimetic).
Physiological Mechanism of Action: Inhaled acetaldehyde and adenosine 5′-monophosphate (AMP) cause bronchoconstriction in asthmatics by a mechanism believed to involve histamine release from airway mast cells.
Application: Characterization of airway function in cases of asthma.
Route of Administration: Inhalation.
Dosage: Progressive increase in dosage concentrations and amount delivered.
Implementation: In asthmatics, the evaluation of airway function can be assessed with the aid of inhlation studies employing acethaldehyde, adenosine 5′-monophosphate, and MCh administered at increasing concentrations. Response to each bronchoconstrictor agent is measured by the PC20 (provocative concentration required to produce a 20% fall in forced expiratory volume over a period of 1 second, or FEV1).
Interpretation and Analysis: The geometric mean (range) PC20 values is approximately 3.1 mmol/l (0.5–46.0 mmol/l) for MCh; 883.1 mmol/l (190.7–1,816.1 mmol/l) for acetaldehyde, and 50.1 mmol/l (3.2–1,152.1 mmol/l) for AMP. Thus, acetaldehyde is 18-fold less potent than AMP in causing bronchoconstriction. A similar correlation can be observed between PC20 acetaldehyde and either PC20 AMP ($r = 0.58$, $p = 0.02$) or PC20 MCh ($r = 0.56$, $p = 0.02$). The challenge procedure with acetaldehyde is moderately repeatable (coefficient of repeatability = ± 1.4 doubling concentrations, intraclass correlation coefficient = 0.64). Inhaled acetaldehyde therefore is less potent than AMP in causing bronchoconstriction in asthma, and the response to inhaled acetaldehyde is repeatable. Furthermore, it has been speculated that acetaldehyde responsiveness and AMP responsiveness are not identifying the same alterations in airway functions.

Airway obstruction induced by inhaled acetaldehyde in asthma: Repeatability relationship to adenosine 5′-monophosphate responsiveness. Prieto L, Gutierrez V, Cervera A, Linana J. J Investig Allergol Clin Immunol 2002; 12 (2), pp. 91–8.

Immune Hypersensitivity: Asthma/Bronchial Responsiveness/Airway Reactivity
Methacholine (MCh)/Corticosteroids/Nitric Oxide (Exhaled)

Cellular/Molecular Mechanism of Action: Methacholine is a parasympathomimetic agent that recognizes, binds to, and activates cholinergic receptor complexes. Corticosteroids inhibit phospholipase A2 biochemical activity in a manner that prevents or severely inhibits the subsequent production of prostaglandin species.
Physiological Mechanism of Action: Methacholine can promote bronchoconstriction of the major airways. Salbutamol promotes bronchodilation. Corticosteroids can exert an anti-inflammatory and immunosuppressive response in part due to their ability to promote inhibition of protein synthesis and stabilization of biological membrane structures.
Background Information: Bronchial reactivity and exhaled nitric oxide (eNO) are not often used to monitor the control and severity of asthma in clinical practice.
Application: Correlation of airway reactivity to MCh to alterations in eNO, which are also related to conditions of atopy.
Implementation: Evaluation of the relationship between asthma and different physiologic measures (pulmonary function, nonspecific bronchial reactivity, and eNO).
Human Model: Cases of asthma in various stages of severity (n = 392; cross-sectional, hospital-based study design).
Interpretation and Analysis: Inhalation of corticosteroids does not influence eNO levels. However, the percentage of predicted forced expiratory volume in 1 second (FEV1) and the provocative dose of MCh causing a 20% decrease in FEV1 are significantly lower with inhaled corticosteroids (ICS group) compared with cases not receiving inhaled corticosteroids (NICS mean, 83.2%; 95% confidence interval [CI], 80.4–86.0%; vs mean, 94.1%; 95% CI, 91.1%–97.1%; $p = 0.001$; and geometric mean, 0.32 mg; 95% CI, 0.23–0.45 mg; vs geometric mean, 0.58 mg; 95% CI, 0.42–0.81 mg; $p = 0.01$, respectively). In conditions with severe bronchial hyperresponsiveness, there is a lower predicted FEV1 percent value ($p < 0.001$) and levels of eNO significantly increase with increasing bronchial hyperresponsiveness ($p < 0.001$). There is no relationship between the percentage of predicted FEV1 and eNO. Conditions of atopy have significantly higher eNO levels than do non-atopic patients (geometric mean, 11.21 ppb; 95% CI, 10.07–12.49 ppb; vs geometric mean, 7.76 ppb; 95% CI, 6.11–9.85 ppb; $p = 0.006$, respectively). Therefore, values for eNO are not related to the degree of airway obstruction but are related to airway reactivity and atopic status independent of inhaled corticosteroid use. Higher values of eNO are seen with increased airway reactivity.

Relationship among pulmonary function, bronchial reactivity, and exhaled nitric oxide in a large group of asthmatic patients. Langley SJ, Goldthorpe S, Custovic A, Woodcock A. Ann Allergy Asthma Immunol 2003 Oct; 91 (4), pp. 398–404.

Immune Hypersensitivity: Asthma/ Bronchoconstriction/Bronchodilation
N-Formyl-Met-Leu-Phe (fMLP)/Ipratropium Bromide/Fenoterol

Cellular/Molecular Mechanism of Action: N-formyl-Met-Leu-Phe is an acylated tripeptide bacterial neutrophil chemotactic factor. Ipratropium bromide functions as an anticholinergic agent, and fenoterol is a β_2 agonist.
Physiological Mechanism of Action: N-formyl-Met-Leu-Phe has been shown *in vivo* to have bronchoconstrictor properties

following inhalation in man. The compound has a putative role in the generation of bronchoconstriction associated with bacterial bronchial infection. Ipratropium bromide promotes bronchodilation through parasympatholytic mechanisms, while fenoterol improves flow through the bronchial tree by sympathomimetic processes.

Application: Detection of asthmatic conditions and characterization of the severity of their condition through the evaluation of respiratory function and response testing.

Route of Administration: N-formyl-Met-Leu-Phe (*f*MLP) is delivered by nebulization.

Implementation: Evaluation of bronchoconstriction responses in non-asthmatic subjects administered nebulized after pretreatment with saline, ipratropium bromide, or fenoterol.

Human Model: Non-asthmatics (n = 10 at 21 to 28 years of age).

Interpretation and Analysis: Approximately 80% of non-asthmatic subjects exposed to nebulized *f*MLP will display a 20% level of bronchoconstriction. In individuals that bronchoconstrict in response to nebulized *f*MLP, there is a significant increase in the concentration of the agent required to reach a 20% fall in forced expiratory volume in 1 second (FEV1/PC20*f*MLP) when either ipratropium bromide or fenoterol has been preadministered. Fenoterol is substantially more effective in this regard than is ipratropium bromide. Comparisons made using absolute fall in FEV1 (including all subjects tested; e.g., n = 10) the same results can be appreciated. Partial inhibition of *f*MLP-induced bronchoconstriction by ipratropium bromide suggests that part of the effect of *f*MLP is vagally mediated. Furthermore, it is proposed that fenoterol acts via the modulation of *f*MLP-induced rises in intracellular free calcium.

The effect of anticholinergic and β agonist pretreatment on bronchoconstriction induced by N-formyl-methionyl-leucyl-phenylalanine. Peters MJ, Breslin AB, Berend N. Eur Respir J 1989 Nov; 2 (10), pp. 946–9.

Immune Hypersensitivity: Asthma/Juvenile Asthma-Like Conditions
Salbutamol

Cellular/Molecular Mechanism of Action: Salbutamol belongs to the class of selective β_2-adrenergic agonists that recognizes, binds to, and activates sympathetic, adrenergic β_2 receptor complexes.

Physiological Mechanism of Action: Salbutamol promotes bronchodilation.

Background Information: A significant response in a bronchodilation test is 1 of the main diagnostic criteria of asthma. However, it is not known what the significant bronchodilator response (BDR) is by the interrupter technique (IR), based on measurements of respiratory resistance (Rint) during tidal breathing.

Route of Administration: Salbutamol (inhalation).

Implementation: Evaluation of asthma conditions following subjection to an 8-minute free running test outdoors. Flow-volume spirometry (FVS) and IR measurements are performed before and 10 minutes after running exercise. Thereafter, a salbutamol inhalation test is performed, and pulmonary function measurements acquired 15 minutes later. Case group types can be divided into 3 categories according to postexercise pulmonary function: FEV1 > 100% of predicted (Type I, n = 15), FEV1 86–99% of predicted (Type II, n = 20), and FEV1 < 85% of predicted (Type III, n = 15).

Human Model: Cases diaplaying signs suggestive of juvenile asthma (n = 50).

Interpretation and Analysis: There are no differences in BDRs between Types I and II; the mean increases in FEV1 are 4–6% and in MMEF 20–23%, and the mean decreases in Rint are 23–26%. The mean change in Type III is significantly higher: 15% (p = 0.004) in FEV1, 55% (p = 0.021) in MMEF, and 38% (p = 0. 014) in Rint. Bronchodilator response is positive (FEV1 rise > 10%) in some cases (e.g., n = 15/50; n = 5/20 Type II; n = 10/15 Type III). For MMEF and Rint, the best combination of sensitivity and specificity is achieved by a limit of 35%. Utilizing this limit, MMEF and Rint can identify positive cases (e.g. n = 14/50 and n = 15/50). The IR technique agrees with both FEV1 and MMEF in the majority of cases (e.g., 73%). The IR technique provides an alternative to conventional ventilatory function measurements in bronchodilation tests in children. A decrease of 35% or more in Rint can serve as diagnostic criteria in postexercise bronchodilation tests.

Evaluation of the interrupter technique in measuring postexercise bronchodilator responses in children. Kannisto S, Vanninen E, Korppi M. Clin Physiol 2000 Jan; 20 (1), pp. 62–8.

Immune Hypersensitivity: Asthma/Predicted Absence
Skin-Prick Test (SPT)/Methacholine (MCh)

Cellular/Molecular Mechanism of Action: The SPT employs a battery of foreign environmental substances that can function as immunogens. The biological preparations applied in the SPT are recognized by the immune system and if they are perceived as allergens, they activate various immune and inflammatory pathways. In classic anaphylactic reactions, antigen is recognized by IgE and the resulting Ag:IgE complex can activate the complement cascade and bind to Fc receptors expressed on the exterior surface membrane of mast cell populations. A wide spectrum of endogenous inflammatory mediators is subsequently released by activated mast cell populations (e.g.. histamine).

Background Information: None of the existing tests for the diagnosis of asthma are considered to be definitive. Certain circumstances require prompt diagnosis, and a test able to predict the absence of asthma would be very useful.

Application: The SPT may be valuable in predicting the probability of asthma in young adults.

Dosage: Methacholine administered at an exposure level capable of causing a 20% fall in forced expiratory volume in 1 second (FEV1) (PC20) at < 4 mg/mL while utilizing < 8 mg/mL as diagnostic cutoff value for asthma in the MCh challenge test (MCT).

Implementation: Evaluation of asthmatic conditions utilizing (i) SPT for the diagnostic introduction of a battery of common aeroallergens; and (ii) MCT. The sensitivity, specificity, positive predictive value, and negative predictive values (NPVs) of the SPT are calculated using provocative concentrations of MCh. The Bayes' formula can be used to determine posttest probabilities of having asthma, both for positive and negative SPT results.

Human Model: Cases suffering from asthma consisting of 3 groups of subjects aged 18–24 years: Group A, asthmatic patients (n = 175); Group B, control subjects (n = 100); and Group C, subjects with suspected asthma (n = 150) with normal spirometry findings and a negative exercise challenge test result.

Interpretation and Analysis: A positive SPT result to at least 1 allergen can be detected in approximately 95.5%, 54%, and 69% of cases classified as asthmatics, reference controls subjects, and suspected asthmatics, respectively. Sensitivity, specificity, and NPVs of the SPT are 90.7%, 52.0%, and 84.8%, respectively, with a cutoff value of PC20 < 8 mg/mL. The lower cutoff, PC20 < 4 mg/mL, increases the sensitivity and NPVs to 98.2% and 97.8%, respectively. A negative SPT result decreases the probability of having asthma by 10-fold to 20-fold in subjects that have a low to moderate pretest probability. Incorporating an SPT into the workup of subjects with suspected asthma can reduce the cost of this process significantly.

The SPT may be used as a simple, fast, safe, inexpensive, and reliable method to predict the absence of asthma in young adults.

Value of a negative aeroallergen skin-prick test result in the diagnosis of asthma in young adults: Correlative study with methacholine challenge testing. Graif Y, Yigla M, Tov N, Kramer MR. Chest 2002 Sep; 122 (3), pp. 821–5.

Immune Hypersensitivity: Asthma/Septic Inflammatory Responses/Alveolar Macrophages and Blood Monocytes/Cytokine Responses: Interleukin (IL)-10, Macrophage Inflammatory Protein-1alpha (MIP-1α), Interferon-Gamma (IFN-γ), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)
Dexamethasone/Lipopolysaccharide/IL-1β

Cellular/Molecular Mechanism of Action: Dexamethasone inhibits phospholipase A2 biochemical activity. Lipopolysaccharide (LPS) is also referred to as bacterial endotoxin. Systemically, it can bind to LPS-binding protein in plasma and membrane-associated LPS receptor complexes (CD14) on the surface of leukocytes and vascular endothelium.

Physiological Mechanism of Action: Dexamethasone functions as an immunosuppressive agent due to its ability to inhibit normal protein synthesis and stabilize cell membranes in a manner that inhibits cell activation and degranulation phenomenon. Bacterial LPS fractions (endotoxin) promote inflammatory responses in primarily leukocyte and endothelial cell populations. The cytokine IL-1β functions as an endogenous mediator of inflammation.

Application: Lipopolysaccharide (bacterial endotoxin) can be utilized to induce septic inflammatory responses. Dexamethasone can be used as a probe to study alterations in the production of endogenous mediators associated with inflammatory and immune responses during allergen-induced reactions.

Implementation: Evaluation of the effect of inhaled corticosteroid, and budesonide, on the release of the anti-inflammatory cytokine, IL-10, and of proinflammatory cytokines, MIP-1α, IFN-γ, and GM-CSF, from blood monocytes and alveolar macrophages.

Human Model: Cases of mild asthma used as donors for alveolar macrophages obtained by bronchoalveolar lavage at the end of each treatment phase (double blind, crossover, placebo-controlled study).

Interpretation and Analysis: Budesonide reduces bronchial hyperresponsiveness and improves baseline forced expiratory volume in 1 second (FEV1). Interleukin-10 from blood monocytes is not altered, but both IL-10 messenger RNA (mRNA) and protein expression from alveolar macrophages stimulated by lipopolysaccharide and IL-1β are increased after corticosteroid therapy. Alternatively, alveolar macrophages release significantly less MIP-1α, IFN-γ, and GM-CSF after steroid challenge. In comparison to alveolar macrophages from normal non-asthmatic reference controls, those from cases suffering from asthma release more MIP-1α, IFN-γ, and GM-CSF but lower amounts of IL-10 particularly at baseline and after IL-1β stimulation. The ability of steroids to inhibit proinflammatory cytokines but to enhance the anti-inflammatory cytokine such as IL-10 may contribute to their beneficial actions in asthma. Asthma is characterized by alveolar macrophages exhibiting both an enhanced capacity to release proinflammatory cytokines and a reduced capacity to produce IL-10.

Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein-1alpha, granulocyte-macrophage colony-stimulating factor, and interferon-gamma release from alveolar macrophages in asthma. John M, Lim S, Seybold J, Jose P, Robichaud A, O'Connor B, Barnes PJ, Chung KF. Am J Respir Crit Care Med 1998 Jan; 157 (1), pp. 256–62.

Immune Hypersensitivity: Asthma/"Skiers Asthma"/ Budesonide Efficacy Evaluation
Methacholine (MCh)

Cellular/Molecular Mechanism of Action: Methacholine is a parasympathomimetic agent that recognizes, binds to, and activates cholinergic receptor complexes.

Background Information: Asthma-like symptoms, MCh hyperresponsiveness, frequent inhaled steroid use, airway inflammation, and increased tenascin expression in the reticular basement membrane have all been reported in competitive cross-country skiers.

Application: Methacholine can be applied to detect bronchial hyperresponsiveness in conditions including various forms of asthma or asthma-like alterations in respiratory function (e.g., skiers asthma) in addition to functioning as a molecular probe for evaluating pharmaceutical efficacy.

Route of Administration: Budesonide (inhaled).

Dosage: Budesonide (400 μg b.i.d.).

Implementation: Delineate the effect of inhaled budesonide on indices of airway inflammation in conditions of skiers asthma. Bronchial biopsy and bronchoalveolar lavage (BAL) analysis of samples is performed to determine the influence of budesonide on levels of cellular inflammation within bronchial mucosal tissues.

Human Model: Competitive cross-country skiers (n = 25; n = 19/25 male; mean age = 18 [16–20] years) with a mean (range) treatment period of 22 (10–32) weeks during the competition season. Cases also previously determined to be affected by skiers asthma defined as asthma-like symptoms within the previous year in addition to bronchial hyperresponsiveness to MCh.

Design: A randomized double blind placebo-controlled parallel group.

Interpretation and Analysis: No changes can be appreciated regarding cellular inflammation in the bronchial mucosa or tenascin expression. In the BAL fluid, both groups have a significant decrease in activated T-suppressor (CD8) lymphocytes and an increase in macrophages, with no differences across the case group types. In cases given budesonide, there is a decrease in IL-2 receptor-activated T-helper lymphocytes and an improvement in forced expiratory volume in 1 second (FEV1). Asthma-like symptoms are unchanged in about 68% of skiers (n = 17/25). Methacholine provocation test is negative in 60% of cases (n = 15/25) and remains positive in a small percentage of cases (n = 5/25 or 20%) in both case group types. Improvement in bronchial responsiveness occurs in both groups and is not accompanied by a decrease in cellular inflammation. Budesonide does not appear to exert any clear beneficial effect in skiers asthma. As changes in training intensity probably account for the spontaneous improvement in bronchial responsiveness, more attention should be directed at reducing environmental stress to the airways than at attempting pharmacological modulation of induced inflammatory changes.

Placebo-controlled study of inhaled budesonide on indices of airway inflammation in bronchoalveolar lavage fluid and bronchial biopsies in cross-country skiers. Sue-Chu M, Karjalainen EM, Laitinen A, Larsson L, Laitinen LA, Bjermer L. Respiration 2000; 67 (4), pp. 417–25.

Immune Hypersensitivity: Asthma (Allergen Identification) Histamine Provocation Test
Bronchial Provocation Test/Radio Allergosorbent Test/ Skin-Prick Test (SPT)

Cellular/Molecular Mechanism of Action: Histamine binds to and activates histamine H_1 and H_2 receptor complexes.

Physiological Mechanism of Action: Histamine directly promotes contraction of smooth muscle in the bronchi and intestine (H_1 receptors), while in small vascular structures it promotes relaxation of smooth muscle tissues (H_1 and H_2 receptor complexes). Depending on the receptor complex, histamine can promote profound inflammatory responses. Gastric secretion is mediated through the H_2 receptor.
Application: Identification of offending asthma-inducing agents.
Implementation: Occupational exposure to allergens can produce marked signs of wheezing that are not appreciated when in a home environment. A strategy than can be employed to identify the offending allergen can include diagnostic procedures that involve acquiring serial peak flow recordings (not diagnostic) in addition to SPTs, radio allergosorbent test (RAST) measurements, and specific bronchial provocation testing. The bronchial provocation testing is performed by a dust tipping method in a single blind manner, with lactose as an inert control and suspected allergen as the active agent. Serial measurements of forced expiratory volume in 1 second (FEV1) are recorded in addition to identification of the histamine provocative concentration that causes a 20% fall in FEV1 (PC20) tests, which are established before and 24 hours after each challenge.
Interpretation and Analysis: Examples of asthmatic reponses include isolated early reactions, isolated late reactions, and early and late reactions. Many cases may demonstrate increases in non-specific bronchial responsiveness after active challenge. In this context, some cases (e.g., n = 4 men) can be identified that suffer from occupational asthma associated with exposure to an allergen (e.g., amylase: *Bacillus licheniformis*-derived contained in detergent washing powders). The introduction of encapsulated enzymes in the detergent industry has produced reductions in the incidence of respiratory sensitization.

Occupational asthma caused by bacillary amylase used in the detergent industry. Hole AM, Draper A, Jolliffe G, Cullinan P, Jones M, Taylor AJ. Occup Environ Med 2000 Dec; 57 (12), pp. 840–2.

Immune Hypersensitivity: Asthma (Allergic vs Non-Allergic)/Aspirin-Sensitive and Aspirin-Insensitive Asthma/Histamine Release (HR)/Sulfidoleukotriene (LTR) Release
Interleukin-3 (IL-3)/Anti-IgE/C5a/Basophil Stimulation Test

Cellular/Molecular Mechanism of Action: The immunoglobulin fraction anti-IgE binds to and neutralizes the biological activity of IgE, which is an antibody isotype that binds to Fe receptors on the surface membrane of basophils and is intimately involved in the initiation of allergic and anaphylactic immune hypersensitivity reponses. The complement fraction C5a among other properties is classified as an anaphylatoxin.
Physiological Mechanism of Action: The interleukin IL-3 appears to be necessary for the development/maturation of mast cell populations. The complement fraction C5a, among its many other properties, functions as an anaphylatoxin related to the initiation of anaphylactic immune hypersensitivity reactions.
Background Information: The differentiation between allergic and non-allergic asthma is a common and important challenge for the clinician. Until now, no *in vitro* diagnostic characteristics have been described to distinguish between these types of asthma.
Application: Differentiation between allergic and non-allergic conditions of asthma, but aspirin-sensitive asthma cannot be distinguished from non-allergic asthma without aspirin tolerance.
Implementation: Characterize and profile mediator release induced by anti-IgE and C5a exposure (basophil stimulation test) in different conditions of bronchial asthma. The *ex vivo* analysis procedure involves the isolation of peripheral blood leukocytes (PBLs) by dextran sedimentation. After priming with IL-3, PBLs are stimulated with anti-IgE and C5a to facilitate the measurement of released histamine (HR) and LTRs in the supernatant. Additionally, purified leukocyte fractions can be evaluated to selectively determine the cellular source of mediator release.

Human Model: Cases of aspirin-sensitive asthma (n = 10); non-allergic asthma without aspirin tolerance (n = 12 NAA); allergic asthma (n = 7 AA); negative healthy reference controls (n = 9).
Interpretation and Analysis: Upon stimulation with anti-IgE, LTRs are slightly, but not significantly, lower in aspirin-sensitive asthma and NAA compared to AA and controls. In contrast, C5a-triggered LTR is significantly higher in ASA (14.4 ± 12.88 pg/105 cells) and NAA (22.9 ± 22.61 pg/105 cells) than in AA (9.6 ± 3.29 pg/105 cells) and controls (7.5 ± 7.19 pg/105 cells) ($p < 0.05$). This difference between aspirin-sensitive asthma and NAA vs AA and controls is even more pronounced when determining the quotient C5a-/anti-IgE-induced LTR ($p < 0.001$). At an optimal cutoff point of 1.0, calculated by relative operating characteristics (ROC) analysis, the positive predictive value for a donor to belong to aspirin-sensitive asthma or NAA is 0.94. No significant differences can be detected in HR between conditions of asthma and healthy controls in either case. As cellular source of LTR and HR in basophils can be determined. Delineation of anti-IgE- and C5a-induced LTR from basophils allows differentiation between allergic and non-allergic asthmatic patients. The quotient C5a-/anti-IgE-induced LTR is diagnostically more significant than considering a single parameter. Aspirin-sensitive asthma cannot be distinguished from NAA.

Differential mediator release from basophils of allergic and non-allergic asthmatic patients after stimulation with anti-IgE and C5a. Abrahamsen O, Haas H, Schreiber J, Schlaak M. Clin Exp Allergy 2001 Mar; 31 (3), pp. 368–78.

Immune Hypersensitivity: Asthma (Aspirin-Induced)/Cysteinyl-Leukotriene Production: Peripheral Blood Leukocytes [Diagnostic Value Limited]
Lysine Acetylsalicylic Acid/fMLP/IL-3

Background Information: Aspirin-induced asthma (AIA) is a clinical syndrome related to cysteinyl leukotriene overproduction in airways. The confirmation of the diagnosis requires inconvenient provocation tests with acetylsalicylic acid (ASA).
Implementation: Evaluate the *in vitro* influence of ASA stimulation of isolated peripheral blood leukocytes on measurements for the release of cysteinyl leukotrienes (cys-LTs) as a potential diagnostic molecular marker for AIA. Potential validation of this theory can be achieved through the use of a cellular allergen stimulation test (CAST) adapted for the measurement of leukotriene release from isolated leukocytes. Isolated cell preparations are stimulated with (i) L-ASA; (ii) N-formyl-Met-Leu-Phe (*f*MLP); or (iii) both *f*MLP and L-ASA, in a buffer containing interleukin-3 cytokine. Results are then compared with human leukemia cell line (Hl-60) responses to L-ASA. Cysteinyl leukotrienes can be measured in cell supernatant fluids by ELISA.

Human Model: Cases of aspirin-tolerant (ATA; n = 32) and aspirin-intolerant (n = 26) asthmatics.
Interpretation and Analysis: Acetylsalicylic acid has a rather weak stimulatory effect on cys-LTs release in conditions of both aspirin-tolerant and aspirin-intolerant asthma. In contrast to findings reported in some related investigations, some studies have detected no significant difference between cys-LTs release by leukocytes from AIA and ATA or by differentiated Hl-60 cells. Measurement of

cysteinyl-leukotriene release by peripheral blood leukocytes pretreated with aspirin has no value for diagnosis of AIA.

A moderate and unspecific release of cysteinyl leukotrienes by aspirin from peripheral blood leucocytes precludes its value for aspirin sensitivity testing in asthma. Pierzchalska M, Mastalerz L, Sanak M, Zazula M, Szczeklik A. Clin Exp Allergy 2000 Dec; 30 (12), pp. 1785–91.

Immune Hypersensitivity: Asthma (Asymptomatic) *Metaproterenol (Aerosol)/Histamine/Methacholine (MCh)/[Quinine Fluorescence]*

Cellular/Molecular Mechanism of Action: Metaproterenol functions as a competitive inhibitor of adrenergic neurotransmitter agents due to binding avidity for β-adrenergic receptor complexes.
Physiological Mechanism of Action: Methacholine is a parasympathomimetic/cholinergic agonist that promotes bronchial constriction.
Application: Induction of bronchoconstriction for the detection of aymptomatic asthma conditions.
Route of Administration: Aerosol inhalation.
Considerations: Studying normal subjects and asthmatics allows the comparison of metaproterenol responsiveness after widely divergent doses of the bronchoprovocative agents.
Implementation: Evaluation of the potential for bronchoprovocative test reagents to achieve the same degree of bronchoconstriction as occurs in aymptomatic cases of asthma. Asymptomatic asthmatics inhale a dry aerosol (mass median aerodynamic diameter, 1.5 microns) with increasing concentrations of MCh or histamine to produce a 35% decrease in specific airway conductance (sGaw), followed by a single inhalation of a metaproterenol aerosol.

Airway deposition of MCh, histamine, and metaproterenol can be measured using a quinine fluorescence technique.
Interpretation and Analysis: At an equivalent degree of bronchoconstriction, metaproterenol responsiveness is influenced by the dose of MCh but not the dose of histamine. The differential metaproterenol response may be related to a functional antagonism between muscarinic and β-adrenergic agonists.

Mean baseline sGaw, metaproterenol responsiveness, and metaproterenol mass deposited are similar in normal subjects and asthmatics. Likewise, mean sGaw after completion of MCh and histamine challenge and subsequently deposited metaproterenol mass are similar in each case group type. After MCh challenge (mean ± SD provocative drug mass causing a 35% decrease in sGaw, PM35: 8.94 ± 5.96 μmol in normal subject and 0.30 ± 0.29 μmol in asthmatics), metaproterenol increases mean sGaw by 89 ± 33% in normal subjects and by 190 ± 55% in asthmatics ($p < 0.05$, 2-way analysis of variance). After histamine challenge (PM35, 2.92 ± 2.49 μmol in normal subjects and 0.17 ± 0.29 μmol in asthmatics), metaproterenol increases mean sGaw by 111 ± 38% in normal subjects and 113 ± 69% in asthmatics (p = not significant).

Metaproterenol responsiveness after methacholine- and histamine-induced bronchoconstriction. Elsasser S, Donna E, Demirozu CM, Danta I, Wanner A. Chest 1996 Sep; 110 (3), pp. 617–23.

Immune Hypersensitivity: Asthma (Grain Dust [GD])/Mast Cells: Tryptase Expression/Neutrophils: Elastase Expression/Interleukin-8 (IL-8) Expression *GD Bronchoprovocation Test/Bronchial Mucosa Biopsy*

Background Information: The immunopathological mechanism for occupational asthma induced by GD remains to be clarified. There have been a few reports that have suggested the involvement of neutrophils and their capacity to induce bronchoconstriction after GD inhalation.
Implementation: Evaluation of the phenotype of leukocytes that infiltrate the bronchial mucosa in conditions of GD-induced asthma. Bronchial biopsy specimens are obtained by fiber-optic bronchoscopy in GD-induced asthmatics. Bronchial biopsy specimens are examined by immunohistochemistry analyses using a panel of monoclonal antibodies to tryptase-containing mast cell (AA1), activated eosinophil (EG2), pan T-lymphocyte (CD3), and neutrophil elastase (NE). Induced sputum is collected before and after initiation of the GD bronchoprovocation test. The IL-8 level in the sputum is also measured using ELISA techniques.

Human Model: Cases of GD-induced asthma ($n = 6$); and house dust mite sensitivity ($n = 6$).
Interpretation and Analysis: There is a significant increase in the number of tryptase-containing mast cell (AA1+) and neutrophil elastase positive (NE+) cells in bronchial mucosa of GD-induced asthma, compared with those of allergic asthma ($p = 0.01$, $p = 0.01$, respectively). No significant differences can be observed in the number of EG2+ and CD3+ cells ($p = 0.13$, $p = 0.15$, respectively). Interleukin-8 is abundant in the sputum of all GD-induced asthma patients and significantly increases after the bronchial challenges compared with baseline values ($p = 0.03$). Such findings support the perspective that neutrophil recruitment together with mast cells may contribute to the bronchoconstriction induced by GD antigen. A possible involvement of IL-8 in such processes has been proposed.
Instrumentation: Fiber-optic bronchoscope.

Neutrophil infiltration and release of IL-8 in airway mucosa from subjects with grain dust-induced occupational asthma. Park HS, Jung KS, Hwang SC, Nahm DH, Yim HE. Clin Exp Allergy 1998 Jun; 28 (6), pp. 724–30.

Immune Hypersensitivity: Asthma (Late Reactions)/(Bronchial Responsive Challenge) *Histamine/Methacholine (MCh)*

Cellular/Molecular Mechanism of Action: Histamine binds to and activates histamine H_1 and H_2 receptor complexes. Methacholine recognizes, binds to, and activates parasympathetic/cholinergic receptor sites.
Physiological Mechanism of Action: Methacholine promotes parasympathomimetic-mediated bronchocontriction. Histamine directly promotes contraction of smooth muscle of the bronchi tree and intestine (H_1 receptors), while in small vascular structures it promotes relaxation of smooth muscle tissues (H_1 and H_2 receptor complexes). Depending on the receptor complex, histamine can promote profound inflammatory responses. Gastric secretion is mediated through the H_2 receptor.
Application: Assess the frequency of changes in bronchial responsiveness in conditions of late asthmatic reactions.
Dosage: Albuterol (200 μg).
Considerations: Late asthmatic reactions generally can be demonstrated to have increased bronchial responsiveness and are considered to respond poorly to inhaled bronchodilators.
Implementation: Assess the frequency of changes in bronchial responsiveness in conditions of late asthmatic reactions and cases with isolated immediate reactions following specific inhalation challenges (e.g., occupational agents).

Human Model: Cases with (i) late asthmatic reactions ($n =$ 101 record review); (ii) isolated immediate reactions after specific inhalation challenges to various occupational agents that had undergone non-specific inhalation challenges to histamine or MCh on a control day and after the late reaction when FEV1 had returned to

± 10% baseline (n = 63); and (iii) late reactions and those administered an inhaled β_2 agent (albuterol, 200 μg) during the late reaction (n = 99 record review).

Interpretation and Analysis: Review of cases with late reactions previously administered an inhaled β_2 agent (albuterol, 200 μg) during the late reaction (e.g., n = 99) reveals that approximately half of these subjects (e.g., n = 57/101 or 56%) with late reactions and those with isolated immediate reactions (e.g., n = 24/63 or 38%) demonstrate a requirement for a 2-fold or greater change in provocative concentration of histamine or MCh in order to cause a 20% change in FEV1 (PC20) from baseline (p = 0.02; odds for the presence of significant changes in PC20 in subjects with late reactions, 56%; odds for the absence of significant changes in PC20 in subjects with immediate reactions, 62%). Changes in FEV1 greater than 20% after administering albuterol at the time of the late reactions occur in approximately 78% of cases tested, and in 66% the FEV1 estimate returns to greater than 90% of baseline. Such retrospective studies demonstrates that changes in bronchial responsiveness after late reactions are not constant and do not appear to distinguish satisfactorily late from immediate reactions. Furthermore, late reactions respond well to β_2 agonist.

Late asthmatic reactions to occupational sensitizing agents: Frequency of changes in non-specific bronchial responsiveness and of response to inhaled β_2-adrenergic agent. Malo JL, Ghezzo H, L'Archeveque J, Cartier A. J Allergy Clin Immunol 1990 May; 85 (5), pp. 834–42.

Immune Hypersensitivity: Asthma (Mild Intermittent Allergic)
Salmeterol/Allergen (Bronchoconstriction)

Physiological Mechanism of Action: Salmeterol provides a protective β_2 agonist effect on early airway response to allergen early airway response (EAR).

Regular use of short-acting β_2 agonists (salmeterol) has been shown to induce tolerance to allergen (EAR) and adenosine 5′-monophosphate challenge.

Application: Assess whether withdrawal from salmeterol for 3 days (72 hours) can restore its bronchoprotective ability on specific bronchial provocative test (sBPT) with allergen.

Route of Administration: Inhalation.

Dosage: Salmeterol, 50 μg/day for 4 days (± allergen).

Example Clinical Trial: Cases undergoing sBPT with allergen after a single dose of inhaled salmeterol (50 μ) (test 1); and then undergo sBPT after 1 week of regular treatment with inhaled salmeterol (50 μ b.i.d.) (test 2); followed by continued inhaled salmeterol treatment for 4 days, then changed to inhaled salmeterol with placebo (2 puffs b.i.d.) for 3 days (72 hours) and subjected to sBPT with allergen after a single dose of salmeterol (50 μ) (test 3).

Considerations: The bronchoprotective effect of salmeterol on allergen-induced EAR, completely lost after 1 week of regular treatment with salmeterol, may be partially restored by withdrawal of salmeterol for 3 days (72 hours). However, the withdrawal time period is not sufficient to recover the baseline bronchoprotective efficacy of 1st-dose salmeterol.

Interpretation and Analysis: The EAR to allergen (Delta forced expiratory volume in 1 second [FEV1] ≥ 20% with respect to postdiluent value) is completely abolished by a single dose of salmeterol (test 1; protection index [PI] ≥ 50%) but is still present after 1 week of regular treatment with salmeterol (test 2; PI < 50%). The maximum FEV1 percentage fall during sBPT with allergen is significantly lower after withdrawal of regular inhaled salmeterol (test 3) than after regular treatment with salmeterol (test 2) (mean, 23% vs 29.5%; range, 4–41% vs 18–49%, respectively; p < 0.05); a similar result is obtained when considering the PI of salmeterol on sBPT with allergen (mean, 44% vs 20%; range, 2–86% vs −11–49%, respectively; p < 0.05). However, the maximum FEV1 percentage fall and PI are significantly different in test 3 than after test 1, and a significant proportion of cases will show (test 3) a PI ≥ 50%.

Tolerance to the protective effect of salmeterol on allergen challenge can be partially restored by the withdrawal of salmeterol regular treatment. Giannini D, Di Franco A, Bacci E, Dente FL, Bartoli ML, Vagaggini B, Paggiaro P. Chest 2001 Jun; 119 (6), pp. 1671–5.

Immune Hypersensitivity: Asthma (Mild to Moderate)/Hyperresponsive Airway/Cellular Airway Infiltration
Methacholine (MCh)/Agonists (β_2)/[Corticosteroids]

Cellular/Molecular Mechanism of Action: Methacholine recognizes, binds to, and activates parasympathetic/cholinergic receptor sites.

Physiological Mechanism of Action: Methacholine promotes parasympathomimetic-mediated bronchocontriction. Corticosteroids like beclomethazone inhibit phospholipase A2 (inhibit prostaglandin/leukotriene production) and stabilize cell membrane structures (inhibit leukocyte degranulation). In addition, like adrenergic agonists and clenbuterol, corticosteroids promote bronchodilation by increasing intracellular adenylcyclase activity (elevates cAMP) within bronchial smooth muscle.

Background Information: Current guidelines for the management of asthma advocate the use of anti-inflammatory agents in all but mild disease conditions. Disease control can be defined in terms of clinical criteria such as lung function and symptoms. However, the relationship between the clinical control of the disease and inflammation of the airways is not clear.

Application: Detection and characterization of the number of infiltrating leukocytes such as mast cells, (activated) eosinophils, CD8+, and CD45RO+ cells in bronchial biopsy specimens in mild to moderate conditions of asthma. These properties appear to correspond with the observation of airway hyperresponsiveness to MCh.

Implementation: Delineate correlations between airway inflammation and measures of clinical control and bronchial hyperresponsiveness (MCh challenge test) in asthmatic conditions during simultaneous steroid inhalation. Biopsy specimens are taken by fiber-optic bronchoscopy from the carina of the right lower and middle lobes, and from the main carina. Immunohistochemical staining is performed on tissue sections with monoclonal antibodies against eosinophil cationic protein (EG1, EG2), mast cell tryptase (AA1), CD45, CD22, CD3, CD4, CD8, CD25, and CD45RO. The number of positively stained cells in the lamina propria is counted twice by using an interactive display system.

Human Model: Cases of mild to moderate asthma (n = 26 atopic adults with baseline forced expiratory volume in 1 second [FEV1] ≥ 50% predicted, concentration of histamine causing a 20% fall in FEV1 [PC20] 0.02–7.6 mg/ml) on regular treatment with inhaled steroids. Histories and bronchoscopic examinations are utilized to determine use of β_2-adrenergic agonists, peak flow variability, and symptom scores.

Investigation Design: Cross-sectional study.

Interpretation and Analysis: No difference exists in cell numbers in biopsy specimens collected from the carina of the right lower and middle lobes, and from the main carina. The PC20 for MCh is inversely related to the average number of total leukocytes, EG1+,

and EG2+ cells, mast cells, CD8+, and CD45RO+ cells in the lamina propria. These relationships are similar for each of the biopsy sites. Symptom scores, β_2 agonist usage, FEV1, and peak flow variability do not appear to consistently correlate with tissue biopsy cell count. Therefore, infiltration of inflammatory cells in the lamina propria of the airways seems to persist in asthmatic outpatients despite regular treatment with inhaled steroids. The number of infiltrating leukocytes such as mast cells, (activated) eosinophils, CD8+, and CD45RO+ cells in bronchial biopsy specimens from these patients appears to be reflected by airway hyperresponsiveness to MCh, but not by symptoms or lung function. These findings may have implications for the adjustment of anti-inflammatory treatment of patients with asthma.

Instrumentation: Cryostat histology sectioning unit.

Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. Sont JK, Han J, van Krieken JM, Evertse CE, Hooijer R, Willems LN, Sterk PJ. Thorax 1996 May; 51 (5), pp. 496–502.

Immune Hypersensitivity: Asthma (Mild to Moderate Degree)/Steroid Therapy: Administration Detection/Neuroendopeptidase/CD10 Expression *Methacholine (MCh)/Histamine/Corticosteroids*

Cellular/Molecular Mechanism of Action: Methacholine recognizes, binds to, and activates parasympathetic/cholinergic receptor sites. Corticosteroids inhibit the biochemical activity of phospholipase A2, thereby diminishing the production of both prostaglandins and leukotrienes.

Physiological Mechanism of Action: Methacholine promotes parasympathomimetic-mediated bronchocontriction. Corticosteroids like beclomethazone inhibit phospholipase A2 (inhibit prostaglandin/leukotriene production) and stabilized cell membrane structures (inhibit leukocyte degranulation). In addition, like adrenergic agonists and clenbuterol, corticosteroids promote bronchodilation through increases in intracellular adenylcyclase activity (increased cAMP) within bronchial smooth muscle.

Background Information: Expression of the endogenous neuropeptide-degrading enzyme neutral endopeptidase (NEP; CALLA, CD10, E.C.3.4.24.11) on cultured human airway epithelial cells can be upregulated by corticosteroids.

Implementation: Evaluation of the potential for NEP expression in the airway epithelium or lamina propria in bronchial biopsies to be enhanced in atopic asthmatics on regular inhaled steroids as compared with those without steroid treatment. Tissue biopsies are taken at (sub)segmental levels from the right lower lobe, the middle lobe, and the main carina. Immunohistochemical staining can be performed on cryostat sections using the VIL-A1 monoclonal antibody against CD10 (NEP).

Human Model: Cases affected by mild to moderate degrees of asthma ($n = 40$ age 19–48 years non-smoking adults; forced expiratory volume in 1 second $\geq$ 50% pred.; histamine PC20 range = 0.02–7.6 mg/ml; $n = 23$ with regular inhaled steroids; $n = 17$ without regular inhaled steroids).

Interpretation and Analysis: In airway epithelium, NEP-positive sites are within the basal layer and, in contrast with studies applying other antibodies, also at apical sites and within the lamina propria. In both the epithelium and lamina propria, NEP expression is not significantly different between the right lower lobe, the middle lobe, and the main carina biopsy sites (Friedman's non-parametric 2-way analysis of variance; $p > 0.68$), nor is expression in the lamina propria associated with inhaled steroid usage (Mann-Whitney U test; $p = 0.98$). However, NEP expression is significantly enhanced in the airway epithelium in patients using inhaled steroids as compared with non-steroid users (mean rank: 23.4 and 15.5, respectively; $p = 0.02$). Among non-steroid-using subjects, NEP expression is related to symptoms and the MCh PC20 (Rs: -0.69 and 0.49, respectively; $p \leq 0.04$). Expression of NEP is therefore enhanced in airway epithelium in bronchial biopsy specimens from patients with atopic asthma who are regularly using inhaled steroids compared with cases that do not. This fits the hypothesis that the anti-inflammatory effect of corticosteroids within the airways is partially mediated by the upregulation of the endogenous neuropeptide-degrading enzyme NEP.

Enhanced expression of neutral endopeptidase (NEP) in airway epithelium in biopsies from steroid- vs nonsteroid-treated patients with atopic asthma. Sont JK, van Krieken JH, van Klink HC, Roldaan AC, Apap CR, Willems LN, Sterk PJ. Am J Respir Cell Mol Biol 1997 May; 16 (5), pp. 549–56.

Immune Hypersensitivity: Inhaled Allergens/ Allergic Reactions/[Interleukin (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, Interferon-Gamma (IFN-γ), RANTES, Tumor Necrosis Factor-Alpha (TNF-α)] *Single Allergen Provocation Test/Nasal Biopsy*

Background Information: The local production and release of a number of cytokines regulates allergic upper airway inflammation. Medication is usually used at the presentation of the 1st symptoms. There are, however, clues that it is advisable to start taking the corticosteroid before the grass pollen season begins.

Application: The single allergen provocation test can be applied to detect natural allergens responsible for inducing hyperactive immune responses *in vivo* in addition to functioning as a tool for evaluating the efficacy of anti-inflammatory and immunosuppressive agents.

Implementation: Evaluation of preventative therapies for inhaled allergic reactions applying the single allergen provocation protocol conducted in autumn (non–hay fever season). Nasal mucosa biopsies are collected twice before provocation (before and after 4 weeks of preventive treatment) and 3 times after allergen provocation (1 hour, 24 hours, and 1 week). Eosinophils and messenger RNA (mRNA) positive cells (in situ hybridization for IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IFN-γ, RANTES, and TNF-α) are then counted in biopsy samples.

Human Model: Cases receiving either fluticasone propionate aqueous nasal spray (FPANS; $n = 10$) or a placebo ($n = 9$).

Interpretation and Analysis: Preventive treatment with FPANS out of season results in a decrease in eosinophils and mRNA positive cells for IL-5 and IL-6. After allergen provocation, levels of most of the measured cytokines (IL-3, IL-5, IL-6, IL-13, IFN-γ, RANTES, and TNF-α) and eosinophils are reduced with corticosteroids. The numbers of cells (eosinophils, IL-3, IL-6, and IL-8) correlate with nasal symptoms. Significant correlations in the early and late allergic phase can be appreciated between eosinophils and cytokines (IL-3, IL-10, and IL-13). These results indicate that preventive treatment with FPANS prior to contact with grass pollen is effective in reducing the increase of cytokine mRNA positive cells in reaction to grass pollen contact.

Preventive treatment of intranasal fluticasone propionate reduces cytokine mRNA expressing cells before and during a single nasal allergen provocation. Kleinjan A, Holm AF, Dijkstra MD, Boks SS, Severijnen L, Mulder PG, Fokkens WJ. Clin Exp Allergy 2000 Oct; 30 (10), pp. 1476–85.

Immune Hypersensitivity and Inflammatory Responses/Asthma: Molecular Pathogenesis of Inflammatory Responses/Sepsis: Molecular Pathogenesis of Inflammatory Responses/Alveolar Macrophages: Septic Inflammatory Responses in Asthma/Cytokine Responses: Interleukin (IL)-10, Macrophage Inflammatory Protein-1alpha (MIP-1α), Interferon-Gamma (IFN-γ), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)
Lipopolysaccharide/Dexamethasone/IL-1β

Cellular/Molecular Mechanism of Action: Lipopolysaccharide (LPS) is also referred to as bacterial endotoxin. Systemically, it can bind to lipopolysaccharide-binding protein and membrane-associated LPS receptor complexes (CD14).
Physiological Mechanism of Action: Bacterial lipopolysaccharide fractions (LPS, endotoxin) promote inflammatory responses in primarily leukocyte and endothelial cell populations.
Background Information: Inflammation in asthma is characterized by a T-helper 2 (Th2) response. In many experimental systems, this response can be regulated by IL-10 and IL-12. Interleukin-10 deactivates T cells, and IL-12 reorients the response toward a Th1 pattern. Alveolar macrophages (AM) can secrete both of these cytokines, and thus regulate T-cell behavior in asthma. They can enhance the Th2 response by turning off their secretion of IL-10 and IL-12 or tend to downregulate it by producing these cytokines.
Application: Delineate the molecular pathogenesis of immune responses and septic inflammatory reactions in addition to functioning as a model agent for evaluating the potential efficacy of candidate anti-inflammatory and immunosuppressive agents.
Implementation: Evaluate IL-10 and IL-12 production in AM populations. Alveolar macrophages are recovered by bronchoalveolar lavage (BAL) from the lung. They are isolated and cultured for 24 hours without stimulation or in the presence of LPS fractions. Assays are then performed for IL-10 and the p40 subunit of IL-12 in BAL fluid samples and in AM culture supernatants by ELISA.

Human Model: Cases of adult asthma (n = 11; n = 4 normal healthy controls, n = 6 asthmatics treated with inhaled corticosteroids).
Interpretation and Analysis: Spontaneous AM IL-10 production is higher in asthmatics, particularly in treated cases. Alveolar macrophage IL-10 production after LPS stimulation is also elevated in asthmatics, but mainly so in untreated cases. Levels of IL-12 are higher in BAL fluids from untreated cases than healthy reference controls. The production of IL-12 in LPS-stimulated AM from such cases is increased. Alveolar macrophages are at least primed for the production of IL-10 and IL-12 in asthma. Such phenomenon suggests that these cells could be involved in the resolution of asthmatic-associated inflammatory processes.

Alveolar macrophage interleukin (IL)-10 and IL-12 production in atopic asthma. Magnan A, van Pee D, Bongrand P, Vervloet D. Allergy 1998 Nov; 53 (11), pp. 1092–5.

Immune Hypersensitivity and Inflammatory Responses/Molecular Pathogenesis/ Bronchoalveolar Lavage Fluid Cell Populations/ Endogenous Inflammatory Mediators: Interleukin (IL)-2, IL-4, IL-5, IL-6, IL-10, Interferon-Gamma (IFN-γ), Inducible Nitric Oxide Synthase (iNOS)
Ovalbumin/Aminophylline

Cellular/Molecular Mechanism of Action: Ovalbumin is the avian equivalent to mammalian serum albumin, and it can function as a potent immunogen/allergen. Aminophylline inhibits phosphodiesterase in a manner that directly inhibits the degradation of cAMP, thereby promoting bronchial smooth muscle relaxation.
Background Information: Evidence has shown that aminophylline has bronchoprotective, anti-inflammatory, and immunomodulatory effects.
Route of Administration: Aminophylline (intraperitoneal/rat).
Dosage: Aminophylline (Group 1 = 25 mg/kg; Group 2 = 5 mg/kg).
Implementation: Evaluation of the effect of different doses of aminophylline on the late-phase reaction, bronchial hyperresponsiveness (BHR), and T cell-related cytokine messenger RNA (mRNA) expression induced by ovalbumin (OA) sensitization.

In this context, aminophylline can be used as a molecular probe for evaluating immune and septic inflammatory reactions. The effect of aminophylline can be evaluated at different concentrations to determine its influence on respiratory function based on the interpretation of pulmonary function tests that are performed at baseline and after varying doses of acetylcholine. Thereafter, bronchoalveolar lavage and histiological analysis of the lungs is performed. Total RNA is extracted from lung tissue and reverse transcriptase-polymerase chain reaction performed using primers for IL-2, IL-4, IL-5, IL-6, IL-10, IFN-γ, iNOS, and β-actin.

Animal Model: Rat (n = 40 Brown Norway strain divided into 4 groups. Groups I, II, and III sensitized and subsequently provoked with OA). Group I (aminophylline at 25 mg/kg); Group II (aminophylline at 5 mg/kg); Group III (intraperitoneal normal saline); Group IV (enhaled aerosol saline control).
Interpretation and Analysis: Group III subjects have worse pulmonary function tests, more severe BHR, and more severe lung inflammation, higher IL-4 and IL-10 cytokine levels in bronchoalveolar lavage fluid, and higher IL-4, IL-5, IL-6, IL-10, tumor necrosis factor-alpha (TNF-α), and iNOS mRNA expression than the other 3 groups. Expression of IL-2 and IFN-γ is significantly reduced in Group III. Aminophylline at both low and high doses is effective in preventing late-phase bronchoconstriction, BHR, and an inflammatory response. Aminophylline decreases T-helper cell 2-related cytokine mRNA expression but increases T-helper cell 1-related cytokine mRNA expression.

Pulmonary function changes and immunomodulation of Th2 cytokine expression induced by aminophylline after sensitization and allergen challenge in Brown Norway rats. Ann Allergy Asthma Immunol 2002 Feb; 88 (2), pp. 215–22.

Immune Hypersensitivity Reactions: Bronchial Responsiveness: Hyperresponsiveness/Allergic Airway Eosinophilia Reactions/Type T-Helper 1 (Th1) and Th2 Cytokine Expression Profiles
Ovalbumin/Dexamethasone/Cyclosporine

Cellular/Molecular Mechanism of Action: Ovalbumin is the avian equivalent of mammalian serum albumin and can be applied as an immunogenic protein fraction. Dexamethasone is a corticosteroid that has an ability to inhibit phospholipase A2 biochemical activity.
Physiological Mechanism of Action: Dexamethasone is a corticosteroid that promotes immunosuppressive properties in part due to inhibition of leukocyte degranulation phenomenon and general inhibition of protein synthesis.
Background Information: Cyclosporine A and dexamethasone exhibit different effects on allergic airway eosinophilia and bronchial hyperresponsiveness (BHR).
Application: Characterization of the molecular pathogenesis of normal and hypersensitivity immune responses including alteration

of cytokine expression such as changes in the profile of Th1- vs Th2-derived cytokines. The investigative strategy can also be applied to delineate the efficacy of various therapeutic agents possessing anti-inflammatory and immunosuppressive properties.

Implementation: Evaluation of the influence of cyclosporine or dexamethasone on the development of immune hypersensitivity reactions to ovalbumin aerosol challenge. Bronchial responsiveness is measured 18–24 hours after aerosol exposure. Airway cellular influx can be determined by bronchoalveolar lavage and tissue immunohistochemistry. Expression of Th1 and Th2 cytokine messenger RNA (mRNA) are then analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR).

Animal Model: Rat (Brown Norway rats sensitized to ovalbumin).

Interpretation and Analysis: Ovalbumin exposure induces significant bronchial hypersensitivity reactions (BHR), with increases in eosinophils, lymphocytes, and neutrophils in bronchoalveolar lavage fluid and an increase in eosinophils, $CD2^+$, and $CD8^+$, but not $CD4^+$ T cells, in the airway submucosa. Interleukin (IL)-2, interferon-gamma (IFN-γ), IL-4, and IL-5 mRNA expression in ovalbumin-sensitized lungs increases ($p < 0.05$) compared with controls. Cyclosporine A does not have a significant effect on BHR and neutrophil accumulation but reduces the number of bronchoalveolar lavage eosinophils ($p < 0.002$), airway submucosal eosinophils, and $CD4^+$ and $CD8^+$ T cells ($p < 0.02$). It also suppresses induced expression of IL-2, IL-4, IL-5, and IFN-γ mRNA transcripts. By contrast, the inflammatory cell influx and mRNA expression of IL-2, IL-4, and IL-5, as well as BHR, is suppressed by dexamethasone. However, increases in IL-10 and IFN-γ mRNA expression can sometimes not be detected depending on the analytical system evaluated. The differential activities of cyclosporine A and dexamethasone on inflammatory cell influx, particularly neutrophils, or cytokine expression such as IL-10 and IFN-γ may underlie their contrasting effects on BHR.

Diagnostic pharmacology: Differential effect of corticosteroids and cyclosporine differential regulation of cytokine expression after allergen exposure of sensitized rats by cyclosporine A and corticosteroids: Relationship to bronchial hyperresponsiveness. Huang TJ, Newton R, Haddad EB, Chung KF. J Allergy Clin Immunol 1999 Sep; 104 (3 Pt 1), pp. 644–52.

Immune Hypersensitivity Reactions: Bronchial Responsiveness: Hyperresponsiveness/Allergic Airway Eosinophilia Reactions/Type T-Helper 1 (Th1) and Th2 Cytokine Expression Profiles
Ovalbumin/Dexamethasone/Cyclosporine

Cellular/Molecular Mechanism of Action: Ovalbumin is the avian equivalent of mammalian serum albumin and can be applied as an immunogenic protein fraction. Dexamethasone is a corticosteroid that has an ability to inhibit phospholipase A2 biochemical activity.

Physiological Mechanism of Action: Dexamethasone is a corticosteroid that promotes immunosuppressive properties in part due to inhibition of leukocyte degranulation phenomenon and general inhibition of protein synthesis.

Background Information: Cyclosporine A and dexamethasone exhibit different effects on allergic airway eosinophilia and bronchial hyperresponsiveness (BHR).

Application: Characterization of the molecular pathogenesis of normal and hypersensitivity immune responses including alteration of cytokine expression such as changes in the profile of Th1- vs Th2-derived cytokines. The investigative strategy can also be applied to delineate the efficacy of various therapeutic agents possessing anti-inflammatory and immunosuppressive properties.

Implementation: Evaluation of the influence of cyclosporine or dexamethasone on the development of immune hypersensitivity reactions to ovalbumin aerosol challenge. Bronchial responsiveness is measured 18–24 hours after aerosol exposure. Airway cellular influx can be determined by bronchoalveolar lavage and tissue immunohistochemistry. Expression of Th1 and Th2 cytokine messenger RNA (mRNA) is then analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR).

Animal Model: Rat (Brown Norway rats sensitized to ovalbumin).

Interpretation and Analysis: Ovalbumin exposure induces significant bronchial hypersensitivity reactions (BHR), with increases in eosinophils, lymphocytes, and neutrophils in bronchoalveolar lavage fluid and an increase in eosinophils, $CD2^+$, and $CD8^+$, but not $CD4^+$ T cells, in the airway submucosa. Interleukin (IL)-2, interferon-gamma (IFN-γ), IL-4, and IL-5 mRNA expression in ovalbumin-sensitized lungs increases ($p < 0.05$) compared with controls. Cyclosporine A does not have a significant effect on BHR and neutrophil accumulation but reduces the number of bronchoalveolar lavage eosinophils ($p < 0.002$), airway submucosal eosinophils, and $CD4^+$ and $CD8^+$ T cells ($p < 0.02$). It also suppresses induced expression of IL-2, IL-4, IL-5, and IFN-γ mRNA transcripts. By contrast, the inflammatory cell influx and mRNA expression of IL-2, IL-4, and IL-5, as well as BHR, is suppressed by dexamethasone. However, increases in IL-10 and IFN-γ mRNA expression can sometimes not be detected depending on the analytical system evaluated. The differential activities of cyclosporine A and dexamethasone on inflammatory cell influx, particularly neutrophils, or cytokine expression such as IL-10 and IFN-γ may underlie their contrasting effects on BHR.

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Immune Hypersensitivity Response/Allergen: Grass and Pollen Immune Response/Late Induction (Nasal Mucosa)/Grass/Pollen Hypersensitivity Immune Responses/Efficacy Evaluation of Anti-Inflammatory/Immunosuppresive Agents
Allergen Challenge Test/Fluticasone Proprionate

Cellular/Molecular Mechanism of Action: Grass/pollen extracts function as allergens that activate immune systems that have been presensitized by exposure to these biological antigens. Fluticasone proprionate is a corticosteroid preparation that can inhibit phospholipase A2 biochemical activity.

Physiological Mechanism of Action: Fluticasone proprionate can exert anti-inflammatory and immunosuppressive properties by decreasing prostaglandin production, inhibition of protein synthesis, and stabilization of cellular protein structures.

Background Information: Allergen-induced late nasal responses are associated with recruitment and activation of T lymphocytes and eosinophils accompanied by preferential messenger RNA (mRNA) expression of T-helper type 2 (Th2) cytokines.

Application: Fluticasone proprionate can be applied as a molecular probe for delineating endogenous inflammatory mediator profiles in conditions of immune hyperreactivity responses and inflammatory reactions in addition to evaluation of the efficacy of immunosuppressive and anti-inflammatory reagents.

Implementation: Detection of allergens responsible for inducing allergic reactions and evaluating the efficacy of potential therapeutic

agents (e.g., anti-inflammatory agents and corticosteroids). An example in this regard is the application of allergen challenge to evaluate the efficacy of topical corticosteroids (fluticasone propionate) and their ability to inhibit late responses by suppressing cellular transcription of Th2-type cytokine mRNA sequences. Nasal biopsies are taken at baseline and repeated 24 hours after local nasal allergen provocation following 6 weeks of treatment (e.g., fluticasone propionate 200 μg or placebo nasal spray twice daily).

Human Model: Cases of grass pollen sensitivity (n = 48 adults) analyzed in a randomized double blind placebo-controlled trial.

Interpretation and Analysis: Baseline mRNA expression for interleukin-4 (IL-4) ($p = 0.01$) and IL-5 ($p = 0.002$) is higher in cases of grass pollen sensitivity. Topical corticosteroid treatment significantly inhibits immediate nasal symptoms, with almost complete inhibition of the late response following allergen challenge. This is associated with a marked decrease in the allergen-induced increases in cells expressing mRNA for IL-4 ($p = 0.002$) but not for IL-5. Inhibition of the late response is also accompanied by decreases in CD25+ cells, presumed T lymphocytes, and eosinophils. A significant correlation exists between decreases in IL-4 mRNA+ cells and in eosinophils after treatment ($r = 0.46$, $p < 0.05$). Therefore, prolonged treatment with topical corticosteroid inhibits allergen-induced early and late nasal responses and the associated tissue eosinophilia, which at least in part may result from inhibition of cells expressing IL-4 mRNA sequences.

Topical glucocorticosteroid (fluticasone propionate) inhibits cells expressing cytokine mRNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. Masuyama K, Jacobson MR, Rak S, Meng Q, Sudderick RM, Kay AB, Lowhagen O, Hamid Q, Durham SR. Immunology 1994 Jun; 82 (2), pp. 192–9.

Immune Hypersensitivity: Rhinitis (Allergic)/(Asthma)
Adenosine 5′-Monophosphate (AMP)/Methacholine (MCh)/[Forced Expiratory Volume in 1 Second (FEV1)]

Physiological Mechanism of Action: Adenosine and its related nucleotide, AMP, both induce bronchoconstriction in asthma and asthma-like conditions. Such events are probably caused by histamine release from airway mast cell populations.

Application: Adenosine 5′-monophosphate can be used in the detection and characterization of conditions including allergic rhinitis and asthma.

Route of Administration: Adenosine 5′-monophosphate is utilized as an inhaled agent.

Dosage: Adenosine 5′-monophosphate.

Implementation: Evaluation of allergic rhinitis conditions detected by challenge with exposure to increasing concentratons of AMP and MCh. Airflow is assessed after the administration of each different concentration of provocative agent. Responses to each bronchoconstrictor agent are then measured pertaining to the provocative concentration required to produce a 20% fall (PC20) in FEV1.

Interpretation and Analysis: A very high percentage of asthma cases (e.g., n = 14/14) and approximately 30% of allergic rhinitis cases (e.g., n = 10/28) display signs of AMP hyperresponsiveness. Subjects with allergic rhinitis have a higher prevalence of hyperresponsiveness to AMP than do healthy controls ($p = 0.038$). Although the prevalence of hyperresponsiveness for MCh and for AMP in subjects with allergic rhinitis is similar (39% vs 36%, respectively), some cases will be hyperresponsive to (i) MCh but not to AMP (e.g., n = 4/28); or (ii) hyperresponsiveness to AMP but not to MCh (e.g., n = 3/28). In this context, inhaled AMP causes airway narrowing in a significantly higher proportion of subjects with allergic rhinitis than healthy volunteers. Furthermore, MCh and AMP hyperresponsiveness are not detected in the same individuals with allergic rhinitis, thus suggesting that responsiveness to the 2 bronchoconstrictor stimuli is not evoking the same type of abnormalities within the airways.

Bronchoconstriction induced by inhaled adenosine 5′-monophosphate in subjects with allergic rhinitis. Prieto L, Gutierrez V, Linana J, Marin J. Eur Respir J 2001 Jan; 17 (1), pp. 64–70.

Immune Hypersensitivity: Rhinitis (Allergic)/Asthma with Airway Inflammation
Adenosine 5′ Monophosphate (AMP)/Methacholine (MCh)/(Exhaled Nitric Oxide)

Background Information: Some studies have reported that the levels of exhaled nitric oxide (ENO) in asthmatics are similar to those in subjects with allergic rhinitis, and it has been postulated that atopic status might be the determinant of enhanced nitric oxide production in asthma.

Implementation: Adenosine 5′-monophosphate and MCh have been employed to determine the differences in ENO levels between asthmatics and subjects with allergic rhinitis (sensitized to the same allergen), in order to correlate nitric oxide concentrations with airway responsiveness. Such clinical investigative strategies have been employed for the purpose of evaluating cases of asthma and allergic rhinitis monosensitized to Parietaria pollen. The response to each bronchoconstrictor agent is measured by the provocative concentration required to produce a 20% fall in forced expiratory volume in 1 second (FEV1) (PC20), and ENO can be measured by the single-exhalation method.

Interpretation and Analysis: The geometric mean (95% confidence interval) ENO values are significantly higher in asthmatics than in subjects with allergic rhinitis: 72.4 p.p.b. (54.9–93.3 p.p.b) vs 44.7 p.p.b. (30.9–64.6 p.p.b., $p = 0.03$). In asthmatics, a significant correlation can be found between ENO and PC20 AMP values ($p = -0.57$, $p = 0.02$), whereas no correlation can be detected between ENO and PC20 MCh ($p = -0.35$, $p = 0.14$). It is therefore suggested that atopy is not the only determinant of increased ENO levels detected in subjects with asthma, and that responsiveness to AMP may be a more sensitive marker for assessing airway inflammation in asthma compared to MCh.

Concentrations of exhaled nitric oxide in asthmatics and subjects with allergic rhinitis sensitized to the same pollen allergen. Prieto L, Gutierrez V, Uixera S, Bruno L. Clin Exp Allergy 2002 Dec; 32 (12), pp. 1728–33.

Immune Hypersensitivity: Rhinitis (Allergic) and Asthma
Acetaldehyde

Background Information: Asthmatic subjects have an exaggerated airway response to inhaled acetaldehyde, but no information is available on airway responsiveness to this bronchoconstrictor agent in subjects with allergic rhinitis.

Application: Characterization of cases suffering from allergic rhinitis.

Route of Administration: Inhalation.

Dosage: Gradient increases with pulmonary function evaluated after each change in concentration inhaled.

Considerations: Minimal information is available about airway responsiveness to acetaldehyde as a bronchoconstrictor agent in subjects with allergic rhinitis.

Implementation: Acetaldehyde and methacholine (MCh) have been employed at gradient concentration in provocative testing procedures to characterize conditions of allergic rhinitis and asthma. The response to each bronchoconstrictor agent is measured by the provocative concentration required to produce a 20% fall in forced expiratory volume over 1 second [FEV1/PC20].

Interpretation and Analysis: The geometric mean PC20 acetaldehyde value for asthmatics is approximately 35.5 mg/ml compared with 67.6 mg/ml in subjects with allergic rhinitis and with 80.0 mg/ml for healthy reference control subjects ($p < 0.001$). The PC20 acetaldehyde values in cases of allergic rhinitis are also significantly lower than in healthy negative reference controls ($p = 0.04$). A large percentage of subjects with allergic rhinitis with an increased responsiveness to acetaldehyde show MCh-induced airway hyperresponsiveness (e.g., n = 43/43). However, some subjects (e.g., n = 9/43) with a hyperresponsiveness to MCh fail to respond to acetaldehyde. Clinical evaluations of acetaldehyde have therefore suggested that subjects with allergic rhinitis are less responsive to inhaled acetaldehyde than cases of asthma, but more so than are healthy reference controls. Furthermore, only approximately half of the cases of allergic rhinitis and airway hyperresponsiveness to MCh exhibit bronchoconstriction with inhaled acetaldehyde, thereby suggesting that airway hyperresponsiveness to MCh may not be the sole factor leading to bronchoconstriction in response to acetaldehyde.

Airway responsiveness to inhaled acetaldehyde in subjects with allergic rhinitis: Relationship to methacholine responsiveness. Prieto L, Sanchez-Toril F, Gutierrez V, Marin MJ. Respiration 2002; 69 (2), pp. 129–5.

Immune Hypersensitivity: Rhinitis/Nasal/(Gastric and Duodenal Ulcers)
Cimetidine and Famotidine/Histamine

Cellular/Molecular Mechanism of Action: Cimetidine functions as an H_2 receptor antagonist that inhibits cellular cAMP synthesis (H_2 receptor agonists promote mast cell membrane stabilization).

Application: Histamine can be applied as an agent for assessing the efficacy/anti-inflammatory properties of candidate agents.

Implementation: Determine the influence of H_2-blockers on nasal hypersensitivity reactions following histamine challenge.

Interpretation and Analysis: A 7-day administration of cimetidine tends to lower the histamine threshold in contrast to famotidine administration, which does not significantly decrease the histamine threshold. Therefore, it has been postulated that famotidine should be preferred to cimetidine as an H_2-blocker in patients with gastric and/or duodenal ulcers.

Effect of H_2-blockers, cimetidine and famotidine, on histamine nasal provocative test. Ogino S, Irifune M, Harada T, Matsunaga T ORL. J Otorhinolaryngol Relat Spec 1992; 54 (3), pp. 152–4.

Immune Hypersensitivity Rhinitis (Allergic)/Type T-Helper 2 (Th2) Cytokine Expression Responses/ Interleukin (IL)-13 Expression
Grass Pollen Extract/Fluticasone Propionate/Nasal Tissue Biopsies

Cellular/Molecular Mechanism of Action: Grass pollen extract can function as an allergen in immunehypersensitivity reactions. Fluticasone proprionate can inhibit phospholipase A biochemical activity.

Physiological Mechanism of Action: Fluticasone proprionate can promote immunesuppressive responses and anti-inflammatory properties in part due to their ability to inhibit both protein synthesis and leukocyte degranulation events.

Background Information: The allergen-induced late nasal response (LNR) is associated with high expression of IL-4 and IL-5 messenger RNA (mRNA) in the nasal mucosa, suggesting a role for Th2-type cytokines in the development of the LNR. Moreover, topical corticosteroid-mediated inhibition of the LNR is accompanied by inhibition of IL-4, but not IL-5 mRNA expression. Interestingly, IL-13 shares a number of functions with IL-4, including IgE switching and vascular cell adhesion molecule-1 (VCAM-1) upregulation.

Application: Grass pollen extract can be used to initiate inflammatory and immune responses in conditions of immunehyperactive immune responses associated with this source of allergen. Fluticasone can be used as a probe to study alterations in the production of endogenous mediators of inflammatory and immune responses to allergen-induced responses.

Implementation: Evaluate the influence of fluticasone propionate on the expression of IL-4 and IL-13 mRNA sequences and immunoreactivity in nasal mucosa tissue biopsy specimens. The design of investigations to address this objective can involve the administration of either topical or inhaled fluticasone propionate in conditions of allergic rhinitis. Nasal biopsies are harvested before treatment and 24 hours after local nasal allergen provocation with a grass pollen extract.

Human Model: Nasal biopsy tissue specimens in conditions of allergic rhinitis (n = 20; n = 10 normal healthy reference control; n = 10/20 enhaled fluticasone propionate; n = 10/20 topical fluticasone propionate b.i.d. for 6 weeks).

Interpretation and Analysis: No significant difference between the allergic rhinitis patients and controls in the expression of IL-13 mRNA and immunoreactivity can be detected prior to treatment.

After allergen provocation/challenge, significant increases in IL-13 mRNA-positive and immunoreactive cells at 24 hours can be detected in the absence of corticosteroid exposure (placebo $p < 0.001$). Inhibition of the LNR after corticosteroid treatment is associated with a marked decrease in allergen-induced IL-13 mRNA-positive ($p < 0.001$) and immunoreactive cells ($p < 0.001$). In the absence of steroid exposure (e.g., placebo), 76.9 ± 5.5% of IL-13 mRNA-positive cells after allergen are $CD3^+$, whereas 11.2 ±2.7% co-express immunoreactivity for mast-cell tryptase. In these cases, increases in cells expressing IL-13 mRNA are greater than for IL-4 mRNA ($p = 0.001$), and double in situ hybridization studies reveal that 100% of the IL-4 mRNA-positive cells co-express IL-13 mRNA, whereas 66.6 ± 10.5% of IL-13 mRNA-positive cells co-express IL-4 transcripts after allergen challenge. Such findings suggest that IL-13 expression is a prominent feature of the LNR, and that inhibition of the LNR following steroid therapy may be partly attributable to inhibition of IL-13 expression.

IL-13 mRNA and immunoreactivity in allergen-induced rhinitis: Comparison with IL-4 expression and modulation by topical glucocorticoid therapy. Ghaffar O, Laberge S, Jacobson MR, Lowhagen O, Rak S, Durham SR, Hamid Q. Am J Respir Cell Mol Biol 1997 Jul; 17 (1), pp. 17–24.

Inflammatory Responses: Molecular Pathogenesis/ Nasal Mucosa Cyclooxygenase (COX)-1 and COX-2 Synthesis
Dexamethasone/Interleukin (IL)-10/Actinomycin-D/ Cyclohexamide/Proinflammatory Mediators (Interferon-Gamma [IFN-γ], IL-1β, and Tumor Necrosis Factor-Alpha [TNF-α])

Cellular/Molecular Mechanism of Action: Actinomycin-D (dactinomycin) intercalates between DNA strands within the DNA

helix at adjacent guanine-cytosine base pairs, resulting in the formation of a stable complex. Dexamethasone inhibits phospholipase A2 biochemical activity.

Physiological Mechanism of Action: Dexamethasone functions as an immunosuppressive and anti-inflammatory agent due to an ability to inhibit normal protein synthesis and stabilize cell membranes in a manner sufficient to inhibit cell activation and leukocyte degranulation events. Actinomycin-D inhibits (blocks) RNA polymerase-mediated transcription events. Alternatively, cyclohexamide inhibits translation events. Interleukin IL-10 exerts relatively profound anti-inflammatory properties.

Background Information: Cyclooxygenase converts arachidonic acid in prostanoids. Cyclooxygenase exists in 2 isoforms; COX-1 is the constitutive, whereas COX-2 is the inducible isoform. The regulation of COX-1 and COX-2 expression in nasal mucosa has not been extensively investigated.

Application: Characterization of the molecular pathogenesis of disease states, immune reactions/responses, and efficacy of various forms of therapeutic intervention. Dexamethasone, therefore, can be utilized as a probe for delineating the role of endogenous inflammatory mediators in immune responses and inflammatory reactions.

Implementation: Evaluation of alterations in endogenous COX-1 and COX-2 production *in vitro* in nasal biopsy tissues applying agents that promote proinflammatory and anti-inflammatory properties, in addition to the utilization of molecular probes that function as inhibitors of transcription and translation events. In this manner, it is possible to gain greater understanding about mechanisms involved in the expression and regulation of COX-1 and COX-2 by cytokines and corticosteroids. The specific design of such investigations can entail employing the use of cultured normal/healthy human nasal explants exposed/incubated with individual agents known to promote (i) proinflammatory properties (IFN-γ, IL-1β, and TNF-α); and (ii) anti-inflammatory properties (dexamethasone; IL-10) and inhibitors of translation (actinomycin-D) and transcription (cyclohexamide) events. Tissue culture preparations are subsequently analyzed for the detection of alterations in COX-1 and COX-2 expression applying semi-quantitative competitive polymerase chain reaction (PCR) and Western blot analyses.

Human *Ex Vivo* Model: Nasal mucosa biopsy tissues.

Interpretation and Analysis: Baseline expression of COX-2 messenger RNA (mRNA) is higher than COX-1 mRNA. In a tissue culture environment, there is a slight spontaneous upregulation of COX-1 and a strong COX-2 mRNA and protein upregulation. Incubation of nasal explants with proinflammatory cytokines increases the expression of COX-2 mRNA and protein, from 1 to 24 hours of incubation in a dose-related manner. Regulation of these effects occurs at the levels of both transcriptional and posttranscriptional phases. Dexamethasone and IL-10 abrogate cytokine-induced COX-2 mRNA and protein expression. Proinflammatory cytokines, dexamethasone, and IL-10 have no effect on COX-1 mRNA expression. Prostanoids therefore appear to have important regulatory effects on immunologically (cytokine) mediated inflammatory responses.

Regulation of cyclooxygenase-1 and -2 expression in human nasal mucosa. Effects of cytokines and dexamethasone. Fernández-Morata JC, Mullol J, Fuentes M, Pujols L, Roca-Ferrer J, Pérez M, Xaubet A, Picado C. Clin Exp Allergy 2000 Sep; 30 (9), pp. 1275–84.

Surfactant: Respiratory/Surfactant Distribution
Fluorescent Bodipy-Labeled Surfactant Protein B

Cellular/Molecular Mechanism of Action: Surfactant lowers the viscosity (surface tension) of pulmonary fluid in a manner that prevents collapse of terminal alveoli.

Application: Fluorescent bodipy-labeled surfactant protein B can be applied to determine the distribution of endotracheally administered surfactant at the alveolar level in an animal model of acute respiratory distress syndrome.

Route of Administration: Intratracheal.

Semi-Synthetic Methods: Covalent conjugation/cross-linking of fluorescent bodipy-labeled surfactant protein B.

Implementation: To measure surfactant distribution *in vitro*, a glass trough mimicking dichotomic lung anatomy can be used to determine the spreading properties of bovine lung surfactant extract supplemented with fluorescent bodipy-labeled surfactant protein B. To measure surfactant distribution *in vivo*, anesthetized rats are exposed to aerosolized lipopolysaccharide (12 mg/kg body weight) to induce lung injury that resembles acute respiratory distress syndrome. In control rats, buffered saline is alternatively aerosolized. Twenty-four hours later rats are anesthetized, tracheotomized, and mechanically ventilated (peak airway pressure = 20 mbar; positive end-expiratory pressure = 6 mbar; inspiration time = expiration time = 0.6 seconds; Fio2 = 50%). Surfactant (bovine lung surfactant extract, supplemented with fluorescent bodipy-labeled surfactant protein B; 50 mg/kg body weight) is given as a bolus; in control rats, a bolus of saline is administered. Rats are then ventilated for 5, 15, 30, or 60 minutes (n = 8 or 9 for each group) and their lungs excised and sliced. Lung slices, divided into aerated (open), underinflated (dystelectatic), or collapsed (atelectatic) alveolar areas are subsequently examined by both light and fluorescence microscopy.

Interpretation and Analysis: Surfactant spreads independent of glass trough geometry and lowers the surface tension to equilibrium values (25 mN/m) within a few seconds in *in vitro* preparations. Administration of surfactant *in vivo* distributes preferentially into underinflated and aerated alveolar areas. Surfactant distribution is not affected by length of mechanical ventilation. When conventional mechanical ventilation is used in lipopolysaccharide-induced lung injury, surfactant preferentially distributes into underinflated and aerated alveolar areas. Because surfactant rarely reaches collapsed alveolar areas, methods aiding in alveolar recruitment (e.g., open lung concept or body positioning) should precede surfactant administration.

I n vitro and *in vivo* intrapulmonary distribution of fluorescently labeled surfactant. Diemel RV, Walch M, Haagsman HP, Putz G. Crit Care Med 2002 May; 30 (5), pp. 1083–90.

BREATH TESTS

Note: Additional breath tests have been included in the hepatic metabolism section (see chapter 6).

Carbon Group Metabolization/Trans-Methylation, Trans-Sulfuration, Remethylation/ Hyperhomocysteinemia: Endstage Renal Failure/Single-Carbon Group Transfer (5,10-Methylenetetrahydrofolate)/Hepatic Cytosol and Mitochondria
Carbon Dioxide Breath Tests/[$^{2}H_3$C-1-[^{13}C]-Methionine/[2,3,3-$^{2}H_3$] Serine

Cellular/Molecular Mechanism of Action: Substrates that can be applied as "probes" to detect alterations in the biochemical activity of specific enzyme systems involved in metabolic pathways.

Application: Estimation of trans-methylation, trans-sulfuration, and remethylation rates, and evaluation of carbon group transfer.

Route of Administration: Intravenous.

Implementation: Evaluation of whole-body trans-methylation, trans-sulfuration, and remethylation rates (e.g., [$^{2}H_3$C-1-[^{13}C]-

methionine). Following administration, carbon radioisotope contained in exhaled air as carbon dioxide is then measured. Preparations of [2,3,3-$^{2}H_3$] serine can be applied to evaluate the transfer of a 1-carbon group from serine via 5,10-methylenetetrahydrofolate in human hepatocyte cytosol and mitochondria, respectively.

Animal Model: Ovine.

Interpretation and Analysis: The amount of carbon radioisotope contained in exhaled air is directly proportional to the rate at which a molecular substrate probe is metabolized *in vivo* following injection. Tissue-free methionine enrichments after infusion of universally labeled [U-^{13}C] methionine show the highest remethylation activity in postmortem investigation of jejunum, liver, and kidney tissue samples, but no such activity can be appreciated in muscle and brain preparations.

Instrumentation: Methods to quantitate 1-carbon acceptor metabolism pathways and folate metabolism have recently become available.

In vivo stable isotope measurements of methyl metabolism: Applications in pathophysiology and interventions. de Meer K, van den Akker JT, Smulders Y, Stam F, Stehouwer CD, Finglas P. Food Nutr Bull 2002 Sep; 23 (3 Suppl), pp. 113–9.

Carboxylic Acid Metabolism/Nitrogen Metabolism/ Gastrectomy, Hepatic Failure, Renal Failure, Myotonic Dystrophy
1-[^{14}C-Alpha-Ketoisovaleric Acid (KIV)/[$^{14}CO_2$ Breath Excretion Test]

Cellular/Molecular Mechanism of Action: 1-[^{14}C]-alpha-ketoisovaleric acid is a keto analogue of valine and can function as a biochemical substrate for mechanisms of carboxylic acid metabolism.

Background Information: The keto analogues of essential amino acids represent a promising therapeutic modality in hereditary and acquired disorders of nitrogen metabolism. The utilization of these substances in humans has been assayed primarily by nitrogen balance studies.

Application: Endogenous biochemical metabolism of KIV can be measured using the $^{14}CO_2$ breath excretion test. In this context, the agent can be applied as a tool for evaluation of certain diet formulations.

Route of Administration: Oral.

Implementation: Assessement of the *in vivo* decarboxylation of KIV measurement of $^{14}CO_2$ using a simple and accurate breath excretion test. Protein diets (5 g) supplemented with essential amino acids and KIV (in place of valine) are then evaluated. Diets of 120 g protein/day are then reevaluated in normal reference controls and postoperative gastrectomy cases.

Human Model: Normal volunteers and diseased cases (n = 6 diseased; gastrectomy, hepatic failure, renal failure, and myotonic dystrophy).

Interpretation and Analysis: In situations of low protein intake, 13–32% of ingested KIV undergoes rapid decarboxylation, and this proportion appears to correlate inversely with damage to organ systems containing the branched-chain keto acid dehydrogenase. With high protein intake, the proportion of decarboxylated increases to 44% to 53%. Such findings confirm that the decarboxylation of KIV in man varies under different conditions of dietary intake and metabolic disease. The $^{14}CO_2$ breath excretion test is applicable to other related analyses of carboxylic acid metabolism in human subjects.

Decarboxylation of alpha-ketoisovaleric acid after oral administration in man. Epstein CM, Chawla RK, Wadsworth A, Rudman D. Am J Clin Nutr 1980 Sep; 33 (9), pp. 1968–74.

Enteropathy: Childhood
Lactose Breath Hydrogen Test/D-Xylose Absorption Test/[Jejunal Biopsy Alternative]

Cellular/Molecular Mechanism of Action: Lactose and D-xylose are normally absorbed across the surface of normal/healthy intestinal mucosa.

Background Information: Three widely used screening tests for enteropathy in childhood are the lactose breath hydrogen test, the 1-hour serum D-xylose absorption test, and the 72-hour fecal fat determination.

Application: Lactose and D-xylose can be applied to detect alterations in the absorptive function of the intestinal mucosa.

Route of Administration: Lactose and D-xylose (oral/per os).

Implementation: Correlate findings from the lactose hydrogen breath test, D-xylose absorption test, and 72-hour fecal fat determination with results from jejunal biopsy evaluations in cases of juvenile enteropathy.

Interpretation and Analysis: Results from D-xylose and fecal fat tests each significantly correlate with jejunal biopsy results (e.g., normal result of either test is highly predictive of a normal biopsy finding). Combining the results of 2 or more screening tests does not improve predictive value. The xylose test is preferable on the basis of greater sensitivity and ease of patient compliance.

Screening tests for enteropathy in children. Levine JJ, Seidman E, Walker WA. Am J Dis Child 1987 Apr; 141 (4), pp. 435–8.

Gastric Emptying/Gastric Motility
[^{13}C]-Acetate/[Breath Test]

Cellular/Molecular Mechanism of Action: [^{13}C]-acetate is a radioactive analog of acetic acid that is absorbed from the gastrointestinal tract, enters the portal circulation, is removed from the portal circulation by the liver, and then is metabolized within hepatocytes in a manner that results in the generation of $^{13}CO_2$, which is then exhaled from the respiratory system.

Background Information: Elderly people frequently have symptoms of fullness and appetite loss due to impaired gastric motor activity. These symptoms may cause malnutrition, immunosuppression, and other complications.

Application: Evaluation of gastric motility by [^{13}C]-acetate breath test in combination with electrogastrography techniques.

Route of Administration: [^{13}C]-acetate (oral ingestion).

Implementation: Electrogastrography evaluation of gastric emptying before and after oral ingestion of [^{13}C]-acetate-mixed liquid meal. Expired air is then sampled every 10 minutes for the measurement of $^{13}CO_2$ concentration.

Human Model: Cases of advanced age (n = 7 active/healthy; n = 7 reduced mental/physical capacities with inactive behaviors; n = 7 young healthy volunteers).

Interpretation and Analysis: The ratio of the incidence of the 3-cpm wave (gastric intrinsic frequency) during the postprandial period compared to the fasting state is reduced in active and inactive geriatric cases compared to young adults. Such reductions are greater in inactive elderly cases than in active elderly cases. The ratio of the amplitude of the peak frequency during the postprandial period to that in the fasting state (power ratio) also decreases with advanced age. The time of peak $^{13}CO_2$ expiration is delayed in the active elderly cases, but more so in inactive elderly. Postprandial peristalsis and gastric contractile force are reduced in the elderly, and gastric emptying is delayed, indicating a reduction in gastric motor activity.

Evaluation of gastric motor activity in the elderly by electrogastrography and the [^{13}C]-acetate breath test. Shimamoto C, Hirata I, Hiraike Y, Takeuchi N, Nomura T, Katsu K. Gerontology 2002 Nov–Dec; 48 (6), pp. 381–6.

Gastric Emptying/Gastric Motility (Delayed)/ Functional Dyspepsia
[^{13}C]-Octanoic Acid/[Breath Test]

Background Information: As a non-invasive modality by which to evaluate the gastric emptying of a solid meal, the [^{13}C]-octanoic acid breath test has recently become more widely used. Previously, ultrasonography has also been reported as another non-invasive and reliable method for assessing gastric motility.
Application: Evaluation of gastric motility.
Route of Administration: [^{13}C]-octanoic acid (oral).
Dosage: [^{13}C]-octanoic acid (100 mg).
Implementation: Evaluation of gastric emptying applying the solid test meal consisting of a scrambled egg labeled with [^{13}C]-octanoic acid (100 mg) served with a bowl of rice and boiled chicken (total 424 kcal). After ingestion of the test meal, examinations are performed in a sitting position. Ultrasonography images are obtained every 15 minutes for 3 hours. Breath sampling schedules follow the same time schedule as for ultrasonographic imaging, with an additional 3 hours of sampling at 30-minute intervals. Results are applied to establish half emptying times ($T_{1/2}$) and lag phase.

Human Model: Cases of functional dyspnea (n = 17 compared to n = 10 normal healthy volunteers).
Interpretation and Analysis: The $T_{1/2}$ values determined by ultrasonography-based methods and the breath test are positively correlated ($r^2 = 0.638$); however, there is no significant agreement between study groups. Both the $T_{1/2}$ and the lag phase are prolonged in cases of functional dyspepsia compared with the healthy volunteers, regardless of the method of measurement. The lag phase significantly correlates ($r^2 = 0.864$) with the $T_{1/2}$ established with breath test methods. Although the [^{13}C]-octanoic acid breath test cannot assess gastric emptying of solids as reliably as ultrasonography, both tests are useful for evaluating functional dyspepsia patients with delayed gastric emptying.

Evaluation of gastric emptying measured with the [^{13}C]-octanoic acid breath test in patients with functional dyspepsia: Comparison with ultrasonography. Aoki S, Haruma K, Kusunoki H, Hata J, Hara M, Yoshida S, Tanaka S, Chayama K. Scand J Gastroenterol 2002 Jun; 37 (6), pp. 662–6.

Gastric Helicobacter Pylori: Intragastric Urease Activity
[^{13}C]-Urea Breath Test

Application: Detection and characterization of *H. pylori* populations residing within the gastrointestinal tract. The reagent can also be applied to evaluate the efficacy of therapeutic agents.
Implementation: Assessment of intragastric urease activity by the [^{13}C]-urea breath test can be performed before and after 1 day of dosing with either De-Noltabs (tripotassium dicitrato bismuthate, 1 tablet 1 q.d.s.), Pepto-Bismol liquid (bismuth salicylate 30 ml q.d.s.), or Roter tablets (bismuth subnitrate, 1 tablet q.d.s.) in 12 *H. pylori*–positive patient volunteers.
Interpretation and Analysis: There is a significant decrease in the excess of [$^{13}CO_2$] after 1 day of dosing with each of the 3 bismuth compounds, but analysis of variance detects no difference between the effects of the 3 compounds. Systemic absorption of bismuth following oral dosing with either Pepto-Bismol or Roter is minimal, yet both compounds have a suppressive effect on *H. pylori* similar to that of De-Noltab. The action of all 3 bismuth compounds is within the gastric lumen, and that systemic absorption of bismuth is not necessary for activity against *H. pylori*.

Comparison of 1-day oral dosing with 3 bismuth compounds for the suppression of Helicobacter pylori assessed by the [^{13}C]-urea breath test. Prewett EJ, Luk YW, Fraser AG, Lam WM, Pounder RE. Aliment Pharmacol Ther 1992 Feb; 6 (1), pp. 97–102.

Gastric Motility
[^{13}C]-Octane Acid/[Breath Test]

Cellular/Molecular Mechanism of Action: [^{13}C]-octane acid is a radioactive analog of octane acid that is absorbed from the gastrointestinal tract and then enters the portal circulation, where it is then removed from the portal circulation by the liver. Hepatocytes metabolize the molecule, resulting in the generation of $^{13}CO_2$ that is then exhaled by the respiratory system.
Background Information: Disordered gastric motility may be a significant factor in the pathogenesis of many equine conditions. Although tests for liquid phase emptying rate have been validated in the horse, there are no effective tests for solid phase emptying measurement that can be performed routinely in the field.
Application: Assessment of a stable isotope technique, the [^{13}C]-octane acid breath test (^{13}C-OABT), for the measurement of gastric emptying of solid ingesta, compared to the optimum method of gastric scintigraphy.
Implementation: A test meal is used containing baked egg yolk labeled with both [^{13}C]-octanoic acid and [^{99m}technetium]-sulphur colloid. Simultaneous, serial lateral gastric scintigraphic images and expiratory breath samples are obtained after voluntary test meal ingestion. Analysis of breath $^{13}CO_2$:$^{12}CO_2$ ratio is then performed by mass spectrometry. Power regression is then used to determine the gastric emptying coefficient, the gastric half-emptying time ($T_{1/2}$), and duration of the lag phase (T_{lag}).

Animal Model: Equine (n = 12 healthy horses).
Interpretation and Analysis: Significant correlations ($p < 0.001$) can be appreciated between the 2 techniques for both $T_{1/2}$ and T_{lag} measurements. In addition, scintigraphic left $T_{1/2}$ significantly correlates with breath test gastric emptying coefficients ($p < 0.001$).

It has been proposed that the [^{13}C]-octanoic acid breath test is a reliable diagnostic procedure for measuring gastric emptying rate of solids in the horse. Being safe, non-invasive, and easy to perform, this test has potential value as both sensitive diagnostic modality and humane research tool for motility studies.
Instrumentation: Continuous flow isotope ratio mass spectrometry.

Validation of the [^{13}C]-octanoic acid breath test for measurement of equine gastric emptying rate of solids using radioscintigraphy. Sutton DG, Bahr A, Preston T, Christley RM, Love S, Roussel AJ. Equine Vet J 2003 Jan; 35 (1), pp. 27–33.

Gastrointestinal: Small Intestine Motility/Orocecal Transit Time: Infants (< 8 months)
Lactose-[^{13}C]-Ureide Breath Test (LUBT)

Background Information: The lactose-[^{13}C]ureide breath test is a novel, non-invasive test to determine orocecal transit time. Lactose ureide resists the enzymatic action of brush border enzymes and is metabolized by colonic bacteria.
Application: Gastrointestinal tract tracer that can be used to determine orocecal transit time in infants < 8 months of age.
Dosage: 500 mg unlabeled lactose ureide; 250 mg lactose-[^{13}C]ureide.

Implementation: Adaptation of the lactose-[^{13}C]ureide breath test for various age groups (children) to determine whether it can be applied in infants, newborns, and preterms to study the development of small intestinal motility. In this manner, a breath test is performed after induction of unlabeled lactose ureide (500 mg) 3 times a day over a 24-hour period. A liquid test meal (chocolate milk) with lactose-[^{13}C]ureide (250 mg) is also ingested. Breath samples are collected every 15 minutes for 10 hours. In a 2nd investigation (age range, 0–3 years), only the *in vitro* stool analysis may be performed for comparison.

Stools are collected from each subject for *in vitro* stool incubation analyses. The procedure involves utilization of a blank stool sample and a sample produced after induction with unlabeled lactose ureide and then incubated with 10 mg lactose-[^{13}C]ureide within small, sealed bottles. Ten-milliliter CO_2 samples are aspirated from the bottles using a needle and a syringe every 30 minutes for 24 hours.

The LUBT results are then compared with findings from *in vitro* stool sample analyses.

Human Model: Children (n = 20 at 3 to 17 years of age; compared to n = 32 of which n = 6 at 1 to 3 years, n = 6 infants 6–12 months, n = 13 infants 0–6 months, n = 7 preterm infants).

Interpretation and Analysis: The mean orocecal transit time in cases 3–17 years of age is 255 minutes (range, 165–390 minutes). Stool incubations demonstrated a clear [$^{13}CO_2$] peak in all infants more than 8 months of age, indicating that their colonic bacterial enzymic activity hydrolyses lactose ureide. However, in all infants < 6 months of age, and in preterm infants, the [$^{13}CO_2$] signal is absent, indicating that those subjects are unable to hydrolyze lactose ureide. Infants < 6 months of age do not host the appropriate bacterial enzymic activity for splitting lactose ureide. In conclusion, the LUBT can be applied in infants more than 8 months of age, after weaning to solid foods, to determine orocecal transit time.

Lactose-[^{13}C]ureide breath test: A new, non-invasive technique to determine orocecal transit time in children. Van Den Driessche M, Van Malderen N, Geypens B, Ghoos Y, Veereman-Wauters G. J Pediatr Gastroenterol Nutr 2000 Oct; 31 (4), pp. 433–8.

Hepatic Biochemical Capacity/Age-Related Alterations/Geriatric Function/Biochemical Capacity: Changes with Age/Geriatric Liver: Changes with Advancing Age
[^{13}C]-Methacetin Breath Test

Cellular/Molecular Mechanism of Action: [^{13}C]-methacetin is a radioactive analog of methacetin that can be metabolized in the liver, yielding [$^{13}CO_2$].

Background Information: The issue of whether or not liver function is compromised in the healthy elderly population remains unsolved. However, it is known that endogenous CO_2 production changes with age, motor activity, and nutrition.

Application: Evaluation of the biochemical capacity of the liver. In this manner it can be used to investigate the putative age-related modifications of hepatic function in the form of the [^{13}C]-methacetin breath test.

Implementation: Evaluation of hepatic biochemical capacity by assessment of CO_2 production following [^{13}C]-methacetin administration. Trans-abdominal echosonography with Doppler pulsed wave analysis of the celiac axis and portal vein can be used to complement the [^{13}C]-methacetin breath test.

Human Model: Geriatric (n = 29 elderly cases; n = 17/12 female/male ratio; mean age = 79.8 +/− 7.9 years); and adults (n = 28 adults; n = 13/15 female/male ratio; mean age = 40.6 ± 12.3 years).

Interpretation and Analysis: Although the [$^{13}CO_2$] peak occurs within 15 to 30 minutes in both elderly and adult subjects, it is significantly decreased in the former (30.66% ± 9.2% vs 38.33% ± 6.05%; $p < 0.001$), as is the cumulative excretion (33.07% ± 7.06% vs 39.81% ± 5.68%; $p < 0.001$). When correcting for the effects of CO_2 excretion by age, the age-related modification of the cumulative dose becomes more evident (elderly group 30.15% ± 6.46% vs adult group 37.97% ± 5.92%; $p < 0.0001$). Elderly cases also show an increase in the intrahepatic resistance index using Doppler pulsed wave analysis that inversely correlates with results from breath tests. Hepatic function is not well preserved in healthy humans throughout life and may be due to an increase in vascular resistance.

Study of liver function in healthy elderly subjects using the [^{13}C]-methacetin breath test. Ciccocioppo R, Candelli M, Di Francesco D, Ciocca F, Taglieri G, Armuzzi A, Gasbarrini G, Gasbarrini A. Aliment Pharmacol Ther 2003 Jan; 17 (2), pp. 271–7.

Hepatic Biochemical Function/Cirrhosis (Alcohol-Induced)/Biochemical Transsulferation Capacity
[$^{2}H_3$]-Methyl-1-[^{13}C]Methionine/[$^{13}CO_2$] Exhalation

Cellular/Molecular Mechanism of Action: [$^{2}H_3$]-methyl-1-[^{13}C]methionine is a radiolabeled analog of methionine that is metabolized within the liver along the same pathways as is the amino acid methionine. In this context, methionine can become a substrate for both trans-sulfuration and remethylation metabolic pathways. Trans-sulfuration through decarboxylation of [$^{2}H_3$]-methyl-1-[^{13}C]methionine to yield [$^{13}CO_2$] is followed by remethylation involving the replacement of the labeled methyl group by an unlabeled methyl group.

Background Information: Disturbances of the methionine cycle may result in liver injury. Patients with alcohol-induced liver disease often exhibit hypermethioninemia and a delayed clearance (CL) of methionine, but the extent to which trans-sulfuration and remethylation pathways of the cyclic methionine metabolism are affected is unknown.

Application: Characterization of trans-sulfuration by its decarboxylation to [$^{13}CO_2$] and remethylation by replacement of the labeled methyl group by an unlabeled one.

Route of Administration: [$^{2}H_3$]-methyl-1-[^{13}C]methionine (oral).

Dosage: [$^{2}H_3$]-methyl-1-[^{13}C]methionine (2 mg/kg).

Implementation: Evaluation of methionine metabolism in conditions of alcohol-induced cirrhosis. Oral administration of 2 mg/kg [$^{2}H_3$]-methyl-1-[^{13}C]methionine.

Human Model: Cases of alcohol-induced cirrhosis (n = 6 cases; n = 7 normal healthy volunteers).

Interpretation and Analysis: Basal plasma concentrations of endogenous methionine (50 + 5 vs 25 + 2 μmol/L, mean ± SEM, $p < 0.001$) are significantly higher in patients with cirrhosis and its clearance is significantly decreased (774 + 103 vs 2,050 + 141 mL/min, $p < 0.001$). Methionine turnover is much slower in conditions of cirrhosis compared to healthy controls (e.g., 27 + 3 vs 42 + 4 μmol/kg/hour, $p < 0.05$) in controls and patients with cirrhosis, respectively. The fraction of administered methionine undergoing remethylation is lower in cirrhosis (7.6 + 1.5 vs 14.1 + 1.1%, $p < 0.005$). However, because of the larger pool of circulating methionine, the total flux of methionine through the remethylation pathway is similar in both cirrhosis and normal reference controls. A significantly lower fraction of the administered dose appears in the form of

[$^{13}CO_2$] in breath in patients with cirrhosis (2.2 + 0.4 vs 11.0 + 0.8%, $p < 0.001$). Cirrhosis of the liver compensates for a decreased activity of remethylating enzymes by operating at higher concentrations of methionine. In contrast, trans-sulfuration is impaired in patients with alcohol-induced cirrhosis such that an assessment of trans-sulfuration by a simple breath test may provide a clinically useful estimate of hepatic function.

Remethylation and transsulfuration of methionine in cirrhosis: Studies with L-[H3-methyl-1-C]methionine. Russmann S, Junker E, Lauterburg BH. Hepatology 2002 Nov; 36 (5), pp. 1190–6.

Hepatic Biochemical Function/Decarboxylation Capacity (Mitochondrial)/Mitochondrial Respiration Uncoupling
[^{14}C]-Alpha-Ketoisocaproic Acid/Exhaled [$^{14}CO_2$]/Ethanol/Sodium Salicylate

Cellular/Molecular Mechanism of Action: Alpha-ketoisocaproic acid is a substrate for enzyme decarboxylation mechanisms mediated by hepatic mitochondria. Ethanol suppresses these biochemical processes. Sodium salicylate uncouples mitochondrial respiration and can therefore promote increased decarboxylation processes.
Application: Alpha-ketoisocaproic acid can be applied in a breath test to assess *in vivo* hepatic mitochondrial function. Ethanol can be applied as a control reagent due to its ability to inhibit hepatic mitochondrial decarboxylation mechanisms.
Route of Administration: 1-[^{14}C]-alpha-ketoisocaproic acid (intraperitoneal).
Implementation: Evaluation of *in vivo* hepatic mitochondrial metabolic capacity as a function of [$^{14}CO_2$] exhalation following 1-[^{14}C]-alpha-ketoisocaproic acid administration.
Animal Model: Rat.
Interpretation and Analysis: Following administration of 1-[^{14}C]-alpha-ketoisocaproic acid, exhaled [$^{14}CO_2$] reaches a peak within 10 to 20 minutes and then declines exponentially, with a half-life of 14.3 minutes. In controls, approximately 38.6% of the administered radioactivity is exhaled within 1 hour. In functionally anhepatic animals, [$^{14}CO_2$] in breath amounted to 23% of that in control animals, indicating that alpha-ketoisocaproic acid decarboxylation reflects mainly *in vivo* hepatic mitochondrial function. Ethanol (3 gm per kg) significantly decreases alpha-ketoisocaproic acid decarboxylation (21.8% of the dose appearing in breath in 1 hour), probably due to the ethanol-induced shift in the NAD+:NADH ratio. In contrast, uncoupling of mitochondrial respiration with sodium salicylate (375 mg per kg), increases decarboxylation of alpha-ketoisocaproic acid (56.3% of the dose recovered as [$^{14}CO_2$] in 1 hour). Mitochondrial damage induced by 4-pentenoic acid decreases decarboxylation of alpha-ketoisocaproic acid but does not affect the microsomal metabolism of antipyrine. Such findings indicate that the alpha-ketoisocaproic acid breath test provides a non-invasive estimate of hepatic mitochondrial function *in vivo* that, when applied to man, might yield clinically useful information.

Assessment of mitochondrial function *in vivo* with a breath test utilizing alpha-ketoisocaproic acid. Michaletz PA, Cap L, Alpert E, Lauterburg BH. Hepatology 1989 Nov; 10 (5), pp. 829–32.

Pancreatic Insufficiency
[^{13}C]-Egg White Breath Test/[Indirect Pancreatic Function Test]

Cellular/Molecular Mechanism of Action: [^{13}C]-egg white can function as a substrate for pancreatic enzyme fractions. The proteolytic peptide fragments are then absorbed in the gastrointestinal tract and metabolized to [$^{13}CO_2$] that is in turn exhaled from the respiratory tract.
Background Information: The recent availability of egg white protein highly enriched with [^{13}C] has allowed breath test technology to be adapted for the study of protein digestion and absorption. Pancreatic trypsin is considered to be the key enzyme in the proteolytic cascade. The aim is to evaluate trypsin activity in the small intestine of healthy volunteers and patients with pancreatic disease by a recently developed [^{13}C]-egg white breath test.
Application: Indirect effect on pancreatic function.
Route of Administration: [^{13}C]-labeled egg protein (oral).
Dosage: [^{13}C]-labeled egg protein (22 g).
Implementation: Evaluation of pancreatic disease states with the ingestion of a test meal consisting of [^{13}C]-labeled egg protein. Breath samples are taken before and after ingestion of the meal and analyzed for [$^{13}CO_2$] concentration. Moreover, pancreatic trypsin output after maximal stimulation is also measured.
Human Model: Cases with pancreatic disease (n = 30; and n = 48 normal volunteers). Pancreatic trypsin output after maximal stimulation measured in n = 13/30 affected cases and n = 9/48 healthy volunteers.
Interpretation and Analysis: The 6-hour cumulative [$^{13}CO_2$] excretion in breath is significantly lower in patients than observed in controls (mean [SEM]: 6.23 + 0.82% vs 19.16 + 0. 58%, $p < 0.0001$). An excellent correlation can be appreciated between the 6-hour cumulative [$^{13}CO_2$] excretion and trypsin activity after maximal pancreatic stimulation. The non-invasive [^{13}C]-egg white breath test is promising as an indirect pancreatic proteolytic function test.

[^{13}C]-egg white breath test: A non-invasive test of pancreatic trypsin activity in the small intestine. Evenepoel P, Hiele M, Geypens B, Geboes KP, Rutgeerts P, Ghoos Y. Gut 2000 Jan; 46 (1), pp. 52–7.

Pulmonary Epithelium Integrity/Viability/Pulmomary Pathophysiology
L-[Guanidino$^{15}N_2$]-Arginine Monohydrochloride/[^{15}NO] Exhalation/[Breath Test]

Cellular/Molecular Mechanism of Action: The amino acid L-[guanidino$^{15}N_2$]-arginine monohydrochloride is resorbed within the lung and partly metabolized to [^{15}NO].
Background Information: Conventional diagnosis of the pulmonary tract uses physical methods such as spirometry and oscillometry. However, the inhalation of a chemical diagnostic agent ought to provide novel ways of more specific diagnosis, for instance of inflammatory states of the bronchial and lung mucosa. The stable isotope technique using a [^{15}N]-labeled substrate appears to be a suitable tool for this application.
Application: The [^{15}NO] yielded after the inhalation of [^{15}N]-labeled arginine could be a potential marker for demonstrating pathophysiological changes in the lung epithelium. This method could establish a new diagnostic principle of "inhalative breath test."
Route of Administration: L-[guanidino$^{15}N_2$]-arginine monohydrochloride (inhaled).
Dosage: L-[guanidino$^{15}N_2$]-arginine monohydrochloride (700 mg of aqueous solution, 20 atom % [^{15}N]).
Implementation: Evaluation of conditions of asthma bronchiale with defined amounts of inhaled amino acid L-[guanidino$^{15}N_2$]-arginine monohydrochloride. The exhaled air is collected under defined conditions in 10-L breath bags and analyzed for NO using chemiluminescence.
Human Model: Cases suffering from asthma bronchiale (n = 7) and healthy/normal volunteers (n = 6).

Interpretation and Analysis: Under standardized test conditions, healthy cases exhale 0.97 ± 0.08 μmol NO/m^3 and asthmatics 1.17 ± 0.14 μmol NO/m^3. A better distinction is expected when comparing affected cases and normal reference controls when using [^{15}NO] exhalation as a diagnostic procedure. Between 30 and 80 minutes after inhaling [^{15}N]arginine, a maximum plateau of [^{15}NO] abundance is found in the exhaled air, at which time healthy and asthmatic cases exhibit clear differences in exhaled [^{15}NO] amounts. Under standardized test conditions, the healthy cases exhale 102.3 ± 6.7 nmol [^{15}NO/m^3], whereas asthmatic cases exhale only 76.1 ± 10.9 nmol [^{15}NO/m^3].

Instrumentation: Cryotrap gas chromatography–mass spectrometry system for analyzing the [^{15}N] abundance of NO gas.

[$^{15}N_2$]arginine as a 1st potential inhaled diagnostic agent to characterize respiratory diseases. Krumbiegel P, Denk E, Russow R, Rolle-Kampczyk U, Metzner G, Herbarth O. Exp Lung Res 2002 Oct–Nov; 28 (7), pp. 535–42.

2 GENETIC DISORDERS, INFECTIOUS DISEASE, NEOPLASIA, AND METABOLIC CONDITIONS

12 Cancer/Neoplastic Disease

GENERAL

Adhesion of Cells to Endothelium: Colon Carcinoma/Intravital Observation of Cancer Cell/Microcirculation Interactions
CalceinAM

Background Information: Organ-specific tumor cell adhesion within the microcirculation of host organs is an important step in the metastatic cascade. Circulating tumor cells have to adhere within the microcirculatory vessels, quickly stabilize their adhesion, and probably leave the circulation to avoid toxic effects of hydrodynamic shear forces of circulating blood.
Application: Intravital observation of colon carcinoma cell adhesion within the hepatic microcirculation.
Implementation: CalceinAM can be used to label colon carcinoma cell populations (e.g., HT-29 [human] and CC531 [rat]) that are then injected intra-arterially as single-cell suspensions. Intravital fluorescence microscopy is then applied to detect adhesive interactions between circulating tumor cells and endothelial surfaces within the hepatic microcirculation. These interactions are then analyzed regarding their time course and site localization within the vascular tree.
Animal Model: Rats (Sprague-Dawley).
Interpretation and Analysis: Autofluorescence of liver parenchyma is sufficient for distinction of hepatic sinusoids. Intravital microscopy is capable of differentiating early events in adhesion formation within hepatic sinosoids, adhesion stabilization, and extravasation of the tumor cells into the liver parenchyma. Tumor cell adhesion occurs almost exclusively within sinusoidal capillaries; however, the diameter of these vessels is usually larger than that of the tumor cells. Colon carcinoma cells rapidly migrate into the liver parenchyma after successful adhesion within the sinusoids. In contrast to common end point assays of the metastatic cascade, this *in vivo* model allows investigations of metastatic colon carcinoma cell adhesion within the liver microcirculation as specific steps during the formation of hematogenous metastasis and their underlying mechanisms.
Instrumentation: Intravital fluorescence microscopy.

An intravital model to monitor steps of metastatic tumor cell adhesion within the hepatic microcirculation. Haier J, Korb T, Hotz B, Spiegel HU, Senninger N. J Gastrointest Surg 2003 May–Jun; 7 (4), pp. 507–14; discussion 514–5.

Angiogenesis/Tumor Dimethylarginine Dimethylaminohydrolase (DDAH) Overexpression
NC100150/Hoechst 33342

Cellular/Molecular Mechanism of Action: The molecular orientation of NC100150 becomes altered within a magnetic field while the agent Hoechst 33342 emits fluorescent light energy.
Background Information: Intracellular factors that regulate nitric oxide (NO) synthesis represent important targets in tumor progression. Overexpression of DDAH, which metabolizes the endogenous inhibitors of NO synthesis (asymmetric dimethylarginine and N-monomethyl-L-arginine), results in C6 gliomas with enhanced growth rate compared with wild types.
Application: NC100150 can be utilized as an intravascular blood pool contrast agent that is detectable by MRI methodologies. Hoechst 33342 can be used as a fluorescent perfusion-marker dye for fluorescent detection systems.
Route of Administration: Utilization of NC100150 and Hoechst 33342 for detection of neoplastic lesion angiogenesis more frequently would require the delivery of these agents intravascularly.
Implementation: Determine the *in vitro* effects of DDAH on tumor vascular morphogenesis by measurement of the transverse relaxation rates R_2* and R_2 in (i) clone D27 gliomas overexpressing DDAH; (ii) C6 wild-type gliomas using intrinsic susceptibility magnetic resonance imaging (MRI) sensitive to changes in endogenous deoxyhemoglobin; (iii) determination of susceptibility contrast-enhanced MRI using NC100150 as an intravascular blood pool contrast agent; and (iv) compare results observed with the perfusion marker Hoechst 33342 applied in combination with the use of tumor fluorescence microscopy analyses.
Interpretation and Analysis: Baseline R_2* is significantly faster in D27 tumors, consistent with a greater vascular development ($p < 0.02$, ANOVA). There is no significant difference between the response of the 2 tumor types to hypercapnia (5% CO_2/95% air), when used as a probe for vascular maturation, or hyperoxia (5% CO_2/95% O_2, when used as a probe for vascular function. NC100150 increases the R_2* and R_2 rates of both tumor types and demonstrates a significantly larger blood volume in D27 tumors ($p < 0.02$, ANOVA). This correlates with a significantly greater uptake of Hoechst 33342 in D27 tumors compared to C6 wild-type tumors ($p < 0.02$, ANOVA). Despite increased tumor blood volume, the Delta R_2*/Delta R_2 ratio, an index of microvessel size, shows that the capillaries in the 2 tumor types are of a similar caliber. Such observations demonstrate the potential of susceptibility MRI-derived quantitative end points to non-invasively assess tumor angiogenesis. Application of intravascular blood pool contrast agents such as NC100150 for such purposes appears very promising. Overexpression of DDAH results in increased *in vivo* neovascularization of C6 gliomas. The lack of significant difference in hypercapnic/hyperoxic response between the C6 and D27 tumors and the similar vessel caliber are also consistent with a role for DDAH in the initial stages of vasculogenesis.
Instrumentation: Magnetic resonance imaging and fluorescence microscopy instrumentation.

Effects of overexpression of dimethylarginine dimethylaminohydrolase on tumor angiogenesis assessed by susceptibility magnetic resonance imaging. Kostourou V, Robinson SP, Whitley GS, Griffiths JR. Cancer Res 2003 Aug 15; 63 (16), pp. 4960–6.

Chemosensitivity
Near-Infrared Fluorescent (NIRF) Annexin V

Background Information: A rapid and accurate assessment of the antitumor efficacy of new therapeutic drugs could speed up drug discovery and improve clinical decision making.

Implementation: Based on the hypothesis that most effective antitumor agents induce apoptosis, the agent NIRF annexin V has been developed for optical sensing of tumor environments. To demonstrate probe specificity, both an active (i.e., apoptosis-recognizing) and an inactive form of annexin V have been developed that have very similar properties (to account for non-specific tumor accumulation).

Animal Model: Murine (nude mice each bearing a cyclophosphamide [CPA] chemosensitive [LLC] and a chemoresistant LLC [CR-LLC] properties).

Interpretation and Analysis: After injection with active annexin V, the tumor-annexin V ratio (TAR; tumor NIRF/background NIRF) for untreated mice is 1.22 ± 0.34 for LLC and 1.43 ± 0.53 for CR-LLC (n = 4). The LLC of CPA-treated mice has significant elevations of TAR (2.56 ± 0.29, p = 0.001, n = 4), but only moderate increases are obtained for CR-LLC (TAR = 1.89 ± 0.19, p = 0.183). The *in vivo* measurements correlate well with terminal deoxyribosyl transferase-mediated dUTP nick end labeling indexes. When inactive Cy-annexin V is used, with or without CPA treatment and in both CCL and CR-CCL tumors, tumor NIRF values range from 0.91 to 1.17 (i.e., tumors are equal to background). Therefore, active Cy-annexin V and surface reflectance fluorescence imaging provide a non-radioactive, semi-quantitative method of determining chemosensitivity in LLC xenografts. The method may be used to image pharmacologic responses in other animal models and, potentially, may permit the clinical imaging of apoptosis with non-invasive or minimally invasive instrumentation.

Optical imaging of apoptosis as a biomarker of tumor response to chemotherapy. Schellenberger EA, Bogdanov A Jr, Petrovsky A, Ntziachristos V, Weissleder R, Josephson L. Neoplasia 2003 May–Jun; 5 (3), pp. 187–92.

Mitosis/Proliferation Rate of Cell Populations/ Neoplastic Lesions: Potential Application
3′-Deoxy-3′-[^{18}F]Fluorothymidine

Cellular/Molecular Mechanism of Action: Thymidine is rapidly assimilated by proliferating cell populations.

Application: 3′-deoxy-3′-[^{18}F]fluorothymidine has been designed as a new agent for positron emission tomography imaging for visualizing cellular proliferation *in vivo* based on the metabolism of thymidine

Route of Administration: Variable but most often intravascular.

Semi-Synthetic Methods: A reliable technique for the radiosynthesis of 3′-deoxy-3′-[^{18}F]fluorothymidine ([^{18}F]-FLT) has been developed based on [^{18}F]10 mCi (370 MBq) of radiochemically pure [^{18}F]1 Ci/μmol (37 GBq/μmol) at EOS within 100 minutes and in 13% radiochemical yield (end of bombardment [EOB]; 7% end of synthesis [EOS]).

Implementation: Evaluation of neoplastic lesions that display relatively rapid rates of proliferation/growth.

Interpretation and Analysis: Detection of increased radioactivity at a specific anatomical location corresponds to a region with a relatively high rate of cellular proliferation.

Radiosynthesis of 3′-deoxy-3′-[^{18}F]fluorothymidine: [^{18}F]FLT for imaging of cellular proliferation *in vivo*. Grierson JR, Shields AF. Nucl Med Biol 2000 Feb; 27 (2), pp. 143–56.

Optical (Near-Infrared [NIR]) Imaging/ Endoscopy/Videoendoscopy
[Bis-Propanoic Acid Cyanine]-IgG

Cellular/Molecular Mechanism of Action: Receptor-specific small peptide molecule that selectively recognizes and physically binds to complexes expressed on the exterior surface membrane of certain neoplastic cell types.

Application: Potential *in vivo* optical imaging of neoplastic lesions using endoscopy/videoendoscopy.

Route of Administration: [Bis-propanoic acid cyanine]-IgG (intravenous).

Semi-Synthetic Methods: Conjugation of the NIR dyes indocyanine green (ICG) and its derivatized analogue, bis-propanoic acid cyanine dye (cypate), to a biological peptide molecule.

Implementation: Evaluation of the ability of an NIR dye-peptide conjugate to function as a tumor-targeting agent.

Animal Model: Rat (n = 6; neoplastic cell type CA20948 that expresses the corresponding biological protein receptor complex).

Interpretation and Analysis: Tumor retention of 2 non-tumor-specific dyes, ICG and its derivatized analogue, bis-propanoic acid cyanine dye (cypate), is negligible. In contrast, the receptor-specific peptide-cypate conjugate (cytate) is retained within CA20948 tumor, with an excellent tumor-to-normal-tissue ratio (e.g., n = 6 rats). Optical detection of tumors with a receptor-targeted fluorescent contrast agent has been demonstrated. These results represent a new direction in cancer diagnosis and patient management.

Instrumentation: A simple continuous-wave optical imaging system, consisting of an NIR laser diode, a cooled charge-coupled device (CCD) camera, and an interference filter.

Novel receptor-targeted fluorescent contrast agents for *in vivo* tumor imaging. Achilefu S, Dorshow RB, Bugaj JE, Rajagopalan R. Invest Radiol 2000 Aug; 35 (8), pp. 479–85.

Visual Detection of Neoplastic Disease
Tumor Receptor Binding Ligand Conjugate/ Indocyanine Green (ICG)/Bis-Propanoic Acid Cyanine Dye (Cypate)

Cellular/Molecular Mechanism of Action: Indocyanine green and bis-propanoic acid cyanine dye (cypate) function as dye marker reagents that when activated emit light energy in the near-infrared (NIR) range. Tumor receptor ligands recognize and physically bind selectively to complexes associated with the exterior surface membrane of cancer cells.

Application: Indocyanine green and bis-propanoic acid cyanine dye reagents can be applied as visible marker molecules to facilitate optical imaging modalities. In this context, they can be conjugated to small peptides that specifically recognize (bind to) tumor-associated receptor complexes.

Route of Administration: Intravenous.

Semi-Synthetic Methods: Covalent conjugation of an NIR dye to a biologically active peptide that recognizes and binds to tumor-associated receptor complexes.

Implementation: Evaluation of the ability of a semi-synthetic NIR dye-peptide conjugate for its ability to selectively localize with tumor lesions. Detection is achieved with a simple continuous-wave optical imaging system.

Animal Model: Rat tumor model (CA20948) known to express receptors for a specific peptide (e.g., n = 6).

Interpretation and Analysis: Tumor retention of 2 non-tumor-specific dyes, ICG and its derivatized analogue, bis-propanoic acid cyanine dye (cypate), is negligible. In contrast, the receptor-specific peptide-cypate conjugate (cytate) is retained by CA20948 tumors, producing an excellent tumor-to-normal-tissue ratio.

Instrumentation: A simple continuous-wave optical imaging system, consisting of an NIR laser diode, a cooled charge-coupled device (CCD) camera, and an interference filter.

Novel receptor-targeted fluorescent contrast agents for *in vivo* tumor imaging. Achilefu S, Dorshow RB, Bugaj JE, Rajagopalan R. Invest Radiol 2000 Aug; 35 (8), pp. 479–85.

Visual Detection of Neoplastic Disease: Selective Recognition
HAT-DO1 Fluorescent Agents

Cellular/Molecular Mechanism of Action: Fluorescent molecular system that can be covalently conjugated to a selective "targeting" agent for the detection of specific biological entities without the simultaneous delivery of photodynamic properties.
Application: Principally used as a sensor or indicator molecular that facilitates external detection of "targeting" modalities designed to selectively interact with a specific biological/pathological entity.
Synthesis and Design Considerations: Various heterodimers linked by various types of "spacers" between a chlorine derivative and its Mn or Cu complex can be synthesized. One representative agent referred to as HAT-DO1 has a molecular formula of m-phthalyl-([13,17-bispropanoic acid-3-ethenyl-8-formylethylidene-7-hydroxy-2,7,12,18-tetramethyl- porphyrinate]-manganese (III))-[3′-ethenyl-8′-formylethylidene-7′-hydroxy-2′,7′,12′,18′-te tramethyl-porphine-13′,17′-bispropanoyl aspartic acid]-bishydrazone.

Tumor-localizing fluorescent diagnostic agents without phototoxicity. Takemura T, Nakajima S, Sakata I. Photochem Photobiol 1994 Mar; 59 (3), pp. 366–70.

CENTRAL NERVOUS SYSTEM

Glioma Cell Type
EtNBA Fluorescent Dye

Cellular/Molecular Mechanism of Action: EtNBA emits a fluorescent color when exposed to certain wavelengths of light energy.
Background Information: Nile blue dyes have been shown to have a higher affinity for tumor tissue, as compared to surrounding normal tissue, and to be relatively non-toxic. The agent EtNBA, a lipophilic, fluorescent benzophenoxazine dye, has been employed in murine models to determine the capacity of this agent to image subcutaneous and intracranial U-87 glioma implants.
Application: The fluorescent dye marker EtNBA can function as a detectable signal molecule that can facilitate the external detection and characterization of neoplastic lesions (e.g., glioma cell types).
Dosage: EtNBA (2.5–5.0 mg/kg).
Implementation: Tumors are illuminated with a 632.8-nm diffuse beam from a helium-neon laser. The resulting video image is processed using an image processor to give real-time pseudocolor and enhanced black and white images.
Animal Model: Mouse (expressing U87-MG glioma solid tumor implants).
Interpretation and Analysis: Following subcutaneous injection of EtNBA dye at doses of 2.5 to 5.0 mg/kg bw, a gradual increase in the fluorescent signal can be observed from tumors that peaks 1–3 hours postinjection with variable selectivity (typically 4:1) for tumor to normal surrounding tissues permitting clear tumor demarcation. The reagent EtNBA is a safe and effective photodiagnostic agent when applied *in vivo* and is capable of demarcating U87-MG solid tumors on a real-time basis (2.5–5.0 mg/kg) for 1 to 3 hours following administration.
Instrumentation: Imaging systems for detecting fluorescence can consist of a silicon-intensified target (SIT) video camera fitted with a zoom microscope-magnifying lens complemented by an image processor to give real-time pseudocolor and enhanced black and white images.

Fluorescent imaging in a glioma model *in vivo*. Nikas DC, Foley JW, Black PM. Lasers Surg Med 2001; 29 (1), pp. 11–7.

Gliosarcoma/Proliferation Rate
Hoechst 33258 Dye/Rhodaminated Long-Circulating Dextran-Coated Iron Oxide (LCDIO)/Green Fluorescent Protein

Cellular/Molecular Mechanism of Action: Hoechst 33258 dye can be applied as an intravital endothelial stain. Rhodaminated LCDIO particles can preferentially localize intracellularly within certain malignant neoplastic cell types (e.g., gliosarcoma).
Application: Detection and characterization of central nervous system neoplastic lesions (e.g., gliosarcoma).
Route of Administration: Intravascular.
Implementation: Determination of the distribution of LCDIO preparation in tumors. Accumulation of LCDIO in tissue sections is evaluated using multichannel fluorescence microscopy with rhodaminated LCDIO, green fluorescent protein as a tumor marker, and Hoechst 33258 dye as an intravital endothelial stain. Uptake into tumor cells is corroborated with results of immunohistochemical analyses and cell culture uptake experiments.
Animal Model: Rodent gliosarcoma.
Interpretation and Analysis: Tumoral accumulation of LCDIO is 0.11% ± 0.06 of the injected dose per gram of tissue in brain tumors and sufficient for detection using magnetic resonance imaging (MRI). In tumor sections, LCDIO preferentially localizes in tumor cells (49.0% ± 4.6) but is also taken up by macrophages in tumors (21.0% ± 3.1) and by endothelial cells in the areas of active angiogenesis (6.5% ± 1.4). In cell culture, LCDIO uptake strongly correlates with the growth rate of tumor cell lines. Tumoral LCDIO accumulation is not negligible and helps explain MRI signal intensity changes observed in clinical trials. Microscopically, LCDIO accumulates predominantly in tumor cells and tumor-associated macrophages. Uptake into tumor cells appears to be directly proportional to cellular proliferation rate.
Instrumentation: Magnetic resonance imaging signal intensity evaluated utilizing a 1.5-T superconducting magnet for the detection of intratumor LCDIO uptake.

Tumoral distribution of long-circulating dextran-coated iron oxide nanoparticles in a rodent model. Moore A, Marecos E, Bogdanov A Jr, Weissleder R. Radiology 2000 Feb; 214 (2), pp. 568–74.

Neuroendocrine Neoplasia/ Somatostatin Receptor Expression
Indotricarbocyanine-Octreotate/(Cyanine Dye: Somatostatin Conjugate)

Cellular/Molecular Mechanism of Action: Octreotate is a biologically functional analog of somatostatin.
Application: Contrast agent for the optical *in vivo* imaging of neoplastic lesions that express somatostatin receptors at relatively high densities so that they can be visualized using endoscopy/videoendoscopy.
Route of Administration: Indotricarbocyanine-octreotate (intravenous).
Implementation: Indotricarbocyanine-octreotate.
Animal Model: Mouse (xenographs of human neuroendocrine neoplastic cell types).

Interpretation and Analysis: During *in vivo* imaging procedures, indotricarbocyanine-octreotate preferentially accumulates in tumors (murine xenografts). The level of fluorescence for tumors rapidly increases and is often more than 3-fold higher than that of normal tissue between 3 and 24 hours after application. The targeting conjugate is internalized by primary human neuroendocrine tumor cells. Imaging applying such an approach combines the specificity of ligand/receptor interaction with near-infrared fluorescence detection, and therefore may be applied in various other fields of cancer diagnosis.

Receptor-targeted optical imaging of tumors with near-infrared fluorescent ligands. Becker A, Hessenius C, Licha K, Ebert B, Sukowski U, Semmler W, Wiedenmann B, Grotzinger C. Nat Biotechnol 2001 Apr; 19 (4), pp. 327–31.

GASTROINTESTINAL TRACT

Carcinoid Syndrome: Midgut/Hepatic Neoplastic Disease: Metastatic Midgut Carcinoid *Pentagastrin/Calcium Infusion/Ketanserin*

Application: Detection of midgut carcinoma conditions.
Background Information: Both 5-hydroxytryptamine (serotonin, 5-HT) and substance-P (SP) are expressed at supranormal plasma concentrations in conditions of midgut carcinoma with hepatic metastasis. The agent 5-HT is important for the development of gastrointestinal symptoms but is not associated with flushing conditions. Substance-P may contribute to but not function as the sole mediator of flushing. Ketanserin may alleviate gastrointestinal symptoms in carcinoid conditions.
Route of Administration: Pentagastrin (IV) and calcium infusion.
Implementation: Evaluation of responses to calcium infusion and pentagastrin as a diagnostic approach to detecting and characterizing carcinoid syndrome conditions.
Interpretation and Analysis: Pentagastrin causes flushing and gastrointestinal symptoms and elevated levels of circulating 5-HT, but not of SP. Pretreatment with a 5-HT2 receptor blocking agent (ketanserin) alleviates gastrointestinal symptoms but has no influence on either 5-HT release or pentagastrin-induced flushing. Calcium infusion induces carcinoid symptoms in only about a third of cases (e.g., n = 2/6). Elevated 5-HT levels can be detected in this case group, in contrast to elevations in SP levels, which are only seen in about 15% of cases (e.g., n = 1/6).

Differential responses to pentagastrin suggest that substance-P is released from a different anatomical location or involves different mechanisms than those associated with 5-HT (serotonin). Substance P and 5-HT do not seem to share a common release mechanism. It appears that PG testing is superior to calcium infusion as a provocative test in patients with the carcinoid syndrome. Based on observations noted in preliminary investigations, the pentagastrin test with measurements of 5-HT levels in peripheral blood seems to be superior to calcium infusion as a provocative test in documenting a diagnosis of carcinoid disease.

Pretreatment with a 5-HT2-receptor blocking agent, ketanserin, abolishes the gastrointestinal effects but has virtually no influence on either 5-HT levels or flushing induced by intravenous pentagastrin. In contrast, calcium infusion induces carcinoid symptoms in only about 30% of cases (e.g., n = 2/6), and this is consistently associated with the stimulation of circulating serotonin levels.
Instrumentation: High-performance liquid chromatography-electron capture and radioimmunoassay methodologies.

The pentagastrin test in the diagnosis of the carcinoid syndrome. Ahlman H, Dahlstrom A, Gronstad K, Jaffe BM, Nilsson O, Oberg K. J Cardiovasc Pharmacol 1985; 7 Suppl 7, pp. S86–8.

Colorectal Cancer/Gastric Cancer *[Indocyanine Green (ICG)]-Anti-MUC1 Mucin*

Cellular/Molecular Mechanism of Action: Monoclonal antibody (IgG) with selective binding properties directed at MUC1 mucin recognizes and physically binds to the corresponding complexes expressed on the exterior surface membrane of certain neoplastic cell types.
Background Information: Videoendoscopy has not significantly advanced diagnostic accuracy beyond that attainable by conventional fiberscopy, with respect to microcarcinomas of the digestive tract. Theoretically, it is possible to selectively label such lesions with agents that can be detected by videoendoscope. Digital processing of the images could facilitate more accurate endoscopic diagnosis of microcarcinomas.
Application: [Indocyanine green]-anti-MUC1 mucin can be utilized to detect and characterize conditions of gastric and colorectal cancer lesions when used in combination with direct visualization using videoendoscopy methodologies.
Route of Administration: [Indocyanine green]-anti-MUC1 mucin (intravenous).
Implementation: Evaluation of the ability of [ICG]-anti-MUC1 mucin to selectively recognize and bind *in vivo* to gastric and colorectal cancer cell types. Validation of findings established by immunohistochemistry analysis using labeled secondary antibody.
Animal Model: Murine (nude mouse with human gastric or colorectal cancer cell types).
Interpretation and Analysis: [Indocyanine green]-anti-MUC1 mucin preferentially accumulates within gastric and colorectal cancer explants in nude mice that can be appreciated on gross examination and by examination of tissue samples applying histochemical analysis. Therefore, antibody labeled with an ICG derivative may be clinically useful in detecting gastrointestinal microcarcinoma by videoendoscopy.
Instrumentation: Videoendoscopy.

Vital immunostaining of human gastric and colorectal cancers grafted into nude mice: A preclinical assessment of a potential adjunct to videoendoscopy. Kusaka Y, Ito S, Muguruma N, Tadatsu M, Bando T, Ii K, Irimura T, Shibamura S. J Gastroenterol 2000; 35 (10), pp. 748–52.

Colorectal Cancer/Terminal α-N-Acetylgalactosaminyl (GalNAc)/Tn Antigen *Fluorescein Isothiocyanate (FITC)-Conjugated GS I-A4/Lectins DBA and LBL*

Cellular/Molecular Mechanism of Action: Lectin GS I-A4 binds to terminal α-N-acetylgalactosaminyl (GalNAc) groups (which include the Tn antigen) but not to the closely related tumor-associated epitope sialylated Tn antigen. The lectin also precipitates asialo OSM but not its native sialylated form.
Application: Lectin histochemistry with human colonic tissues reveals that GS I-A4 specifically stains specimens of colon cancer and colonic tissues from individuals with FAP; however, normal colonic tissues from patients without colonic disease are rarely stained with this lectin.
Dosage: A dose-response study conducted using 10–160 μg/ml of GS I-A4 demonstrates significant dose-related toxicities against LS174t and SW1116 cells.
Interpretation and Analysis: Glycoconjugates bound by GS I-A4 can be observed on the surface membranes of LS174t and SW1116 human colon cancer cell lines when exposed to FITC-conjugated GS I-A4. GS I-A4 is toxic to these 2 human colon cancer cell lines in monolayer culture. In this context, at concentrations > 80

μg/ml, > 99% of LS174t and > 90% of SW1116 cells are killed. At 4 mM, GalNAc specifically inhibits the cytotoxic effect of GS I-A4 ($p < 0.001$), whereas 4mM N-acetylglucosamine (GlcNAc) has no effect. In the light of these findings, it has been speculated that GS I-A4 may have potential use as a diagnostic agent against colorectal cancer. Other lectins that recognize terminal α-GalNAc residues including DBA and LBL are significantly less cytotoxic to colon cancer cells than GS I-A4.

The lectin Griffonia simplicifolia I-A4 (GS I-A4) specifically recognizes terminal alpha-linked N-acetylgalactosaminyl groups and is cytotoxic to the human colon cancer cell lines LS174t and SW1116. Chen YF, Boland CR, Kraus ER, Goldstein IJ. Int J Cancer 1994 May 15; 57 (4), pp. 561–7.

Colorectal Carcinoma/Intraoperative Lesion Localization
[^{125}I]-B72.3 Anti-Colorectal Carcinoma Monoclonal Antibody (MAb)

Cellular/Molecular Mechanism of Action: [^{125}I]-B72.3 MAb selectively binds to antigenic complexes expressed by lesions of colorectal carcinoma.
Application: Intraoperative detection of tumor location using a handheld gamma probe.
Route of Administration: [^{125}I]-B72.3 MAb (intravenous).
Examples of Clinical Implementation: Evaluation of a new intraoperative approach to tumor localization using radiolabeled MAb B72.3 has been devised that involves the use of a handheld gamma-detecting probe (GDP). Surgical procedures can be performed between 5 and 35 days postadministration of [^{125}I]-labeled MAb B72.3 IgG preparations. Analysis of resected tumor and normal tissues involves measurement of relative [^{125}I]-labeled MAb B72.3 IgG (e.g., injected dose/gram tissue).
Interpretation and Analysis: Applying a handheld GDP, surgeons intraoperatively can successfully localize lesions 68% of the time (e.g., n = 21/31). Resected tumor radiolocalization indices range from 0.5 to 543 with radiolocalization indices of 3.0 or higher in 71% (e.g., n = 22/31) of the tumor specimens. Sixty-eight percent of carcinoma lesions positively detected intraoperatively with a handheld GDP will also have levels of tumor antigen expression that can be detected with an immunoperoxidase-based assay, while approximately 8% of lesions that cannot be detected intraoperatively are also antigen negative. Alternatively, about 24% of carcinoma lesions cannot be localized intraoperatively based on the use of the GDP, while immunohistopathology analysis reveals positive expression of detectable levels of tumor antigen. The [^{125}I]-labeled MAb B72.3 IgG reagent recognizes all histologic grades of colorectal cancer. In most situations, higher *in vivo* probe ratios and radiolocalization indices can be observed when intraoperative handheld gamma scans are performed 20–35 days following injection of MAb and preparations are successful in detecting colorectal carcinoma lesions that have metastasized to the mesenteric and peri-aortic lymph nodes, liver, lung, and peri-rectal soft tissue. A strong degree of correlation ($p = 0.001$) can be appreciated between the intraoperative detection of tumorous lesions with increased MAb B72.3 localization, and the *in vitro* measurement of cpm/g of tumor tissue compared to normal/healthy tissue samples.

Intraoperative radioimmunolocalization of colorectal carcinoma with a handheld gamma probe and MAb B72.3: Comparison of *in vivo* gamma probe counts with *in vitro* MAb radiolocalization. Tuttle SE, Jewell SD, Mojzisik CM, Hinkle GH, Colcher D, Schlom J, Martin EW. Int J Cancer 1988 Sep 15; 42 (3), pp. 352–8.

Colorectal Carcinoma Cell Adhesion to Endothelium/Intravital Observation of Cancer Cell/Microcirculation Interactions
CalceinAM

Background Information: Organ-specific tumor cell adhesion within the microcirculation of host organs is an important step in the metastatic cascade. Circulating tumor cells have to adhere to the luminal surface of microcirculatory vessels, quickly stabilize their adhesion, and then leave the circulation to avoid toxic effects of hydrodynamic shear forces of circulating blood.
Application: Intravital observation of colon carcinoma cell adhesion within the hepatic microcirculation.
Implementation: CalceinAM is used to label colon carcinoma cell populations (e.g., HT-29 [human] and CC531 [rat]) that are then injected intra-arterially as single-cell suspensions. Intravital fluorescence microscopy is then applied to detect adhesive interactions between circulating tumor cells and endothelial surfaces within the hepatic microcirculation. These interactions are then analyzed regarding their time course and site localization within the vascular tree.
Animal Model: Rats (Sprague-Dawley).
Interpretation and Analysis: Autofluorescence of liver parenchyma is sufficient for distinction of hepatic sinusoids. Intravital microscopy is capable of differentiating early events in adhesion formation within hepatic sinosoids, adhesion stabilization, and extravasation of the tumor cells into the liver parenchyma. Tumor cell adhesion occurs almost exclusively within sinusoidal capillaries; however, the diameter of these vessels is usually larger than that of the tumor cells. Colon carcinoma cells rapidly migrate into the liver parenchyma after successful adhesion within the sinusoids. In contrast to common end point assays of the metastatic cascade, this *in vivo* model allows investigations of metastatic colon carcinoma cell adhesion within the liver microcirculation as specific steps during the development of hematogenous metastasis and their underlying mechanisms.
Instrumentation: Intravital fluorescence microscopy.

An intravital model to monitor steps of metastatic tumor cell adhesion within the hepatic microcirculation. Haier J, Korb T, Hotz B, Spiegel HU, Senninger N. J Gastrointest Surg 2003 May–Jun; 7 (4), pp. 507–14; discussion 514–5.

Esophogeal Squamous Cell Carcinoma/Elevated Epidermal Growth Factor Receptor (EGFR) Expression
Anti-EGFR Coated Supermagnetic Particles

Cellular/Molecular Mechanism of Action: Anti-EGFR monoclonal antibody recognizes and physically binds to membrane-associated EGFR.
Background Information: Epidermal growth factor receptors are overexpressed in conditions of esophageal squamous cell carcinoma.
Application: Enhanced detection of esophageal squamous cell carcinoma employing an endoluminal surface coil.
Route of Administration: Anti-EGFR-coated supermagnetic particles (intravascular).
Semi-Synthetic Methods: Coating superparamagnetite particles with monoclonal antibodies (MAbs) directed against EGFRs.
Animal Model: Rat (athymic bearing TE8 or H69 tumors).
Human Model: Cases of esophageal cancer (n = 30).
Interpretation and Analysis: In terms of depth of cancer invasion, the accuracy rate of magnetic resonance imaging (MRI) using

the endoluminal surface coil is 83%. In conclusion, MRI with an endoluminal surface coil will be a useful examination strategy for esophageal cancer in the future. Anti-EGFR-coated supermagnetic particles possess EGFR-specific MRI contrast detection characteristics *in vivo* in tumor-bearing rats. Immunospecific MRI using magnetite particles coated with MAbs against EGFR seems to be useful in the diagnosis of squamous cell carcinoma of the esophagus.

Instrumentation: Endoluminal 1.5-T serconductive MR system.

What's new in imaging? New magnetic resonance imaging of esophageal cancer using an endoluminal surface coil and antibody-coated magnetite particles. Ozawa S, Imai Y, Suwa T, Kitajima M. Recent Results Cancer Res 2000; 155, pp. 73–87.

Gastric Carcinoma: Metastasis/Retrovirus Delivery
Green Fluorescent Protein (GFP)

Cellular/Molecular Mechanism of Action: Retroviral agents expressing the GFP gene can insert their genome into certain mammalian cell populations. Cells that have been transfected by such retroviral agents express the GFP gene and emit a green color when exposed to certain wavelengths of light.

Application: Visualization of future regional and distant metastases.

Route of Administration: Intraperitoneal (other routes possible).

Implementation: Green fluorescent protein retroviral supernatants are injected intraperitoneally (i.p.) from day 4 to day 10 after implantation of gastric cancer cells. Tumor and metastasis fluorescence are then visualized every other week with the use of fluorescence optics via a laparotomy.

Animal Model: Mouse (intraperitoneally growing human gastric neoplasia).

Interpretation and Analysis: At 2 weeks after retroviral GFP delivery, GFP-expressing tumor cells can be observed in gonadal fat, greater omentum, and intestine, indicating that these primary i.p. growing tumors are efficiently transduced by the GFP gene and could be visualized by its expression. During 2nd and 3rd laparotomies, GFP-expressing tumor cells can be observed spreading to lymph nodes in the mesentery and other regional sites. At the 4th laparotomy, widespread tumor growth is visualized by GFP expression, including liver metastasis. Normal tissues are rarely transduced by the GFP retrovirus. Thus, reporter gene transduction of the primary tumor can enable detection of its subsequent metastasis. This gene therapy model could be applied to primary tumors before resection or other treatment to have a fluorescent early detection system for metastasis and recurrence.

In vivo tumor delivery of the green fluorescent protein gene to report future occurrence of metastasis. Hasegawa S, Yang M, Chishima T, Miyagi Y, Shimada H, Moossa AR, Hoffman RM. Cancer Gene Ther 2000 Oct; 7 (10), pp. 1336–40.

Gastrinoma: Zollinger-Ellison Syndrome
Secretin and [Ca^{2+}]

Cellular/Molecular Mechanism of Action: Induces gastrin secretion.

Application: To evaluate criteria of positivity for and usefulness of both the secretin and calcium gastrin-provocative tests in patients with the Zollinger-Ellison syndrome. Design: Prospective trial in consecutive patients.

Route of Administration: Kabi-secretin (intravenous bolus); calcium gluconate (10% intravenous).

Dosage: Kabi-secretin (2 U/kg bolus); calcium gluconate 10% (54 mg/kg/hr as 5 mg/kg/hr of calcium continuously infused for 3 hours).

Considerations: The distinct advantages (short test period, low patient morbidity, and relatively great potency) of the calcium + secretin test make it an attractive alternative to other previously described provocative tests for the diagnosis of gastrinoma. There are usually no false-positive or false-negative tests with the calcium + secretin.

Implementation: Evaluation of suspected cases of gastrinoma as a function of response to Kabi-secretin and calcium gluconate (10%) (54 mg/kg/hr [5 mg/kg/hr of calcium]) given by continuous intravenous infusion for 3 hours. Serum gastrin is measured at −15 and −1 minutes before, and 2, 5, 10, 15, 20, and 30 minutes after secretin, or every 30 minutes for 3 hours during calcium infusion. Serum calcium and serum gastrin are measured simultaneously during calcium infusion. Criterion for diagnosis is either a 50% increase or a 200 pg/ml increase above the basal gastrin level.

Human Model: Cases suspected of having Zollinger-Ellison syndrome (n = 80).

Interpretation and Analysis: There is no significant difference in the responses of cases with different extents or locations of the tumor, presence or absence of multiple endocrine neoplasia, type-I, or with fasting gastrin $<$ or $>$ 1,000 pg/mL. In cases with fasting gastrin of $<$ 1,000 pg/mL, sensitivity of the secretin test using the criterion of an increase in gastrin of at least 110 pg/mL is 93% (CI, 76–99%) and for an increase of 200 pg/mL it is 85% (CI, 66–96%) ($p > 0.05$). With the calcium infusion test, the sensitivity using the criterion of an increase of 395 pg/mL is 43% (CI, 23–66%) and for an increase of 50% is 74% (CI, 52–90%) ($p < 0.01$). The calcium infusion test is positive in 33% of cases with a negative secretin test. Applying the secretin test, 75% of cases have a positive response by 5 minutes, 95% by 10 minutes, 100% by 15 minutes, and 6% only at 2 minutes. Utilizing calcium infusions, cases have positive responses at 120 to 180 minutes. The secretin test is preferred over the calcium test because of its greater sensitivity and simplicity. The recommended criteria are a 200 pg/mL increase for the secretin test and a 395 pg/mL increase for the calcium test. The calcium test should be reserved for patients having a negative secretin test, gastric acid hypersecretion, and a strong clinical suspicion of the Zollinger-Ellison syndrome.

Instrumentation: Laboratory facilities and instrumentation for immunodetection methodologies (RIA, ELISA).

Secretin and calcium provocative tests in the Zollinger-Ellison syndrome. A prospective study. Frucht H, Howard JM, Slaff JI, Wank SA, McCarthy DM, Maton PN, Vinayek R, Gardner JD, Jensen RT. Ann Intern Med 1989 Nov 1; 111 (9), pp. 713–22.

Gastrinoma: Zollinger-Ellison Syndrome (Persistent)
Methylene Blue

Route of Administration: Intra-arterial.

Considerations: Localizing studies can include ultrasonography, computed tomography, magnetic resonance imaging, duodenal endoscopy, endoscopic ultrasonography, and intraoperative endoscopic transillumination of the duodenum (all negative postop in this case report).

Implementation: Evaluation of Zollinger-Ellison syndrome lesions using methylene blue injection is used to identify a 6-mm gastrinoma in the duodenum that has been locally excised.

Human Model: Case of Zollinger-Ellison syndrome (persistent postsurgical excision of a 4-cm duodenal gastrinoma; 40 years old).

Interpretation and Analysis: Methylene blue injection facilitated the identification of an excisable 6-mm gastrinoma in the duodenum.

Selective intra-arterial methylene blue injection: A novel method of localizing gastrinoma. Ko TC, Flisak M, Prinz RA. Gastroenterology 1992 Mar; 102 (3), pp. 1062–4.

Gastrinoma/[Insulinoma]/[Intestinal Carcinoid Syndrome]/Zollinger-Ellison Syndrome
Calcium/Pentagastrin/Secretin

Background Information: Calcium infusion has been advocated as a provocative test for the diagnosis of some endocrine tumors of the pancreas and gastrointestinal tract (gastrinoma, insulinoma, intestinal carcinoids).
Application: The calcium infusion test is recommended in Zollinger-Ellison syndrome when the results of secretin stimulation test are equivocal.
Route of Administration: Intravenous injection.
Interpretation and Analysis: The release of gastrin from gastrinoma tissue is very sensitive to alterations in the serum calcium level. The calcium provocative test in the detection of insulinoma and carcinoid tumors is less reliable than other safer and simpler procedures. Intravenous injection of calcium followed by pentagastrin stimulates the release of somatostatin in cases with somatostatinoma and offers a reliable means for establishing the diagnosis of this tumor type. Calcium administration has not proven to be useful in the diagnosis of other endocrine tumors of the digestive system.

Use of calcium provocative test in the diagnosis of gastroenteropancreatic endocrine tumors. Vezzadini C, Poggioli R, Casoni I, Vezzadini P. Panminerva Med 1996 Dec; 38 (4), pp. 255–8.

Intestinal and Colon Microcarcinomas/Colorectal Cancer/Gastric Cancer
[Indocyanine Green (ICG)]-Anti-MUC1 Mucin

Cellular/Molecular Mechanism of Action: Monoclonal antibody (IgG) with selective binding properties directed at MUC1 mucin recognizes and physically binds to the corresponding complexes expressed on the exterior surface membrane of certain neoplastic cell types.
Background Information: Videoendoscopy has not significantly advanced diagnostic accuracy beyond that attainable by conventional fiberscopy, with respect to microcarcinomas of the digestive tract. Theoretically, it is possible to selectively label such lesions with agents that can be detected by videoendoscope. Digital processing of the images could facilitate more accurate endoscopic diagnosis of microcarcinomas.
Application: [Indocyanine green]-anti-MUC1 mucin can be utilized to detect and characterize conditions of gastric and colorectal cancer lesions when used in combination with direct visualization using videoendoscopy methodologies.
Route of Administration: [Indocyanine green]-anti-MUC1 mucin (intravenous).
Implementation: Evaluation of the ability of [ICG]-anti-MUC1 mucin to selectively recognize and bind *in vivo* to gastric and colorectal cancer cell types. Validation of findings established by immunohistochemistry analysis using labeled secondary antibody.
Animal Model: Murine (nude mouse with human gastric or colorectal cancer cell types).
Interpretation and Analysis: [Indocyanine green]-anti-MUC1 mucin preferentially accumulates within gastric and colorectal cancer explants in nude mice that can be appreciated on gross examination and by histochemical analysis. Therefore, antibody labeled with an ICG derivative may be a clinically useful strategy for detecting gastrointestinal microcarcinoma by videoendoscopy.
Instrumentation: Videoendoscopy.

Vital immunostaining of human gastric and colorectal cancers grafted into nude mice: A preclinical assessment of a potential adjunct to videoendoscopy. Kusaka Y, Ito S, Muguruma N, Tadatsu M, Bando T, Ii K, Irimura T, Shibamura S. J Gastroenterol 2000; 35 (10), pp. 748–52.

Intestinal/Colon Carcinoma/Optical Lesion Imaging
Indotricarbocyanine (ITCC)/ITCC-Transferrin/ ITCC-Human Serum Albumin

Cellular/Molecular Mechanism of Action: Indotricarbocyanine is a dye that can serve as a detectable marker molecule in biological systems.
Background Information: Macromolecules accumulate in solid tumors and can thus be used as carriers for the delivery of attached contrast agents to tumors.
Application: Indotricarbocyanine can be applied as a contrast agents for optical imaging of neoplastic lesions.
Route of Administration: Intravascular.
Semi-Synthetic Methods: Production of serum protein-dye conjugates consisting of transferrin (Tf) or human serum albumin (HSA) covalently linked to ITCC derivative.
Implementation: Evaluation of the ability of semi-synthetic ITCC-HSA and ITCC-Tf conjugates pertaining to (i) photophysical properties; (ii) ability to bind *in vitro* to tumor cells; and (iii) *in vivo* for their potential to detect/characterize experimental tumors.
Model: Human HT29 colon cancer cell types propagated *in vitro* in a tissue culture environment and HT29 tumor-bearing nude mice.
Interpretation and Analysis: In contrast to ITTC-HSA, ITCC-Tf displays *in vitro* receptor-mediated uptake by HT29 human colon cancer cells. After intravenous injection, ITCC-HAS and ITCC-Tf both induce increased *in vivo* fluorescence contrast of tumors. After 24 hours the contrast between tumor and normal tissue is significantly higher for ITCC-Tf than for HAS-ITCC-HSA. Dye-induced fluorescence can be found to be predominantly located in perinecrotic areas of tumor lesions. Furthermore, ITCC-Tf produces fluorescence of viable tumor cells, whereas ITCC-HAS fluorescence can be detected along connective tissue. Therefore, ITCC-labeled Tf and HSA can serve as macromolecular contrast agents for the optical imaging of tumors, with ITCC-Tf showing higher efficiency.

Macromolecular contrast agents for optical imaging of tumors: Comparison of indotricarbocyanine-labeled human serum albumin and transferrin. Becker A, Riefke B, Ebert B, Sukowski U, Rinneberg H, Semmler W, Licha K. Photochem Photobiol 2000 Aug; 72 (2), pp. 234–41.

Oral Cancer Lesions/Differentiation: Premalignant vs Malignant Lesions
5-Aminolevulinic Acid (5-ALA)-Induced Protoporphyrin IX (PPIX) Fluorescence

Cellular/Molecular Mechanism of Action: 5-aminolevulinic acid is capable of promoting fluorescence from PPIX compounds.
Background Information: 5-aminolevulinic acid induces fluorescence of PPIX that provides a sensitivity level of 95 to 100% for the diagnosis of oral cancer with a corresponding specificity of only approximately 50–60%.
Application: Differentiation of premalignant from malignant forms of oral cancer applying PPIX fluorescence endoscopy and fluorescence image quantification.

Route of Administration: Topical.
Implementation: Characterization of oral premalignant and malignant lesions applying PPIX fluorescence endoscopy and fluorescence image quantification. To improve diagnostic specificity, 5-ALA mediated digitized fluorescence endoscopic imaging systems have been designed that enable on-line image acquisition, analysis, and fluorescence quantification for early detection of oral cavity neoplasms.

Human Model: Cases of suspected premalignant and malignant lesions of the oral cavity (e.g., n = 16).
Interpretation and Analysis: Head and neck clinical trials have shown that the red-to-blue intensity ratio of malignant tissue is larger than that of benign tissue. By applying the intensity ratio as a diagnostic algorithm, both high specificity and sensitivity can be achieved for discriminating malignant tissue from benign tissue, and the capability of classifying different histopathological stages of oral lesions has also been demonstrated. Red fluorescence intensity distribution in lesion areas could also be obtained to better understand PPIX tissue accumulations. Our initial results indicate that the digitized endoscopic imaging system combined with the fluorescence image quantification method and the ratio diagnostic algorithm has the potential to significantly improve the non-invasive *in vivo* diagnosis of early oral neoplasms.

Detection of neoplasms in the oral cavity by digitized endoscopic imaging of 5-aminolevulinic acid-induced protoporphyrin IX fluorescence. Zheng W, Soo KC, Sivanandan R, Olivo M. Int J Oncol 2002 Oct; 21 (4), pp. 763–8.

Oral Lesions/Differentiation: Oral Premalignant vs Malignant Squamous Cell Carcinoma
5-Aminolevulinic Acid-Induced Protoporphyrin IX (PPIX) Fluorescence

Background Information: Studies of 5-aminolevulinic acid-induced PPIX fluorescence have shown a sensitivity of 95 to 100% for oral cancer diagnosis, but the specificity is only about 50 to 60%.
Application: Quantifying PPIX fluorescence imaging for specific diagnostic detection of early oral lesions (differentiation of premalignant from malignant disease states).
Implementation: Detection and characterization of suspected premalignant or malignant oral cavity lesions utilizing quantifying PPIX fluorescence endoscopic imaging modalities. Biopsy samples are also collected from tissue sites imaged for evaluation using histological analysis. The red-to-blue and red-to-green intensity ratios are then calculated from fluorescent images for correlation with histology analysis.

Human Model: Cases with known or suspected premalignant or malignant oral cavity lesion (e.g., n = 28 and n = 70 biopsy specimens).
Interpretation and Analysis: Suspicious lesions display bright reddish fluorescence, while normal mucosal tissues exhibit blue color background in the fluorescence images. The red-to-blue and red-to-green intensity ratios of malignant tissues are larger than those of benign tissues. Combining the 2 ratio diagnostic algorithms yields a sensitivity and specificity of 95% and 97%, respectively, exceeding each diagnostic algorithm alone for discriminating malignant tissue from benign tissue. Quantifying PPIX fluorescence endoscopic images combined with ratio diagnostic algorithms has the potential to significantly improve the *in vivo* non-invasive diagnosis of oral cavity lesions.
Instrumentation: Fluorescence endoscopy.

Detection of squamous cell carcinomas and pre-cancerous lesions in the oral cavity by quantification of 5-aminolevulinic acid induced fluorescence endoscopic images. Zheng W, Soo KC, Sivanandan R, Olivo M. Lasers Surg Med 2002; 31 (3), pp. 151–7.

Pancreas: Insulin (Islet Cell) Adenoma
Calcium

Application: Diagnosis of insulin-secreting tumors, including islet cell adenoma, that are undetectable during the course of preoperative morphologic (imaging) modalities.
Route of Administration: Calcium test (selective intra-arterial catheterization).
Dosage: Calcium gluconate (4 mg/kg/hour) is infused over a 2-hour period of time.
Considerations: Calcium ion *in vitro* plays a fundamental role in regulating insulin secretion but the influence of calcium excess on insulin release *in vivo* has not been clearly defined. The described test is not of diagnostic value for the stated purpose.
Implementation: Evaluate the efficacy of the intra-arterial calcium test for the purpose of localizing sources of hyperinsulinism that remain undetectable at preoperative morphologic studies (e.g., ultrasound, computed tomography, magnetic resonance imaging, endoscopic US, abdominal arteriography). The calcium test is performed, whereby insulin concentrations are measured in hepatic venous blood after selective intra-arterial calcium stimulation.

Human Model: Cases of clinically proven endogenous hyperinsulinism due to tumorous insulin production (e.g., n = 24). Of these cases, approximately 30% (n = 7/24) will have negative morphologic findings (n = 5/7 females, n = 2/7 males; age range, 30–66 years; mean age, 47 years).
Interpretation and Analysis: Approximately 30% of affected cases will have negative results from morphological analyses (e.g., n = 7/24). Surgical intervention may lead to the detection of solitary insulinomas (e.g., n = 6 with a mean diameter, 0.73 cm) and nodular hyperplasia (e.g., n = 1). Calcium tests allow accurate localization of the pathologic source of insulin secretion (e.g., n = 7/7). In less than 50% of cases, results of arterial calcium stimulation with hepatic venous sampling (ASVS) may affect intraoperative management (e.g., n = 3/7). An increase in insulin concentration after stimulation in the hepatic artery is not observed, making hepatic metastases unlikely. Arterial calcium stimulation with hepatic venous sampling, which is procedurally simpler than trans-hepatic pancreatic venous sampling, is effective for localizing sources of hyperinsulinism not detected with preoperative morphologic studies.

Other clinical trials have reported mean calcium plasma levels can increase from 9.6 ± 0.4 to 11.6 ± 0.8 mg/100 ml. During calcium infusion, blood glucose and plasma insulin concentrations can remain unchanged. Observations noted in these case-based studies suggest that calcium fails to stimulate basal insulin secretion even in cases of organic hyperinsulinism, and that calcium infusion is not helpful as a provocative test in the diagnosis of insulinoma.

Insulinoma and islet cell hyperplasia: Value of the calcium intra-arterial stimulation test when findings of other preoperative studies are negative. Pereira PL, Roche AJ, Maier GW, Huppert PE, Dammann F, Farnsworth CT, Duda SH, Claussen CD. Radiology 1998 Mar; 206 (3), pp. 703–9.

Pancreatic Insulin (Islet Cell) Adenoma/ [Lack of Diagnostic Efficacy]
Calcium Gluconate

Cellular/Molecular Mechanism of Action: Calcium gluconate *in vivo* does not promote the release of insulin from insulinoma lesions.

Background Information: Calcium ion *in vitro* plays a fundamental role in regulating insulin secretion but the influence of calcium excess on insulin release *in vivo* has not been clearly defined. The described test is not of diagnostic value for the stated purpose.
Application: Calcium gluconate *in vitro* promotes the release of insulin but *in vivo* it is ineffective in this capacity and is therefore of no diagnostic benefit in the detection and diagnosis of insulin-secreting tumors.
Route of Administration: Calcium gluconate (intravenous).
Dosage: Calcium gluconate (4 mg/kg/hr infused over a period of 2 hours).
Interpretation and Analysis: Mean calcium plasma levels increase from 9.6 ± 0.4 to 11.6 ± 0.8 mg/100 ml. During calcium infusion, blood glucose and plasma insulin concentrations remain unchanged. These observations suggest that calcium fails to stimulate basal insulin secretion even in cases of organic hyperinsulinism. They show that calcium infusion is not helpful as a provocative test in the diagnosis of insulinoma.

Lack of effect of calcium *infusion on blood glucose and plasma insulin levels in patients with insulinoma.* De Palo C, Sicolo N, Vettor R, Federspil G. J Clin Endocrinol Metab 1981 Apr; 52 (4), pp. 804–6.

Pancreatic Tumors: Vasoactive Intestinal Peptide (VIP) Secreting/VIPoma Syndrome: VIP/Pancreatic Peptide/Neurotensin/Enhanced Immunohistopathology Accuracy
Calcium, Pentagastrin

Diagnostic Application: Detection of patients suffering from pancreatic tumors consistent with VIPoma syndrome.
Background Information: Pancreatic endocrine tumors (PETs) may secrete a variety of peptide hormones, either alone or in combination, and intravenously administered provocative agents have been used to stimulate hormone release to aid in the diagnosis and localization of lesions in suspected cases.
Route of Administration: Intravenous injection.
Dosage: Pentagastrin (0.5 mg/kg/5 sec or 0.5 mg/kg/min); rapid calcium infusion (2 mg/kg/min).
Semi-Synthetic Methods: Plasma hormone radioimmunoassays and immunohistochemical studies are performed for a panel of peptide hormones, including VIP, neurotensin, and pancreatic polypeptide (PP). Acid alcohol extracts of tumor specimens are analyzed for the peptide hormones as well.
Implementation: Before surgical intervention, 4 different provocative test regimens can be administered intravenously after an overnight fast: (i) pentagastrin (0.5 mg/kg/5 sec); (ii) rapid calcium infusion (2 mg/kg/min); (iii) a combination of calcium (2 mg/kg/min) followed by pentagastrin (0.5 μg/kg/min); and (iv) secretin (2 clinical units/kg bolus). Blood samples are collected before each test and 1, 2, 3, 5, and 10 minutes after infusions.

Case Example: Detailed biochemical investigations and provocative testing in a 26-year-old man with a 5-cm VIP-secreting tumor located at the head of the pancreas.
Interpretation and Analysis: Increases in the production/release of vasoactive intestinal peptide, neurotensin, and PP can be detected in both serum samples and within tumor biopsies by immunohistochemical analysis. Coexpression of VIP and neurotensin can be seen immunohistochemically within some individual tumor cells. Provocative testing results in maximal stimulation of VIP and neurotensin secretion with pentagastrin administration, which produces increases in plasma levels of VIP and neurotensin over basal levels of 81% and 87%, respectively. After surgery, plasma levels of VIP, neurotensin, and PP are undetectable before and after administration of pentagastrin. Such observations emphasize the importance of comprehensive biochemical evaluation in cases of VIPoma syndrome to detect production of a range of peptide hormones. Administration of intravenous pentagastrin appears to stimulate release of VIP and neurotensin and should be evaluated further as a provocative agent for the diagnosis and follow-up of cases with these tumors.

Stimulation of vasoactive intestinal peptide and neurotensin secretion by pentagastrin in a patient with VIPoma *syndrome.* Brunt LM, Mazoujian G, O'Dorisio TM, Wells SA Jr. Surgery 1994 Mar; 115 (3), pp. 362–9.

ENDOCRINE

Adrenal Incidentaloma/Cushing's Syndrome: Preclinical/21-Hydroxylase and 11β-Hydroxylase Biochemical Activity/Tetrahydrocortisol, Tetrahydrocortisone, Tetrahydroaldosterone/ Potential to Distinguish between Non-Functioning Adrenocortical Adenoma (NFI) and Preclinical Cushing's Syndrome (PCS)/Hepatic Cortisol 5β-Reduction/Cytochrome 450 CYP21B Gene Expression
1-24 Adenocorticotripic Hormone (1-24ACTH) Stimulation Test

Background Information: Adrenal incidentalomas are mostly NFIs. However, in 5 to 12% of patients, a PCS is present with autonomous tumor cortisol production. Urinary free cortisol excretion is not sensitive enough to detect subclinical conditions of hypercortisolism. A limited amount of information is known about daily aldosterone production.
Route of Administration: 1-24ACTH stimulation test (intravenous).
Dosage: 1-24ACTH stimulation test (250 μg).
Implementation: Delineation of the relative sensitivity of tetrahydrocortisol, tetrahydrocortisone, and urinary free cortisone together with urinary free cortisol concentration as markers for characterizing increases in daily cortisol production recognized in conditions of adrenal incidentalomas. Note: Urinary free cortisol excretion is not sensitive enough to determine subclinical hypercortisolism. Complementary analyses involve the measurement of excreted aldosterone metabolites. Determination of the types of steroidogenic enzymes that have altered activities is done with the aid of the 1-24ACTH stimulation test, combined with the determination of multiple steroids. In situations where it is assumed that a 21-hydroxylase deficiency, or even an 11β-hydroxylase deficiency, may be involved in adrenal tumourigenesis, the prevalence of germline CYP21B and CYP11B1 mutations can be studied in the same cases that have been evaluated applying the ACTH stimulation test. Note: The ACTH stimulation test can detect increased responses in the form of precursor production within the glucocorticoid and mineralocorticoid pathway.

Human Model: Cases of adrenal incidentalomas (n = 35; n = 29 NFI; n = 6 PCS; n = 35 healthy controls). Performance of the 1-24ACTH stimulation test (n = 25 cases; n = 18 healthy controls).
Interpretation and Analysis: Glucocorticoid metabolites are within the normal range in a small percentage of NFI cases (e.g., n = 3/29). As a group, cases have subtle alterations in cortisol metabolism. Tetrahydrocortisol excretion is elevated in NFI and PCS compared to normal subjects (2.1 ± 0.2 and 2.5 ± 0.5 vs 1.5 ± 0.1 mg/24 h; $p < 0.05$). Accordingly, a 2-fold elevation of the tetrahydrocortisol/free cortisol ratio indicates increases in 5β-reduction

of cortisol in the liver. Tetrahydroaldosterone and aldosterone-18-glucuronide excretions are not different relative to controls. In incidentaloma cases, an increased response to ACTH can be appreciated as a function of changes in the concentrations of 17-hydroxyprogesterone (595 ± 133 vs 160 ± 25 ng/dL), 21-desoxycortisol (105 ± 25 vs 29 ± 9 ng/dL), and 11-desoxycortisol (401 ± 40 vs 293 ± 17 ng/dL).

In a small percentage of cases (n = 1/25 or 4%), a heterozygous deletion in exon 3 of the CYP21B gene can be detected. Therefore, even the excretion of the main glucocorticoid metabolites appears not to be a marker that is sensitive enough to distinguish between NFI and PCS. However, it is also possible that alterations in cortisol secretion are qualitative rather than quantitative. Zona glomerulosa function is not influenced. The elevation of 21-desoxycortisol represents evidence that argues against an impairment of 11β-hydroxylase and is instead better explained by decreased 21-hydroxylase activity. The remainder of cases evaluated will have wild-type sequences of both genes. In conclusion, neither 21-hydroxylase deficiency nor 11β-hydroxylase deficiency are predisposing factors for adrenal tumourigenesis.

Metabolism of glucocorticoids and mineralocorticoids in patients with adrenal incidentalomas. Maser-Gluth C, Reincke M, Allolio B, Schulze E. Eur J Clin Invest 2000 Dec; 30 Suppl 3, pp. 83–6.

Aldosterone-Producing Adenoma (APA)/Adrenal Mineral Corticoid Function/Dopamine Excretion: Urinary
Metoclopramide/High-Salt Intake/Low-Salt Intake

Physiological Mechanism of Action: Metoclopramide enhances cholinergic activity at muscarinic synapses and suppresses/antagonizes central nervous system (CNS) dopaminergic activity. Normally, dopaminergic input inhibits aldosterone secretion.
Background Information: Impairment of renal dopamine excretion on high-salt diet (HS) may account for increased blood pressure (BP) in hypertensive subjects. Whether such impairment of dopaminergic activity exists in the adrenal gland is unknown.
Route of Administration: Metoclopramide (intravenous).
Dosage: Metoclopramide (10 mg).
Implementation: Delineate the degrees of dopaminergic inhibition of aldosterone secretion in conditions of an APA. More specifically, evaluate APA conditions on diets containing different levels of salt at the end of which a 24-hour ambulatory BP recording is obtained in concert with measurements of daily urine catecholamine and the performance of a metoclopramide test.

Human Model: Cases of unilateral APA (n = 15) fed a low-salt diet (LS) for 1 week, followed by another week on a high-salt diet. At the end of each diet period, 24-hour ambulatory BP recording, daily urine catecholamine measurement, and a metoclopramide test are performed.
Interpretation and Analysis: A high-salt diet increases both daytime and nighttime blood pressure (BP at $p < 0.001$), and urine dopamine excretion ($p < 0.01$). Intravenous metoclopramide injection increases plasma aldosterone concentrations (PAC) on both diets. The area-under-the-curve for PAC between LS and HS is not different, but the area-under-the-curve for PAC increment is greater on HS ($p < 0.05$). Less than half of affected cases with increment areas on HS greater than those on LS by 50% are termed "suppressible" (n = 6/15), and the remainder are referred to as "non-suppressible." On a high-salt diet, so-called "suppressible" cases have greater urine dopamine and less urine norepinephrine excretions ($p < 0.05$). The "non-suppressible" cases have a greater percentage increase of nighttime BP by HS than the suppressible (for systolic BP, 13.1% vs 4.5%, $p < 0.01$; for mean BP, 12.0% vs 5.1%, $p < 0.01$, respectively), but no difference in daytime blood pressure.

Two subtypes of APA can be defined according to their responses to metoclopramide during salt manipulation. On a high-salt diet, the non-suppressible subjects, with less dopaminergic inhibition of aldosterone secretion, have less urinary dopamine excretion and greater BP elevation. The renal and adrenal dopaminergic activities are regulated in a parallel fashion.

Dopaminergic modulation of aldosterone secretions on changes of sodium intake in aldosterone-producing adenoma. Wu KD, Chen YM, Chu TS, Chueh SC, Tsai GC, Tseng YZ, Hsieh BS. Am J Hypertens 2002 Jul; 15 (7 Pt 1), pp. 609–14.

Multiple Endocrine-Related Neoplasias/Multiple Endocrine Neoplasia Type 2A Syndrome (MEN 2A)/Multiple Endocrine Neoplasia Type 2B (MEN 2B)/Familial Medullary Thyroid Carcinoma (FMTC)
Pentagastrin and Calcium Chloride/[Pentagastrin and Calcium Gluconate]

Cellular/Molecular Mechanism of Action: Combined influence of pentagastrin and calcium chloride ($CaCl_2$) promotes the release of calcitonin from certain types of endocrine neoplasias.
Application: Characterization and detection of MEN 2A. The pentagastrin provocative test and biochemical screening can be applied to rule out either hyperparathyroidism or pheochromocytoma.

Medullary thyroid carcinoma (MTC) is a malignancy of the thyroid C-cells that comprises 5–10% of all thyroid cancers. It occurs in both sporadic and familial forms, the latter making up 25% of all MTCs and being comprised of 3 distinct syndromes that have been recognized, including MEN 2A, MEN 2B, and FMTC. To date, screening for MTC has been performed using the pentagastrin stimulation test, which is a provocative test for calcitonin release.
Route of Administration: Pentagastrin (intravenous bolus), calcium chloride (intravenous over 5 minutes).
Dosage: Pentagastrin (0.5 μg/kg); calcium infusions (2 mg/kg).
Considerations: RET proto-oncogene analysis can be carried out by automatic DNA sequencing in concert with adequate digestion of polymerase chain reaction (PCR) amplified products for exons 10 and 11. Two distinct mutations in the RET proto-oncogene have been identified. A T >A transition at position 1783 (codon 618) in exon 10 has been detected in 1 family, and a G >A replacement at position 1832 (codon 634) in exon 11 in others. Germline mutations in the RET proto-oncogene have been identified in families manifesting these syndromes and genetic screening of individuals at risk of 1 of these syndromes has become integral to their clinical management. The majority of the mutations associated with MEN 2A and FMTC are tightly clustered in a cysteine-rich region of the RET receptor. A single mutation associated with MEN 2B is in the tyrosine kinase domain of the RET receptor.
Implementation: Evaluation of MEN 2A as a function of calcitonin release in response to pentagastrin and calcium chloride administration.

Human Model: Cases (n = 44) related to 3 individuals with proven MEN 2A and negative reference controls.
Interpretation and Analysis: Peak calcitonin (CT) levels are usually reached within 2 to 5 minutes after administration of provocative stimulus. Normal subjects have no significant difference in the mean basal CT levels between males (54.8 ± 21.7 pg/ml) and females (56.5 ± 34.8 pg/ml). Mean peak response values for males

is 146.3 ± 120.6 pg/ml, which is significantly different from the mean value of females, namely 71.6 ± 39.0 pg/ml. No significant correlations exists between the basal CT level, peak CT response, and age.

Calcium infusions (2 mg/kg/5 min) in normal individuals (e.g., n = 18 males; n = 37 females) yields a significantly greater secretory response than does pentagastrin (0.5 μg/kg bolus). In cases with medullary thyroid carcinoma (e.g., n = 12), pentagastrin is a better secretagogue than calcium ($p < 0.001$). Therefore, some recommendations have suggested routine measurement of plasma silica-extractable calcitonin and pentagastrin injection as the provocative test of choice for the detection and management of MTC. The short calcium-infusion test is a good alternative.

In cases from families with a history of MEN 2A, approximately 20% display exaggerated calcitonin responses to combined pentagastrin/$CaCl_2$ sufficient to warrant thyroidectomy. Histological examination can confirm the presence of C-cell hyperplasia (CCH) (n = 1/9) or MTC (n = 8/9). A relatively high percentage of cases (33%) will have high basal plasma calcitonin. Postoperatively, basal plasma calcitonin levels decline and response to pentagastrin/$CaCl_2$ produces a flat baseline concentration profile. Screening of cases with histological proof of MTC or CCH for a genetic linkage to the gene for MEN 2A using an MCK2 probe usually shows some correlation.

Several families (n = 10) FMTC due to V804M mutation have been reported in the literature. Some reports have also described kindreds with not only heterozygous but also homozygous members with the V804M mutation. In gene carriers with the V804M mutation (e.g., n = 4 homozygous; n = 22 heterozygous) the pentagastrin stimulation test result is within normal range for the vast majority of heterozygous gene carriers (thyroidectomy not indicated). Therefore, in some cases, the V804M mutation in heterozygous cases seems not to be enough to express the full disease.

In non-gene carriers, false-positive results for basal calcitonin (6.6%) and for the pentagastrin provocative test (15.4%) can be observed. Based on these observations, the specificity of basal and pentagastrin-stimulated calcitonin is rather limited and RET proto-oncogene analysis is considered by many investigators and clinicians to be the 1st-line screening procedure of choice for the identification of gene carriers.

Experience with provocative and genetic tests strongly supports the concept of the indolent behavior of V804M RET proto-oncogene mutation. Counseling for preventive total thyroidectomy, the specific mutation of RET proto-oncogene, and also the natural history of the disease within a particular family should be considered.

Instrumentation: Scintilation counter and minor laboratory instrumentation necessary for the utilization of a commercial radioimmunoassay for the measurement of serum calcitonin. Radioimmunoassays for human calcitonin in whole plasma are limited in sensitivity and specificity; basal values of calcitonin are often undetectable in normal plasma, and non-specific increases are occasionally found in seemingly healthy persons. Such problems can be avoided by applying a silica-cartridge extraction-concentration technique for calcitonin assay, and the effectiveness of 2 calcitonin stimulation tests in healthy volunteers and patients with medullary thyroid carcinoma is then compared. The radioimmunoassay can be improved by using a new antiserum and a sequential incubation procedure that reduces the previously used sample-volume requirement and incubation period substantially. The method can be used to measure mean basal plasma levels of calcitonin (± SD) in 45 normal men (8.2 ± 5 pg/ml) and 47 normal women (4.8 ± 4 pg/ml) ($p < 0.001$).

Pars Intermedia Pituitary Adenoma (PIPA)/ Cushing's Syndrome

Dexamethasone Suppression/Thyrotropin-Releasing Hormone (TRH)

Cellular/Molecular Mechanism of Action: Dexamethasone suppresses hypothalamic corticotropin-releasing hormone (CRH) synthesis/release, which in turn decreases adenocorticotripic hormone (ACTH) synthesis.

Application: Detection and characterization of horses affected by PIPA. To evaluate results of a combined dexamethasone suppression/TRH stimulation test in horses suspected clinically of having a PIPA.

Route of Administration: Dexamethasone (intravenous); TRH (intravenous).

Dosage: Dexamethasone (40 μg/kg [18 μg/lb] of body weight); TRH (1.1 mg).

Implementation: Evaluation of changes in cortisol concentrations in conditions of PIPA induced by dexamethasone administration. Blood samples are collected at baseline prior to dexamethasone suppression and then at 3 hours postinjection. A dose of TRH is then administered, followed by the collection of serial blood samples at 15, 30, 45, 60, and 90 minutes and 21 hours after TRH administration (24 hours after dexamethasone injection). Cortisol concentrations are measured in all blood samples collected for analysis.

Animal Model: Equine (n = 5 horses suspected of having PIPA; n = 7 healthy adult horses).

Interpretation and Analysis: Baseline cortisol concentrations are significantly lower in horses suspected of having a PIPA compared to healthy horses. Cortisol concentrations are suppressed by dexamethasone in both groups; however, after TRH administration, cortisol concentrations returned to baseline values in horses suspected of having a PIPA, but not in healthy horses. Concentrations are still less than baseline values 24 hours after dexamethasone administration in healthy horses.

The combined dexamethasone suppression/TRH stimulation test may be a useful diagnostic test in horses suspected of having a PIPA. For clinical application, collection of a blood sample 30 minutes after TRH administration is recommended.

Results of a combined dexamethasone suppression/thyrotropin-releasing hormone stimulation test in healthy horses and horses suspected to have a pars intermedia pituitary adenoma. Eiler H, Oliver JW, Andrews FM, Fecteau KA, Green EM, McCracken M. J Am Vet Med Assoc 1997 Jul 1; 211 (1), pp. 79–81.

Pheochromocytoma

Glucagon

Background Information: Pheochromocytoma cases frequently have elevated plasma concentrations of adrenaline and noradrenaline in addition to increases in urinary vanillylmandelic acid, metanephrine, and/or normetanephrine.

Glucagon-receptor messenger RNA (mRNA) can be detected by reverse transcription-polymerase chain reaction in human pheochromocytomas, but not in normal adrenal medullas. Quantitative autoradiography can detect the presence of abundant [^{125}I]-$Thyr_{10}$ glucagon binding sites in pheochromocytomas, which are recognized with cold glucagon and the glucagon receptor antagonist $Des\text{-}His_1[Glu_9]$glucagon amide (GR-A). The adrenal medulla is weakly labeled, and the binding is not displaced by GR-A. Glucagon enhances epinephrine and norepinephrine release by pheochromocytoma slices, minimal and maximal effective concentrations being

10^{-8} M and 10^{-6} M. Adrenomedullary slices have a weak catecholamine response only to 10^{-5} M glucagon. GR-A abolishes the secretory response to glucagon of pheochromocytomas, but not of adrenal medullas. Collectively, these observations indicate that (human) pheochromocytomas, but not adrenal medulla, express glucagon receptors and possess a marked secretory response to glucagon, thereby providing the rationale to explain the specificity of the glucagon provocative test in the diagnosis of pheochromocytoma.

Application #1: Study of pig adrenal medulla or human pheochromocytoma to determine the influence of glucagon infusion. In the 1st experiment a continuous flow incubation system has been developed in which the secretory response to these drugs is characterized by a serial fluorimetric assay of catecholamines in the effluent medium.

Application #2: Characterize the *in vivo* effect of glucagon on blood pressure and urinary catecholamine levels of healthy control subjects and of patients suffering from essential hypertension, thyroid disease, diabetes mellitus, and acromegaly.

Route of Administration: Pheochromocytoma and chromaffin granules of the pig adrenal medulla in experimental *ex vivo*/*in vitro* scenarios.

Dosage: Glucagon (10^{-4}) infusion for 10 minutes following 2 hours of preincubation.

Considerations: Most patients suffering from pheochromocytoma are diagnosed by measuring urine concentrations of vanillylmandelic acid, metanephrine, and/or normetanephrine in addition to plasma catecholamine concentrations. However, a subset of patients suffering from the condition will not have elevated concentrations of the metabolites in the plasma or urine.

Administration of phenotolamine (blocking agent) prior to histamine challenge test in order to protect against hypotension or catecholamine-induced arrythmias.

Implementation (Clinical): Evaluation of conditions of suspected pheochromocytoma as a function of catecholamine and blood pressure response to glucagon challenge.

Human Model: Extra-adrenal pheochromocytoma.

Interpretation and Analysis: During *in vitro* preparations, glucagon (10^{-4}) exposure for 10 minutes following a 2-hour incubation period results in abrupt increases in adrenaline and noradrenaline (epinephrine to 0.08 μg/mg, and norepinephrine to 0.07 μg/mg). Systemically (*in vivo*), there is initially a massive release of catecholamines that declines to basal levels (0.02 μg/mg) after 1.5 hours. During the course of *in vivo* administration trials, glucagon induces a slight but constant increase in blood pressure. By contrast no significant urinary catecholamine elevation may be evoked. Adjunct diagnostics can include detection using [^{131}I]-metaiodobenzylguanidine (MIBG) scintigraphy.

Human pheochromocytomas, but not adrenal medulla, express glucagon-receptor gene and possess an *in vitro* secretory response to glucagon**.** Albertin G, Aragona F, Gottardo L, Malendowicz LK, Nussdorfer GG. Peptides 2001 Apr; 22 (4), pp. 597–600.

Pheochromocytoma *[Metaclopramide]/[^{131}I]-Metaiodobenzylguanidine (MIBG)*

Background Information: Pheochromocytoma accounts for approximately 25% of incidentally discovered adrenal masses. Certain diagnostic procedures (e.g., adrenal arteriography, needle biopsy of an adrenal mass), anesthesia, and abdominal surgery may cause a sudden release of catecholamines from a pheochromocytoma and induce paroxysmal attacks of hypertension. In addition, pheochromocytoma is well known to cause unsuspected operating room deaths. Therefore, it is important to carefully separate this functioning neoplasm from other types of adrenal masses.

Pheochromocytoma cases frequently have elevated plasma concentrations of adrenaline and noradrenaline in addition to increases in urinary vanillylmandelic acid, metanephrine, and/or normetanephrine.

Application: Detection of pheochromocytoma.

Route of Administration: Metaclopramide (IV); [^{131}I]-MIBG (IV).

Considerations: In situations where results from these assays are negative, both the metaclopramide (provocative test) and [^{131}I-] or [^{123}I]-MIBG (scintigraphy) can be performed as an alternative diagnostic strategy.

Implementation: Compare results of non-invasive tests including (i) assay of urinary catecholamines and their metabolites; (ii) a provocative pharmacologic test using metoclopramide (MCP test); and (iii) [^{131}I]-MIBG scintigraphy to screen for pheochromocytoma.

Human Model: Cases with an incidentally discovered adrenal mass (n = 10; n = 6 pheochromocytomas, n = 4 non-functioning adrenocortical adenomas).

Interpretation and Analysis: Urinary excretion of catecholamines, metanephrines, and vanillylmandelic acid may be positive in only 50% of cases (e.g., n = 5/10; n = 4 negative; n = 1 false-negative; sensitivity = 83%, specificity = 100%). The [^{131}I/^{123}I]-MIBG reagent uptake within adrenal lesions is increased to the extent they can be visualized in diagnostic nuclear medicine studies. The MCP test may be positive in < 50% of cases (e.g., n = 3/7; n = 3/7 negative; n = 1 false-negative; sensitivity = 75%, specificity = 100%). Metaiodobenzylguanidine scintigraphy may similarly be positive in over 50% of cases (e.g., n = 4/7; n = 1/7 negative; n 2/7 false-negative; sensitivity = 67%, specificity = 100%). Such observations suggest that all patients with an incidentally discovered adrenal mass should undergo a determination of the 24-hour urinary excretion of catecholamines and their metabolites, including metanephrines. If this urine assay is negative, other non-invasive tests including the MCP test and MIBG scintigraphy should be considered in selected patients with radiographic characteristics of pheochromocytoma.

Non-invasive screening for pheochromocytoma in patients with an incidentally discovered adrenal mass: Usefulness of provocative test with metoclopramide and [^{131}I]-metaiodobenzylguanidine scintigraphy. Yokoyama H, Tsuji Y. Hinyokika Kiyo 1999 Oct; 45 (10), pp. 677–80.

Pheochromocytoma (Silent/Subclinical) *Glucagon/[^{131}I]-Metaiodobenzylguanidine (MIBG)*

Application: Diagnosis of subclinical/silent pheochromocytoma.

Route of Administration: Glucagon (IV) and [^{131}I]-MIBG (IV).

Considerations: Adrenal masses can sometimes be incidentally discovered during abdominal ultrasonography and computed tomography procedures in cases that are normotensive, asymptomatic, and have nearly normal plasma catecholamine levels. However, urinary levels of catecholamine metabolites are often slightly elevated in such cases.

Implementation: Evaluation of suspected conditions of pheochromocytoma. Note: A cutoff at 4.0 cm of tumor mass size can be set for differentiating benign and malignant tumors.

Interpretation and Analysis: A glucagon provocative test and [^{131}I]-MIBG scintigram can be useful for diagnosing atypical pheochromocytoma. In cases classified as incidentaloma, 82.5% are benign masses, of which 35.0% (n = 36/85) are non-functional adre-

nal cortical adenomas, and 11.7% (n = 12/85) are pheochromocytoma. Cases with malignant masses are significantly younger than patients with adenomas ($p < 0.05$), and adenomas are significantly smaller than malignant lesions ($p < 0.001$). During the diagnosis of atypical incidentaloma, it's essential to evaluate hormonal activity and assess the risk for malignancy. If the mass size is 4 cm or larger, the risk of malignancy increases. More attention should be paid to subclinical hyperfunctional state.

A clinical analysis of 103 cases of adrenal incidentaloma. Tao H, Lu ZL, Li HZ. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2003 Apr; 25 (2), pp. 172–5.

Hypertension following minor trauma: A rare presentation of pheochromocytoma. Dueck A, Poenaru D, Kamal I. Pediatr Surg Int 1999; 15 (7), pp. 508–9.

Thyroid Cancer
Thyroid-Stimulating Hormone (TSH)

Background Information: Detection of recurrent thyroid cancer tumor requires TSH stimulation for radioiodine scanning and thyroglobulin (Tg) measurement. Temporary thyroid hormone withdrawal has previously been used, but causes hypothyroidism and, rarely, tumor progression.
Implementation: Evaluation of recombinant thyrotropin (rTSH) assessed in 2 randomized clinical trials in which cases have had [^{131}I] and Tg testing twice: (i) initially after rTSH, and (ii) after thyroid hormone withdrawal. Note: Quality of life comparisons have subsequently been made in preliminary investigations.
Interpretation and Analysis: In trials with positive-scan cases (49% of total), rTSH is equivalent to withdrawal in 66%, superior in 5%, and inferior in 29% ($p < 0.05$), suggesting a lesser sensitivity for rTSH scans. In a 2nd trial employing enhanced techniques, patients with positive scans (49%), there is no difference in the number of superior rTSH and withdrawal scans. Furthermore, among all cases with withdrawal study evidence of residual thyroid tissue, 74% of all patients with residual thyroid tissue and 100% of cases with tumor metastases have rTSH-stimulated thyroglobulin values above 2 ng/mL. Naturally, cases experience significantly more symptoms and diminished quality of life in a hypothyroid state than after rTSH. Therefore, combined rTSH-stimulated radioiodine and Tg testing is as sensitive as thyroid hormone withdrawal to detect recurrent thyroid cancer, and causes less morbidity.

Recombinant thyrotropin for detection of recurrent thyroid *cancer*. Ladenson PW. Trans Am Clin Climatol Assoc 2002; 113, pp. 21–30.

Thyroid Cancer/Medullary Thyroid Carcinoma (MTC)/(Induced Calcitonin Secretion)
Histamine/Calcium/Pentagastrin Infusion/Glucagon

Cellular/Molecular Mechanism of Action: Histamine promotes a secretagogue effect on calcitonin in conditions of MTC.
Application: Characterization of cases of MTC due to its ability to induce calcitonin secretion. Glucagon and calcium are other known stimuli for calcitonin secretion in cases of MTC. Histamine will not replace these agents in provocative tests but can provide better insight into the behavior of this neoplastic cell type.
Implementation: Determination of the relative effectiveness of histamine, calcium/pentagastrin, and glucagon as calcitonin secretagogues in cases of MTC.
Interpretation and Analysis: In cases of MTC with undetectable levels of basal plasma calcitonin (e.g., n = 7/15), calcium and pentagastrin can promote detectable elevations in plasma calcitonin concentrations. Similar responses cannot be appreciated for histamine or glucagon administration. Alternatively, in cases of MTC that do express detectable baseline levels of calcitonin (e.g., n = 8/15), significant responses can be seen in response to glucagon, calcium/pentagastrin, and histamine but the calcium/pentagastrin combination induces the most potent secretagogue effect. Blockade of H_2-receptor complexes with cimetidine has no effect on basal calcitonin concentrations and does not suppress responses to histamine, calcium, or pentagastrin. The variable secretagogue effect of histamine may be mediated through H_1-receptors, through non-specific vascular dilation "washing out" preformed calcitonin, or through its destruction to varying degrees by histaminase, present in most medullary thyroid tumors. Histamine is unlikely to replace calcium/pentagastrin as the most discriminating provocative diagnostic agent in MTC. However, correlation of secretory responses with tissue histaminase concentrations and attempted blockade with differing antihistamines will further understanding of this tumor type.

Some laboratories have proposed that a definitive diagnosis can be established by measuring calcitonin and carcinoembryonic antigen (CEA) levels in sera. In cases with normal levels of calcitonin and CEA, the provocative test (Ca-gluconate plus pentagastrin) can be useful [e.g., (pt. no. 11,K.N.)].

Histamine and calcitonin release from medullary thyroid carcinoma. Farndon JR, Lewis KR, Dale JK, Hinshaw WM, Dilley WG, Wells SA Jr. Cancer 1983 Apr 1; 51 (7), pp. 1221–5.

Thyroid Cancer (Differentiated)/Metastatic Disease
Recombinant Thyrotropin

Cellular/Molecular Mechanism of Action: Recombinant thyrotropin stimulates iodine uptake in thyroid tissue.
Application: Detection of differentiated thyroid cancer (± metastasis).
Considerations: Recombinant thyrotropin is a safe and effective diagnostic agent for cases that require radioiodine scanning for routine follow-up. The use of recombinant thyrotropin has fewer adverse effects than does the alternative, which is withdrawal of thyroid hormone replacement, although nausea and headache have been reported. Recombinant thyrotropin is relatively expensive.
Interpretation and Analysis: A serum thyroglobulin > 2 ng/mL and/or a positive whole-body scan after recombinant thyrotropin stimulation suggest residual thyroid tissue or neoplastic disease. The combined application of measured serum thyroglobulin concentrations and whole-body radioiodine scintigraphy can identify virtually all patients with distant metastatic disease.

The use of recombinant thyrotropin in the follow-up of patients with differentiated thyroid cancer. Basaria M, Graf H, Cooper DS. Am J Med 2002 Jun 15; 112 (9), pp. 721–5.

Thyroid Carcinoma: Medullary Thyroid Carcinoma (MTC; Hereditary)/(C-Cell Disease)
Omeprazole/Pentagastrin

Cellular/Molecular Mechanism of Action: Pentagastrin and omeprazole both induce the synthesis/release of calcitonin that becomes excessively increased within the plasma in conditions of MTC.
Application: Diagnosis of MTC through the induction of increased serum calcitonin.
Route of Administration: Omeprazole (oral).
Dosage: Omeprazole (20 mg twice a day for 4 days); pentagastrin (0.5 μg/kg of body weight).
Implementation and Validation: Evaluation of conditions of hereditary MTC.

Interpretation and Analysis: In conditions of hereditary MTC, pentagastrin administration produces a significantly higher increase in serum calcitonin than does omeprazole. Occasional unpleasant side effects are associated with the administration of pentagastrin by injection, including substantial "tightness" being appreciated in almost all cases. Alternatively, omeprazole is not associated with inducing any significant adverse side effects. A direct positive correlation exists between calcitonin-% (ratio of calcitonin peak to basal value × 100) and gastrin-% (ratio of gastrin peak to basal value × 100) during the omeprazole test in MTC patients ($r = 0.73$; $p < 0.001$). In spite of several adverse effects, pentagastrin remains the best provocative test for the diagnosis of MTC. Omeprazole may be useful when pentagastrin is contraindicated or refused because of the unpleasant side effects, but further validation is needed.
Instrumentation: Laboratory facilities and reagents necessary for calcitonin radioimmunoassay or ELISA-based methodologies.

Comparison of 2 provocative tests for calcitonin in medullary thyroid carcinoma: Omeprazole vs pentagastrin. Vitale G, Ciccarelli A, Caraglia M, Galderisi M, Rossi R, Del Prete S, Abbruzzese A, Lupoli G. Clin Chem 2002 Sep; 48 (9), pp. 1505–10.

Thyroid Medullary Carcinoma/ (Familial Chromaffinomatosis)
Oral Whiskey/Pentagastrin

Cellular/Molecular Mechanism of Action: Induces or promotes the secretion of calcitonin.
Background Information: Familial medullary carcinoma of thyroid (MCT) can be diagnosed early by measuring stimulated plasma calcitonin following provocation with oral whiskey or pentagastrin. The calcitonin release, however, can be variable following both stimuli.
Diagnostic Application: Detection of cases associated with thyroid medullary carcinoma.
Route of Administration: Whiskey (oral ingestion); pentagastrin (intravenous).
Dosage: Whiskey (50 ml); pentagastrin (0.5 μg/kg).
Implementation: Determine whether a humoral mechanism might be involved in mediating calcitonin release assessed by measuring 9 gut peptides, histamine, alcohol, calcium, and calcitonin plasma concentrations.

Human Model: Cases affected by multiple endocrine neoplasia Type II (MEN II) syndrome ($n = 8$).
Interpretation and Analysis: Pentagastrin produces significant rises in plasma calcitonin, histamine, and pancreatic polypeptide. Whiskey induces a variable rise in plasma alcohol but significant rises in plasma calcitonin, histamine, and pancreatic polypeptide, as well as insulin ($p < 0.05$). Such observations suggest that histamine may be the mediator of calcitonin release following provocation by either pentagastrin or alcohol. Histamine infusion requires evaluation as a screening test for MCT in MEN II syndrome.

Multiple endocrine neoplasia type II: The role of gastrointestinal humoral factors in calcitonin release following alcohol and pentagastrin stimulation. Fletcher DR, Gamvros O, Man WK, Ahmed Y, Trayner I, Adrian T. Aust N Z J Surg 1984 Jun; 54 (3), pp. 271–5.

Pentagastrin, calcium and whisky stimulated serum calcitonin in medullary carcinoma of the thyroid. Emmertsen KK, Nielsen HE, Mosekilde L, Hansen HH. Acta Radiol Oncol 1980; 19 (2), pp. 85–9.

Whisky: A new provacative test for calcitonin secretion. Dymling JF, Ljungberg O, Hillyard CJ, Greenberg PB, Evans IM, MacIntyre I. Acta Endocrinol (Copenh) 1976 Jul; 82 (3), pp. 500–9.

IMMUNE SYSTEM

Lymph Node: Sentinel Mapping/ Adenocarcinoma Colorectal Cancer
Isosulfan Blue Dye

Cellular/Molecular Mechanism of Action: Isosulfan blue is a pigment-based dye reagent.
Background Information: Sentinel lymph node (SLN) biopsy is a widely accepted method for staging breast cancer and melanoma, and it has recently been proposed as a means of improving staging in colorectal cancer. However, lymphatic mapping in colorectal cancer has been plagued by studies demonstrating high false-negative rates.
Application: Evaluate possible mechanisms for high false-negative rates after SLN biopsy in colorectal cancer. Poor accuracy may be due to bulky tumor mass or complete replacement of lymph nodes by tumor lesions.
Route of Administration: Isosulfan blue dye (intralesional injection).
Dosage: Isosulfan blue dye (1 ml volumetric).
Implementation: Intraoperative injection of isosulfan blue dye into colorectal adenocarcinoma lesions for the purpose of lymphatic mapping using either an *in vivo* or *ex vivo* technique. Routine pathological evaluations are then performed. Sentinel node are examined by hematoxylin and eosin stains, and if they are negative they are subsequently evaluated by cytokeratin immunohistochemistry. The patient's age, operation type, tumor stage, tumor diameter, method of SLN detection, presence of palpable nodes, and pathological description of nodes completely replaced by tumor are recorded.

Human Model: Cases undergoing colorectal cancer resection (mean age = 62.8 years, 50% male).
Interpretation and Analysis: Evaluation of lymphatic mapping procedures for the assessement of colorectal cancer conditions. Right- and left-sided colorectal resections are almost equally distributed (48% vs 42%). Sentinel lymph nodes can be successfully identified in a large percentage of cases (e.g., n = 47/51 or 92%). The mean number of SLNs obtained from each specimen is approximately 1.5 (range, 1–5). Routine pathological evaluation demonstrates lymph node metastasis in less than half of cases (e.g., n = 20/47 or 43%) that have SLN identified. The SLN is positive for metastasis in about half of these cases (e.g., n = 10/20). Approximately half of cases with metastasis have a negative SLN resulting in a false-negative rate of 50% (e.g., n = 10/20 cases). The rate of false-negatives is significantly higher in patients undergoing left-sided procedures vs right-sided procedures. Differences among gender, tumor stage, tumor diameter, method of SLN detection, presence of palpable nodes, and pathological description of nodes completely replaced by tumor are not associated with a higher false-negative rate.

Does tumor burden limit the accuracy of lymphatic mapping and sentinel lymph node biopsy in colorectal cancer? Broderick-Villa G, Ko A, O'Connell TX, Guenther JM, Danial T, DiFronzo LA. Cancer J 2002 Nov–Dec; 8 (6), pp. 445–50.

Lymphoma/Leukemia Nucleoli Targeting/ Gene Expression
Fluorescein-Labeled Phosphorothioate (PS)-Triple Helix-Forming Oligonucleotide (TFO)/Antisense Oligonucleotides

Cellular/Molecular Mechanism of Action: Oligonucelotide sequences can be designed that recognize and bind to specific gene/messenger RNA (mRNA) sequences.

Physiological Mechanism of Action: Selective inhibition of the transcription/translation of specific genes.
Background Information: The c-myc gene is frequently deregulated and overexpressed in human cancers, and strategies designed to inhibit c-myc expression in cancer cells may have considerable therapeutic value.
Application: Detection and characterization of lymphoma/leukemia cell types based on the detection of the homopurine-homopyrimidine sequence in the P2 promoter of the c-myc gene. Resistance to intra- and extracellular nucleases is a property that is required for the design of oligonucleotides intended for *in vivo* testing and therapeutic applications.
Route of Administration: Potentially variable (intravenous, intralesional).
Semi-Synthetic Methods: Triple helix-forming oligonucleotide can be synthesized with PS internucleotide linkages to confer resistance to intra- and extracellular nucleases. The resulting preparation can be conjugated with fluorescein as a tracer agent to identify selective accumulations within certain tissues and subcellular organelles.
Implementation: Characterize the antigen and antiproliferative activity of a TFO targeted to a homopurine-homopyrimidine sequence in the P2 promoter of the c-myc gene.
Interpretation and Analysis: Phosphorothioate-modified triple helix-forming oligonucleotide forms triplex DNA complexes with affinity and specificity comparable with that of the corresponding phosphodiester TFO, as delineated by gel mobility shift and footprinting assays. Fluorescence microscopy and polyacrylamide gel analysis reveal that fluorescein-labeled PS-TFO accumulates in the nuclei of CEM leukemia cells and remains intact for a duration of at least 72 hours. Incubation of CEM cells with PS-TFO reduces c-myc RNA and protein levels. A single exposure of leukemia cells to the PS-TFO is sufficient to induce dose-dependent growth inhibitory effects. Growth inhibition correlates with accumulation of cells in S phase and with induction of cell death by apoptosis. Preparations of PS-TFO are also effective in other leukemia and lymphoma cell lines. Control oligonucleotides have minimal effects in all assays. These data indicate that c-myc-targeted PS-TFO is an effective antigene and antiproliferative agent, with potential as an agent for *in vivo* testing as a novel approach to cancer therapy/detection.

Antigene and antiproliferative effects of a c-myc-targeting phosphorothioate triple helix-forming oligonucleotide in human leukemia cells. McGuffie EM, Pacheco D, Carbone GM, Catapano CV. Cancer Res 2000 Jul 15; 60 (14), pp. 3790–9.

Lymphoma/Mammary Carcinoma
Fluorescein-Somatostatin Receptor-Avid Peptide/ Fluorescein-Bombesin/Carbocyanine-Somatostatin Receptor-Avid Peptide/Carbocyanine-Bombesin

Cellular/Molecular Mechanism of Action: Somatostatin receptor-avid peptides and bombesin recognize and possess binding avidity for somatostatin receptor complexes.
Background Information: Site-specific delivery of drugs and contrast agents to tumors protects normal tissues from the cytotoxic effects of drugs and enhances the contrast between normal and pathologic tissues. One approach to achieve selective detection is to target overexpressed receptors on the exterior surface membranes of tumor cells and to visualize the tumors by a non-invasive optical imaging method.
Application: Detection and characterization of neoplastic lesions that express increased densities of membrane-associated somatostatin receptor complexes.
Semi-Synthetic Methods: Fluorescein and carbocyanine dyes can be conjugated to somatostatin and bombesin receptor-avid peptides. Receptor binding avidities and selectivity can be determined using whole cell or membrane preparations. Dual-imaging probes consisting of a bioactive peptide for tumor targeting, a biocompatible dye for optical imaging, and a radioactive or paramagnetic metal chelator can be prepared for potential use as scintigraphic or magnetic resonance imaging agents.
Interpretation and Analysis: Carbocyanine derivatives of somatostatin and bombesin analogues retain high binding for their respective receptors. Further evaluation of representative molecules in rats bearing somatostatin- and bombesin-positive tumors show selective uptake of the agents by the tumor cells. Unlike carbocyanine derivatives, receptor binding of fluorescein-somatostatin peptide conjugates is highly sensitive to the type of linker and the site of fluorescein attachment on the non-receptor binding region of the peptide. In general, the presence of flexible linkers disrupts binding affinity, possibly due to the interaction of the linker's thiourea group with the peptide's cyclic disulfide bond. While the receptor binding affinity of the dual probes is not dependent on the type of chelating group examined, it is affected by the relative positions of fluorescein and chelator on the lysine linker. For somatostatin compounds, best results are obtained when the chelator is on the alpha-amino lysine linker and fluorescein is on the epsilon-amino group. In contrast, conjugation of the chelator to epsilon-amino and fluorescein to the alpha-amino lysine linker of bombesin peptides results in high receptor binding. These findings indicate that despite their small size, conjugation of dyes to truncated somatostatin and bombesin peptide analogues results in the generation of promising diagnostic agents that retain high *in vitro* receptor binding activity. These contrast agents can selectively and specifically localize in receptor-positive tumors in rat models.

Synthesis, *in vitro* receptor binding, and *in vivo* evaluation of fluorescein and carbocyanine peptide-based optical contrast agents. Achilefu S, Jimenez HN, Dorshow RB, Bugaj JE, Webb EG, Wilhelm RR, Rajagopalan R, Johler J, Erion JL. J Med Chem 2002 May 9; 45 (10), pp. 2003–15.

INTEGUMENTARY SYSTEM

Carcinoma of the Dermus: Differentiation/Basal Cell Carcinoma (BCC)/Squamous Cell Carcinoma (SCC)/Precancerous Lesions/Benign Lesions
Nitrogen/Dye Laser (410 nm)

Cellular/Molecular Mechanism of Action: Induced fluorescence of neoplastic tissues.
Application: Laser-induced fluorescence spectroscopy for *in vivo* diagnosis of non-melanoma skin cancers in a manner that can accurately differentiate normal tissues from basal cell carcinoma, squamous cell carcinoma, precancerous lesions, and benign lesions based on the interpretation of emissions profiles.
Implementation: Cases are classified as having either skin types I, II, or III and biopsy samples from abnormal tissues collected by biopsy for evaluation. Each analysis measurement is designated as being either normal, BCC, SCC, precancerous, or benign. The total emission photon count is used as a discriminating index. A threshold value is calculated to separate normal tissue indices from indices of cancer tissues. The classification accuracy of each data point is determined using a threshold value.
Human Model: Cases with either skin types I, II, or III (n = 49 total; n = 279 analyses on normal and abnormal tissues).
Interpretation and Analysis: Applying the described classification/categorization scheme, cancers can be classified 93%, 89%,

and 78% correctly in cases with skin types I, II, and III, respectively. Normal tissues can be classified 93%, 88%, and 50% correctly in cases with skin types I, II, and III, respectively. Using the same threshold, precancerous spectra can be classified 78% and 100% correctly in skin types I and III, respectively. Benign lesions can be classified 100%, 46%, and 27% correctly in patients with skin types I, II, and III, respectively. Therefore, *in vivo* laser-induced fluorescence spectroscopy at 410 nm (excitation) and using the intensity of emission signal is an effective approach for detecting BCC, SCC, and actinic keratosis, especially in patients with light-colored skin.

Instrumentation: Nitrogen/dye laser tuned at 410 nm, an optical multichannel analyzer, and a fiber optic probe for excitation of tissue and collection of fluorescence emission.

Laser-induced fluorescence spectroscopy for *in vivo* diagnosis of non-melanoma skin cancers. Panjehpour M, Julius CE, Phan MN, Vo-Dinh T, Overholt S. Lasers Surg Med 2002; 31 (5), pp. 367–73.

Melanoma/Non-Invasive Transcutaneous Detection
Fluorescein-Isothiocyanate-Labeled Antimelanoma IgG/Texas Red-Labeled Dextran

Cellular/Molecular Mechanism of Action: Fluorescein-isothiocyanate and Texas Red emit fluorescent color when exposed to light energy of a specific wavelength.

Application: Fluorescein-isothiocyanate and Texas Red can be used as visual color marker or tracer molecules in biological systems. Fluorescein-isothiocyanate-labeled antimelanoma and Texas Red-labeled dextran can be applied for the transcutaneous detection of melanoma lesions.

Route of Administration: Variable.

Semi-Synthetic Methods: Covalent conjugation and cross-linking conjugation strategies for the semi-synthetic production of fluorescein-isothiocyanate-labeled antimelanoma IgG and Texas Red-labeled dextran.

Implementation: Determination of the relative effectiveness of 3 different fluorescein-isothiocyanate-labeled antimelanoma antibodies to selectively localize within melanoma lesions. Detection is achieved *in vivo* by applying a single-channel confocal imaging system.

Animal Model: Mice (athymic BALB/c strain) with human melanoma cells (n = 3 cell lines) culture from human skin implanted intradermally into the haunch skin of anesthetized mice and allowed to grow to a maximum size of 2 mm in diameter.

Interpretation and Analysis: Fiber-optic confocal imaging performed following intravenous administration of fluorescent-labeled antibodies and Texas Red-dextran facilitates the *in vivo* detection of melanoma and surrounding blood vessels in athymic mice. Using non-invasive techniques, the overall *in vivo* melanoma detection rate for tumors within 0.2 mm of the skin surface is 84% (27 of 32 tumors). Normal cultured human skin cells have little or no fluorescence after administration of the fluorescein-isothiocyanate-labeled antibodies and tumors are not labeled by an isotype control antibody. Dual-channel *in vivo* imaging of implanted melanoma tumors and surrounding dermal vasculature reveals increased blood vessel density at the melanoma site. Detection *in vivo* of melanoma tumors up to 0.2 mm below the skin surface by fiber-optic confocal imaging can be confirmed by conventional immunoperoxidase histology.

Instrumentation: Single-channel confocal imaging system.

In vivo detection of small subsurface melanomas in athymic mice using non-invasive fiber optic confocal imaging. Anikijenko P, Vo LT, Murr ER, Carrasco J, McLaren WJ, Chen Q, Thomas SG, Delaney PM, King RG. J Invest Dermatol 2001 Dec; 117 (6), pp. 1442–8.

Melanoma: Sentinel Lymph Nodes/Guide for Sentinel Lymphadenectomy
Isosulfan Blue Dye/[^{99m}Tc]-Human Serum Albumin (HSA)/[^{99m}Tc]-Sulfur Colloid (SC)

Cellular/Molecular Mechanism of Action: Isosulfan blue dye serves as a fluid pigment-based marker system. [^{99m}Tc]-HSA and [^{99m}Tc]-SC can be phagocytized by reticuloendothelial cell populations residing within peripheral lymph nodes.

Background Information: Radiopharmaceutical agents appear to improve the accuracy of sentinel node (SN) identification in patients with early-stage melanoma, but the optimal radiopharmaceutical agent and its timing from injection to surgery remain controversial.

Application: [^{99m}Tc]-SC and [^{99m}Tc]-HSA can be used intraoperatively as probes for lymphatic mapping in radiopharmaceutical-directed sentinel lymphadenectomy (LM/SL) procedures.

Route of Administration: Isosulfan blue dye, [^{99m}Tc]-SC, and [^{99m}Tc]-HSA can be administered intraoperatively into lymphatic vessels.

Implementation: Cutaneous lymphoscintigraphy (LS) performed before LM/SL utilizing 1 of 3 techniques including (i) [^{99m}Tc]-HSA injected at least 24 hours before LM/SL (n = 124 patients); (ii) filtered [^{99m}Tc]-SC injected no more than 4 hours before LM/SL (same-day SC, n = 95 patients); or (iii) [^{99m}Tc]-SC injected at least 18 hours before LM/SL (prior-day SC, 28 patients). At the time of LM/SL, isosulfan blue dye is injected alone (SC groups) or with a 2nd dose of HSA (HSA group). A handheld gamma probe is used to determine the radioactive (hot) counts of blue-stained and non-stained nodes, and the *in vivo* and *ex vivo* node-to-background count ratios of the nodes compared.

Human Model: Melanoma (n = 247; American Joint Committee on Cancer stage I or II).

Interpretation and Analysis: Preoperative LS can identify a large percentage of drainage basins (e.g., n = 299 in 247 cases). Lymphatic mapping with radiopharmaceutical-directed LM/SL is capable of identifying at least 1 sentinal lymph node in a large percentage of cases. Specific examples include (i) 119 (98%) of 121 basins using same-day SC; (ii) 142 (97%) of 146 basins using HAS; and (iii) 32 (100%) of 32 basins using prior-day SC. There is no difference ($p = 0.62$) in the accuracy rate between the 3 techniques. In the scenario described, a total of 463 sentinel nodes in 247 cases. Same-day SC yields higher intraoperative node-to-background count ratios than do either of the other techniques ($p < 0.0001$). Same-day SC also has the greatest relative change in radioactivity between the blue sentinel node and the postexcision basin ($p < 0.0001$), and the highest rate of SNs that are both blue and hot (*in vivo* or *ex vivo* ratio ≥ 2, $p = 0.05$). Therefore, LS and LM/SL performed on the same day with a single injection of filtered [^{99m}Tc]-SC serves as the most useful method for probe-directed LM/SL. The technique demonstrates the highest *in vivo* and *ex vivo* count ratios, fall-off of radioactivity between the excised nodes and postexcision basin, and concordance between blue dye and hot nodes. It should be recommended as the method of choice for probe-directed LM/SL.

Standardized probe-directed sentinel node dissection in melanoma. Essner R, Bostick PJ, Glass EC, Foshag LJ, Haigh PI, Wang HJ, Morton DL. Surgery 2000 Jan; 127 (1), pp. 26–31.

MUSCULOSKELETAL SYSTEM

Osteosarcoma: Hydroxyapatite Deposition/ Osteoblastic Activity/Atherosclerosis
Near-Infrared (NIR) Light-Emitting Probes/ Fluorescent Bisphosphonate Derivatives

Background Information: In vertebrates, the development and integrity of the skeleton requires hydroxyapatite (HA) deposition by osteoblasts. Sites of osteoblastic activity have most commonly been imaged *in vivo* using gamma-emitting radioisotopes. The scan times required are long, and the resultant radioscintigraphic images suffer from relatively low resolution.
Application: Hydroxyapatite deposition is a marker of, or a participant in, processes as diverse as cancer and atherosclerosis.
Semi-Synthetic Methods: Production of an NIR fluorescent bisphosphonate derivative.
Implementation: Evaluation of the ability for NIR fluorescent bisphosphonate derivatives to physically bind to hydroxyapatite deposits.
Interpretation and Analysis: Near-infrared light-based probes like fluorescent bisphosphonate derivatives possess an ability to bind to hydroxyapatite under *in vitro* conditions, in addition to recognizing osteoblastic activity in living animals. Such biotechnology can be applied to study skeletal development, osteoblastic metastasis, coronary atherosclerosis, and other disease states.

In vivo near-infrared fluorescence imaging of osteoblastic activity. Zaheer A, Lenkinski RE, Mahmood A, Jones AG, Cantley LC, Frangioni JV. Nat Biotechnol 2001 Dec; 19 (12), pp. 1148–54.

RENAL/URINARY SYSTEMS

Urinary Bladder Cancer
5-Aminolevulinic Acid (5-ALA)/ 5-ALA Methyl Ester (AME)

Cellular/Molecular Mechanism of Action: 5-aminolevulinic acid and its AME derivative emit fluorescent light energy. Certain neoplastic cell populations preferentially accumulate these agents intracellularly.
Background Information: The use of 5-ALA and its esters to induce endogenous porphyrins for the purpose of detecting epithelial cancers has been studied extensively at many centers around the world. The challenge is to prepare an efficacious formulation for the purpose of cancer detection. Photodynamic diagnosis of cancer using 5-ALA and its ester derivatives is being actively investigated.
Application: 5-aminolevulinic acid and AME can be used as fluorescent imaging agents for the detection of exterior neoplastic lesions such cancers of the urinary bladder.
Implementation: Compare 5-ALA with AME derivative in terms of protoporphyrin IX (PPIX) fluorescence intensity in *in vitro* and *in vivo* systems of bladder carcinoma. Complementary studies involve comparison evaluation of intravenous and topical application modes for drug administration in *in vivo* systems with RT112 xenografts. An endoscopy system is used to obtain macroscopic fluorescence images. The macroscopic images are further analyzed for fluorescence intensity distribution.
Animal Model: Rodent (tumor-bearing RT112 xenographs of a human bladder cancer cell type).
Interpretation and Analysis: For intravenous administration, over all time points studied (1, 3, 6 hours), AME-PPIX fluorescence is lower than ALA-PPIX fluorescence and is cleared at a faster rate than ALA-PPIX when administered intravenously. Topical application with 2 different polymers, Gantrez and Polyvinyl pyrrolidone (PVP), which are fast-releasing polymers, has been comparable for inducing PPIX fluorescence. Topical AME-PPIX fluorescence is comparable with ALA-PPIX fluorescence. The agent AME also appears to be applicable as a viable diagnostic agent.
Instrumentation: Karl Storz fluorescence endoscopy system.

A study of 5-aminolevulinic acid and its methyl ester used in *in vitro* and *in vivo* systems of human bladder cancer. Manivasager V, Heng PW, Hao J, Zheng W, Soo KC, Olivo M. Int J Oncol 2003 Feb; 22 (2), pp. 313–8.

REPRODUCTIVE SYSTEM

Mammary Carcinoma/Lymphoma
Fluorescein-Somatostatin Receptor-Avid Peptide/ Fluorescein-Bombesin/Carbocyanine-Somatostatin Receptor-Avid Peptide/Carbocyanine-Bombesin

Cellular/Molecular Mechanism of Action: Somatostatin receptor-avid peptides and bombesin recognize and possess binding avidity for somatostatin receptor complexes.
Background Information: Site-specific delivery of drugs and contrast agents to tumors protects normal tissues from the cytotoxic effects of drugs and enhances the contrast between normal and pathologic tissues. One approach for achieving selectivity is to target overexpressed receptors on the membranes of tumor cells and to visualize the tumors by non-invasive optical imaging methods.
Application: Detection and characterization of neoplastic lesions that express an increased density of membrane-associated somatostatin receptor complexes.
Semi-Synthetic Methods: Conjugation of fluorescein and carbocyanine dyes to somatostatin and bombesin receptor-avid peptides. Binding avidity and selectivity for receptor complexes can be assessed applying whole-cell or membrane preparations. In this context, it is possible to prepare dual-imaging probes consisting of a bioactive peptide for tumor targeting, a biocompatible dye for optical imaging, and a radioactive or paramagnetic metal chelator for scintigraphic or magnetic resonance imaging of tumors.
Interpretation and Analysis: Carbocyanine derivatives of somatostatin and bombesin analogs retain high binding characteristics for their respective receptors. Further evaluation of representative molecules in rats bearing somatostatin- and bombesin-positive tumors shows selective uptake of the agents by tumor cells. Unlike carbocyanine derivatives, receptor binding of fluorescein-somatostatin peptide conjugates is highly sensitive to the type of linker and the site of fluorescein attachment on the non-receptor binding region of the peptide. In general, the incorporation of flexible linkers disrupts binding affinity, possibly due to the interaction of the linker's thiourea group with the peptide's cyclic disulfide bond. While the receptor binding affinity of the dual probes is not dependent on the type of chelating group examined, it is affected by the relative positions of both fluorescein and chelator on the lysine linker. For somatostatin compounds, best results are obtained when the chelator is on the alpha-amino lysine linker and fluorescein is on the epsilon-amino group. In contrast, conjugation of the chelator to epsilon-amino and fluorescein to the alpha-amino lysine linker of bombesin peptides results in high receptor binding. These findings indicate that despite their small size, conjugation of dyes to truncated somatostatin and bombesin peptide analogs results in the generation of promising diagnostic agents that retain high *in vitro* receptor binding activity. These contrast agents can selectively and specifically localize in receptor-positive tumors in rat models.

Synthesis, *in vitro* receptor binding, and *in vivo* evaluation of fluorescein and carbocyanine peptide-based optical contrast agents. Achilefu S, Jimenez HN, Dorshow RB, Bugaj JE, Webb EG, Wilhelm RR, Rajagopalan R, Johler J, Erion JL. J Med Chem 2002 May 9; 45 (10), pp. 2003–15.

Mammary Carcinoma Ex-Plants
Glucamine-Substituted Cyanine/ Gluosamine-Substituted Cyanine

Application: Glucamine and gluosamine-substituted cyanine are dyes structurally related to indocyanine green (ICG) that can be applied as contrast-enhancing optical imaging agents for the detection of neoplastic lesions (e.g., human mammary carcinoma explants in rats) utilizing near-infrared modalities.
Route of Administration: Intravenous.
Semi-Synthetic Methods: Production of a group of glucamine and gluosamine-substituted cyanine dyes structurally related to ICG.
Implementation: Characterization of glucamine and gluosamine-substituted cyanine dyes structurally related to ICG for their potential as contrast agents for biomedical optical imaging. Derivatives with the highest hydrophilicity are evaluated for their efflux from tumor and normal tissue by monitoring with intensity-modulated diffuse optical spectroscopy after intravenous injection.

Animal Model: Rat (tumor-bearing).

Interpretation and Analysis: Glucamine and gluosamine-substituted cyanine dyes exhibit increased hydrophilicity and less plasma protein binding ($< 50\%$) compared to ICG. Accordingly, they also have different pharmacokinetic properties. Measurable enhancement in fluorescence quantum yields (7–15%) can be detected in a physiological environment compared to ICG. The derivative 5a has the highest level of hydrophilicity. In comparison with ICG, derivative 5a exhibits considerably enhanced tissue-efflux half-life (73 minutes vs less than 10 minutes for ICG in tumor tissue) and a 2-fold higher initial tissue absorption coefficient compared to ICG, and it generates an elevated tumor-to-tissue concentration gradient up to 1 hour after injection. In conclusion, compounds such as 5a are promising contrast agents for optical imaging and can facilitate highly sensitive and specific detection of breast cancer or other malignancies by utilizing mechanisms similar to contrast-enhanced magnetic resonance imaging or computerized tomography.

Hydrophilic cyanine dyes as contrast agents for near-infrared tumor imaging: Synthesis, photophysical properties and spectroscopic *in vivo* characterization. Licha K, Riefke B, Ntziachristos V, Becker A, Chance B, Semmler W. Photochem Photobiol 2000 Sep; 72 (3), pp. 392–8.

Mammary Gland Disease Differentiation/ Optical Imaging
Indocyanine Green (ICG)/Gadolinium/ Near-Infrared (NIR) Diffuse Optical Tomography

Cellular/Molecular Mechanism of Action: Indocyanine green is an albumin-bound absorbing dye.
Application: Indocyanine green is an absorbing dye commonly used as a marker system within the plasma compartment. Under certain conditions, ICG possesses the potential to differentiate disease based on the quantified enhancement of suspicious lesions using MRI concurrently with NIR diffuse optical tomography (contrast enhancement properties).
Route of Administration: Intravascular.
Implementation: Near-infrared diffuse optical tomography (DOT) analysis of the mammary gland applied in combination/concurrently with magnetic resonance imaging (MRI). Results are then correlated with histopathological evaluation of suspicious lesions.

Human Model: Cases scheduled for excision biopsy or surgery.

Interpretation and Analysis: The ICG-enhanced optical images co-register accurately with gadolinium-enhanced magnetic resonance images, thereby validating the ability of DOT to image breast tissue. In contrast to simple transillumination, DOT appears to provide for localization and quantification of exogenous tissue chromophore concentrations. Additionally, ICG has demonstrated a potential to differentiate disease based on the quantified enhancement of suspicious lesions.

Concurrent MRI and diffuse optical tomography of breast after indocyanine green enhancement. Ntziachristos V, Yodh AG, Schnall M, Chance B. Proc Natl Acad Sci U S A 2000 Mar 14; 97 (6), pp. 2767–72.

13 Genetics and Nucleic Acids

Anemia: Fanconi's Syndrome
Nitrogen Mustard (NTM)/[Diepoxybutane (DEB)]

Cellular/Molecular Mechanism of Action: Nitrogen mustards and DEB can function as DNA alkylating agents.
Background Information: Fanconi's anemia (FA) is an autosomal recessive inherited disorder that is associated with a variety of congenital anomalies. These include morphometric abnormalities involving mainly the head and face, skeletal malformations particularly of the radial ray, growth retardation, abnormal skin pigmentation, deafness, and renal, ocular, genital, and cardiac defects. The cardinal clinical feature is a severe progressive pancytopenia.
Application: Compare 2 different alkylating agents that would permit rapid and unequivocal detection of FA.
Implementation: Evaluate the potential to detect conditions of FA) rapidly and accurately applying alkylating agents as screening agents. In this context, NTM and DEB tests are performed in concert with the determination of baseline chromosomal breakage analyses.
Human Model: Cases suspected of suffering from Fanconi's syndrome anemia (n = 271).
Interpretation and Analysis: Chromosomal breakage analyses can facilitate the detection of cases with FA (e.g., n = 72) in contrast to cases unaffected by the condition (e.g., n = 136). Studies of family members of affected FA cases (e.g., n = 63 individuals) reveal that NTM seems more specific to identify chromosomal breakages in FA parents than is possible with DEB.

Differential diagnosis of Fanconi's anemia by nitrogen mustard and diepoxybutane. Deviren A, Yalman N, Hacihanefioglu S. Ann Hematol 2003 Apr; 82 (4), pp. 223–7.

DNA Intercalation/Neoplastic Disease: Topoisomerase I & II/Adenocarcinoma (HT29)/ Gliobastoma (U87MG)/Melanoma (A375M Antigen Positive)/Rational Chemotherapeutic Selection
[^{11}C]-9-Methoxyphenazine-1-Carboxamide/[^{11}C]2-(4-Pyridyl)Quinoline-8-Carboxamide/[^{11}C]DACA/ [Topoisomerases I & II]

Cellular/Molecular Mechanism of Action: The tricyclic carboxamide N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA) is a DNA-intercalating agent capable of inhibiting both topoisomerases I and II enzyme fractions.
Background Information: The tricyclic carboxamide DACA is currently in Phase II clinical trial. Many related analogues have been developed, but despite their potent *in vitro* cytotoxicities, they exhibit poor extravascular distribution.
Application: Delineation of rational drug selection.
Semi-Synthetic Methods: Each candidate compound shares a common structural feature, N-dimethyl side chain, which is radiolabeled with [^{11}C] functioning as a positron-emitting radioisotope.
Implementation: Evaluate the biodistribution and metabolite profiles of the prototype compound, DACA, with 3 analogues to aid rational drug selection in order to identify agents that are "minimal intercalators" that have lower DNA association constants. In this fashion, promising candidates will be identified in emerging preclinical studies in animals that can ultimately be evaluated in humans using positron emission tomography (PET).
Animal Model: Murine (mice bearing human tumor xenografts).
Interpretation and Analysis: Acridine DACA, phenazine SN 23490, pyridoquinoline SN 23719, and dibenzodioxin SN 23935 are cytotoxic when evaluated with *in vitro* assays utilizing HT29, U87MG, and A375M cell lines (IC50 of 1.4 to 1.8 μM, 0.4 to 0.6 μM, 1.3 to 1.6 μM, and 24 to 36 μM, respectively). *Ex vivo* biodistribution studies with [^{11}C]-radiolabeled compounds in mice bearing human tumor xenografts show rapid clearance of [^{11}C] radioactivity (parent drug and metabolites) from blood and major organs. Rapid hepatobiliary clearance and renal excretion can also be observed. There is low (< 5% of injected dose/g [%ID/g]) and variable uptake of [^{11}C] radioactivity in 3 tumor types for all of the compounds. Tumor (U87MG) to blood [^{11}C] radioactivity for [^{11}C]DACA, [^{11}C]-9-methoxyphenazine-1-carboxamide (SN 23490), [^{11}C]2-(4-pyridyl) quinoline-8-carboxamide (SN 23719), and [^{11}C]dibenzo[1,4]dioxin-1-carboxamide (SN 23935) at 30 minutes is approximately 2.9 ± 1.1, 2.3 ± 0.6, 2.6 ± 0.6, and 0.7 ± 0.2, respectively. For SN 23719, the distribution of [^{11}C] radioactivity in normal tissues and tumors determined *ex vivo* is in broad agreement with that determined *in vivo* by whole-body PET scanning. [^{11}C]DACA is rapidly and extensively metabolized to several plasma metabolites and a major tumor metabolite. In contrast, [^{11}C]SN 23935, [^{11}C]SN 23490, and [^{11}C]SN 23719 show less extensive metabolism. In tumor samples, parent [^{11}C]DACA and [^{11}C]SN 23935 are present at between 0.3 and 1.5%ID/g, whereas [^{11}C]SN 23490 and [^{11}C]SN 23719 is present at between 1.5 and 2.8%ID/g. Therefore, by using a strategy employing [^{11}C] labeling, it is possible to determine the tissue distribution and metabolic stability of novel tricyclic carboxamides.

Given the objective of identifying analogs with potentially improved *in vivo* activity against solid tumors, the agents SN 23490 and SN 23719 have more favorable distribution and metabolic stability compared with DACA and SN 23935. Such observations suggest that further development and evaluation are warranted. The radiolabeling strategy used allows *ex vivo* and *in vivo* evaluation of promising anticancer agents in animals and offers the potential of rapid translation to studies in humans using PET.

Comparative biodistribution and metabolism of carbon-11-labeled N-[2-(dimethylamino)ethyl]acridine-4-carboxamide and DNA-intercalating analogues. Osman S, Rowlinson-Busza G, Luthra SK, Aboagye EO, Brown GD, Brady F, Myers R, Gamage SA, Denny WA, Baguley BC, Price PM. Cancer Res 2001 Apr 1; 61 (7), pp. 2935–44.

DNA Synthesis: Neoplastic Disease/Cellular Proliferation Rate/Thymidine Kinase Expression
5-Iodo-4′-Thio-2′-Deoxyuridine (ITdU)

Cellular/Molecular Mechanism of Action: 5-Iodo-4′-thio-2′-deoxyuridine is a metabolically stable nucleoside that can function as a substrate for cellular thymidine kinase biochemical activity.

Application: Radioiodinated forms of ITdU and 5-iodo-2′-deoxyuridine (IUdR) can be applied to detect elevations in DNA synthesis for cell populations that positively express thymidine kinase activity. In this capacity, both ITdU and IUdR can serve as indicator molecules for measuring alterations in cellular proliferation especially relevant to the characterization of neoplastic disease states.

Route of Administration: Usually ITdU or IUdR would most frequently be administered intravenously/intravascularly. Theoretically, it may be possible to effectively image lesions if the agents are administered intrathecally for central nervous system (CNS) tumors, by direct injection into tumors, or into the arterial network supplying a suspected neoplastic lesion.

Semi-Synthetic Methods: Radiolabeling of ITdU and 5-iodo-1-(4-thio-β-D-arabinofuranosyl)uracil (ITAU) with [^{125}I] utilizing a destannylation reaction of the trimethylstannyl precursor of each nucleoside. The products can be isolated in high yields and with a > 99% level of radiochemical purity.

Implementation: Evaluation of ITdU and ITAU for their ability to assess cell growth based on DNA content.

Ex Vivo Model: Thymidine kinase-expressing L-M cell rather than a thymidine kinase-deficient mutant L-M (TK$^-$) cell.

Interpretation and Analysis: [^{125}I]-ITdU is effectively phosphorylated by cytosolic nucleoside kinases and specifically incorporated into a thymidine kinase-expressing L-M cell rather than a thymidine kinase-deficient mutant L-M (TK$^-$) cell. In addition, *in vitro* cell metabolism studies employing [^{125}I]-ITdU reveal that [^{125}I]-ITdU is effectively and specifically incorporated into a DNA fraction (> 90% at 60 minutes). Therefore, [^{125}I]-ITdU appears to be as effective of a DNA synthesis marker system as is IUdR. In contrast, [^{125}I]-ITAU is neither remarkably phosphorylated by cytosolic nucleoside kinases nor extensively incorporated into an L-M cell rather than an L-M (TK$^-$) cell. [^{125}I]-ITdU and [^{125}I]-ITAU show a higher resistance to phosphorolytic cleavage by recombinant thymidine phosphorylase than does [^{125}I]-IUdR. Furthermore, biodistribution of [^{125}I]-ITdU and [^{125}I]-ITAU display better *in vivo* stability of radioiodination than do [^{125}I]-IudR preparations. [^{125}I]-ITdU also displays a significantly higher uptake in proliferating organs (thymus, spleen, small intestine, and bone) than in non-proliferating organs (brain, muscle, liver, and lung), as does [^{125}I]-IUdR, at 18 hours postinjection. As indicated by *in vitro* studies, [^{125}I]-ITAU does not show any significant uptake in proliferating organs. Therefore, radioiodinated ITdU is potentially useful as a proliferation-imaging agent, and further studies should focus on clarifying the usefulness of this compound as a single photon emission computed tomography (SPECT) tumor-imaging agent.

Rationale of 5-[^{125}]I-iodo-4′-thio-2′-deoxyuridine as a potential iodinated proliferation marker. Toyohara J, Hayashi A, Sato M, Tanaka H, Haraguchi K, Yoshimura Y, Yonekura Y, Fujibayashi Y. J Nucl Med 2002 Sep; 43 (9), pp. 1218–26.

Gene Therapy Assessment Model/Neoplastic Disease: Leukemia Nucleoli Targeting/c-myc Gene Overexpression

Fluorescein-Labeled Phosphorothionate-Triple Helix-Forming Oligonucleotide (PS-TFO)/Antisense Oligonucleotides/Homopurine-Homopyrimidine P2 Promoter Sequence for c-myc

Cellular/Molecular Mechanism of Action: Oligonucelotide sequences can be designed that recognize and bind to specific gene/messenger RNA (mRNA) sequences.

Physiological Mechanism of Action: Selective inhibition of the transcription/translation of specific genes.

Background Information: The c-myc gene is frequently deregulated and overexpressed in human cancers, and strategies designed to inhibit c-myc expression in cancer cells may have considerable therapeutic value.

Application: Characterize the antigene and antiproliferative activity of a triple helix-forming oligonucleotide (TFO) targeted to a homopurine-homopyrimidine sequence in the P2 promoter of the c-myc gene.

Route of Administration: Potentially variable (intravenous, intralesional).

Semi-Synthetic Methods: Triple helix-forming oligonucleotide can be synthesized with phosphorothioate (PS) internucleotide linkages to confer resistance to intra- and extracellular nucleases. The resulting preparation can be conjugation with fluorescein as a tracer agent to identify selective accumulation within certain tissues and subcellular organelles.

Implementation: Delineation of the affinity of fluorescein-labeled PS-TFO.

Interpretation and Analysis: Resistance to intra- and extracellular nucleases is a property that is required for the design of oligonucleotides intended for *in vivo* testing and therapeutic applications.

Phosphorothioate-modified triple helix-forming oligonucleotides form triplex DNA complexes with affinity and specificity comparable with that of the corresponding phosphodiester TFO, as delineated by gel mobility shift and footprinting assays. Fluorescence microscopy and polyacrylamide gel analysis reveal that fluorescein-labeled PS-TFO accumulates in the nuclei of CEM leukemia cells and remains intact for a duration of at least 72 hours. Incubation of CEM cells with PS-TFO reduces c-myc RNA and protein levels. A single exposure of leukemia cells to the PS-TFO is sufficient to induce dose-dependent growth inhibitory effects. Growth inhibition correlates with S-phase accumulation of cells and with induction of cell death by apoptosis. Preparations of PS-TFO are also effective in other leukemia and lymphoma cell lines. Control oligonucleotides have minimal effects in all assays. Such findings indicate that c-myc-targeted PS-TFO is an effective antigene and antiproliferative agent, with potential for testing *in vivo* as a novel approach for the diagnosis and therapy of neoplastic disease states.

Antigene and antiproliferative effects of a c-myc-targeting phosphorothioate triple helix-forming oligonucleotide in human leukemia cells. McGuffie EM, Pacheco D, Carbone GM, Catapano CV. Cancer Res 2000 Jul 15; 60 (14), pp. 3790–9.

Malignant Hyperthermia (MH)/Genomic Analyses and Provocative Testing Correlation/Arginine-614 → Cysteine (Arg614Cys: RYR1 C1840T)

Caffeine/Halothane

Physiological Mechanism of Action: In conditions of MH, exposure of muscle tissue to caffeine/halothane promotes an exaggerated contractile response.

Background Information: Malignant hyperthermia is an important cause of anesthesia-induced death. The caffeine/halothane contracture test (CHCT) is highly invasive, expensive, and lacks 100% specificity. Genetic and biochemical evidence provide strong support for the view that the substitution of cysteine for arginine 614 (Arg614Cys) in the human ryanodine receptor gene is 1 of several mutations that are likely to cause human MH.

Application: Detection and characterization of MH conditions.

Route of Administration: Traditionally applied as a topic exposure for an *ex vivo* tissue-based challenge study.

Implementation: Delineation of a possible genetic basis for a predisposition to MH pertaining to the presence of Arg614Cys (RYR1 C1840T) mutations detected through a combination of polymerase chain reaction and restriction endonuclease digestion. Blood samples are utilized as a source of biological material for DNA analyses. Alternatively, detection of MH susceptibility can be established using the *in vitro* CHCT that employs fresh muscle biopsy specimens. Results from CHCT and DNA-based diagnostic parameters are then compared to determine their potential value in predicting MH susceptibility in large MH families that possess Arg614Cys mutations.

Human Model: Cases selected from a large Mennonite malignant hyperthermia kindred identified by an index patient that died at age 45 years of an MH crisis after general anesthesia. Case population: N = 68 family members, n = 19/68 muscle biopsies, n = 1/681 documented crisis without biopsy. Family members can be classified as MH-susceptible or MH-normal on the basis of the CHCT.

Interpretation and Analysis: In some family cohorts, as high as 33% of cases can be found to be heterozygous for the Arg614Cys mutation (n = 22/68) and slightly < one-fourth have prior positive CHCT results (e.g., n = 5/22), while a small percentage may have an MH crisis without a biopsy procedure. Utilizing DNA testing, it is possible to detect a relatively large number of cases within a related kindred that are normal for the allele (e.g., n = 44). Of these, slightly < 25% can be classified as MH-normal, while some may be determined to be MH-susceptible (e.g., n = 5) on the basis of CHCT evaluations. Based on the interpretation of the data obtained in earlier CHCT diagnoses, the condition of the muscle is poor, with no twitch for approximately 60% of cases of individuals homozygous for the normal allele but originally classified as MH-susceptible (e.g., n = 3/5) and for a small percentage that are homozygous for the normal allele and originally classified as MH-normal. Observations from caffeine/halothane contraction test results performed for such individuals (e.g., n = 4) can often be considered invalid. The twitch response is good for a small number of cases (e.g., n = 2 remaining) that are homozygous for the normal allele but classified as MH-susceptible if contracture can be observed with appropriately low levels of both caffeine and halothane.

An absolute correlation between DNA test results and CHCT assignment can often not be established. Possible explanations for discordance are that the Arg614Cys mutation is not linked to MH, that a 2nd MH mutation is segregating in the family, or that there are errors in the CHCT. Because there is strong evidence supporting the causal nature of the Arg614Cys mutation, the discordant cases are not closely related within the pedigree as they would be if a 2nd MH mutation was segregated, and the observed CHCT not 100% accurate. It has therefore been proposed that the observed discordance between DNA test results and CHCT assignment in 1 example kindred results from 2 false-positive diagnoses by the CHCT.

Comparison of the segregation of the RYR1 C1840T mutation with segregation of the caffeine/halothane contracture test results for malignant hyperthermia susceptibility in a large Manitoba Mennonite family. Serfas KD, Bose D, Patel L, Wrogemann K, Phillips MS, MacLennan DH, Greenberg CR. Anesthesiology 1996 Feb; 84 (2), pp. 322–9.

Methylation of RNA (Guided) *snoRNA-2 Gene*

Background Information: Trypanosomatids are the causative agent of several major parasitic diseases including African trypanosomiasis, American trypanosomiasis, and leishmaniasis. These parasites possess unique RNA-processing mechanisms including trans-splicing of pre-mRNA and RNA editing of mitochondrial transcripts.

Implementation: A novel group of trypanosomatid small nucleolar RNAs has been identified that belongs to the box C/D snoRNA, which has been shown to guide ribose methylation on rRNA. Three snoRNA genes include snoRNA-2 carrying a single snoRNA and g2 and b2 coding for single or multiple snoRNAs, respectively. Mapping of the methylation sites guided by snoRNA-2 using 2 different approaches suggest that snoRNA-2 has the potential to guide methylation on both 5.8S and 18S rRNAs. The trypanosomes follow the same guide-methylation rule established for yeast and for mammals. As a 1st attempt to change the methylation pattern of target RNAs, trans-genic parasites have been generated that carry the B2 and snoRNA-2, which have been engineered to shift the methylation site on rRNA.

Interpretation and Analysis: Despite efficient expression of these tagged snoRNAs, novel methylation sites have been very difficult to generate. However, efficient expression of tagged snoRNAs in trans-genic parasites opens the possibility of engineering novel methylation sites on different target RNAs *in vivo*.

Novel trypanosomatid small nucleolar RNAs that guide methylation: Their genome organization, expression and potential use to direct specific methylation on target RNA molecules. Xu YX, Liu L, Michaeli S. Isr Med Assoc J 2000 Jul; 2 Suppl, pp. 58–62.

Rheumatoid Arthritis: Severe vs Mild-Moderate/ Prediction of Disease Severity: G Allele (TT vs TG/GG Genotypes)/Tumor Necrosis Factor-Alpha Receptor Type II (TNFRII): Exon 6 Polymorphism *Anti-TNF-α Response/Methotrexate*

Application: Characterizing severe vs mild-moderate conditions of rheumatoid arthritis as a function of exon 6 TNFRII gene polymorphism. The G allele appears to have utility in predicting the degree of responsiveness to anti-TNF-α treatment, while the GG genotype for the exon 6 TNFRII polymorphism may be useful in predicting more aggressive forms of rheumatoid arthritis.

Implementation: Characterization of exon 6 TNFRII gene polymorphism in cases of (i) mild-moderate synovitis classified as responders to methotrexate therapy (e.g., n = 97) for comparison with (ii) "non-responder" cases receiving anti-TNF-α treatment due to severe and aggressive disease (e.g., n = 78), and (iii) cases with severe rheumatoid arthritis monitored for 6 months during anti-TNF-α therapy. Results are evaluated pertaining to response as it relates genotype and compared to matched age, sex, and ethnic reference controls (e.g., n = 84).

Interpretation and Analysis: A distinct trend exists toward increases in the frequency of the GG genotype in patients with severe rheumatoid arthritis (6.4%) compared to cases of mild-moderate disease (3.1%) and reference controls (1.2%). Evaluation of the response to anti-TNF-α therapy after 12 weeks of treatment reveals that 37.8% of the TT genotype pass from high to medium-low disease activity. Such observations are in contrast to the 10.7% of the TG/GG genotype that pass from high to medium-low disease activity ($p = 0.03$).

Collective evaluation of laboratory results therefore suggests that in cases of rheumatoid arthritis classified according to response to the conventional therapy and by disease severity, there is a discernable trend toward a higher prevalence of the GG genotype for the exon 6 TNFRII polymorphism in less responsive cases displaying a more aggressive form of disease. In addition, the G allele tends to promote a lower degree of response to anti-TNF-α treatments.

Tumor necrosis factor-alpha receptor II polymorphism in patients from southern Europe with mild-moderate and severe rheumatoid arthritis. Fabris M, Tolusso B, Di Poi E, Assaloni R, Sinigaglia L, Ferraccioli G. J Rheumatol 2002 Sep; 29 (9), pp. 1847–50.

GENE DELIVERY AND TARGETING

Gene Delivery
Cationic Liposome-Mediated Gene Delivery/ Mechanisms of Intracellular Incorporation

Cellular/Molecular Mechanism of Action: Protection and/or selective delivery of diagnostic pharmaceuticals and biological/ genetic agents.
Application: Cationic liposome-mediated gene delivery as an alternative approach for promoting internalization of large lipoplex particles.
Interpretation and Analysis: Significant changes are observed in particle size and zeta potential of liposomes and their structures (assessed by atomic force microscopy) are created upon addition of DNA sequences. These alterations appear to be dependent on the relative (±) charge ratio that develops. Atomic force microscopy images reveal that lipoplexes are formed from extensively fused and apparently homogeneous lipid particles encapsulating DNA. Lipoplexes are internalized into cells by processes of endocytosis. Lipoplex-cell fusion occurs mainly at the plasma membrane level; however, this lipoplex-cell membrane fusion is essential for the uptake of the large particles.
Instrumentation: Atomic force microscopy.

Cationic liposome-mediated gene delivery: Biophysical study and mechanism of internalization. Almofti MR, Harashima H, Shinohara Y, Almofti A, Baba Y, Kiwada H. Arch Biochem Biophys 2003 Feb 15; 410 (2), pp. 246–53.

Gene Delivery
Cationic Liposomes

Background Information: Cationic liposomes are useful to transfer genes into eukaryotic cells *in vitro* and *in vivo*. However, liposomes with good transfection efficiency are often cytotoxic and also require serum-free conditions for optimal activity.
Route of Administration: Potentially variable.
Semi-Synthetic Methods: Formulation of cationic liposomes containing DC-6-14, O,O′-ditetradecanoyl-N-(α-trimethylammonioacetyl)diethanolamine chloride, dioleoylphosphatidylethanolamine, and cholesterol.
Interpretation and Analysis: Liposomes containing DC-6-14, O,O′-ditetradecanoyl-N-(α-trimethylammonioacetyl)diethan olamine chloride, dioleoylphosphatidylethanolamine and cholesterol dispersed in 5% serum-containing growth medium, efficiently deliver plasmid DNA for green fluorescent protein (GFP) into more than 80% of cultured human cell hybrids derived from HeLa cells and normal fibroblasts. Flow cytometric analysis reveals an efficiency of the GFP gene expression of 40 to 50% in tumor-suppressed cell hybrids, while it is greatly reduced in the tumorigenic counterpart. The enhanced GFP expression in tumor-suppressed cell hybrids is quantitatively well correlated with a prolonged presence of the plasmid DNA that has been labeled with the fluorescent probe ethidium monoazide within cells. Newly developed cationic liposomes appear to be useful for gene delivery into human cells bathed in serum-supplemented media, and the stability of the plasmid DNA inside the cell is a crucial step in liposome-mediated gene expression.

A new cationic liposome for efficient gene delivery with serum into cultured human cells: A quantitative analysis using 2 independent fluorescent probes. Serikawa T, Suzuki N, Kikuchi H, Tanaka K, Kitagawa T. Biochim Biophys Acta 2000 Aug 25; 1467 (2), pp. 419–30.

Gene Delivery/Hepatic Asialoglycoprotein
Glycosylated Cationic Liposomes

Cationic liposomes have been considered as a potential non-viral vector for gene delivery because they possess low immunogenicity, unlike viral vectors. The gene transfer efficiency of cationic liposomes is lower than that of viral vectors, but recent advances have shown that it is possible to enhance the gene expression levels for cationic liposomes. The main problem with cationic liposomes seems to be the lack of organ or cell selectivity because the lung has the highest level of gene expression after intravenous injection. Applying cell-specific targeting technology to liposomes would improve *in vivo* gene delivery and reduce any unexpected side effects. Both liver parenchymal and non-parenchymal cells exclusively express large numbers of high-affinity asialoglycoprotein and mannose receptors, respectively. Receptor-mediated gene delivery systems are able to introduce foreign DNA into specific cell types *in vivo*. However, the authors of the study discussed in the referenced paper have confirmed that not only the nature of the ligands grafted to carriers but also the overall physicochemical properties of the complexes need to be optimized for effective cell-selective targeting of plasmid DNA. In this article, the authors attempt to evaluate a gene delivery system based on the physicochemical properties of plasmid DNA/glycosylated cationic complexes.

Glycosylated cationic liposomes for cell-selective gene delivery. Kawakami S, Yamashita F, Nishida K, Nakamura J, Hashida M. Crit Rev Ther Drug Carrier Syst 2002; 19 (2), pp. 171–90.

Gene Delivery/Endothelium of Hepatic Sinusoids/ Antisense Oligonucleotide/[*In Vivo* Genetic Messenger RNA (mRNA) Expression]
Albumin-Coated Cationic Lipoplexes/Anti ICAM-1/ Antisense Oligonucleotides (ODN)/Cationic Lipid (DOTAP)/Polyethylene Glycol (PEG)/Aconitylated Albumin (AcoHSA)/Anti-ICAM-1-ODN-[DOTAP]-PEG-Neutral Lipid (Aco-HAS)

Background Information: Massive *in vivo* uptake of surface-modified (negatively charged) liposomes including AcoHSA preparations by liver sinusoidal endothelial cells (ECs) has been previously reported.
Route of Administration: Albumin-coated cationic lipoplexes (intravascular).
Semi-Synthetic Methods: Anti ICAM-1 ODN is complexed with the cationic lipid DOTAP and the complex coated by an excess of neutral lipids including a lipid-anchored poly(ethylene glycol). Aco-HSA is coupled to the coated cationic lipoplexes (CCLs).
Implementation: Plasma disappearance and organ and intrahepatic distribution of Aco-HSA modified CCLs can be determined using [^{3}H]-cholesteryl oleyl ether and [^{32}P]-labeled ODN as markers.
Animal Model: Rat.
Interpretation and Analysis: Aco-HSA coupled CCLs are $<$ 160 nm in size and contain 1.03 ± 0.35 nmol ODN and 54 ± 18 μg Aco-HSA per μmol total lipid. Such CCLs are rapidly eliminated from plasma with about 60% of the injected dose of [^{3}H]- or [^{32}P]-label entering the liver after 30 minutes. Within the liver, the ECs account for two-thirds of total liver uptake. Control non-targeted CCLs are eliminated very slowly because after 30 minutes, $>$ 90% of the particles are still present in the blood. Our results demonstrate efficient targeting of antisense ODN to EC *in vivo*, employing plasma-

stable coated cationic lipoplexes, surface modified with negatively charged albumin. Forty percent of the injected ODN is delivered to predetermined "target cells" within 30 minutes.

Massive and selective delivery of lipid-coated cationic lipoplexes of oligonucleotides targeted *in vivo* to hepatic endothelial cells. Bartsch M, Weeke-Klimp AH, Meijer DK, Scherphof GL, Kamps JA. Pharm Res 2002 May; 19 (5), pp. 676–80.

Gene Delivery/Endothelium of Vascular Structures and Melanoma
Liposomes: Artificial Virus (Vector) Particles

Semi-Synthetic Methods: A semi-synthetic liposome has been designed that can function as a vector in part due to its composition being formulated to mimic the properties of retroviral envelope structures (serum-resistant, non-toxic, $\leq$ 200 nm in size). The DNA is condensed using low molecular weight branched PEI.
Interpretation and Analysis: Addition of a cyclic Arg-Gly-Asp (RGD) peptide ligand to these particles as targeting device (i) renders them selective for tumor endothelial and melanoma cells that express high levels of $\alpha v\beta 3$-integrins; and (ii) allows for an efficient delivery of the enclosed genetic material. The specificity of the vector system for melanoma cells theoretically can be further improved by using a melanocyte-specific tyrosinase promoter to drive transgene expression.

A new colloidal lipidic system for gene therapy. Fahr A, Muller K, Nahde T, Muller R, Brusselbach S. J Liposome Res 2002 Feb–May; 12 (1–2), pp. 37–44.

Gene Delivery/Ophthalmic
PEO-PPO-PEO Non-Ionic Copolymeric Micelles/Plasmid DNA: lacZ Gene

Cellular/Molecular Mechanism of Action: Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) or PEO-PPO-PEO non-ionic copolymeric micelles can be used as a delivery modality for genetic DNA strands.
Application: Poly(ethylene oxide)-poly(propylene oxide)-poly (ethylene oxide) non-ionic copolymeric micelles can be applied as an *in vivo* carrier for eyedrop gene delivery of plasmid DNA (e.g., lacZ gene).
Implementation: Pyrene fluorescence probe methods, zeta potential, and dynamic light scattering test (DLS) can be applied to characterized micelle formation of PEO-PPO-PEO block copolymers with plasmids. Gene expressions can be visualized by both the quality of an enzymatic color reaction using X-gal staining and by the quantification of the substrate chlorophenol red galactopyranoside (CPRG) in enucleated eyes on day 2 after gene transfer. In addition, microscopy can be used to identify the types of cells showing uptake and expression of the transferred gene sequence.
Interpretation and Analysis: Block polymeric micelles are formed above 0.1% (w/v) of block copolymer with a size of 160 nm and a zeta potential of -4.4 mV. After 2 days of topical delivery 3 times a day, the most intense gene expression is observed on days 2 and 3. Reporter expression is detected around the iris, sclera, conjunctiva, and lateral rectus muscle of rabbit eyes and also in intraocular tissues of nude mice upon *in vivo* topical application for 48 hours with a DNA/polymeric micelle formulation. Furthermore, after 2 enhancement treatments, the transport mechanisms of the block copolymeric micelles are found through endocytosis in tissues by enhancement through the tight junction pathway. Thus, efficient and stable transfer of the functional gene can be achieved with PEO-PPO-PEO polymeric micelles through topical delivery in mice and rabbits. These *in vivo* experiments indicate the possible potential use of block copolymers for DNA transfer.

In vivo gene delivery into ocular tissues by eyedrops of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) polymeric micelles. Liaw J, Chang SF, Hsiao FC. Gene Ther 2001 Jul; 8 (13), pp. 999–1004.

Gene Delivery (Oligonucleotides)/ Neoplasia (Solid Tumor)
Liposome: Cationic (100–200 nm)/Porphyrin Derivatives ($<$ 1 nm)/Branched-Chain Dendrimers (10 nm)/Polyethylenimine Polymers (10 nm)/ Nanoparticles (20–1,000 nm)/Microspheres ($>$ 1 μm)/ Cyclodextrins (CyDs 1.5 nm)

Background Information: The vasculature of a tumor provides the most effective route by which neoplastic cells may be reached and eradicated by drugs. The fact that a tumor's vasculature is relatively more permeable than healthy host tissue should enable selective delivery of drugs to tumor tissue. Such delivery is relevant to carrier-mediated delivery of genetic medicine to tumors. Cationic liposomes have been used for the introduction of nucleic acids into mammalian cells for more than a decade.
Application: Nucleic acid delivery into cells (*in vitro* and *in vivo*).
Semi-Synthetic Methods: Cationic liposomes are generally 100–200 nm in diameter, whereas cyclodextrins (CyDs) typically span 1.5 nm across.

Evaluate the potential of delivering therapeutic ONs to tumors using cationic liposomes and CyDs, and evaluate the major hindrances posed by the tumor itself on such delivery.
Interpretation and Analysis: Cyclodextrin molecules are routinely used as agents that engender cholesterol efflux from lipid-laden cells, thus having an efficacious potential in the management of atherosclerosis. A recent trend is to employ these oligosaccharide molecules for delivering nucleic acids in cells both *in vitro* and *in vivo*. Comparisons have been made with other oligonucleotide delivery agents, such as porphyrin derivatives ($<$ 1 nm), branched-chain dendrimers (approximately 10 nm), polyethylenimine polymers (approximately 10 nm), nanoparticles (20–1,000 nm), and microspheres ($>$ 1 μm), in the context of delivery to solid tumor. The chemical and physical properties of these carriers and how they may affect the uptake of oligonucleotides into cells, particularly *in vivo*, is of critical importance.

Vehicles for oligonucleotide delivery to tumors. Dass CR. J Pharm Pharmacol 2002 Jan; 54 (1), pp. 3–27.

Gene Delivery: Central Nervous System (Hippocampus)/Neuronal Nucleoli Entry
Anionic Liposomes/Liposome-Encapsulated Cy3-Labeled Oligonucleotides

Background Information: Anionic liposomes can efficiently introduce foreign DNA into postmitotic neurons and other cell types.
Application: Liposome-encapsulated Cy3-labeled oligonucleotides can be applied to deliver DNA sequences into the nucleus of CNS neurons.
Implementation: Evaluate the mechanism of liposome uptake established through characterization of internalization processes for anionic liposome-encapsulated Cy3-labeled oligonucleotides (AL-Cy3ONs) by hippocampal neurons.

Interpretation and Analysis: Uptake of AL-Cy3ONs is widespread and a function of time- and temperature-dependent variables, indicative of receptor-mediated endocytosis. The low-density lipoprotein receptor-related protein (LRP) is crucial for anionic liposome endocytosis because the receptor-associated protein or an anti-LRP antibody inhibits internalization, and fibroblasts lacking LRP do not internalize AL-Cy3ONs. Using selective endocytosis inhibitors, it can be determined that liposome endocytosis and intracellular transport require clathrin, dynamin, an intact cytoskeletal network, and phosphatidylinositol 3-kinase activity. Cy3ONs does not significantly co-localize with recycling endosomal/lysosomal markers and enter neuronal nuclei within 1 to 3 hours of incubation. Approximately 50% of the internalized liposomal phospholipids are recycled back to the cell surface, in keeping with the fluidity of their acyl chains. Liposome endocytosis does not require heparan sulfate proteoglycans or cause calcium influx into neurons. Thus, constitutive endocytosis of anionic liposomes by LRP utilizes only 1 component, in contrast to the more involved heparan sulfate proteoglycan-LRP pathway implicated in the pathogenesis of Alzheimer's disease.
Instrumentation: Confocal microscopy.

Low-density lipoprotein receptor-related protein mediates the endocytosis of anionic liposomes in neurons. Lakkaraju A, Rahman YE, Dubinsky JM. J Biol Chem 2002 Apr 26; 277 (17), pp. 15085–92.

Gene Delivery: *In Vivo*/Potential Strategy for the Detection of Messenger RNA (mRNA) Expression
Antisense Oligonucleotides Liposomes

Cellular/Molecular Mechanism of Action: Antisense constructs can be designed to bind to either specific sequences within genomic DNA or mRNA strands.
Physiological Mechanism of Action: Antisense constructs can be designed to block/inhibit either transcription (genomic DNA → mRNA) or translation (mRNA → protein) processes.
Background Information: Mice with a targeted mutation of the Hoxa10 gene demonstrate uterine factor infertility. It is unclear if the defect in the uterine environment arises due to the absence of Hoxa10 expression during embryonic development or in the adult. It has recently been demonstrated that HOXA10 expression in human endometrium rises dramatically at the time of implantation, suggesting maternal expression of Hoxa10/HOXA10 may be essential to the process.
Application: Delivery of DNA sequences (e.g., uterus).
Route of Administration: Intrauterine infusion.
Semi-Synthetic Methods: Oligonucleotide constructs that correspond to the "opposite-strand" of either specific genomic DNA or mRNA sequences.
Implementation: Utilization of DNA/liposomes to deliver oligonucleotide constructs capable of altering maternal Hoxa10 expression prior to implantation.
Animal Model: Mouse (gestation period = day 2).
Interpretation and Analysis: Transfection with a Hoxa10 antisense oligodeoxyribonucleotide significantly decreases the number of implantation sites. Transfection with a plasmid that constitutively expresses Hoxa10 optimizes survival of implanted embryos, resulting in increased litter size. Maternal Hoxa10 expression is therefore essential for implantation and is the 1st report of the maternal alteration of a gene known to affect implantation specifically. Furthermore, DNA/liposome complexes containing the same Hoxa10 constructs that alter fertility in mice can also affect Hoxa10 expression in a human endometrial cell line. Alteration of human endometrial HOXA10 via liposome-mediated gene transfection is a potential contraceptive agent or fertility treatment.

Alteration of maternal Hoxa10 expression by *in vivo* gene transfection affects implantation. Bagot CN, Troy PJ, Taylor HS. Gene Ther 2000 Aug; 7 (16), pp. 1378–84.

Gene Delivery: Macrophages/Kupffer Cells (KCs)/ Transgene Expression (Enhanced/Prolonged)/ Enhanced Adenoviral Vector Delivery
Clodronate Liposomes

Background Information: Tissue macrophages, in particular hepatic KCs, contribute to early inflammatory responses following adenoviral vector administration.
Implementation: Evaluate the effect of selective and transient (3 days) depletion of hepatic KCs by a single injection of clodronate liposomes on the *in vivo* performance of high-capacity adenoviral (HC-Ad) vectors.
Interpretation and Analysis: Interestingly, in the majority of animals of both strains, depletion of KCs is sufficient to prevent the generation of anti-hAAT antibodies, resulting in prolonged transgene expression. Thus, short-term and selective depletion of hepatic macrophages at the same time significantly increases hepatic transgene expression and reduces the humoral immune response to the transgenic protein.

Selective depletion or blockade of Kupffer cells leads to enhanced and prolonged hepatic transgene expression using high-capacity adenoviral vectors. Schiedner G, Hertel S, Johnston M, Dries V, van Rooijen N, Kochanek S. Mol Ther 2003 Jan; 7 (1), pp. 35–43.

Gene Delivery: Neoplasia/Ovarian Carcinoma/ Plasmid Transfection/Non-Viral Vector Platform/ Green Fluorescent Protein (GFP)/DDC/DNA Complex
Cationic DOTAP:DOPE:Cholin Liposomes

Application: Various attempts have been made to design strategies and efficient protocols for ovarian cancer gene therapy, but there has been little progress in their clinical application.
Semi-Synthetic Methods: Preparations of DDC liposome are produced by mixing DOTAP:DOPE:Cholin at a 1:0.7:0.3 molar ratio using the extrusion method.
Implementation: Plasmid DNA (pEGFP-C1) and DDC are complexed at various weight ratios to find the optimum efficacy. The percentage of transfected cells is determined by selecting a GFP expressing cells using flow cytometry instrumentation. The transfection efficiency of the DDC liposome is compared with 3[N-(N,N-dimethylaminoethylene) carbamoyl] cholesterol (DC-Chol)/DOPE liposome and commercially available lifopectin.
Interpretation and Analysis: Evaluation of the capacity for cationic liposomes of dioleoyltrimethylaminopropane (DOTAP), 1,2-dioleoyl-3-phosphophatidylethanolamine (DOPE), and cholesterol (Chol)(DDC) to facilitate plasmid DNA transfer into ovarian cancer cells. The optimal transfection of plasmid DNA can be achieved at a 1:4 (w/w) ratio of DDC to DNA. The DDC/DNA complex exhibits higher transfection efficiency in human ovarian cancer cells (OVCAR-3 and SK-OV-3 cells) compared to that in other types of cell lines (NCI-NIH:522 and HepG2 cells). Flow cytometric analysis reveals that the DDC/DNA complex exhibits an over 4-fold increase in GFP expression levels compared with DC-Chol/DOPE or lipofectin in OVCAR-3 cells. Such findings can be further confirmed by confocal microscopy and reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Therefore, cationic liposome (DDC) appears to be a promising non-viral vector for treating ovarian adeno-

carcinoma because of its selective high gene transfer ability in ovarian cancer cells.

Instrumentation: Flow cytometry.

Non-viral vector for efficient gene transfer to human ovarian adenocarcinoma cells.Kim CK, Haider KhH, Choi SH, Choi EJ, Ahn WS, Kim YB. Gynecol Oncol 2002 Jan; 84 (1), pp. 85–93.

Gene Delivery: Neoplasia Oncogene Detection Strategy/Melanoma c-myc Oncogene/ Neuroblastoma Disialoganglioside (GD_2)
Cationic Immuno-Lipsomes/Anti-Disialoganglioside (GD_2)

Background Information: Melanoma is a highly malignant and increasingly common tumor. Because the cure rate of metastatic melanoma by conventional treatment is very low, new therapeutic approaches are needed. Reports have previously described that coated cationic liposomes (CCL) targeted with a monoclonal antibody against the GD_2 and containing c-myb antisense oligodeoxynucleotides (asODNs) result in a selective inhibition of the proliferation of GD_2-positive neuroblastoma cells *in vitro*.

Application: Selective delivery to melanoma and neuroblastoma neoplastic cell types.

Route of Administration: Intravascular.

Implementation: Evaluation of the selective *in vivo* delivery capabilities of antisense liposomal formulations with antineoplastic properties that target the c-myc oncogene of melanoma, a neuroectodermal tumor sharing with neuroblastoma the expression of GD_2.

Interpretation and Analysis: Preparations of stable GD_2-targeted liposomes can be produced that entrap 90% of added c-myc asODNs. Such liposomes show a selective binding for GD_2-positive melanoma cells *in vitro*. Melanoma cell proliferation is inhibited to a greater extent by GD_2-targeted liposomes containing c-myc asODNs (aGD_2-CCL-myc-as) than by non-targeted liposomes or free asODNs. The pharmacokinetic results obtained after intravenous injection of [^{3}H]-myc-asODNs, free or encapsulated in non-targeted CCLs or GD_2-targeted CCLs, reveal that free c-myc-asODNs are rapidly cleared, with less than 10% of the injected dose remaining in blood at 30 minutes after injection. Sequences of c-myc-asODNs encapsulated within either CCL or aGD_2-CCL demonstrate a more favorable profile in blood, with about 20% of the injected dose of each preparation remaining *in vivo* at 24 hours after injection. In an *in vivo* melanoma experimental metastatic model, aGD_2-CCL-myc-as, at a total dose of only 10 mg of asODN per kilogram, significantly inhibits the development of microscopic metastases in the lung compared with animals treated with myc-asODNs, free or entrapped in non-targeted liposomes, or aGD_2-CCL encapsulating scrambled asODNs ($p < 0.01$). Moreover, mice bearing established subcutaneous human melanoma xenografts treated with aGD_2-CCL-myc-as exhibits significantly reduce tumor growth and increase survival ($p < 0.01$ vs control mice). The mechanism for the antitumor effects appears to be associated with a downregulation of c-myc protein expression and interruption of c-myc-mediated signaling: induction of p53 and inhibition of Bcl-2 proteins, leading to extensive tumor cell apoptosis. Such observations suggest that inhibition of c-myc proto-oncogene by GD_2-targeted antisense therapy could provide an effective approach for the treatment of melanoma in an adjuvant setting.

Targeted liposomal c-myc antisense oligodeoxynucleotides induce apoptosis and inhibit tumor growth and metastases in human melanoma models. Pastorino F, Brignole C, Marimpietri D, Pagnan G, Morando A, Ribatti D, Semple SC, Gambini C, Allen TM, Ponzoni M. Clin Cancer Res 2003 Oct 1; 9 (12), pp. 4595–605.

Gene Delivery: Neoplastic Disease/Prostatic Carcinoma: Potential Messenger RNA (mRNA) Detection Strategy
[Gd]-Anti-mRNA Oligonucleotide Strand/Trans-Membrane Carrier Peptide

Cellular/Molecular Mechanism of Action: The physical properties/orientation of gadolinium becomes altered when placed within a magnetic field. Trans-membrane carrier proteins are transported across cell membrane structures by specific membrane-associated complexes. Oligonucleotides can be designed as the complementary sequence to specific mRNA strands and the resulting cDNA/mRNA duplex effectively prevents translation of the mRNA strand. Diagnostically, complementary strands of cDNA theoretically would accumulate intracellularly within cells that are actively transcribing the corresponding mRNA sequence that cDNA strands have been designed to recognize.

Background Information: The term "molecular imaging" can be broadly defined as the *in vivo* characterization and measurement of biological processes at the cellular and molecular level.

Application: Concept and methodology strategy for diagnostically detecting *in vivo* gene expression by magnetic resonance imaging (MRI). Example: Specific oncogenes within neoplastic cell types.

Route of Administration: Intravenous may be the preferred method of delivery.

Semi-Synthetic Methods: Development of a novel intravital and intracellular MRI contrast agent composed of a gadolinium complex, an oligonucleotide sequence (peptide nucleic acid [PNA]), and a trans-membrane carrier peptide that is composed of a peptide sequence similar to that of the homeodomain of the Antennapedia protein.

Implementation: Determine if [Gd]-oligonucleotide complexes (i) can accumulate in tumor cells *in vitro* and *in vivo*; and (ii) whether the specificity of a PNA for the upregulated c-myc mRNA in the cell's cytoplasm has an effect on contrast agent retention within tumor cells.

Tissue Culture Model: HeLa cell lines.

Animal Model: Rat (Dunning R3327 AT1 rat prostate adenocarcinoma).

Intracellular visualization of prostate cancer using magnetic resonance imaging. Heckl S, Pipkorn R, Waldeck W, Spring H, Jenne J, von der Lieth CW, Corban-Wilhelm H, Debus J, Braun K. Cancer Res 2003 Aug 15; 63 (16), pp. 4766–72.

Gene Delivery-Expression/Neoplasia (Green Fluorescent Protein [GFP])/Detection of Future Regional and Distant Metastases
Retrovirus

Cellular/Molecular Mechanism of Action: Retroviral agents expressing the GFP gene are capable of inserting their genome into certain mammalian cell populations. Cells that have been transfected by such retroviral agents express the GFP gene and emit a green color when exposed to certain wavelengths of light.

Application: Visualization of future regional and distant metastases.

Route of Administration: Intraperitoneal (other routes possible).

Implementation: Green fluorescent protein retroviral supernatants are injected intraperitoneally from day 4 to day 10 after implantation of gastric cancer cells. Tumor and metastasis fluorescence are then visualized every other week with the use of fluorescence optics via a laparotomy on the tumor-bearing animals.

Animal Model: Mouse (intraperitoneally growing human gastric neoplasia).

Interpretation and Analysis: At 2 weeks after retroviral GFP delivery, GFP-expressing tumor cells can be observed in gonadal fat, greater omentum, and intestine, indicating that these primary intraperitoneally growing tumors can be efficiently transduced by the GFP gene and can be visualized by its expression. At 2nd and 3rd laparotomies, GFP-expressing tumor cells can be observed spreading to lymph nodes in the mesentery and other regional sites. At the 4th laparotomy, widespread tumor growth is visualized by GFP expression, inducing liver metastasis. Normal tissues are rarely transduced by the GFP retrovirus. Thus, reporter gene transduction of the primary tumor can enable detection of its subsequent metastasis. This gene therapy/diagnostic model could be applied to primary tumors before resection or other treatment to have a fluorescent early detection system for metastasis and recurrence.

In vivo tumor delivery of the green fluorescent protein gene to report future occurrence of metastasis. Hasegawa S, Yang M, Chishima T, Miyagi Y, Shimada H, Moossa AR, Hoffman RM. Cancer Gene Ther 2000 Oct; 7 (10), pp. 1336–40.

Gene Delivery-Transfer/Central Nervous System (CNS)
Liposome/Plasmid DNA Complexes/[Lipoplexes]

Background Information: Acute cerebral ischemia causes hypoxic neuronal cell death by necrosis and apoptosis. Expression of antiapoptotic transgenes in ischemic brain may provide a useful therapeutic strategy for alleviation of postischemic damage.

Route of Administration: Lipoplexes (intrathecal).

Implementation: Evaluation of the potential for liposomes to mediate transfer of human bcl-2 protein into the CNS during conditions of focal transient cerebral ischemia. Two different types of plasmid vectors can be used for bcl-2 expression: (i) 1 driven by the constitutive cytomegalovirus promoter (pCMV), and (ii) a 2nd based on the hypoxia-inducible human vascular endothelial growth factor promoter (pHRE). Cationic liposome/plasmid DNA complexes (lipoplexes) are injected directly into the cerebrospinal fluid (CSF) of rats immediately after middle cerebral artery (MCA) occlusion. The brains of treated and control animals are analyzed 48 hours later. Infarct volumes and numbers of apoptotic cells are then quantified.

Animal Model: Rat (focal transient ischemia created by MCA occlusion).

Interpretation and Analysis: Occlusion of the MCA results in ipsilateral cerebral infarcts. Transfer of the bcl-2 gene results in high-level widespread protein expression in the case of the pCMV-bcl2 plasmid, while animals treated with the pHRE-bcl2 vector show lower expression levels of bcl2 that are limited to areas of ischemia. Treatment with pCMV-bcl2, but not with pHRE-bcl2, is able to significantly reduce infarct volumes (109 ± 8 mm^3 for pCMV-bcl2; 152 ± 29 mm^3 for pHRE-bcl2; and 155 ± 18 mm^3 for control animals). Animals transfected with either of the 2 vectors have significant reductions in apoptotic cell numbers within infarcts and penumbra area compared to controls. There are no short-term neurological side effects from CSF lipoplex injection or bcl-2 expression. Hypoxia-inducible bcl-2 expression mediated by intrathecal lipoplexes may represent a novel, biologically safe, and lesion-selective diagnostic and therapeutic approach for detection and neuroprotection after acute cerebral ischemia.

Liposome-mediated transfer of the bcl-2 gene results in neuroprotection after *in vivo* transient focal cerebral ischemia in an animal model.Cao YJ, Shibata T, Rainov NG. Gene Ther 2002 Mar; 9 (6), pp. 415–9.

Gene Delivery-Transfer/Endothelium: Capillaries in Central Nervous System (CNS)/Gene Transfer Model
Hemagglutination Virus of Japan (HVJ)-Liposomes/ Liposome Encapsulated β-Gal Plasmid

Background Information: Bioactive proteins or peptides cannot be effectively delivered into brain capillary endothelial cells (BCECs) or brain parenchyma.

Route of Administration: Superselective internal carotid artery catheterization.

Implementation: Evaluation of the effectiveness of transferring the *Escherichia coli* β-galactosidase gene (β-gal) as a model gene into BCECs utilizing the HVJ-liposomes. HVJ-liposomes encapsulating a β-gal plasmid can be used *in vitro* to transfect mouse brain capillary endothelial cells (MBEC4) cells and also delivered *in vivo* by selective administration.

Animal Model: Rat via the internal carotid artery.

Interpretation and Analysis: Success of the procedure is confirmed by the detection of 116 kDa β-gal protein in transfected MBEC4 cells and in brain capillaries isolated from transfected rats, by Western blot analysis and histological staining. The enzymatic activities of β-gal are 5- to 10-fold and 20-fold higher than when β-gal-containing liposomes without fusogenic activity (uncoated liposomes) or plasmid alone are employed *in vitro* and *in vivo*, respectively. Thus, HVJ-liposomes have demonstrated applicability as a vector for the transfer of foreign genes into brain capillary endothelium *in vivo* (transluminal route).

Transluminal gene transfer into brain capillary endothelial cells *in vivo* with HVJ-liposomes.Jiang C, Matsuo H, Koyabu N, Ohtani H, Fujimoto H, Yonemitsu Y, Kaneda Y, Narro M, Tsuruo T, Sawada Y. J Drug Target 2002 Jun; 10 (4), pp. 345–52.

Gene Delivery-Transfer/Endothelium: Central Nervous System (CNS) Capillaries/Gene Transfer Model
Hemagglutination Virus of Japan (HVJ)-Liposomes/ Liposome Encapsulated β-Gal Plasmid

Application: Gene delivery into the CNS (e.g., evaluation of the protective effect for dopamine neuron against a retrograde 6-hydroxydopamine [6-OHDA]).

Route of Administration: HVJ-liposomes (internal carotid artery).

Semi-Synthetic Methods: The gene encoding mouse glial cell-derived neurotrophic factor (mGDNF gene) can be transfected into brain capillary endothelial cells (BCECs) with the aim of delivering the gene product extensively into the brain parenchyma by making use of BCEC secretory function. First, the mGDNF gene is transfected *in vitro* into cultured BCECs (mouse brain capillary endothelial cells [MBEC4]). The amount of mGDNF protein secreted from the transfected cells into the medium is about 1,500 to 3,200 pg/mg of cell protein/day, or about 7-fold higher than that accumulated intracellularly. Furthermore, the basolateral-directed secretion of mGDNF protein from the transfected MBEC4 cells is 5-fold higher than the apical-directed secretion.

Implementation: Evaluation of the capacity of the HVJ-liposomes to deliver an encapsulated gene (e.g., mGDNF) into the CNS.

Animal Model: Rat.

Interpretation and Analysis: Transfected rats show a marked increase in the brain level of GDNF as assessed by means of ELISA and Western blotting on day 3 after the administration, and the level remains significantly elevated for at least 12 days. Furthermore, im-

munohistochemical staining reveals an increase in GDNF immunoreactivity throughout the transfected forebrain. Such profiles indicate that the gene can be successfully transferred *in vivo* from HVJ-liposomes into BCECs, where it is expressed, and the gene product is secreted into the brain. Using this delivery method, the protective effect for dopamine neuron against a retrograde 6-hydroxydopamine (6-OHDA) lesion can be evaluated and assessed as a function of behavioral and neurochemical indices.

In vivo delivery of glial cell-derived neurotrophic factor across the blood-brain barrier by gene transfer into brain capillary endothelial cells. Jiang C, Koyabu N, Yonemitsu Y, Shimazoe T, Watanabe S, Naito M, Tsuruo T, Ohtani H, Sawada Y. Hum Gene Ther 2003 Aug 10; 14 (12), pp. 1181–91.

Gene Delivery-Transfer/Neoplasia: Central Nervous System (CNS) Gliosarcoma
TEAPC-Chol/DOPE Cationic Liposome/Precondensing Plasmid DNA with Poly-L-Lysine/Polyethylene Glycol (PEG)

Application: Delivery and transfection of genetic DNA sequences into cells within the CNS.
Semi-Synthetic Methods: Cationic liposome can be prepared from triethyl aminopropane carbamoyl cholesterol (TEAPC-Chol) and a helper lipid, dioleoylphosphatidylethanolamine (DOPE).
Implementation: Evaluation of a strategy for transfecting genes into gliosarcoma cell lines (9L) using an original cholesterol-based cationic liposome as carrier.
Animal Model: Murine (nude mice).
Interpretation and Analysis: Liposomes at specific concentrations are not cytotoxic based on 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) analysis. TEAPC-Chol/DOPE liposomes allow plasmids encoding reporter genes to enter the nucleus as observed both by electron microscopy and functionality tests using fluorescence detection of green fluorescent protein (GFP) and luminometric measurements of luciferase activity. By changing the cationic lipid/DNA molar charge ratio, optimal conditions can be determined. Further, improvement of the transfection level can be obtained by either precondensing plasmid DNA with poly-L-lysine or by adding PEG in the transfection medium. The optimal conditions determined are different depending on whether the transfection is made with cells in culture or with tumors induced by subcutaneous injection of cells in nude mice. For *in vivo* assays, a simple method to overcome the interference of hemoglobin with the chemiluminescence intensity of luciferase has been used. These results would be useful for gaining knowledge about the potential for the cationic liposome TEAPC-Chol/DOPE to transfect brain tumors efficiently.

Evaluation and optimization of DNA delivery into gliosarcoma 9L cells by a cholesterol-based cationic liposome.Lesage D, Cao A, Briane D, Lievre N, Coudert R, Raphael M, Salzmann J, Taillandier E. Biochim Biophys Acta 2002 Aug 31; 1564 (2), pp. 393–402.

Gene Delivery-Transfer/Neoplasia: Leukemia (Hairy-Cell Type)
Human Papilloma Virus/Epstein-Barr Virus/Antisense Nucleic Acids

Cellular/Molecular Mechanism of Action: Antisense oligonucleotides, are small chains of nucleic acids capable of binding to cellular RNA sequences (hybridization).
Application: Antisense oligonucloetides can be applied as diagnostic and therapeutic pharmaceuticals. In the field of nuclear imaging, radiolabeled antisense oligonucleotides can be applied as a diagnotic imaging modality.
Considerations: The use of anti-sense oligonucleotides as *in vivo* diagnostic agents is attractive because it brings molecular imaging to a level of detecting real-time gene expression.
Interpretation and Analysis: Oligonucleotides are non-canonical radiopharmaceuticals and much progress is needed to adapt them to *in vivo* imaging. The requirements to reach this goal include improvements in radiosynthesis, stability, targeting, and specific and non-specific binding.

Simultaneous detection by non-isotopic *in situ* hybridization of human papilloma viruses and Epstein-Barr virus during the lytic cycle in oral hairy leukoplakia lesions. Courtade M, Brousset P, Delsol M, Gorguet B, Viraben R, Voigt JJ, Delsol G. Ann Pathol 1992; 12 (6), pp. 353–7.

Gene Delivery-Transfer-Expression/Lymphocytes/ Hunter's Syndrome: Iduronate-2-Sulfatase (IDS)
Peripheral Lymphocyte-Mediated Delivery/Retroviral Vectors

Cellular/Molecular Mechanism of Action: Utilization of the natural (native) homing properties of peripheral blood lymphocytes to selectively deliver diagnostic agents.
Application: Example: Hunter's syndrome (peripheral blood lymphocytes (PBL[MPS]).
Route of Administration: Intravenous.
Biological Preparation and Analysis: Utilizing PBLs from Hunter's syndrome patients (PBL[MPS]), the expression of IDS can be induced through the use of 3 different promoter segments associated with 4 retroviral vectors (i.e., the long-term repeat, or LTR, in vectors L2SN and L2, avian β-actin promoter in LB2, and the cytomegalovirus [CMV] early promoter in LNC2). The production of PBL(MPS) in this manner involves exposure to packaging cell supernatant resulting in transduction frequencies ranging 10-fold between 5 and 49%.

Surprisingly, IDS activities were equally high in all transduced lymphocyte populations: 515 U/mg/hr in PBL(MPS)-L2SN, 734 in PBL(MPS)-LB2, 352 in PBL(MPS)-L2, and 389 in PBL(MPS)-LNC2 compared to controls (< 10 in PBL(MPS)-LXSN or PBL (MPS)). The half-life of endocytosed IDS in PBL(MPS) is approximately 1.9 days. However, the level of lymphocyte IDS activity from proviral expression is only a fraction of the total, a large portion being derived from reuptake of enzyme from murine packaging cells, or a "2nd source" of enzyme.
Considerations: Measurement of transgene lysosomal enzyme soon after exposure of target cells to vector supernatant may yield a gross overestimate of long-term transgene expression by transduced cells. Nevertheless, patient fibroblasts co-cultured with transduced PBL(MPS) have reduced [$^{35}SO^{4}$]-GAG accumulation, levels similar to those of normal fibroblasts.
Interpretation and Analysis: Cells can be charged with a lysosomal enzyme to levels much higher than those found in nature. By "supercharging" cells with a lysosomal protein (or other molecule bearing the mannose-6-phosphate ligand), such cells may be exploited as vehicles for systemic delivery of therapeutic or diagnostic agents.

Combined ultrafiltration -transduction in a hollow-fiber bioreactor facilitates retrovirus-mediated gene transfer into peripheral blood lymphocytes from patients with mucopolysaccharidosis type II.Pan D, Shankar R, Stroncek DF, Whitley CB. Hum Gene Ther 1999 Nov 20; 10 (17), pp. 2799–810.

Gene Delivery-Transfer-Expression/Pulmonary Expression: Cystic Fibrosis (CF)/Trans-Membrane Conductance Regulator Gene
(CFTR-DNA)-DC-Chol/DOPE Cationic Liposomes/ Genetic DNA Sequences

Background Information: The major cause of mortality in patients with CF is lung disease. Expression of the CF trans-membrane conductance regulator (CFTR) gene product in the airways is a potential treatment. Clinical studies in which the CFTR cDNA is delivered to the respiratory epithelia of CF patients have resulted in modest, transient gene expression. It seems likely that repeated administration of the gene transfer vector will be required for long-term gene expression.

Application: Delivery of genetic DNA sequences (*in vitro* and *in vivo*) into cell populations to induce selective gene expression.

Route of Administration: DNA/liposome formulation (intravascular).

Implementation: Evaluation of multiple doses of a DNA/liposome formulation for their ability to serve as a delivery platform into nasal epithelium (double blinded study). Complementary *in vivo* investigations can employ the administration of plasmid DNA expressing the CFTR cDNA complexed with DC-Chol/DOPE cationic liposomes (e.g., n = 10; n = 2 placebo controls). Each case receives 3 doses, administered 4 weeks apart.

***Ex Vivo* Model:** Nasal epithelium harvested from cases of CF.

Human Model: Cases of CF.

Interpretation and Analysis: There is no evidence of inflammation, toxicity, or an immune response toward the DNA/liposomes or the expressed CFTR. Nasal epithelial cells are collected 4 days after each dose for a series of efficacy assays including quantitation of vector-specific DNA and messenger RNA (mRNA), immunohistochemistry of CFTR protein, bacterial adherence, and detection of halide efflux *ex vivo*. Airway ion transport is also assessed *in vivo* by repeated nasal potential difference measurements. On average, 60% of the treated subjects can be expected to be positive for CFTR gene transfer after each dose. All subjects positive for CFTR function are also positive for plasmid DNA, plasmid-derived mRNA, and CFTR protein.

Repeat administration of DNA/liposomes to the nasal epithelium of patients with cystic fibrosis.Hyde SC, Southern KW, Gileadi U, Fitzjohn EM, Mofford KA, Waddell BE, Gooi HC, Goddard CA, Hannavy K, Smyth SE, Egan JJ, Sorgi FL, Huang L, Cuthbert AW, Evans MJ, Colledge WH, Higgins CF, Webb AK, Gill DR. Gene Ther 2000 Jul; 7 (13), pp. 1156–65.

Gene Delivery-Transfer-Expression/Stem Cells: Hematopoietic/EGFP and LNGFR Hematopoietic Stem Cell (HSC) Populations
Retroviral Vectors

Background Information: Retroviral vectors are widely used to deliver foreign genes to HSCs. Improvement of marking protocols allows for reporter genes to be rapidly detected and efficient selection of transduced cells. Both EGFP and LNGFR hold great potential as reporter systems and can be used simultaneously to compare their properties using the same retroviral backbone and the same gene transfer procedures.

Application: Delivery of genetic sequences for their expression in mammalian cell systems. Such strategies could theoretically be utilized to deliver sense or antisense nucleotide strands for various diagnostic purposes.

Implementation: The EGFP and LNGFR coding sequences are separately cloned into an MFG retroviral vector backbone. A cloning strategy that assures that both genes utilize the same ATG as the start codon can be applied. Marker gene expression, viral titers, transduction efficiency, and vector stability can be evaluated in expanded amphotropic packaging clones and human hematopoietic cell lines by flow cytometry and polymerase chain reaction (PCR) analysis. Vectors are also tested for their ability to transduce CD34+ peripheral blood cells.

Interpretation and Analysis: A significantly larger number of MFG-LNGFR packaging clones are obtained that produced high viral titers. A direct correlation between viral titer and marker gene expression in packaging clones can be demonstrated for both constructs. Similar expression kinetics and absence of *in vitro* toxicity in transduced cells are also observed for both constructs. Successful infection of CD34+ cells can be achieved after only a short time of exposure to recombinant viruses.

The marker genes EGFP and LNGFR have been demonstrated to be equally useful for a rapid, specific, and non-toxic detection of transduced cells. The MFG-EGFP construct appears useful to optimize gene transfer protocols *in vitro*. On the other hand, the MFG-LNGFR construct, for making possible a more efficient selection of high titer producer clones, as well as for safety and adaptability to the *in vivo* use, is more suitable for clinical applications.

A comparative evaluation of gene transfer into blood cells using the same retroviral backbone for independent expression of the EGFP and delta LNGFR marker genes. Giaretta I, Madeo D, Bonaguro R, Cappellari A, Rodeghiero F, Giorgio P. Haematologica 2000 Jul; 85 (7), pp. 680–9.

Gene Transfer (Selective): Neoplastic Disease
Plasmid/Transferrin-PEG-Polyethylenimine/TNF-cDNA

Considerations: Tumor-targeting DNA complexes that can readily be generated by the mixing of stable components and freeze-thawed would be very advantageous for their subsequent application as diagnostic/therapeutic products.

Semi-Synthetic Methods: Complexes are formulated by the mixing of plasmid DNA, linear polyethylenimine (PEI22, 22 kDa) as the main DNA condensing agent, PEG-PEI (poly(ethylene glycol)-conjugated PEI) for surface shielding, and Tf-PEG-PEI (transferrin-PEG-PEI) to provide a ligand for receptor-mediated cell uptake.

Within the shielding conjugates, PEG chains of varying size (5, 20, or 40 kDa) are conjugated with either linear PEI22 (22 kDa) or branched PEI25 (25 kDa). The 3 polymer components are mixed together at various ratios with DNA; particle size, surface charge, *in vitro* transfection activity, and systemic gene delivery to tumors for subsequent detection. In general, increasing the proportion of shielding conjugate in the complex reduces surface charge, particle size, and *in vitro* transfection efficiency in transferrin receptor-rich K562 cells.

Interpretation and Analysis: The particle size or surface charge of the complexes containing the PEG-PEI conjugate does not significantly change after freeze-thawing, while complexes without the shielding conjugate aggregate. Complexes containing PEG-PEI conjugate efficiently transfected K562 cells after freeze-thawing. Furthermore, the systemic application of freeze-thawed complexes exhibits *in vivo* tumor targeted expression. For complexes containing the luciferase reporter gene the highest expression is found in tumor tissue of mice. An optimum formulation for *in vivo* application, PEI22/Tf-PEG-PEI/PEI22-PEG5, containing plasmid DNA encoding for the tumor necrosis factor-alpha (TNF-α), inhibits tumor growth in 3 different murine tumor models. These new DNA complexes offer simplicity and convenience, with tumor targeting activity *in vivo* after freeze-thawing.

Novel shielded transferrin-polyethylene glycol-polyethylenimine/DNA complexes for systemic tumor-targeted gene transfer. Kursa M, Walker GF, Roessler V, Ogris M, Roedl W, Kircheis R, Wagner E. Bioconjug Chem 2003 Jan–Feb; 14 (1), pp. 222–31.

GENE EXPRESSION

Gene Expression
Phosphoric Diester-Modified Oligonucleotides

Cellular/Molecular Mechanism of Action: Oligonucleotide phosphorothioates are modified oligonucleotides. Some of these incorporate "hairpin" structures at the 3′ end or are "chimeras" that bear 2 modified oligonucleotides. To further enhance their effectiveness, bioreversible analogs of oligonucleotides can be synthesized. Several isosteric analogs of phosphoric diester oligonucleotides with neutral achiral backbones, and other novel molecules, such as peptide nucleic acids and circular oligonucleotides, are also reported to form stable duplexes with complementary RNA/DNA strands.
Interpretation and Analysis: Oligonucleotides can be synthesized that carry a variety of fluorescent tags that are effective as probes in a manner that makes them suitable as potential diagnostic agents.

Modified oligonucleotides as therapeutic and diagnostic agents. Agrawal S, Iyer RP. Curr Opin Biotechnol 1995 Feb; 6 (1), pp. 12–9.

Gene Expression/Double-Strand DNA Selective Targeting
Circular Triplex-Forming Oligonucleotides (CTFOs)

Cellular/Molecular Mechanism of Action: Circular triplex-forming oligonucleotides possess tight binding avidity for short single-stranded homopurine DNAs in a sequence-specific manner. Recent investigations have demonstrated their ability to bind to double-helical DNA segments.
Considerations: Binding experiments with DNA show that a CTFO can recognize its homopurine target when the target is embedded in a long duplex. Unlike their linear oligonucleotides, CTFOs bind the double helix in 2 topologically distinct forms. The more stable of the 2 complexes is found to be a pseudorotaxane, having the same topology as the sliding clamp protein subunits associated with some DNA and RNA polymerases.
Interpretation and Analysis: Circular triplex-forming oligonucleotides have been shown to bind the DNA double helix in a topological manner that is unprecedented among synthetic ligands. This novel binding motif allows a synthetic CTFO to be irreversibly locked onto a circular double-stranded DNA target without covalently modifying the target.

Triplex-directed self-assembly of an artificial sliding clamp on duplex DNA. Ryan K, Kool ET. Chem Biol 1998 Feb; 5 (2), pp. 59–67.

Gene Expression: Altered Gene Function/Mutation
Mu-lac Transducing Phage

Application: Assay for transcription of genes to detect alterations in gene function, regulation, and mutation. Potential model for mammalian genome systems.
Interpretation and Analysis: Bacterial lactose structural genes without the lactose promoter can be incorporated into the bacteriophage Mu genome to form a Mu-lac specialized transducing phage. This phage also carries a gene encoding resistance to ampicillin (Ap)[Mu(Ap, lac)]. After infection and upon establishment of lysogeny, the Mu(Ap, lac) genome can integrate into apparently random sites within the *Escherichia coli* chromosome. When integration occurs within a gene in the orientation of its transcription, the lactose structural genes are so situated that they become expressed solely from the promoter of that gene. Thus, expression of the lactose genes of Mu(Ap, lac) can be used as an assay for transcription of that gene and for functional and mutational studies of gene regulation.

Lactose genes fused to exogenous promoters in 1 step using a Mu-lac bacteriophage: *In vivo* probe for transcriptional control sequences. Casadaban MJ, Cohen SN. Proc Natl Acad Sci U S A 1979 Sep; 76 (9), pp. 4530–3.

Gene Expression: Cardiac/Myocardium/Imaging the Location, Magnitude, and Time Course of Gene Expression
Adenovirus-Expressing Firefly Luciferase/D-Luciferin

Cellular/Molecular Mechanism of Action: D-luciferin is a fluorescent substrate for firefly luciferase enzyme fractions.
Background Information: Studies of cardiac gene transfer rely on postmortem analysis using histologic staining or enzyme assays. Non-invasive imaging of the temporal and spatial characteristics of cardiac gene expression in the same subject offers significant advantages.
Application: Fluorescent marker system for validating the positive detection of gene-specific transcription processes.
Route of Administration: D-luciferin can be injected intraperitoneally following adenovirus-facilitated firefly luciferase gene expression.
Implementation: Direct myocardial injection via left thoracotomy with adenovirus-expressing firefly luciferase (Ad-CMV-Fluc; n = 30). The reporter substrate D-luciferin can be injected intraperitoneally. Serial images are acquired by use of a cooled charged-coupled device (CCD) camera. Results are expressed as relative light unit per minute (RLU/min). To assess the detection sensitivity, serially diluted titers of Ad-CMV-Fluc can be injected: 1×10^9 (195,393 ± 14,896), 1×10^8 (33,777 ± 18,179), 1×10^7 (417 ± 91), 1×10^6 (185 ± 64), 1×10^5 (53 ± 1), and control (54 ± 1) ($p < 0.05$ for 1×10^9, 1×10^8, and 1×10^7 plaque-forming units vs control adenovirus-expressing mutant thymidine kinase [Ad-CMV-HSV1-sr39tk]; n = 3).
Animal Model: Rat.
Interpretation and Analysis: Rats transduced with 1×10^9 plaque-forming units show decremental cardiac luciferase activity over time: 152,070 ± 21,170 (day 2), 195,806 ± 62,630 (day 5), 7,250 ± 2,941 (day 8), and 2,040 ± 971 RLU/min (day 14). Rats are sacrificed, and *in vitro* luciferase activity correlated with *in vivo* CCD signals ($r^2 = 0.92$). Such observations demonstrate the feasibility of imaging the location, magnitude, and time course of cardiac reporter gene expression in living rats. Cardiac gene therapy studies could be aided with wider application of this approach.

Optical imaging of cardiac reporter gene expression in living rats. Wu JC, Inubushi M, Sundaresan G, Schelbert HR, Gambhir SS. Circulation 2002 Apr 9; 105 (14), pp. 1631–4.

Gene Expression: Central Nervous System (CNS)/ Huntington's Disease (HD): Huntingtin RNA Genetic Targeting/Macromolecule CNS Delivery/Transferrin Receptor Complexes
[^{125}I]-Peptide Nucleic Acid-[Anti-Transferrin Receptor IgG]/Antisense Gene Targeting/In Vivo Gene Expression Quantification

Cellular/Molecular Mechanism of Action: The peptide nucleic acid (PNA) agent is a 16-mer PNS that is the antisense strand

that corresponds to the segment around the methionine initiation codon of the huntingtin gene of HD. In this context, it recognizes and physically binds to huntingtin RNA (HD exon-1 messenger RNA).

Background Information: Disease-specific genes of unknown function can be imaged *in vivo* with antisense radiopharmaceuticals, providing the transcellular transport of these molecules is enabled with drug-targeting technology.

Route of Administration: [^{125}I]-PNA/8D3 conjugate (intravenous).

Semi-Synthetic Methods: Biotinylation of PNA. Covalent cross-linking of strepavidin and rat 8D3 monoclonal antibody (mAb) to the mouse transferrin receptor (TfR). The latter moiety contains a tyrosine residue that can facilitate [^{125}I] radioiodination. The reformulated PNA antisense radiopharmaceutical conjugated to 8D3 mAb is designated as [^{125}I]-PNA/8D3.

Implementation: Evaluation of biotinylated PNA that can be rapidly captured by conjugates of covalently cross-linked strepavidin and [anti-mouse transferring receptor] IgG. Complementary *in vivo* studies in mice can be used to determine the biodistribution of the PNA analog, [^{125}I]-PNA/8D3, through harvest of tissue/organ systems 6 hours postadministration for frozen sectioning of the brain and quantitative autoradiography.

Animal Model: Murine (littermate control mice or to R6/2 transgenic mice that express the exon 1 of the human HD gene for HD).

Interpretation and Analysis: Despite covalent cross-linking of the PNA analog, [^{125}I]-PNA/8D3 to monoclonal antibody, it can gain access to endogenous transferrin transport pathways at both the blood-brain barrier and the brain cell membrane and undergo both import from the blood to the brain and export from the brain to the blood through the TfR complex. In this manner, it is able to hybridize with "target" huntingtin RNA based on analysis from both cell-free translation assays and ribonuclease protection assays.

Approximately a 3-fold increase in sequestration *in vivo* can be appreciated for antisense [^{125}I]-PNA/8D3 radiopharmaceutical conjugates in the brains of HD transgenic mice. Such observations are consistent with the selective expression of the HD exon-1 messenger RNA in these animals. Therefore, gene expression *in vivo* can be quantitated with antisense radiopharmaceuticals, providing these molecules are reformulated with drug-targeting technology. Drug targeting enables access of the antisense agent to endogenous transport pathways, which permits passage across the cellular barriers that separate blood and intracellular compartments of target tissues.

Imaging gene expression in the brain *in vivo* in a transgenic mouse model of Huntington's disease with an antisense radiopharmaceutical and drug-targeting technology. Lee HJ, Boado RJ, Braasch DA, Corey DR, Pardridge WM.J Nucl Med 2002 Jul; 43 (7), pp. 948–56.

Gene Expression: Detection *In Vivo* (General Concept)
Antisense Oligonucleotides

Cellular/Molecular Mechanism of Action: Selective *in vivo* detection of messenger RNA (mRNA) gene expression. Antisense oligonucleotides bind to cellular RNA sequences by hybridization mechanisms.

Application: Non-invasive, *in vivo* detection modality, imaging agent, and as selective therapeutic delivery platform.

Route of Administration: Intravenous.

Considerations: Labeled oligonucleotides can function as non-canonical radiopharmaceuticals and much progress needs to be made in adapting them to *in vivo* imaging methodologies. The requirements to reach this goal include improvements in radiosynthesis, stability, targeting, and specific and non-specific binding.

Reagent Design and Validation: *In vitro*, antisense oligonucleotides are widely used as reagents to detect or block specific RNA sequences. The use of antisense as an *in vivo* diagnostic agent is attractive because it advances molecular imaging modalities to the level of evaluating gene expression.

Interpretation and Analysis: Signal labeled antisense oligonucleotides can function as a valuable molecular modality for detecting and characterizing "real-time" *in vivo* gene expression.

In vivo antisense imaging.Tavitian B. Q J Nucl Med 2000 Sep; 44 (3), pp. 236–55.

Gene Expression: Neoplasia/HSV1-tk(+) Reporter Gene/[Oncogenes]/[Hereditary Disorders]
[^{123}I]-2′-Fluoro-2′-Deoxy-1-β-D-Arabinofuranosyluracil/[^{125}I]-2′-Fluoro-2′-Deoxy-1-β-D-Arabinofuranosyluracil

Cellular/Molecular Mechanism of Action: The molecular agent 2′-fluoro-2′-deoxy-5-iodo-1-β-D-arabinofuranosyluracil has been designed to function as a substrate for herpes simplex virus type 1 thymidine kinase (HSV1-tk) biochemical activity.

Background Information: Previous studies have shown that HSV1-tk, in combination with effective radiolabeled substrates (e.g., [I*]-2′-fluoro-2′-deoxy-5-iodo-1-β-D-arabinofuranosyluracil, or I*-FIAU, where the asterisk indicates the use of any radioactive iodine isotopes), can be used as a reporter gene for *in vivo* monitoring of gene transfer and expression.

Application Model: Potential gene expression imaging. In this context, [^{123}I]-2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyluracil and [^{125}I]-2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyluracil can be applied as radiolabeled substrates for HSV1-tk gene expression. Note: The *in vivo* detection of HSV1-tk gene expression utilizing [^{123}I]-2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyluracil and [^{125}I]-2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyluracil represents a model feasibility study designed to determine optimum conditions and methodologies that can be extrapolated to *in vivo* detection of altered expression of other unrelated genetic sequences (e.g., oncogenes, hereditary disorders).

Route of Administration: [^{123}I]-2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyluracil and [^{125}I]-2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyluracil (intravenous).

Semi-Synthetic Methods: The molecular agent 2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyluracil (FAU) can be radioiodinated (e.g., [^{123}I] or [^{125}I]) using the iodogen method. High-performance liquid chromatography purification results in a yield of product with a high specific activity and radiochemical purity for both tracers ([^{123}I]-FIAU and [^{125}I]-FIAU).

Implementation: Evaluate the early kinetics of [^{123}I] or [^{125}I]-labeled 2′-fluoro-2′-deoxy-5-iodo-1-β-D-arabinofuranosyluracil (I*-FIAU) and delineate the potential utilization of [^{123}I]-labeled FIAU for imaging of gene expression. Fibrosarcoma CMS-5 cells can be transduced *in vitro* with the retroviral vector STK containing the HSV1-tk gene. Complementary investigations can be conducted to determine the biodistribution profiles for individual radiopharmaceutical agents established by gamma camera imaging at 0.5, 1, 2, and 4 hours postinjection. In addition, the genomic DNA of the tumors can be isolated for the measurement of accumulated activity resulting from the [^{125}I]-FIAU incorporation.

Animal Model: Murine (fibrosarcoma cells are transduced *in vitro* with the retroviral vector STK containing the HSV1-tk gene.

BALB/c mice are inoculated subcutaneously with HSV1-tk(+) and tk(−) cells into both flanks).

Interpretation and Analysis: Biodistribution studies 0.5 hours postinjection reveal tumor/blood and tumor/muscle ratios of 3.8 and 7.2, respectively, for the HSV1-tk(+) tumors, and 0.6 and 1.2, respectively, for negative control tumors. Fast renal elimination of the tracer from the body results in rapidly increasing tumor/blood and tumor/muscle ratios that reach values of 32 and 88 at 4 hours postinjection, respectively. Tracer clearance from blood is biexponential, with an initial half-life of 0.6 hours followed by a half-life of 4.6 hours. The tracer half-life in herpes simplex viral thymidine kinase-expressing tumors is 35.7 hours. The highest activity accumulation (20.3% ± 5.7% ID/g) in HSV1-tk(+) tumors can be observed 1 hour postinjection. At that time, 46% of the total activity found in HSV1-tk(+) tumors is incorporated within genomic DNA. Planar gamma camera imaging reveals distinct tracer accumulation as early as 0.5 hours postinjection, with an increase in contrast over time. Such findings suggest that sufficient tumor/background ratios for *in vivo* HSV1-tk expression imaging with [^{123}I]-FIAU are reached as early as 1 hour postinjection.

In vivo imaging of herpes simplex virus type 1 thymidine kinase gene expression: Early kinetics of radiolabeled FIAU. Haubner R, Avril N, Hantzopoulos PA, Gansbacher B, Schwaiger M. Eur J Nucl Med 2000 Mar; 27 (3), pp. 283–91.

Gene Expression: Neoplasia/Mammary Carcinoma Prognostic Marker
RECK Messenger RNA (mRNA)

Cellular/Molecular Mechanism of Action: Reversion-inducing cysteine-rich protein with Kazal motifs (RECK) functions as a tumor suppressor gene.

Background Information: The recently described RECK inhibits membrane Type 1 matrix metalloproteinase (MMP-14), MMP-2, and MMP-9 secretion and enzymatic activity. Its expression is essential for normal vasculogenesis. Downregulation of RECK has been implicated in tumor angiogenesis and progression.

Application: Determination of the prognosis for human mammary carcinoma and possibly a target for the molecular design of a new class of imaging agent.

Interpretation and Analysis: The expression of RECK mRNA as determined by real-time polymerase chain reaction (PCR) in breast carcinoma tissue specimens (n = 278) on day 1 and on a 2nd date sometime between 2 to 169 months later reveals a degree of accuracy in predicting a longer recurrence-free survival time (RFS; p = 0.037) at the optimal cutoff value (hazard ratio, 0.66; 95% confidence interval, 0.44–0.98). Cases with tumors exhibiting low levels of RECK (n = 100) have a mean RFS time of 80.4 months and a 61.8% 5-year RFS rate, whereas cases with tumors with high RECK expression (n = 178) have a mean RFS time of 91.2 months and a 73.0% 5-year RFS rate. The expression of RECK mRNA maintains a significant independent prognostic value for RFS time (p = 0.047) based on multivariate Cox regression analysis. No relevant associations of RECK with clinicopathologic factors or treatment regimens can be appreciated.

Due to its desirable characteristics, RECK could be applied as a template for the molecular design of pharmaceutical mimetics, or drugs activating endogenous RECK expression, as possible diagnostic, therapeutic, and preventive agents for breast carcinoma.

Instrumentation: Real-time quantitative reverse transcriptase-polymerase chain reaction.

Matrix metalloproteinase inhibitor reversion-inducing cysteine-rich protein with Kazal motifs: A prognostic marker for good clinical outcome in human breast carcinoma. Span PN, Sweep CG, Manders P, Beex LV, Leppert D, Lindberg RL. Cancer 2003 Jun 1; 97 (11), pp. 2710–5.

Gene Expression: Neoplasia/Pulmonary Non-Small Cell Lung Carcinoma
Modified Oligonucleotide Phosphorothionates

Cellular/Molecular Mechanism of Action: Selective recognition or detection of specific messenger RNA (mRNA) strands or DNA gene segments through the formation of stable heteroduplexes (cDNA/mRNA) or homoduplexes (cDNA/DNA).

Application: Oligonucleotides carrying a variety of fluorescent tags have been used as probes with potential for diagnostic applications. Represents a possible modality capable of facilitating the detection of various pathological conditions precipitated by infectious agents (human immunodeficiency virus) or associated with conditions of neoplastic disease.

Considerations: Some of the most extensively investigated agents in this class of compounds are the oligonucleotide phosphorothioates. Many of these incorporate hairpin structures at the 3′ end and "chimeras" that bear 2 modified oligonucleotides, all are potentially of value as diagnostic/therapeutic agents. To further enhance their applicability, bioreversible analogs of oligonucleotides have been synthesized. Several isosteric analogs of phosphoric diester oligonucleotides with neutral achiral backbones, and other novel molecules, such as peptide nucleic acids and circular oligonucleotides, are also reported to form stable duplexes with complementary RNA/DNA and are currently under investigation.

Additionally, oligonucleotides carrying a variety of fluorescent tags have been used as probes for diagnostic applications.

Activation of peroxisome proliferator-activated receptor-gamma stimulates the growth arrest and DNA-damage inducible 153 gene in non-small cell lung carcinoma cells. Satoh T, Toyoda M, Hoshino H, Monden T, Yamada M, Shimizu H, Miyamoto K, Mori M, Yamada M, Mori M. Oncogene 2002 Mar 28; 21 (14), pp. 2171–80.

Growth enhancement of normal human keratinocytes by the antisense oligonucleotide of retinoblastoma susceptibility gene. Koike M, Ishino K, Ikuta T, Huh N, Kuroki T. Oncogene 1995 Jan 5; 10 (1), pp. 117–22.

Gene Expression: Neoplasia (General Cancer)/ HSV-tk
2′-Deoxy-2′-Flouro-5-Methyl-1-β-D-Arabinofuranosyluracil (FMAU)/9-[(3-[^{18}F]-Fluoro-1-Hydroxy-2-Propoxy)Methyl]-Guanine ([^{18}F]-FHPG)/ 9-(4-[^{18}F]-Fluoro-3-Hydroxy-Methylbutyl)Guanine ([^{18}F]-FHBG)

Application: 2′-deoxy-2′-flouro-5-methyl-1-β-D-arabinofuranosyluracil (FMAU) has demonstrated potential utilization as a positron emission tomography (PET) radiotracer for imaging HSV-tk gene expression.

Interpretation and Analysis: *In vitro* experiments demonstrate that the accumulation of [^{14}C]-FMAU in HSV-tk-expressing cells is 2.4-fold ($p < 0.02$), 4.0-fold ($p < 0.001$), and 5.3-fold ($p < 0.001$) higher than the wild-type cells at 1, 3, and 5 hours, respectively. *In vivo* studies reveal that the tumor uptake in HSV-tk-expressing cells is 2.3-fold ($p < 0.001$), 3.0-fold ($p < 0.001$), and 5.5-fold ($p < 0.001$) higher than the control cells at 1, 2, and 5 hours, respectively. FMAU is more sensitive compared to earlier studies using [^{18}F]-FHPG and [^{18}F]-FHBG in the same cell lines, although the specificity is less than FHBG. While FMAU labeled with PET isotopes may be useful for imaging HSV-tk-expressing tumors *in vivo*, multitracer

studies across additional tumor models are necessary in order to identify an optimal PET radiotracer.

Evaluation of 2′-deoxy-2′-flouro-5-methyl-1-β-D-arabinofuranosyluracil as a potential gene imaging agent for HSV-tk expression *in vivo*. Alauddin MM, Shahinian A, Gordon EM, Conti PS. Mol Imaging 2002 Apr–Jun; 1 (2), pp. 74–81.

Gene Expression: Neoplasia Chemotherapeutic Resistance Potential/Radioresistance: Mammary Carcinoma

Antisense Insulin-Like Growth Factor-I Receptor (IGF-IR)/IGF-IR Messenger RNA (mRNA)

Cellular/Molecular Mechanism of Action: The IGF-IR plays a critical role in cell growth regulation and transformation. Antisense IGF-IR nucleotides inhibit the transcription of IGF-IR mRNA gene sequences.

Application: Expression of IGF-IR mRNA can serve as a prognostic marker pertaining to the potential of antisense IGF-IR to reverse resistance to radiation and chemotherapy in neoplastic cell populations (e.g., mammary carcinoma). Strategies designed to disrupt the biological expression of IGF-IR may be a fruitful avenue for achieving enhanced resolution of neoplastic disease states.

Route of Administration: Antisense IGF-IR nucleotide (intravenous).

Dosage: Antisense IGF-IR nucleotide.

Implementation: Evaluation of the radiosensitivity of fibroblasts (e.g., NIH 3T3 cell line) that overexpress either wild-type or mutant IGF-IR complexes (e.g., IGF-IR). Supplementary analysis can entail immunohistochemical analysis of primary mammary carcinoma neoplastic lesions to detect the expression of IGF-IR complexes.

Interpretation and Analysis: High levels of wild-type IGF-IR confer radioresistance, and mutational analysis has revealed that this effect correlates with the transforming capacity but not the mitogenic activity of the receptor. The radioresistant phenotype is reversed when the cells are incubated with antisense oligonucleotides targeted to IGF-IR mRNA, demonstrating that IGF-IR directly influences radioresistance. Immunohistochemical analysis of primary breast tumors reveals that high levels of IGF-IR in tumor samples highly correlates with ipsilateral breast tumor recurrence (IBTR) following lumpectomy and radiation therapy ($p = 0.001$). Subgroup analysis reveals that, for early breast tumor relapses (within 4 years of initial breast tumor diagnosis), elevated levels of IGF-IR are strongly associated with IBTR ($p = 0.004$), but IGF-IR expression is not prognostic for IBTR from breast cancer patients with late relapses (p value not significant).

Insulin-like growth factor-I receptor overexpression mediates cellular radioresistance and local breast cancer recurrence after lumpectomy and radiation. Turner BC, Haffty BG, Narayanan L, Yuan J, Havre PA, Gumbs AA, Kaplan L, Burgaud JL, Carter D, Baserga R, Glazer PM. Cancer Res 1997 Aug 1; 57 (15), pp. 3079–83.

Genetic Expression: Oligonucleotide (Hybridization)

Magnetic Bead-Linked Oligonucleotide Probes

Cellular/Molecular Mechanism of Action: Oligonucelotides incorporated into magnetic bead-linked oligonucleotide probes will hybridize (e.g., form cDNA/cDNA diamers or messenger RNA [mRNA]/cDNA heteriomers).

Application: Methodology for the detection of gene expression that could potentially be developed or adapted as a technique for the non-invasive detection of gene expression events in both *ex vivo* or *in vivo* environments.

Route of Administration: Intravascular most commonly (selective and non-selective delivery).

Implementation: Hybridization of a target oligonucleotide to magnetic bead-linked oligonucleotide probes is followed by binding of the streptavidin-coated metal nanoparticles to the captured DNA, dissolution of the nanometer-sized gold tag, and potentiometric stripping measurements of the dissolved metal tag at single-use thick-film carbon electrodes.

An advanced magnetic processing technique is used to isolate the DNA duplex and to provide low-volume mixing. The influence of relevant experimental variables, including the amounts of the gold nanoparticles and the magnetic beads, the duration of the hybridization and gold dissolution steps, and the parameters of the potentiometric stripping operation upon the hybridization signal have been optimized in several laboratories.

Interpretation and Analysis: Transmission electron microscopy micrographs indicate that the hybridization event leads to the bridging of the gold nanoparticles to the magnetic beads. Further signal amplification and lowering of the detection limits to the nanomolar and picomolar domains are achieved by precipitating gold or silver, respectively, onto the colloidal gold label. The new electrochemical stripping metallogenomagnetic protocol couples the inherent signal amplification of stripping metal analysis with discrimination against non-hybridized DNA, the use of microliter sample volumes, and disposable transducers and, hence, offers great promise for decentralized genetic testing.

Metal nanoparticle-based electrochemical stripping potentiometric detection of DNA hybridization. Wang J, Xu D, Kawde AN, Polsky R. Anal Chem 2001 Nov 15; 73 (22), pp. 5576–81.

14 Inflammation, Infection, and Sepsis

GENERAL ABSCESS FORMATION, SEPSIS, AND INFLAMMATION

General Abscess Formation: Non-Septic Inflammation
[^{99m}Tc]-Interleukin-8 (IL-8)

Cellular/Molecular Mechanism of Action: Interleukin-8 recognizes and binds to IL-8 receptor complexes expressed on the exterior surface membrane of neutrophil and monocyte populations.

Physiological Mechanism of Action: Interleukin-8 can function as a neutrophil chemotactic agent but this has little relevance to the use of this agent as an *in vivo* diagnostic agent in the field of nuclear medicine.

Route of Administration: [^{99m}Tc]-IL-8 (intravenous).

Implementation: Evaluation of the specificity of [^{99m}Tc]-IL-8 for detecting focal non-septic abscess lesions as a function of biodistribution patterns and pharmacokinetics profiles. Complimentary analyses include determination of the interaction of [^{99m}Tc]-IL-8 with circulating blood cells achieved through characterization of the radiolabel in peripheral leukocytes, red blood cells, and plasma. The *in vivo* kinetics of [^{99m}Tc]-IL-8 can be studied by quantitative analysis of whole-body images acquired between 0 and 6 hours postinjection. The results of this analysis (*in vivo* biodistribution) can be validated by *ex vivo* counting of radioactivity in dissected tissues.

Animal Model: Rabbit (turpentine-induced abscesses in neutropenic and normal rabbits).

Interpretation and Analysis: The abscess uptake (percentage of injected dose per gram of tissue [%ID/g] $\pm$ standard error of measurement [SEM]) in immunocompetent rabbits (0.41 $\pm$ 0.05) is 10 times higher than that in neutropenic rabbits (0.038 $\pm$ 0.014) and demonstrates specificity of target uptake for [^{99m}Tc]-IL-8. Abscess-to-muscle ratios $\pm$ SEM are also 10 times higher (110 $\pm$ 10 vs 10 $\pm$ 5). Lung and spleen uptake is 3-fold less with conditions of neutropenia compared to normal reference controls. The blood clearance of the radiolabel in conditions of neutropenia is similar to that in normal reference controls. In the vascular compartment, most of the [^{99m}Tc]-IL-8 (70%) is found in the plasma. Less than one-third is associated with red blood cells, and only a very low percentage ($<$ 2.5%) is associated with white blood populations. Image analysis reveals a gradual increase in abscess uptake over time up to $>$ 15%ID, which can be confirmed by *ex vivo* gamma counting of infected muscle. The highest increases in abscess uptake can be observed $>$ 2 hours postinjection when most of the [^{99m}Tc]-IL-8 has cleared from the blood, suggesting specific neutrophil-mediated accumulation of [^{99m}Tc]-IL-8 within abscesses. Furthermore, region-of-interest (ROI) analysis reveals that gradual accumulation of [^{99m}Tc]-IL-8 in abscesses is accompanied by a simultaneous clearance of activity from the lungs, suggesting that neutrophil-associated [^{99m}Tc]-IL-8 that was initially trapped in the lungs migrates to abscess lesions at later time points, favoring neutrophil-bound transportation from the lungs to the abscess. Substantial evidence supports the hypothesis that [^{99m}Tc]-IL-8 localizes within abscesses, primarily due to physical binding to peripheral neutrophils. Accumulation in abscesses is a highly specific, neutrophil-driven process. As assessed by *in vivo* and *ex vivo* analyses, the total fraction that accumulates in inflamed tissue is extremely high (up to $>$ 15 %ID) compared with that of other agents used for imaging infection and inflammation.

Kinetics of [^{99m}Tc]-labeled interleukin-8 in experimental inflammation and infection. Rennen HJ, Boerman OC, Oyen WJ, Corstens FH. J Nucl Med 2003 Sep; 44 (9), pp. 1502–9.

General Abscess Formation: Septic Inflammation
Anti-DTPA Antibody/[In]-DTPA-Bovine Serum Albumin/ [^{111}In]-DTPA (Bivalent)/[3-Phase Injection Scheme]

Application: A new 3-step approach to imaging infectious and inflammatory foci has been developed and optimized in a rat model. The approach relies on the non-specific localization of an anti-diethylenetriaminepentaacetic acid (DTPA) antibody in inflamed tissue. In this study, the 3-step strategy can be enhanced by selecting the most suitable radiolabeled hapten and optimizing the dosing schedule.

Route of Administration: Anti-DTPA antibody, [^{111}In]-DTPA-bovine serum albumin, and [^{111}In]-diDTPA (intravenous).

Semi-Synthetic Methods: An N2S2 core is attached to the diDTPA compound, thereby allowing [^{99m}Tc] radiolabeling.

Implementation: Evaluation of focal septic inflammatory lesions by initial "priming" with the anti-DTPA antibody DTIn-1 (0.67, 2, or 6 nmol per rat). In the 2nd phase (1–24 hours later), the anti-DTPA activity within the intravascular department is blocked with unlabeled [In]-DTPA-bovine serum albumin (0.3, 1, or 3 nmol per rat). In the 3rd step (5–30 minutes later), the radiolabeled hapten (monovalent or bivalent [^{111}In]-DTPA) is administered. The *in vivo* distribution of the radiolabel is monitored by scintigraphic imaging and by *ex vivo* counting of dissected tissues.

Animal Model: Rat (Wistar strain) injected with *Staphylococcus aureus* in the left calf muscle.

Interpretation and Analysis: Scatchard analysis reveals that the affinity of DTIn-1 for bivalent [^{111}In]-diDTPA is 6 times higher than its affinity for monovalent [^{111}In]-DTPA ($K_a = 0.87 \times 10^{-9}$ mol/L vs 5.3×10^{-9} mol/L). The uptake of the bivalent chelate within abscesses is 2.5-fold higher than that for monovalent [^{111}In]-DTPA. Most important, the bivalent chelate is completely retained in the abscess over time. Using the bivalent chelate, the optimal dosing scheme can be determined with respect to the DTIn-1 dose (2 nmol per rat), the blocking agent dose (1 nmol per rat), and radiolabeled chelate dose (40 pmol per rat). The procedure is rapid with infectious foci clearly visualized at 1 hour postinjection of [^{111}In]-labeled diDTPA (5 hours after administration of the anti-DTPA antibody). The non-targeted radiolabel is rapidly cleared into the urine and is only retained within abscess and the kidney (4–6% of injected dose). The 3-step diagnostic protocol enables rapid imaging of infectious foci with minimal uptake in non-inflamed tissues.

A 3-step pretargeting strategy to image infection. Boerman OC, van Eerd J, Oyen WJ, Corstens FH. J Nucl Med 2001 Sep; 42 (9), pp. 1405–11.

General Abscess Formation (Septic) *[^{99m}Tc]-HYNIC-C5adR/[^{99m}Tc]-HYNIC-C5a*

Cellular/Molecular Mechanism of Action: Complement fraction C5a and its natural metabolite C5a des Arg(74) (C5adR) recognize and bind to receptor complexes expressed on the exterior surface membrane of neutrophil and monocyte populations.

Application: The complement anaphylatoxin C5a and its natural metabolite C5adR are involved in several stages of the inflammatory process. Both act on a common receptor expressed by different cell types, including neutrophils and monocytes. The receptor binding affinity of C5a is in the nanomolar range and exceeds that of C5adR by 1 to 2 orders of magnitude. The biologic potency of C5a is considerably higher than that of C5adR.

Semi-Synthetic Methods: Labeling of proteins can be achieved utilizing [^{99m}Tc] following initial conjugation with an appropriate moiety such as hydrazinonicotinamide (HYNIC).

Implementation: Evaluation of intramuscular septic abscesses induced by bacterial injection utilizing [^{99m}Tc]-HYNIC-C5a and [^{99m}Tc]-HYNIC-C5adR as imaging agents. Biodistribution of the radiolabel can be determined by gamma camera imaging and by counting dissected tissues at 5 hours postinjection.

Interpretation and Analysis: Complement fractions [^{99m}Tc]-HYNIC-C5a and [^{99m}Tc]-HYNIC-C5adR display *in vivo* abscess uptake values of 0.12 and 0.025 percentage injected dose (%ID)/g, abscess/muscle ratios of 76 and 14, abscess/blood ratios of 9.1 and 2.6, and region-of-interest (ROI)-derived target-to-background ratios of 5.9 and 2.1, respectively, at 5 hours postinjection. Therefore, [^{99m}Tc]-HYNIC-C5a provides superior capabilities as an agent for *in vivo* imaging of foci of sepsis (e.g., abscess formation). However, C5a is a very bioactive protein, and this biological property impedes its clinical use as an infection imaging agent. The naturally occurring partial agonist C5adR has less biological effect but shows suboptimal imaging characteristics. Therefore, for adequate localization of a receptor binding ligand, the affinities for the receptor in the nanomolar range are required.

Tc-99m-labeled C5a and C5a des Arg74 for infection imaging. Rennen HJ, Oyen WJ, Cain SA, Monk PN, Corstens FH, Boerman OC. Nucl Med Biol 2003 Apr; 30 (3), pp. 267–72.

General Abscess Formation (Septic) *[^{99m}Tc]-HYNIC-Interleukin-8 (IL-8)*

Cellular/Molecular Mechanism of Action: Interleukin-8 recognizes and binds to IL-8 receptor complexes expressed on the exterior surface membrane of neutrophil and monocyte populations.

Background Information: Previous investigations have demonstrated that IL-8 can be labeled with [^{99m}Tc] using hydrazinonicotinamide (HYNIC) as a bifunctional coupling agent and tricine as a co-ligand. Such preparations possess excellent characteristics for imaging infections (e.g., rabbit model of soft-tissue infection).

Route of Administration: [^{99m}Tc]-HYNIC-IL-8 (intravenous).

Semi-Synthetic Methods: Propylaldehyde hydrazone formulations of HYNIC can be utilized to stabilize HYNIC-IL-8 conjugation products. [^{99m}Tc]-HYNIC-IL-8 can be prepared using 5 different co-ligand formulation protocols. In this context, HYNIC-conjugated IL-8 can be labeled with [^{99m}Tc] in the presence of either (i) tricine; (ii) ethylenediaminediacetic acid (EDDA); (iii) tricine and trisodium triphenylphosphinetrisulfonate (TPPTS); (iv) tricine and nicotinic acid (NIC); or (v) tricine and isonicotinic acid (ISONIC). These preparations can be characterized *in vitro* by (i) reverse phase high-performance liquid chromatography (RP-HPLC) analysis; (ii) measurement of octanol/water partition coefficient parameters; (iii) establishing molecular stability; and (iv) receptor affinity analysis (ligand binding assays).

Implementation: Evaluation of the *in vivo* biodistribution of [^{99m}Tc]-HYNIC-IL-8 in conditions of induced localized sepsis (*Escherichia coli*–induced soft-tissue infection) and evaluated by gamma camera imaging as well as by tissue counting at 6 hours postinjection.

Animal Model: Rabbit (*E. coli*–induced soft-tissue septic lesions).

Interpretation and Analysis: Specific activity (MBq/μg) is highest for (ISO)NIC (up to 80) > TPPTS (40) > tricine (15) > EDDA (7). Reverse phase high-performance liquid chromatography and octanol/water partition coefficients show a shift toward higher lipophilicity for TPPTS preparations. The leukocyte receptor binding fractions are around 40 to 55% for all preparations except for TPPTS, which displays predominantly non-specific binding. All preparations are stabile in serum, but the stability in PBS is highest for NIC and TPPTS > EDDA > ISONIC > tricine. Profiles for *in vivo* biodistribution show highest abscess/muscle for NIC and ISONIC (> 200) > EDDA and tricine (approximately 100) > TPPTS (< 40). Gamma camera imaging rapidly visualizes abscesses from 2 hours postinjection onward for all formulations. The abscess/background (A/B) at 6 hours postinjection for ISONIC is significantly higher ($p < 0.05$) than that of tricine and the A/B of TPPTS is significantly lower ($p < 0.05$). Interleukin-8 can be rapidly and easily labeled with [^{99m}Tc] using HYNIC as a chelator in combination with various co-ligands. The most optimal infection imaging characteristics can be appreciated with formulations using nicotinic acid/tricine as a co-ligand system, which simultaneously provides high specific activity and high *in vitro* stability with high abscess/muscle ratios (> 200) and high abscess/background ratios (> 20). Protein doses can be administered as low as 70 ng/kg bodyweight. At these low protein doses, side effects are not to be expected in the human system.

Instrumentation: Gamma camera imaging.

Effects of co-ligand variation on the *in vivo* characteristics of Tc-99m-labeled interleukin-8 in detection of infection. Rennen HJ, van Eerd JE, Oyen WJ, Corstens FH, Edwards DS, Boerman OC. Bioconjug Chem 2002 Mar–Apr; 13 (2), pp. 370–7.

General Abscess Formation (Septic): *Escherichia Coli*–Induced/Comparison of Radioiodination Methods/Plasma Kinetic Profiles: $T_{1/2}$ *[^{123}I]-Interleukin-8 (IL-8)*

Background Information: Several small receptor-binding agents have been tested for imaging of infection and inflammation. The potential of chemotactic peptides and of interleukins is promising and superior to that of conventional agents.

Application: Detection and characterization of septic and potentially non-septic inflammatory lesions.

Route of Administration: [^{123}I]-IL-8 (intravenous).

Dosage: [^{123}I]-IL-8 (18.5 MBq).

Semi-Synthetic Methods: Radiolabeling of biological protein fractions with radioiodine [^{123}I, ^{124}I, ^{125}I, ^{127}I] facilitated by the application of Bolton-Hunter reagent.

Implementation: Imaging of septic abscesses can be performed 24 hours postinduction with [^{123}I]-IL-8 preparations. Gamma camera images can be obtained at 5 minutes and at 1, 4, and 8 hours after injection. Biodistribution is determined 8 hours after injection. Gamma camera imaging is then performed at 5 minutes and at 1, 4, and 8 hours postinjection with biodistribution profiles established at 8 hours postinjection.

Animal Model: Rabbits (*E. coli*–induced abscesses in the left thigh muscle).

Interpretation and Analysis: Radioiodinated [^{123}I]-IL-8 is rapidly cleared from the blood. Accumulation of [^{123}I]-IL-8 in abscesses is visible as early as 1 hour postinjection. The highest abscess uptake can be appreciated 4 hours after injection (2.6 ± percentage injected dose [%ID]), whereas [^{123}I]-IL-8 rapidly clears from all other tissues. Approximate increases in abscess-to-background ratios are 13.0 ± 0.7 (8 hours postinjection), as determined by quantification of the images. In tissue biodistribution investigations (8 hours postinjection), abscess uptake is 0.057 ± 0.011 %ID/g with abscess-to-contralateral muscle ratios of 114.7 ± 23.0. The radioiodination method clearly affects the *in vivo* biodistribution of IL-8 because IL-8 iodinated using the Iodo-Gen method cleared significantly slower from the blood and most other organs, resulting in poor visualization of the abscess. The superior characteristics of IL-8 radioiodinated using the Bolton-Hunter method—that is, high abscess uptake and rapid background clearance within a few hours—make IL-8 a promising agent for imaging septic (infection) and inflammatory lesions.

Radiolabeled interleukin-8: Specific scintigraphic detection of infection within a few hours. van der Laken CJ, Boerman OC, Oyen WJ, van de Ven MT, van der Meer JW, Corstens FH. J Nucl Med 2000 Mar; 41 (3), pp. 463–9.

General Abscess Formation (Septic): Systemic Foci/Leukotriene B_4 (LTB_4) Receptor Complexes *[^{99m}Tc]-RP517*

Cellular/Molecular Mechanism of Action: The agent [^{99m}Tc]-RP517 recognizes and physically binds to LTB_4 receptor complexes.

Background Information: In a search for a rapid and accurate imaging agent for scintigraphic detection of infection and inflammation, an LTB_4 receptor antagonist, [^{99m}Tc]-RP517, has been developed that contains a hydrazinonicotinamide moiety.

Application: Detection and characterization of systemic septic foci including abscess formation.

Route of Administration: [^{99m}Tc]-RP517 (intravenous).

Implementation: Determination of the *in vivo* behavior of [^{99m}Tc]-RP517 in an abscess model. Detection of created abscesses can be established by gamma camera imaging, which can additionally be utilized to establish the *ex vivo* biodistribution of the agent several hours postinjection. Complementary studies can entail cannulation of the choledochal duct to facilitate quantitative monitoring of hepatobiliary radiopharmaceutical clearance.

Animal Model: Rabbit abscesses (induced by intravenous injection of *Escherichia coli* bacteria).

Animal Model: Rabbit (choledochal duct cannulation).

Interpretation and Analysis: The *ex vivo* receptor-bound fraction of radiolabeled RP517 exceeds 70%. Accumulation of [^{99m}Tc]-RP517 within abscesses can be visualized as early as 1 hour postinjection. Due to rapid blood clearance ($t_{1/2}$ alpha = 18 ± 0.6 minutes, $t_{1/2}$ beta = 6.5 ± 0.4 hours) and high abscess uptake, the abscess-to-muscle ratios increase with time from 7.0 ± 2.3 at 1 hour postinjection to 44.3 ± 4.6 at 20 hours postinjection. The agent is mainly cleared via hepatobiliary excretion: 50% of the radiolabel can be recovered in the small bowel at 1 hour postinjection, whereas 85% can be found in the cecum and sigmoid at 20 hours postinjection. In conclusion, [^{99m}Tc]-RP517 can facilitate rapid visualization of *E. coli* abscesses in rabbits. The agent rapidly clears from the blood, mainly via the hepatobiliary route. Considerably high abscess-to-background target ratios can be achieved. Accumulation within the intestines may limit the applicability of the agent for detecting infectious processes within the abdominal cavity. The development of a more hydrophilic analogue of [^{99m}Tc]-RP517 could improve the clinical applicability of this agent.

A [^{99m}Tc]-labeled leukotriene B4 receptor antagonist for scintigraphic detection of infection in rabbits. Brouwers AH, Laverman P, Boerman OC, Oyen WJ, Barrett JA, Harris TD, Edwards DS, Corstens FH. Nucl Med Commun 2000 Nov; 21 (11), pp. 1043–50.

General Sepsis: Bacterial Infection *[^{99m}Tc]-Labeled Ciprofloxacin/[^{99m}Tc]-Hexamethylpropyleneamine Oxime (HMPAO)-Labeled White Blood Cells (WBCs)*

Cellular/Molecular Mechanism of Action: Ciprofloxacin (infecton) when applied as an imaging agent localizes in high concentrations within living bacteria.

Application: [^{99m}Tc]-labeled ciprofloxacin (infecton) has been developed for detecting infectious foci.

Route of Administration: [^{99m}Tc]-labeled ciprofloxacin and [^{99m}Tc]-HMPAO-labeled WBC preparations are injected intravenously.

Dosage: [^{99m}Tc]-HMPAO-labeled WBC (185–200 MBq). [^{99m}Tc]-labeled ciprofloxacin (370–400 MBq).

Considerations: Previous reports have described greater specificity of radiolabeled ciprofloxacin for bacterial infections compared to radiolabeled WBC scans.

Implementation: Performance of scintigraphic image analysis in suspected cases of bone or joint sepsis using [^{99m}Tc]-labeled ciprofloxacin and [^{99m}Tc]-WBC preparations (e.g., n = 51; n = 21/51 prosthetic implant materials). Procedures that can complement or validate findings observed in scintigraphic image analyses include biochemical, radiographic, and microbiologic data in addition to clinical history (e.g., outcomes). Scintigraphic images are performed at 1 and 4 hours postinjection of 370–400 MBq [^{99m}Tc]-labeled ciprofloxacin or 185–200 MBq [^{99m}Tc]-HMPAO-WBCs. At least a 2-day and at most a 7-day lag-time period occurs between the imaging agent administration and the acquisition of scintigraphic images.

Interpretation and Analysis: Scintigraphic imaging with [^{99m}Tc]-labeled ciprofloxacin and [^{99m}Tc]-HMPAO-labeled WBC populations in cases of bone or joint sepsis produces a variety of image assessment classifications including true-positives (e.g., n = 30/32 vs n = 20/32); false-positives (e.g., n = 4/24 vs n = 1/24); true-negatives (e.g., n = 20/24 vs 23/24), and false-negatives (e.g., n = 2/22 vs 12/22), respectively. Corresponding values for sensitivity, specificity, and accuracy are approximately 94%, 83%, and 89% ([^{99m}Tc]-labeled ciprofloxacin), and 63%, 96%, and 77% ([^{99m}Tc]-HMPAO-labeled WBC), respectively. Differences between the 2 agents are statistically significant ($p < 0.001$). [^{99m}Tc]-labeled ciprofloxacin and WBC scan results are in general concordance for 77% of sites (n = 43/56). [^{99m}Tc]-HMPAO-labeled WBC results for vertebral infections are the most notable. [^{99m}Tc]-labeled ciprofloxacin scans are positive in a large percentage of patients with vertebral osteomyelitis (e.g., n = 83% or 5/6). White blood cell scans can show photon-deficient areas in some of these cases (e.g., n = 4/6), but normal distribution in the remaining scenarios (e.g., n = 2/6). [^{99m}Tc]-labeled ciprofloxacin is a useful agent for detecting infectious foci in bones and joints. Moreover, the [^{99m}Tc]-labeled ciprofloxacin scans seem to be a more powerful tool in diagnosing vertebral infections than WBC scintigraphy.

Usefulness of [^{99m}Tc]-ciprofloxacin (infecton) scan in diagnosis of chronic orthopedic infections: Comparative study with [^{99m}Tc]-HMPAO leukocyte scintigraphy. Sonmezoglu K, Sonmezoglu M, Halac M, Akgun I, Turkmen C,

Onsel C, Kanmaz B, Solanki K, Britton KE, Uslu I. J Nucl Med 2001 Apr; 42 (4), pp. 567–74.

General Sepsis: Bacterial Infection (Focal) *[^{18}F]-2-Deoxy-2-Fluoro-D-Glucose (FDG)/[^{3}H]-2-Deoxy-2-Fluoro-D-Glucose (FDG)/[^{125}I]-Human Serum Albumin (^{125}I-HSA)/L-[^{11}C]-Methionine/L-[^{3}H]-Methionine/[^{3}H]-Thymidine*

Application: Detection of focal sites of septic inflammation within extravascular tissues applying positron emission tomography (PET) imaging modalities.

Implementation: Determination of the relative biodistribution of [^{18}F]- or [^{3}H]-FDG, [^{3}H]-thymidine, L-[^{11}C]- or [^{3}H]-methionine, gallium-67 citrate ([^{67}Ga]-citrate), and iodine-125 human serum albumin ([^{125}I]-HSA) in cases of focal sepsis (e.g., rat left calf muscles infected with *Escherichia coli* suspension). Uptake of [^{3}H]-FDG within infectious foci is evaluated by autoradiography of histological sections.

Interpretation and Analysis: Although [^{18}F]-FDG, [^{67}Ga]-citrate, and [^{125}I]-HSA showed comparatively high uptake in the infected muscle (the percentage activity of injected dose [ID] per gram of tissue normalized for rat weight in kilogram [%ID/g] $\times$ kg at 2 hours postinjection is as follows: [^{18}F]-FDG, 0.184$\pm$ 0.026 to 0.218 $\pm$ 0.046; [^{67}Ga]-citrate, 0.221 $\pm$ 0.016; [^{125}I]-HSA, 0. 198 $\pm$ 0.019), the infected muscle-to-blood ratio is much higher for [^{18}F]-FDG than for [^{67}Ga]-citrate or [^{125}I]-HSA ([^{18}F]-FDG, 10.31 $\pm$ 0.76 to 14.89 $\pm$ 2.26; [^{67}Ga]-citrate, 1.24 $\pm$ 0.67; [^{125}I]-HSA, 0.20 $\pm$ 0.02). The draining reactive lymph nodes also show higher accumulation of [^{18}F]-FDG than of [^{67}Ga]-citrate or [^{125}I]-HSA. The uptake of [^{3}H]-thymidine and L-[^{11}C]-methionine or [^{3}H]-methionine in the infected muscle is lower than that of [^{18}F]-FDG or [^{3}H]-FDG (at 2 hours postinjection, [^{3}H]-thymidine = 0. 039 $\pm$ 0.005 and L-[^{3}H]-methionine = 0.063 $\pm$ 0.007 [%ID/g] $\times$ kg). Autoradiographs reveal that the highest [^{3}H]-FDG uptake occurs in the area of inflammatory cell infiltration surrounding necrotic regions. In conclusion, [^{18}F]-FDG, which rapidly accumulates in sites of bacterial infection and in reactive lymph nodes with a high target-to-background ratio, appears to be a promising infection detection agent.

Uptake of positron emission tomography tracers in experimental bacterial infections: A comparative biodistribution study of radiolabeled FDG, thymidine, L-methionine, [^{67}Ga]-citrate, and [^{125}I]-HSA. Sugawara Y, Gutowski TD, Fisher SJ, Brown RS, Wahl RL. Eur J Nucl Med 1999 Apr; 26 (4), pp. 333–41.

General Sepsis: Differentiated from Non-Septic Inflammation *[^{99m}Tc]-UBI 29-41*

Cellular/Molecular Mechanism of Action: A [^{99m}technetium]-labeled peptide derived from ubiquicidine, referred to as [^{99m}Tc]-UBI 29-41, targets bacterial and fungal infections, but not sterile inflammatory processes, in experimental animals.

Application: Differentiation between conditions of septic inflammation due to bacterial pathogens vs non-septic inflammatory responses utilizing [^{99m}Tc]-UBI 29-41 or [^{99m}Tc]-Sc-UBI 29-41.

Route of Administration: [^{99m}Tc]-UBI 29-41 and [^{99m}Tc]-Sc-UBI 29-41 (intravenous).

Semi-Synthetic Methods: Radiochemical analyses of [^{99m}Tc]-UBI 29-41 and a radiolabeled scrambled version of this peptide, that is, [^{99m}Tc]-Sc-UBI 29-41, reveal that both peptides can be (i) labeled rapidly (within 10 minutes); (ii) generate little colloid formation (less than 5% of the total radioactivity); and (iii) produce very little free pertechnetate (or radioactive intermediates) in the preparations containing radiolabeled peptide.

Implementation: Delineation of the relative capacity of [^{99m}Tc]-UBI 29-41 and [^{99m}Tc]-Sc-UBI 29-41 to differentiate between septic inflammatory responses and conditions of non-septic inflammation. In this manner, greater insight can be achieved in delineating the regions of the amino acid sequence for UBI 29-41 that are the most critical to selectively imaging bacterial infections.

Animal Model: Murine (*Staphylococcus aureus*–infected mice).

Interpretation and Analysis: The molecular interaction of peptides with bacteria can be enhanced utilizing a relative excess of unlabeled peptide and this physical association has been shown to be a temperature-dependent process. Based on *in vitro* observations of this nature, labeling of peptides with [^{99m}Tc] by a direct method of this type is rapid, efficient, and safe. Scintigraphy demonstrates that radioactivity is rapidly removed from the circulation (half-lives of UBI 29-41 and Sc-UBI 29-41 are 16 and 21 minutes, respectively) and primarily by renal clearance mechanisms. Analysis of murine blood reveals that only a small proportion of the intravenously injected [^{99m}Tc]-peptides is associated with blood cells. Although both types of radiolabeled peptides accumulate rapidly at sites of infection, the values for [^{99m}Tc]-UBI 29-41 are higher ($p < 0.05$) than for [^{99m}Tc]-Sc-UBI 29-41. Moreover, injection of excess unlabeled UBI 29-41, but not Sc-UBI 29-41, in scenarios of *S. aureus* infections prior to injection of [^{99m}Tc]-UBI 29-41 significantly ($p < 0.05$) reduces the accumulation of this radiopharmaceutical at the site of sepsis infiltration. In addition, significantly ($p < 0.01$) higher amounts of [^{99m}Tc]-UBI 29-41 can be appreciated at the site of infection in mice using a carrier-free formulation of radiolabeled UBI 29-41 peptide in contrast to unpurified preparations containing radiolabeled UBI 29-41 peptide. Such *in vivo* data implies that the amino acid sequence of [^{99m}Tc]-UBI 29-41 contributes to its accumulation at sites of infection.

Radiochemical and biological characteristics of [^{99m}Tc]-UBI 29-41 for imaging of bacterial infections. Welling MM, Mongera S, Lupetti A, Balter HS, Bonetto V, Mazzi U, Pauwels EK, Nibbering PH. Nucl Med Biol 2002 May; 29 (4), pp. 413–22.

General Sepsis: Differentiated from Sterile Inflammation *[^{99m}Tc]-Labeled Ubiquicidin (UBI) Peptides*

Cellular/Molecular Mechanism of Action: Human UBI is an antimicrobial peptide that physically binds to bacterial organisms.

Application: Discrimination between bacterial infections and sterile inflammatory reactions.

Route of Administration: [^{99m}Tc]-labeled ubiquicidin (intravascular).

Implementation: Evaluate the *in vitro* binding of various [^{99m}Tc]-labeled natural or synthetic peptides (based on the sequence of the human antimicrobial peptide UBI or human lactoferrin [hLF]) to bacteria and leucocytes. Complementary investigations can entail the *in vivo* evaluation of these same radiolabeled peptides injected into mice intraperitoneally infected with *Klebsiella pneumoniae* in order to identify sequences that preferentially bind to bacteria compared to host cell populations. The amount of radioactivity associated with bacteria and leukocytes is then quantified. Discovery of the ability for candidate [^{99m}Tc]-labeled peptides to discriminate between bacterial infections and sterile inflammatory processes can be established in mice intramuscularly infected with various bacteri-

al organisms (e.g., multi-drug-resistant *Staphylococcus aureus*) and in animals injected with lipopolysaccharides (LPS) of bacterial origin (sterile inflammatory process). Complementary validation studies can entail determination of [^{99m}Tc]-labeled UBI 29-41 and UBI 18-35 distribution in rabbits having an experimental thigh muscle infection induced with the introduction of *K. pneumoniae,* and in rabbits injected with LPS.

Animal Model: Murine (mouse sepsis: intraperitoneal injection of *K. pneumoniae*).

Animal Model: Rabbit (intramuscular injection of *K. pneumoniae* (septic foci model) and lipopolysacchairde fractions (sterile LPS inflammatory model).

Interpretation and Analysis: In both *in vitro* and *in vivo* binding assays, the 2 peptides, UBI 29-41 and UBI 18-35, are 2 candidates platforms for image analysis of infection. The radiolabeled peptides can detect infections with both gram-positive and gram-negative bacteria in mice as early as 5–30 minutes after injection, with a target-to-non-target (T/NT) ratio between 2 and 3; maximum T/NT ratios can be seen within 1 hour after injection. In rabbits, high T/NT ratios ($>$ 5) for [^{99m}Tc]-labeled UBI 29-41 are observed from 1 hour after injection. No accumulation of candidate [^{99m}Tc]-labeled UBI-derived peptides can be observed in thighs of mice and rabbits previously injected with LPS. Scintigraphic investigations into the biodistribution of [^{99m}Tc]-labeled UBI peptides reveal that these peptides are rapidly removed from the circulation by renal excretion. Similar data can be observed for [^{99m}Tc]-labeled defensin 1-3. Preliminary investigations utilizing [^{99m}Tc]-labeled hLF and related peptides indicate that these compounds are less favorable for infection detection. Taken together, [^{99m}Tc]-labeled UBI 18-35 and UBI 29-41 enable discrimination between bacterial infections and sterile inflammatory processes in both mice and rabbits. Based on their characteristics and properties, these peptides are candidate platforms for the preferential detection of bacterial infections.

Technetium-99m labeled antimicrobial peptides discriminate between bacterial infections and sterile inflammations. Welling MM, Paulusma-Annema A, Balter HS, Pauwels EK, Nibbering PH. Eur J Nucl Med 2000 Mar; 27 (3), pp. 292–301.

General Sepsis: *Escherichia Coli* Targeting/E. Coli Envelope Proteins *Sulfosuccinimidyl-6-Biotinamido-Hexanoate (NHS-LC-Biotin)/Succinimidyl-6-Biotinamido-Hexanoate (NHS-A-Biotin)*

Cellular/Molecular Mechanism of Action: Succinimidyl-6-biotinamido-hexanoate and NHS-LC-biotin function as (covalent) primary amine coupling reagents.

Considerations: High sucrose concentrations appear to chemically change components such as outer membrane protein-F (OmpF) found in the outer membrane structure of *E. coli* bacteria to the extent that they can no longer be recognized applying NHS-A-biotin as a detection agent.

Interpretation and Analysis: Both hydrophobic NHS-A-biotin and hydrophilic NHS-LC-biotin preferentially label outer membrane, periplasmic, and inner membrane proteins in *E. coli* preparations. NHS-A- and NHS-LC-biotin can also label a specific inner membrane marker protein (Tet-LacZ). Neither hydrophobic NHS-A-biotin nor hydrophilic NHS-LC-biotin probes label a cytosolic marker (the omega fragment of beta-galactosidase). Applying labeling procedures, *E. coli* populations grown in low-salt Luria broth medium reveal that both reagents react with outer membrane protein-A (OmpA) and outer membrane protein-C (OmpC) when bacteria are grown in the presence of sucrose formulated at gradient concentrations (e.g., 0%, 10%, and 20% sucrose supplementation). Conversely, only NHS-A-biotin labels OmpF in media containing 0 and 10% sucrose, and neither reagent detects this outer membrane protein fraction at 20% sucrose concentrations. A difference in the amount of OmpF contained in *E. coli* populations grown at 0%, 10%, and 20% cannot be detected in Coomassie-stained acrylamide gels. However, similar quantities of OmpF can be detected in *E. coli* cells grown at all 3 sucrose concentrations.

In vivo labeling of *Escherichia coli* cell envelope proteins with N-hydroxysuccinimide esters of biotin. Bradburne JA, Godfrey P, Choi JH, Mathis JN. Appl Environ Microbiol 1993 Mar; 59 (3), pp. 663–8.

General Septic Inflammation and Disease/ Radiolabeling Efficiency/Radiolabel Stability: *In Vivo*/Kinetic Profile: In Vivo/Dosimetry: *In Vivo Stabilized [^{99m}Tc]-Exametazime-Labeled Leukocytes*

Background Information: Labeling leukocytes with [^{99m}Tc]-exametazime is a validated technique for imaging infection and inflammation. A new radiolabeling technique has been described that enables leukocyte labeling with a more stable form of [^{99m}Tc]-exametazime.

Application: Detection and characterization of inflammatory and septic disease states.

Route of Administration: Stabilized [^{99m}Tc]-exametazime-labeled leukocytes (intravascular).

Semi-Synthetic Methods: Labeling of autologous leukocyte populations with [^{99m}Tc] using stabilized exametazime.

Implementation: Evaluation of the *in vivo* radioactive distrubution, kinetic profile, and dosimetry of stabilized [^{99m}Tc]-exametazime-labeled autologous leukocytes. Serial whole-body imaging and blood sampling are performed up to 24 hours after injection. Cell-labeling efficiency and *in vivo* viability, organ dosimetry, and clearance calculations are obtained from the blood samples and imaging data as well as samples of urine and stool collected up to 36 hours after injection.

Human Model: Cases in normal health (n = 10 volunteers).

Interpretation and Analysis: Cell-labeling efficiencies of 87.5% $\pm$ 5.1% can be achieved, which is similar to or better than that reported with the standard preparation of [^{99m}Tc]-exametazime. *In vivo* stability of the radiolabeled leukocytes is also similar to *in vitro* results with stabilized [^{99m}Tc]-exametazime and better than previously reported for the *in vivo* stability of non-stabilized [^{99m}Tc]-exametazime-labeled leukocytes. Organ dosimetry and radiation-absorbed doses are similar with a whole-body absorbed dose of 1.3 $\times$ 10^{-3} mGy/ MBq. Urinary and fecal excretion of activity is minimal, and visual assessment of images shows little renal parenchymal activity and no bowel activity up to 2 hours after injection.

Cell labeling and *in vivo* stability appear to be improved compared to leukocytes labeled with the non-stabilized [^{99m}Tc]-exametazime preparations. Advantages of the methodology include increased preparation cost-effectiveness of stabilized [^{99m}Tc]-exametazime and an extended window for clinical usage, with good visualization of abdominal structures on early images. No significant increase in specific organ and whole-body dosimetry estimates has been noted compared with previous estimates using non-stabilized [^{99m}Tc]-exametazime-labeled leukocytes.

Biodistribution and radiation dosimetry of stabilized [^{99m}Tc]-exametazine-labeled leukocytes in normal subjects. Robins PD, Salazar I, Forstrom LA, Mullan BP, Hung JC. J Nucl Med 2000 May; 41 (5), pp. 934–40.

ORGAN- AND TISSUE-ASSOCIATED SEPSIS AND INFLAMMATORY RESPONSES

Cardiovascular/Vascular Permeability: Septic Inflammation (Osteomyelitis) *[^{99m}Tc]-Sulesomab/[^{99m}Tc]-Human Serum Albumin (HSA)*

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-sulesomab and [^{99m}Tc]-HSA have similar molecular weights (molecular mass). Human serum albumin within the intravascular compartment is primarily responsible for maintaining plasma oncotic pressure. However, when it is labeled and applied for diagnostic purposes, it per se exerts not true biological/molecular mechanism of action while functioning like a "blood-pool" agent.

Background Information: [^{99m}Tc]-sulesomab, the Fab fragment of anti-NCA-90, is used as an *in vivo* granulocyte labeling agent for imaging inflammation. It is not clear to what extent it can target cells that have already migrated into the interstitial space of an inflammatory lesion as opposed to circulating cells. The signal contribution of radioprotein diffusion in scenarios of increased vascular permeability has also been poorly documented.

Application: Detection and characterization of septic osteomyelitis conditions.

Route of Administration: [^{99m}Tc]-sulesomab and [^{99m}Tc]-HSA (intravascular).

Implementation: Evaluation of orthopedic infections (e.g., septic osteomyelitis) utilizing [^{99m}Tc]-sulesomab and [^{99m}Tc]-HSA as a function of their local kinetic profiles. [^{99m}Tc]-sulesomab and [^{99m}Tc]-HSA are administered in sequence separated by an interval of 2 to 6 days. Images are obtained 1, 3, 4, and 6 hours after injection, and multiple venous blood samples obtained for the calculation of blood clearance parameters. Patlak-Rutland (P-R) analysis is performed to measure lesion and control tissue protein clearance. Target-to-background tissue (T/Bkg) ratios are calculated for each radioprotein and compared with the T/Bkg ratio for [^{111}In]-leukocytes. [^{99m}Tc]-sulesomab binding to granulocytes can be measured *in vitro*, in *ex vivo* preparations, and *in vitro* to primed and activated granulocytes.

Human Model: Cases of septic osteomyelitis (n = 7) already previously detected as being clearly positive on [^{111}In]-leukocyte scintigraphy analysis.

Interpretation and Analysis: After intravenous injection, $<$ 5% of the circulating radioactivity is cell-bound for both radioproteins so that the P-R curves can therefore be assumed to represent extravascular uptake of free protein. The blood clearance (mean $\pm$ SD) of sulesomab is 23.4 $\pm$ 11.7 mL/min, which is approximately 5 times greater than that of HSA (4.8 $\pm$ 3.1 mL/min). Likewise, clearance into the lesion by sulesomab is consistently higher than for HSA (on average about 3 times as high). Nevertheless, the T/Bkg ratios for sulesomab and HSA are similar, except at 6 hours when that of HSA (2.14 $\pm$ 0.6) is higher than that of sulesomab (1.93 $\pm$ 0.5; p approximately 0.01). Both values are considerably less than the T/Bkg ratio on the [^{111}In]-leukocyte images, which, at 22 hours, is 12.3 $\pm$ 5.3. Moderate clearance of sulesomab, but not HSA, occurs in control tissue regions. Granulocytes bind significantly more [^{99m}Tc]-sulesomab *in vitro* when primed or activated. In conclusion, (i) sulesomab does not localize in inflammation as a result of binding to circulating granulocytes; (ii) sulesomab is cleared into inflammation non-specifically via increased vascular permeability; nevertheless, it may be cleared after local binding to primed granulocytes or bind to activated granulocytes that have migrated extravascularly; and (iii) HSA produces a similar or higher T/Bkg ratio than sulesomab because sulesomab is cleared into normal tissues and because image positivity in inflammation is significantly dependent on local blood-pool expansion.

Mechanism of accumulation of [^{99m}Tc]-sulesomab in inflammation. Skehan SJ, White JF, Evans JW, Parry-Jones DR, Solanki CK, Ballinger JR, Chilvers ER, Peters AM. J Nucl Med 2003 Jan; 44 (1), pp. 11–8.

Cardiovascular: Endocarditis (Septic/Bacterial)/ Platelet GP IIb/IIIa Receptor Expression Postactivation *DMP444*

Cellular/Molecular Mechanism of Action: The agent DMP444 recognizes GP IIb/IIIa receptors expressed by activated platelet populations.

Background Information: Bacterial endocarditis is an important clinical problem that may result in persistent bacteremia and irreversible cardiac damage. The condition is also characterized by the aggregation of activated platelets, fibrin, and bacteria.

Application: Scintigraphic detection and characterization of bacterial endocarditis conditions.

Route of Administration: [^{99m}Tc]-DMP444 (37 MBq/kg).

Dosage: [^{99m}Tc]-DMP444 (intravenous).

Implementation: Evaluation of the expression of GP IIb/IIIa receptors by activated platelets utilizing [^{99m}Tc]-labeled DMP444, which functions as a high-affinity antagonist of activated platelet GP IIb/IIIa receptor complexes. At 24 hours postinjection of bacterial populations, 37 MBq/kg [^{99m}Tc]-DMP444 is administered (IV). A non-specific control agent that can be applied in conditions of focal sepsis (e.g., 37 MBq/kg [^{99m}Tc]-IgG). Image analysis can be performed up to 4 hours postinjection. Samples are then obtained for tissue counting, microbiology, and histology.

Animal Model: Canine (n = 7 beagles [11–15 kg] with catheterization of their left ventricle through the right carotid artery; n = 2 complete sham procedures [reference controls]). One hour following catheterization, a dose of 5×10^7 colony-forming units of *Staphylococcus aureus* bacteria is injected intracardially (n = 5/7 beagles). Half an hour later, catheters are removed.

Interpretation and Analysis: At or greater than 1 to 2 hours postinjection, there is clear focal accumulation of DMP444 in the aortic valve region when endocarditis lesions exist, with an observed accumulation of agent over time. The non-infected and the [^{99m}Tc]-IgG injected models show only persistent blood pool activity without evidence of focal abnormality. At 4 hours postinjection, the *in vivo* valve-to-blood pool ratios are 1.87 $\pm$ 0.18 in endocarditis, 1.01 $\pm$ 0.05 in non-infected controls, and 1.09 $\pm$ 0.02 in [^{99m}Tc]-IgG injected dogs ($p < 0.05$). Targeting of activated platelets with the [^{99m}Tc]-labeled GP IIb/IIIa antagonist DMP444 allows determining a final diagnosis of experimental bacterial endocarditis within 4 hours facilitated by high, specific, and rapid *in vivo* uptake.

Scintigraphic detection of acute experimental endocarditis with the technetium-99m labeled glycoprotein IIb/IIIa receptor antagonist DMP444. Oyen WJ, Boerman OC, Brouwers FM, Barrett JA, Verheugt FW, Ruiter DJ, Corstens FH, van der Meer JW. Eur J Nucl Med 2000 Apr; 27 (4), pp. 392–9.

Central Nervous System (CNS): Septic and Non-Septic Inflammation/Glucocorticoid Influence on Inflammatory Responses/Multiple Sclerosis: Potential Applications/Leukocytes: *In Vivo* Labeling of Neutrophil and Macrophage Tuftsin Receptors *[^{99m}Tc]-RP128*

Cellular/Molecular Mechanism of Action: The agent RP128 functions as a chelation agent for [^{99m}Tc] in addition to recognizing

and binding to tuftsin receptors expressed by neutrophils and monocyte-macrophages.

Application: The agent [^{99m}Tc]-RP128 can be applied for the *in vivo* labeling of neutrophil and macrophage tuftsin receptor complexes for the purpose of detecting and characterizing focal lesions of septic inflammation. Additionally, the agent may be potentially applicable for the detection and characterization of human multiple sclerosis.

Route of Administration: Intravascular.

Implementation: Administration of [^{99m}Tc]-RP128 for the *in vivo* detection of CNS inflammation (e.g., experimental allergic encephalomyelitis).

Animal Model: Murine (experimental allergic encephalomyelitis [EAE] utilized as a reference disease model for human multiple sclerosis).

Interpretation and Analysis: The radiopharmaceutical [^{99m}Tc]-RP128 can be detected at significantly increased levels within EAE-diseased CNS tissues at 0.5, 1.0, and 3.0 hours postinjection using a dual radioisotope technique to correct for non-extravasated tracer ($p < 0.05$). Moreover, extravascular accumulation of [^{99m}Tc]-RP128 can clearly be demonstrated in inflamed tissues with minimal loss of sensitivity when the secondary isotopic correction for blood volume is omitted. In addition, [^{99m}Tc]-RP128 can successfully monitor glucocorticoid suppression of inflammation ($p < 0.05$), detected as a typical dose-response to increasing steroid concentration. Clearly, [^{99m}Tc]-RP128 can quantitatively detect CNS inflammation and assess responses to therapy, indicating potential value as an imaging agent both clinically and as a research aid. Furthermore, the rapid *in vivo* labeling by [^{99m}Tc]-RP128 of specific inflammatory cells combined with the ability to monitor the progress of anti-inflammatory therapeutics may validate the use of this cellular radiolabeling agent for detecting a variety of inflammatory conditions.

The detection and quantitation of inflammation in the central nervous system during experimental allergic encephalomyelitis using the radiopharmaceutical [^{99m}Tc]-RP128. Paul C, Peers SH, Woodhouse LE, Thornback JR, Goodbody AE, Bolton C. J Neurosci Methods 2000 May 15; 98 (1), pp. 83–90.

Gastrointestinal: Diarrhea: *Escherichia Coli* Enterotoxin-Induced Diarrhea/Intestinal Absorption *[^{51}Cr]-EDTA/D-Xylose*

Cellular/Molecular Mechanism of Action: [^{51}Cr]- ethylenediaminetetraacetic (EDTA) is an organic chelate of radioactive ^{51}chromium that is normally not significantly absorbed across intact, normal intestinal mucosa. Alternatively, D-xylose is absorbed across the surface of the small intestine mucosa.

Application: Detection and characterization of alterations in the intestinal permeability ([^{51}Cr]-EDTA) and absorptive capacity, including conditions associated with *E. coli* enterotoxin-induced diarrhea.

Route of Administration: D-xylose and [^{51}Cr]-EDTA can be administered orally for the purpose of evaluating intestinal absorption and permeability.

Implementation: Evaluation of alterations in intestinal absorptive capacity in diarrhea induced by the administration of *E. coli* enterotoxins. Intestinal absorption is evaluated using D-xylose and [^{51}Cr]-EDTA absorption tests.

Animal Model: Murine (*E. coli* heat-stable, heat-labile, and both heat-stable and heat-labile enterotoxin-induced diarrhea).

Interpretation and Analysis: The absorption of D-xylose is greatest 45 minutes after ingestion in both control and enterotoxin-treated groups. There is a significant ($p < 0.01$) decrease in xylose absorption at 45 minutes following exposure to all 3 entertoxin formulations compared to reference controls. Such findings may be due to changes in membrane fluidity and hence changes in membrane permeability, whereas [^{51}Cr]-EDTA absorption is at its maximum 15 minutes after ingestion in both controls and following enterotoxin exposure. No significant ($p < 0.05$) increase can be appreciated for [^{51}Cr]-EDTA absorption in enterotoxin-treated groups when compared to reference controls. Findings of this nature suggest a change in tight junctional permeability that may be due to alterations in cytoskeletal microfilaments. Both these tests do, however, demonstrate that *E. coli* enterotoxin-induced diarrhea results in absorption and permeability changes.

[^{51}Cr]-labeled ethylenediaminetetraacetic acid and D-xylose absorption test in *Escherichia coli* enterotoxin-induced diarrhea in mice. Verma M, Ganguly NK, Majumdar S, Walia BN, Verma M, Ganguly NK, Majumdar S, Walia BN. Scand J Gastroenterol 1995 Sep; 30 (9), pp. 886–91.

Gastrointestinal and Musculoskeletal Inflammation/ Intestinal Lesions: Non-Steroidal Anti-Inflammatory Drug (NSAID)-Associated/Intestinal Lesions: Crohn's Ileitis/Musculoskeletal: Rheumatoid Arthritis *[^{111}In]-Leukocytes/Tauro 23 [^{75}Se]-Selena-25-Homocholic Acid/[^{58}Co]-Labeled Cyanocobalamine*

Cellular/Molecular Mechanism of Action: The agent tauro 23 [^{75}Se]-selena-25-homocholic acid is a bile acid analog, while [^{58}Co]-labeled cyanocobalamine is an analog of vitamin B_{12}. Radiolabeled [^{111}In]-leukocytes behave *in vivo* like endogenous leukocytes of the same cell type.

Application: The agents tauro 23 [^{75}Se]-selena-25-homocholic acid and [^{58}Co]-labeled cyanocobalamine can be applied to evaluate intestinal absorption. Conversely, [^{111}In]-leukocytes can be used to detect foci of inflammation and sepsis.

Route of Administration: Intravenous.

Implementation: Evaluation of residual inflammatory lesions following discontinuation of NSAIDs using [^{111}In]-leukocyte scintigraphy imaging methodologies.

Human Model #1: Cases of rheumatoid arthritis ($n = 90$) and osteoarthritis ($n = 7$).

Human Model #2: Cases treated with NSAIDs ($n = 19$), healthy controls ($n = 20$), and Crohn's ileitis ($n = 13$).

Interpretation and Analysis: Using a [^{111}In]-leukocyte imaging technique reveals that cases of rheumatoid arthritis and osteoarthritis administered NSAIDs are associated with gastrointestinal lesions. More specifically, approximately two-thirds of all cases evaluated on long-term treatment display inflammatory lesions that may persist for up to 16 months following discontinuation. The prevalence and magnitude of the intestinal inflammation is unrelated to the type and dose of non-steroidal drugs and previous or concomitant 2nd-line drug treatment. There is a significant inverse correlation ($r = -0.29$, $p < 0.05$) between fecal [^{111}In] excretion and hemoglobin levels in cases treated with NSAIDs. The kinetics of fecal [^{111}In] excretion in cases treated with NSAIDs are almost identical to that of patients with small bowel Crohn's disease. Small bowel radiological examination of cases on NSAIDs reveals that approximately one-sixth have asymptomatic ileal disease with ulceration and strictures (e.g., $n = 3/18$ cases). Evaluation of cases on anti-inflammatory agents, normal healthy controls, and cases of Crohn's ileitis with tauro 23 [^{75}Se]-selena-25-homocholic acid and [^{58}Co]-labeled cyanocobalamine reveals several abnormalities. On day 4, more than half the cases with rheumatoid arthritis have evidence of bile acid malabsorption, but the ileal dysfunction is much milder than recognized with conditions of Crohn's ileitis.

Non-steroidal anti-inflammatory drug-induced intestinal inflammation in humans. Bjarnason I, Zanelli G, Smith T, Prouse P, Williams P, Smethurst P, Delacey G, Gumpel MJ, Levi AJ. Gastroenterology 1987 Sep; 93 (3), pp. 480–9.

Gastrointestinal Inflammation/Intestinal Lesions: Human Immunodeficiecy Virus (HIV) vs Acquired Immune Deficiency Syndrome (AIDS)/Intestinal Lesions: Crohn's Ileitis
[^{111}In]-Leukocytes/Tauro 23 [^{75}Se]-Selena-25-Homocholic Acid/[^{58}Co]-Labeled Cyanocobalamine

Cellular/Molecular Mechanism of Action: The agent tauro 23 [^{75}Se]-selena-25-homocholic acid is a bile acid analog, while [^{58}Co]-labeled cyanocobalamine is an analog of vitamin B_{12}. Alternatively, radiolabeled [^{111}In]-leukocytes behave *in vivo* like endogenous leukocytes of the same cell type.

Background Information: Previous investigations have examined small intestinal absorption-permeability, intestinal inflammation, and ileal structure and function (e.g., alterations in HIV-positive male homosexuals).

Application: The agents tauro 23 [^{75}Se]-selena-25-homocholic acid and [^{58}Co]-labeled cyanocobalamine can be applied to evaluate intestinal absorption. Conversely, [^{111}In]-leukocytes can be used to detect foci of inflammation and sepsis.

Implementation: Evaluation of intestinal absorption permeability and [^{111}indium] leukocyte distribution studies in order to detect and characterize intestinal inflammation in HIV-positive conditions. Complementary studies can be designed to characterize AIDS cases with a dual radioisotopic ileal function test (whole-body retention of tauro 23 [^{75}Se]-selena-25-homocholic acid and [^{58}cobalt]-labeled cyanocobalamine). Such cases can be evaluated using ileocolonoscopy in concert with terminal ileal biopsy.

Human Model #1: Cases of HIV that are seropositive ($n = 30$ at various stages of disease).

Human Model #2: Cases of AIDS ($n = 26$; $n = 17/26$ evaluated by ileocolonoscopy and terminal ileal biopsy).

Interpretation and Analysis: Normal intestinal absorption-permeability can be appreciated in well, HIV-infected cases, but both functions are impaired upon the development of AIDS. The median fecal excretion of [^{111}indium] in well patients (0.66%) does not differ significantly ($p > 0.5$) from controls (0.46%), but subjects with AIDS who are well or who have had diarrhea have significant ($p < 0.005$) intestinal inflammation (1.33% and 2.18%, respectively). The median 7-day retention of tauro 23-[^{75}Se]-selena-25-homocholic acid in well patients with AIDS (38.9%) does not differ significantly ($p > 0.2$) from controls (39.3%), whereas the absorption of [^{58}cobalt]-labeled cyanocobalamine is significantly ($p < 0.05$) lower than observed in controls (32.1% and 59.4%). Patients with AIDS-diarrhea have significant ($p < 0.001$) malabsorption of both the bile acid (7.7%) and vitamin B_{12} (8.9%), which is more severe than in Crohn's ileitis (14.2% and 30.3%, respectively). Morphometric analyses of ileal biopsies is unremarkable in AIDS. Such findings depict the presence of low-grade enteropathy in conditions of AIDS, severe ileal malabsorption in cases with AIDS diarrhea, and relatively minor ileal morphologic changes. Malabsorption of bile acids may play a pathogenic role in patients with AIDS and diarrhea.

Intestinal inflammation, ileal structure and function in HIV. Bjarnason I, Sharpstone DR, Francis N, Marker A, Taylor C, Barrett M, Macpherson A, Baldwin C, Menzies IS, Crane RC, Smith T, Pozniak A, Gazzard BG. AIDS 1996 Oct; 10 (12), pp. 1385–91.

Gastrointestinal Inflammation: Colitis (Acute Chemically Induced)
[^{99m}Tc]-HYNIC-Interleukin-8 (IL-8)/[^{99m}Tc]-Granulocytes

Cellular/Molecular Mechanism of Action: Interleukin-8 binds to its corresponding membrane-associated receptor complex expressed at relatively high densities by neutrophil and monocyte cell types. Granulocytes are chemotactically attracted to sites of septic and non-septic inflammation.

Physiological Mechanism of Action: Interleukin-8 can function as a chemotactic agent for granulocyte populations.

Implementation: Evaluation of acute colitis conditions facilitated by scintigraphic image analysis with [^{99m}Tc]-HYNIC-IL-8 and [^{99m}Tc]-granulocytes (purified cell populations). Gamma camera images are acquired at 2 minutes and at 1, 2, and 4 hours postinjection. At 4 hours after injection in animal models (e.g., rabbits), the uptake of the radiolabel in dissected tissues can be determined. The dissected colon is imaged and the inflammatory lesions are scored macroscopically. For each affected colon segment, the colitis index (affected colon-to-normal colon uptake ratio, CI) can be calculated and correlated with the macroscopically scored severity of inflammation.

Animal Model: Rabbit (chemically induced acute colitis).

Interpretation and Analysis: Both agents can facilitate visualization of colitis conditions within 1 hour after injection. [^{99m}Tc]-HYNIC-IL-8 images of colonic abnormalities are more accurate and the intensity of uptake in regions of affected colon continuously increase up to 4 hours after injection, whereas no further increases can be appreciated scintigraphically 1 hour after injection of [^{99m}Tc]-hexamethylpropyleneamine oxime (HMPAO)-granulocytes. Absolute uptake within affected colon is much higher for IL-8 than for radiolabeled granulocytes with the percentage of injected dose per gram equal to (%ID/g) 0.41 ± 0.04 %ID/g and 0.09 ± 0.05 4 %ID/g hour after injection, respectively. With increasing severity, the CI at 4 hours after injection for [^{99m}Tc]-HYNIC-IL-8 is approximately 4.4 ± 0.6, 13.5 ± 0.5, and 25.8 ± 1.0; for granulocytes, the CI at 4 hours after injection is 1.5 ± 0.1, 3.4 ± 0.2, and 6.4 ± 0.5, respectively. The CI correlates with the severity of the inflammation ($r = 0.95$, $p < 0.0001$ for IL-8; $r = 0.95$, $p < 0.0001$ for granulocytes). Therefore, within 1 hour after injection, visualization of the extent of colon inflammation *in vivo* is possible with [^{99m}Tc]-HYNIC-IL-8 and [^{99m}Tc]-HMPAO-granulocytes. Within 2 hours after injection, [^{99m}Tc]-IL-8 allows a good evaluation, and within 4 hours after injection, a meticulous evaluation of the severity of inflammatory bowel disease (IBD). Although [^{99m}Tc]-HMPAO-granulocytes are able to delineate the extent of IBD within 2 hours after injection, an accurate estimation of the severity of inflammation is not possible. [^{99m}Tc]-HYNIC-IL-8 is an inflammation-imaging agent that has demonstrated promising results in this study. [^{99m}Tc]-IL-8 can be prepared off-the-shelf and yields excellent imaging with high target-to-background ratios.

Instrumentation: Gamma camera imaging capabilities.

Rapid imaging of experimental colitis with [^{99m}Tc]-interleukin-8 in rabbits. Gratz S, Rennen HJ, Boerman OC, Oyen WJ, Corstens FH. J Nucl Med 2001 Jun; 42 (6), pp. 917–23.

Gastrointestinal Septic Foci/Appendicitis/Diverticulitis
Leukocytes/[^{99m}Tc]-HMPAO-Labeled Neutrophils

Cellular/Molecular Mechanism of Action: The preparation [^{99m}Tc]-hexamthylpropyleneamineoxide (HMPAO) functions as a radiolabeling agent for leukocytes.

Physiological Mechanism of Action: Radiolabeled neutrophils preferentially localize at sites of inflammation and sepsis in response to chemotactic stimuli in a manner highly analogous to if not identical to "native" neutrophil populations.
Application: Identification of septic and inflammatory foci (e.g., appendicitis). Neutrophils are the cell type most widely used for such diagnostic purposes.
Considerations: Diagnostic imaging with [^{99m}Tc]-HMPAO-labeled leukocytes is valuable for the detection of appendicitis and other intra-abdominal septic/inflammatory conditions. In 1 *in vivo* trial, 8 scans interpreted as negative for acute appendicitis were alternatively positive for other conditions, including 5 positive cases of diverticulitis, 1 case of neoplastic disease, 1 case of tubo-ovarian abscessation, and 1 case of small intestinal infarction.
Interpretation and Analysis: In *in vivo* clinical trials, the sensitivity of [^{99m}Tc]-HMPAO leukocyte scans is 87%, while their specificity rate is 92% and overall accuracy rate 89% for the detection of septic/inflammatory foci.

Gastrointestinal Septic Inflammatory Foci/ Appendicitis
[^{99m}Tc]-Labeled Anti-CD15 IgM Immunoglobulin

Cellular/Molecular Mechanism of Action: Anti-CD15 immunoglobulin M monoclonal antibody recognizes and binds to CD-15 complexes expressed on the exterior surface membrane of polymorphonuclear neutrophils. The difference between IgG and IgM immunoglobulin is that IgM is the principal immunoglobulin produced after the 1st exposure to an antigen while IgG is the principal immunoglobulin produced after 2nd exposure to the same antigen. IgM is composed of a pentomer of IgG-like molecules and generally has less specific antigen-binding properties than does IgG, but IgM has a higher propensity to generate multimolecular antigen/antibody complexes.
Application: Detection and monitoring of septic foci and lesions.
Route of Administration: Anti-CD15 immunoglobulin M monoclonal antibody (LeuTech: intravenous).
Dosage: Anti-CD15 immunoglobulin M monoclonal antibody (LeuTech: 10–20 mCi of Tc-99m).
Semi-Synthetic Methods: Preparations of anti-CD15 immunoglobulin M monoclonal antibody (e.g., LeuTech) avidly bind to circulating and sequestered human polymorphonuclear neutrophils *in vivo,* eliminating the need for *in vitro* cell labeling and the risks of blood handling.
Implementation: Evaluation of the safety and efficacy of anti-CD15 immunoglobulin M monoclonal antibody (LeuTech) imaging in cases of suspected acute appendicitis. An effective procedure includes the acquisition of serial dynamic and static planar images for up to 3 hours after intravenous administration. Scans are read as positive or negative for acute appendicitis or other intra-abdominal (septic) infection. The institutional diagnosis can be validated by surgery and histopathology of the appendix, results of other diagnostic studies, or 2-week clinical follow-up.

Human Model: Cases presenting with equivocal clinical signs (e.g., n = 99).
Interpretation and Analysis: Scans are usually positive in a very high percentage of cases of suspected appendicitis with validation of the condition at surgery (e.g., n = 39/40, sensitivity 98%). Conversely, images are negative for appendicitis in a large percentage of cases found not to have appendicitis (e.g., n = 49/58 = specificity 84%). Accuracy, positive predictive, and negative predictive values are approximately 90, 81, and 98%, respectively. In patients with appendicitis and positive scans, more than 50% of the images are positive at 4 minutes, and essentially all images are positive by 1 hour. Mean time of 1st positive image is about 15 minutes. Serious adverse reactions are generally rare. Imaging with anti-CD15 immunoglobulin M monoclonal antibody (LeuTech) is considered to be a highly sensitive diagnostic agent for detecting appendicitis in equivocal cases. Advantages of this agent over the other currently used radiotracers include greater convenience and time to diagnosis, particularly the expedient nature with which acute appendicitis will be visualized on scintigraphic images.

Scintigraphic determination of equivocal appendicitis. Rypins EB, Kipper SL. Am Surg 2000 Sep; 66 (9), pp. 891–5.

Histamine Receptor Agonist: H_3 Selective Avidity
Histamine H_3 Ligand

Cellular/Molecular Mechanism of Action: Highly selective H_3 receptor agonist with virtually no H_4 subtype receptor affinity.
Application: Evaluation of histamine function and image analysis of histamine receptor site distribution.
Semi-Synthetic Methods: A series of cyclopropane-based conformationally restricted analogues of histamine, the "folded" *cis*-analogues—that is, (1S,2R)-2-(aminomethyl)-1-(1H-imidazol-4-yl) cyclopropane (11), (1S,2S)-2-(2-aminoethyl)-1-(1H-imidazol-4-yl) cyclopropane (13), and their enantiomers ent-11 and ent-13, and the "extended" *trans*-analogues, that is, (1R,2R)-2-(aminomethyl)-1-(1H-imidazol-4-yl)cyclopropane (12) and its enantiomer ent-12—have been designed as histamine H_3 receptor agonists. These targeting compounds are synthesized from the versatile chiral cyclopropane units, (1S,2R)- and (1R,2R)-2-(tert-butyldiphenylsilyloxy)methyl-1-formylcyclopropane (14 and 15, respectively) or their enantiomers ent-14 and ent-15.
Interpretation and Analysis: Among the conformationally restricted analogues, the "folded" analogue 13 (AEIC) having the *cis*-cyclopropane structure has been identified as a potent H_3 receptor agonist, which shows significant binding affinity (K_i = 1.31 ± 0.16 nM) and has an agonist effect (EC_{50} value of 10 ± 3 nM) on the receptor. The compound owes its importance to being the 1st highly selective H_3 receptor agonist to have virtually no effect on the H_4 subtype receptor. Preliminary studies have therefore demonstrated that the *cis*-cyclopropane structure is very effective in the conformational restriction of histamine to improve the specific binding to the histamine H_3 receptor.

Cyclopropane-based conformational restriction of histamine. (1S,2S)-2-(2-aminoethyl)-1-(1H-imidazol-4-yl)cyclopropane, a highly selective agonist for the histamine H_3 receptor, having a cis-cyclopropane structure. Kazuta Y, Hirano K, Natsume K, Yamada S, Kimura R, Matsumoto S, Furuichi K, Matsuda A, Shuto S. J Med Chem 2003 May 8; 46 (10), pp. 1980–8.

Musculoskeletal: Osteomyelitis
[^{99m}Tc]-PEG Liposomes/[^{99m}Tc]-HYNIC-IgG/ [^{99m}Tc]-MDP/[^{111}In]-Granulocytes/[^{67}Ga]-Citrate

Background Information: Assessment of disease activity and disease extent in chronic osteomyelitis remains a difficult diagnostic problem. Radiographic imaging is not a particularly sensitive diagnostic modality for this particular purpose. Scintigraphic techniques can be more helpful, but routinely available agents lack specificity (e.g., [^{99m}Tc]-methylene diphosphonate (MDP); [^{67}Ga]-citrate) or are laborious to prepare (e.g., [^{111}In]-leukocytes).
Route of Administration: [^{99m}Tc]-PEG liposomes (intravenous); [^{99m}Tc]-HYNIC-IgG (intravenous); and [^{99m}Tc]-MDP (intravenous); [^{111}In]-granulocytes (intravenous); or [^{67}Ga]-citrate (intravenous).

Dosage: [^{99m}Tc]-PEG liposomes (37 MBq/rabbit); [^{99m}Tc]-HYNIC-IgG (37 MBq/rabbit); and [^{99m}Tc]-MDP (37 MBq/rabbit); [^{111}In]-granulocytes (18 MBq/rabbit); or [^{67}Ga]-citrate (18 MBq/rabbit).

Implementation: Scintigrahic image analysis of osteomyelitis lesions of the femur 4 weeks after bacterial infusion applying [^{99m}Tc]-PEG liposomes, [^{99m}Tc]-HYNIC-IgG, and [^{99m}Tc]-MDP on 3 consecutive days and imaged up to 4 hours (MDP) or 22 hours (liposomes and IgG) postinjection. On day 4, preparations of [^{111}In]-granulocytes or [^{67}Ga]-citrate can be injected and imaged up to 44 hours after injection. Uptake in the septic femur is determined within regions of interest. Ratios of infected-to-sham-operated femur can be calculated. In animal models, the left and right femur scored for microbiologic and histopathologic evidence of osteomyelitis can be established after acquiring the last scintigraphic image.

Animal Model: Chronic osteomyelitis is induced by inserting *Staphylococcus aureus* into the right reamed and washed femoral canal (rabbits). The canal is closed with cement and a sham operation performed on the left femur. Routine radiographs are obtained immediately after surgery and before scintigraphy.

Interpretation and Analysis: [^{99m}Tc]-PEG liposomes and [^{99m}Tc]-HYNIC-IgG can correctly identify lesions of osteomyelitis in a high percentage of cases (e.g., n = 6/6). In a small percentage of cases, [^{111}In]-granulocytes and [^{67}Ga]-citrate can produce equivocal results (e.g., 15% or n = 1/6), and [^{99m}Tc]-MDP may miss lesions of osteomyelitis (e.g., 15% or n = 1/6). Uptake in affected regions does not differ significantly between the imaging agents, although [^{99m}Tc]-MDP tends to produce higher values (MDP, 4.75 ± 1.23 percentage injected dose per gram [%ID/g]; [^{67}Ga], 2.05 ± 0.54 %ID/g; granulocytes, 1.56 ± 0.83 %ID/g; liposomes, 1.75 ± 0.76 %ID/g, and IgG, 1.96 ± 0.27 %ID/g). The ratios of infected-to-normal femur are also not significantly different for the respective radiopharmaceuticals. Radiography visualizes only severe osteomyelitis. [^{99m}Tc]-PEG liposomes and [^{99m}Tc]-HYNIC-IgG perform at least as well as [^{111}In]-granulocytes and [^{67}Ga]-citrate in the localization of chronic osteomyelitis (e.g., rabbits). The ease of preparation, the better image quality, and the lower radiation dose suggest that [^{99m}Tc]-PEG liposomes and [^{99m}Tc]-HYNIC-IgG might be suitable alternatives for [^{67}Ga]-citrate and [^{111}In]-granulocytes in the scintigraphic evaluation of osteomyelitis.

Scintigraphic evaluation of experimental chronic osteomyelitis. Dams ET, Nijhof MW, Boerman OC, Laverman P, Storm G, Buma P, Lemmens JA, van der Meer JW, Corstens FH, Oyen WJ. J Nucl Med 2000 May; 41 (5), pp. 896–902.

Musculoskeletal: Osteomyelitis
[^{67}Ga]-Citrate/[^{99m}Tc]-Methylene Diphosphonate (MDP)/[^{99m}Tc]-Interleukin-8 (IL-8)

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-MDP is believed to be assimilated by active ostoblasts/osteoclasts at sites of dynamic bone growth and destruction.

Background Information: Early and accurate diagnosis of osteomyelitis remains a clinical problem. Acute osteomyelitis often occurs in infants and most often is located in the long bones. Radiologic images show changes only in advanced stages of disease. Scintigraphic imaging with [^{99m}Tc]-MDP, or bone scanning, is much more sensitive in detecting acute osteomyelitis but lacks specificity.

Application: Detection and characterization of septic osteomyelitis lesions.

Route of Administration: [^{99m}Tc]-MDP (intravenous), [^{99m}Tc]-IL-8 (intravenous).

Dosage: [^{99m}Tc]-MDP (18.5 MBq), [^{99m}Tc]-IL-8 (18.5 MBq).

Implementation: Scintigraphic characterization of osteomyelitis lesions 10 days following induction (e.g., n = 10 rabbits; n = 5/group) utilizing injections of either 18.5 MBq [^{111}In]-granulocytes or 18.5 MBq [^{67}Ga]-citrate. Images are acquired at both 24 hours and 48 hours postinjection. On day 12, serial images are acquired at 0, 1, 2, 4, 8, 12, and 24 hours after injection of either 18.5 MBq [^{99m}Tc]-MDP or 18.5 MBq [^{99m}Tc]-IL-8 preparations. Uptake in the infected femur is determined by establishing regions of interest (ROIs). Ratios of infected femur (target) to sham-operated femur (background) (T/Bs) are then calculated. In animal models, the right femur can be harvested postmortem to facilitate dissection and analysis of tissue samples for microbiological and histopathological evidence of osteomyelitis.

Animal Model: Acute pyogenic osteomyelitis can be induced in rabbits (e.g., n = 10) by inserting sodium morrhuate and *Staphylococcus aureus* into the medullary cavity of the right femur. The cavity is then closed with liquid cement. A sham operation is performed on the left femur. Routine radiographs are obtained just before scintigraphy imaging. Acute osteomyelitis develops in a high percentage of rabbits (e.g., n = 8/10).

Interpretation and Analysis: All imaging agents can correctly detect acute osteomyelitis in a very high percentage of cases (e.g., n = 8/8). The extent of infection can be optimally visualized with [^{67}Ga]-citrate and by delayed bone scanning, whereas diaphyseal photopenia can be detected with both [^{99m}Tc]-IL-8 and [^{111}In]-granulocytes. Some false-negative (e.g., n = 1/8) and false-positive (e.g., n = 1/8) image results can be observed with [^{111}In]-granulocytes and [^{99m}Tc]-MDP, respectively. Quantitative analysis of the images reveals that the uptake in the infected region is highest with [^{67}Ga]-citrate (4.9 ± 0.8 percentage injected dose [%ID]) and [^{99m}Tc]-MDP (4.7 ± 0.7 %ID), whereas the uptake in the infected area is significantly lower with [^{99m}Tc]-IL-8 (2.2 ± 0.2 %ID) and [^{111}In]-granulocytes (0.8 ± 0.2 %ID) ($p < 0.0042$). In contrast, the T/Bs are significantly higher for [^{99m}Tc]-IL-8 (T/B, 6.2 ± 0.3 at 4 hours after injection) than for [^{67}Ga]-citrate, [^{99m}Tc]-MDP, and [^{111}In]-granulocytes, which have ratios of 1.5 ± 0.4, 1.9 ± 0.2, and 1.4 ± 0.1, respectively ($p < 0.0001$). Radiography correctly reveals acute osteomyelitis in only a relatively small percentage of cases (e.g., n = 2/8). In experimentally induced osteomyelitis, [^{99m}Tc]-IL-8 clearly detects the osteomyelitic lesion. Although the absolute uptake in the osteomyelitic area is significantly lower than that obtained with [^{99m}Tc]-MDP and [^{67}Ga]-citrate, the T/Bs are significantly higher for [^{99m}Tc]-IL-8 because of fast background clearance. The ease of preparation, good image quality, and lower radiation burden suggest that [^{99m}Tc]-IL-8 may be a suitable imaging agent for the scintigraphic evaluation of acute osteomyelitis.

[^{99m}Tc]-interleukin-8 for imaging acute osteomyelitis. Gratz S, Rennen HJ, Boerman OC, Oyen WJ, Burma P, Corstens FH. J Nucl Med 2001 Aug; 42 (8), pp. 1257–64.

Musculoskeletal: Osteomyelitis
[^{99m}Tc]-Labeled Interleukin-8 (IL-8)

Cellular/Molecular Mechanism of Action: The cytokine IL-8 binds with high affinity to the CXCR1 and CXCR2 receptors on neutrophils.

Application: Previous investigations have shown that [^{99m}Tc]-IL-8 can rapidly and effectively delineate foci of infection and inflammation in rabbit models of intramuscular infection, colitis, and osteomyelitis.

Semi-Synthetic Methods: A derivative of hydrazinonicotinamide (HYNIC) can be used as a bifunctional coupling agent to label protein fractions with the [^{99m}Tc] radioisotope.

Implementation: Delineation of [^{99m}Tc]-IL-8 pharmacokinetic profiles in conditions of neutropenia compared to normal states and determination of the ability of [^{99m}Tc]-IL-8 to detect septic abscesses. To investigate the interaction of [^{99m}Tc]-IL-8 with blood cells in circulation (e.g., normal rabbits), the distribution of the radiolabel relative to circulating white and red blood cells and plasma is determined. The *in vivo* kinetics of [^{99m}Tc]-IL-8 is established by quantitative analysis of whole-body images acquired between 0 and 6 hours after injection. The results of such analyses (*in vivo* biodistribution) are validated by *ex vivo* counting of radioactivity residing within dissected tissue samples.

Animal Model: Turpentine-induced abscesses in both neutropenic and normal rabbits.

Interpretation and Analysis: The abscess uptake (percentage of injected dose per gram of tissue [%ID/g] ± standard error of measurement [SEM]) in immunocompetent rabbits (0.41 ± 0.05) is 10 times higher than that in neutropenic rabbits (0.038 ± 0.014), demonstrating specificity of the target uptake of [^{99m}Tc]-IL-8. Abscess-to-muscle ratios ± SEM are also 10 times higher (110 ± 10 vs 10 ± 5). Lung and spleen uptake in normal rabbits is 3 times higher than that in neutropenic rabbits. The blood clearance of the radiolabel in neutropenic rabbits is similar to that in normal rabbits. In circulation, most of the [^{99m}Tc]-IL-8 (70%) is found within the plasma fraction. Less than one-third is associated with red blood cells, and only a very low percentage (< 2.5%) is associated with white blood cells. Image analysis reveals a gradual increase in abscess uptake over time up to > 15%ID, which can be confirmed by *ex vivo* gamma counting of infected muscle tissue. The highest increase in uptake in abscesses is observed after 2 hours following injection, when most [^{99m}Tc]-IL-8 is cleared from the blood, suggesting specific neutrophil-mediated accumulation of [^{99m}Tc]-IL-8 within abscesses. Furthermore, region-of-interest analysis demonstrates gradual accumulation of [^{99m}Tc]-IL-8 within abscesses that is accompanied by a simultaneous clearance of activity from the lungs, suggesting that neutrophil-associated [^{99m}Tc]-IL-8 initially trapped in the lungs migrates to the abscess at later time points, favoring neutrophil-bound transportation from the lungs to the abscess. Substantial support exists for the hypothesis that [^{99m}Tc]-IL-8 localizes in the abscess, mainly bound to peripheral neutrophils. Accumulation within abscesses is a highly specific, neutrophil-driven process. As assessed by *in vivo* and *ex vivo* analysis, the total fraction that accumulates in the inflamed tissue is extremely high (up to > 15 %ID) compared with that of other agents used for imaging infection and inflammation.

Kinetics of [^{99m}Tc]-labeled interleukin-8 in experimental inflammation and infection. Rennen HJ, Boerman OC, Oyen WJ, Corstens FH. J Nucl Med 2003 Sep; 44 (9), pp. 1502–9.

Musculoskeletal: Osteomyelitis
[^{99m}Tc]-Labeled Antigranulocyte Antibody Fragments

Cellular/Molecular Mechanism of Action: Antigranulocyte antibody possesses binding avidity for membrane-associated antigens that are unique to neutrophil populations. Neutrophils are chemotactically attracted to areas of (septic) inflammation. The Fab fragment of IgG has the advantage of being devoid of the Fc fragment. Therefore, it minimally binds to membrane-associated Fc receptor complexes expressed by the mononuclear phagocytic system residing within the liver and spleen and is generally associated with a lower level of non-specific binding.

Application: Detection and characterization of septic inflammatory lesions.

Route of Administration: [^{99m}Tc]-labeled antigranulocyte antibody fragment (intravenous).

Dosage: [^{99m}Tc]-labeled antigranulocyte antibody fragment (740 MBq [20 mCi]).

Implementation: Immunoscintigrams performed with [^{99m}Tc]-labeled antigranulocyte antibody fragments for the detection of focal lesions of septic inflammation. Validation of findings can be established by histopathology, magnetic resonance imaging, computed tomography, and monitoring of clinical signs.

Interpretation and Analysis: The percentage of true-positive images obtained with [^{99m}Tc]-labeled antigranulocyte antibody fragments for the detection of septic inflammation is approximately 67% (e.g., n = 20/30). The number of false-positive results is relatively low (e.g., n = 3/30). Approximately 20% of images will be true-negatives (e.g., n = 6/30). Consequently, [^{99m}Tc]-labeled antigranulocyte antibody fragments can achieve high levels of sensitivity (e.g., 95%), moderately high specificity (e.g., 67%), and high diagnostic accuracy (e.g., 86%). Therefore, [^{99m}Tc]-labeled monoclonal antibody-Fab fragments are suitable for the detection of osteomyelitis. Their clinical application is simple, and their use guarantees a reliable and accurate diagnostic result just 1–2 hours after injection, making a late scan unnecessary. Coxarthrosis or hyperostosis can lead to false-positive results. A perforating ulcer of the foot may result in a false-negative conclusion.

Imaging osteomyelitis with Tc-99m-labeled antigranulocyte antibody Fab fragments. Von Rothenburg T, Schaffstein J, Ludwig J, Vehling D, Koster O, Schmid G. Clin Nucl Med 2003 Aug; 28 (8), pp. 643–7.

Musculoskeletal: Osteomyelitis/Sepsis: Inflammatory Reponses/[Diabetes Mellitus]
[^{131}I]-Interleukin-8 (IL-8) (Recombinant)/[^{99m}Tc]-Hydroxyethylene Diphosphonate

Cellular/Molecular Mechanism of Action: Neutrophils perceive IL-8 as a chemotactic factor and, therefore, express membrane-associated IL-8 receptor complexes.

Background Information: The chemotactic cytokine IL-8 plays an important role in the attraction and activation of polymorphonuclear leukocytes in infection and inflammation.

Application: Detection and characterization of septic inflammatory disease states. In this context, [^{131}I]-human recombinant IL-8 (rhIL-8) accumulates rapidly within infected foci in osteomyelitis and cellulites.

Route of Administration: [^{131}I]-rhIL-8 (intravenous).

Semi-Synthetic Methods: Radioiodination of rhIL-8 with [^{131}I] (specific activity, 0.4–0.7 MBq [11–18 μCi] [^{131}I]/μg IL-8).

Implementation: Detection and characterization of septic disease states applying [^{131}I]-rhIL-8 as an imaging agent.

Human Model: Cases of diabetes mellitus (n = 8 active foot infections with evidence of osteomyelitis; n = 2 successfully treated osteomyelitis; n = 1 cellulitis of the thumb).

Interpretation and Analysis: Focal accumulation of [^{131}I]-rhIL-8 can be seen in a high percentage of cases with active foot infection (e.g., n = 8/8). In other affected appendages, the image profile may appear diffuse (e.g., n = 1/1 thumb cellulitis). In cases successfully treated for osteomyelitis, multiphase [^{99m}Tc]-hydroxyethylene diphosphonate bone scans may be negative early, but late-phase (> 3 hours) uptake can detect degenerative lesions that are not initially detected by imaging with [^{131}I]-rhIL-8 (e.g., n = 2/2 cases). [^{131}I]-rhIL-8 accumulates rapidly within infected foci in

osteomyelitis and cellulitis but not in successfully treated infections or degenerative joint disease.

Imaging of human infection with [^{131}I]-labeled recombinant human interleukin-8. Gross MD, Shapiro B, Fig LM, Steventon R, Skinner RW, Hay RV. J Nucl Med 2001 Nov; 42 (11), pp. 1656–9.

Musculoskeletal Abscess Formation/Septic Inflammation and Infection
[^{111}In]-DPC11870-11/Leukotriene B_4 (LTB_4) Receptor-Binding Ligands

Cellular/Molecular Mechanism of Action: The agent DPC11870-11 is an antagonist of LTB_4 at its respective membrane-associated receptor complex.

Background Information: Several radiolabeled chemotactic peptides have been tested for their suitability to show infection and inflammation. Leukotriene B_4 receptor-binding ligands could be useful agents for revealing neutrophilic infiltrations because the LTB_4 receptor is abundantly expressed on neutrophils after an inflammatory stimulus.

Route of Administration: [^{111}In]-DPC11870-11 (intravenous).

Implementation: Detection of septic foci applying scintigraphic imaging techniques facilitated by radiolabeling the LTB_4 antagonist DPC11870-11 with [^{111}In]. Complementary investigations entail that delineation of the pharmacokinetics and biodistribution of [^{111}In]-DPC11870-11 established with serial scintigraphic imaging (0–24 hours after injection) and by *ex vivo* counting of dissected tissues (6 and 24 hours after injection). Receptor-mediated *in vivo* localization of the radiobiopharmaceutical agent can also be more precisely determined following the administration of an excess of non-radioactive indium-labeled agent 2 minutes before the administration of the [^{111}In]-labeled LTB_4 antagonist.

Animal Model: Rabbit (n = 3 New Zealand White rabbits with *Escherichia coli* infection induced by local injection into the left thigh muscle).

Interpretation and Analysis: In rabbits with intramuscular *E. coli* infection, abscesses can be visualized as early as 2 hours after injection. Accumulation in the abscess increases with time, resulting in the acquisition of excellent images at 6 hours postinjection. Blood clearance is rapid in the 1st hours after injection (alpha-half-life = 30 ± 6 minutes, 85%; beta-half-life = 25.7 ± 0.8 hours, 15%). Abscess-to-background ratios, as derived from the region-of-interest analysis, increases to 34 ± 7 at 24 hours after injection. Images of both groups show moderate uptake in the liver, spleen, kidneys, and bone marrow. No activity can be appreciated in the bladder, indicating almost complete retention in the kidneys. Accumulation within the abscess can be blocked completely by the injection of an excess of non-radioactive agent, indicating the occurrence of specific receptor-ligand interactions of the radiolabeled agent in infected/septic tissues. Biodistribution data show that after saturation of the LTB_4 receptor, the abscess uptake, in percentage injected dose per gram, is significantly reduced (0.03 ± 0.02 vs 0.24 ± 0.06, p = 0.008). The modified LTB_4 antagonist identifies/detects infectious foci rapidly after injection because of specific receptor-ligand interaction. Because of the high abscess-to-background ratios that are obtained and the fact that no accumulation of radioactivity is observed in the gastrointestinal tract, this compound has excellent characteristics for revealing infectious and inflammatory foci.

A bivalent leukotriene B_4 antagonist for scintigraphic imaging of infectious foci. van Eerd JE, Oyen WJ, Harris TD, Rennen HJ, Edwards DS, Liu S, Ellars CE, Corstens FH, Boerman OC. J Nucl Med 2003 Jul; 44 (7), pp. 1087–91.

Renal and Hepatic Cyst Formation/Septic vs Non-Septic Disease Differentiation/Polycystic Kidney Disease: Autosomal Dominant
[^{18}F]-Fluorodeoxyglucose (FDG)

Background Information: Infection of a renal or hepatic cyst is a serious complication of autosomal dominant polycystic kidney disease (ADPKD). Although crucial for successful management, early diagnosis is difficult, largely because of non-specific symptoms and limitations of conventional imaging techniques. Because of an increased metabolic rate, inflammatory cells take up large amounts of glucose. [^{18}F]-FDG, therefore, represents a promising agent for detection of cyst infections using positron emission tomography (PET).

Implementation: Performance of [^{18}F]-FDG PET scan imaging in cases of ADPKD suspected of being affected by renal or hepatic cyst infection (e.g., n = 7 FDG PET scans in 3 ADPKD cases suspected of renal or hepatic cyst infection; n = 2 PET scans of case A (PET 1 and 2); n = 1 PET scan of case B (PET 3); and n = 4 PET scans of case C (PET 4, 5, 6, and 7).

Interpretation and Analysis: [^{18}F]-FDG PET can identify infected cysts in conditions of (i) renal cyst infection; (ii) hepatic cyst infection; and (iii) simultaneous renal and hepatic cyst infection. Evaluations utilizing FDG PET imaging modalities have proven to be very helpful in establishing diagnoses and in excluding renal and hepatic cyst infections. Based on experience with FDG PET, this diagnostic strategy has promise as an imaging technique enabling early identification of renal and hepatic cyst infections in ADPKD patients.

Diagnosis of renal and hepatic cyst infections by [^{18}F]-fluorodeoxyglucose positron emission tomography in autosomal dominant polycystic kidney disease. Am J Kidney Dis 2003 Jun; 41 (6), pp. E18–21.

Respiratory Disease: Inflammation/Acute Respiratory Distress Syndrome (ARDS)
[^{111}In]-Labeled Neutrophils/[^{111}In]-aICAM-1/[^{111}In]-Labeled Normal Mouse IgG (nmIgG)/[^{111}In]-Labeled Rat Serum Albumin (RSA)

Cellular/Molecular Mechanism of Action: Fractions of aICAM-1 recognize and physically bind to ICAM-1 complexes (intracellular adhesion molecule-1). Neutrophils through mechanisms of chemotactic activity migrate to anatomical regions of inflammation and sepsis.

Background Information: Previous study of the bleomycin-induced lung injury model suggested that [^{111}In]-labeled anti-rat intercellular adhesion molecule-1 (aICAM-1) might be a useful diagnostic agent for detecting and characterizing conditions of ARDS.

Application: Detection and characterization of inflammation in the lung including ARDS.

Implementation: Evaluation of the diagnostic agent biodistribution, scintigraphic image profiles, and lung ICAM-1 upregulation utilizing [^{111}In]-labeled polymorphonuclear leukocytes (PMNs), [^{111}In]-aICAM-1, nmIgG, and [^{111}In]-labeled RSA injected 18–24 hours before euthanasia.

Animal Model: Rat neutrophils (polymorphonuclear leukocytes or PMNs) and rat *in vivo* radioactive distribution studies following induced injury with oleic acid.

Interpretation and Analysis: [^{111}In]-RSA and [^{111}In]-nmIgG localize in inflamed lung at 5 minutes postinjury (PI). [^{111}In]-PMN uptake increases significantly only at 24 hours PI. [^{111}In]-aICAM-1

localization increases significantly (30–60%) at 1 hour postinjury and remains elevated up to 24 hours postinjury. Lung/blood ratios (L/B) at 1 and 4 hours postinjury are very low (< 0.6) for [^{111}In]-nmIgG and [^{111}In]-PMN rats; however, for [^{111}In]-aICAM-1 rats, they are > 1 and 25–60% higher than those observed in reference control samples. A low L/B suggests poor detection of inflammation on acquired images. Images and region-of-interest analysis confirm that only [^{111}In]-aICAM-1 can distinguish inflamed lungs at 4 hours postinjury. ICAM-1 is up-regulated at 4 and 24 hours postinjury. Under certain circumstances, [^{111}In]-aICAM-1 can detect lung inflammation very early in the course of the disease and suggests that [^{111}In]-aICAM-1 could be a very early, highly specific ARDS diagnostic agent and may be useful to detect a wide range of inflammatory conditions.

Early detection of oleic acid-induced lung injury in rats using [^{111}In]-labeled antirat intercellular adhesion molecule-1. Weiner RE, Sasso DE, Gionfriddo MA, Thrall RS, Syrbu S, Smilowitz HM, Vento J. J Nucl Med 2001 Jul; 42 (7), pp. 1109–15.

Viral Infections: Human Immunodeficiency Virus (HIV)/Biological Proteins: Selective Binding
Aptamers (SELEX-Derived Small Oligonucleotides)/ [Systematic Evolution of Ligands by Exponential Enrichment]

Cellular/Molecular Mechanism of Action: Aptamers have been developed (designed/evolved) to bind biological proteins that are associated with a number of disease states. In this fashion, powerful antagonists of such biological proteins can be developed.

Application: Strategic development of powerful antagonists against biological proteins associated with the pathogenesis of certain disease states. Example: Detection of HIV infections.

Example: Human immunodeficiency virus.

Route of Administration: Intravenous (most often).

Dosage: Designed to directly establish a correlation between the specific biological activity (efficacy) of aptamer preparations and the corresponding pathological condition.

Considerations: Aptamers are small oligonucleotides derived from an *in vitro* evolution process called SELEX. The modest chemical functionality of nucleic acids poses some limits on their versatility as binders and catalysts. In addition, the sensitivity of pure RNA- and DNA-based aptamers to nucleases restricts their use as therapeutic and diagnostic agents.

Synthetic chemistry strategies have been established for modifying nucleotides to enhance aptamer affinity for targets and increase their stability in biological fluids. Implementation of *in vitro* selections with modified nucleotides promises to be an elegant technique for the creation of ligands with novel physical and chemical properties and is anticipated to have a significant impact on biotechnology, diagnostics, and drug development.

Although very effective *in vitro*, only a few examples are available showing their potential *in vivo*. In order for these antagonists to work in animal models of disease and in humans, it is necessary to modify aptamers. Sugar modifications of nucleoside triphosphates are necessary to render the resulting aptamers resistant to nucleases found in serum. Changing the 2′OH groups of ribose to 2′F or 2′NH_2 groups yields aptamers that have a plasma half-life of a relatively prolonged duration. The low molecular weight of aptamers (8,000–12,000) leads to rapid clearance from the blood. Conjugation to larger molecular weight compounds or delivery modalities can be utilized to extend the pharmacokinetic profile of aptamers within the intravascular compartment.

Synthetic Design: Combinatorial library selections through the systematic evolution of ligands by exponential enrichment (SELEX) technique identify so-called nucleic acid aptamers that bind with high affinity and specificity to a wide range of selected molecules.

Interpretation and Analysis: When modified conjugated aptamers are injected into animals, they inhibit physiological functions known to be associated with their target proteins. Aptamer arrays on solid surfaces will become available because SELEX protocols have been successfully automated. The use of photo-cross-linkable aptamers will allow the covalent attachment of aptamers to their cognate proteins, with very low background levels appreciated from other proteins found in body fluids. Finally, protein staining with any reagent that distinguishes functional groups of amino acids from those of nucleic acids (and the solid support) will give a direct readout of proteins on the solid support.

The influence of a well-characterized pseudoknot RNA aptamer selected for tight binding to HIV type 1 reverse transcriptase on HIV replication has been demonstrated in preliminary investigations. Transient intracellular expression of a chimeric RNA consisting of the human initiator tRNA(Met) (tRNA(Meti))/aptamer sequence in human 293T cells shows inhibition of HIV particle release by $>$ 75% when the cells are co-transfected with proviral HIV-1 DNA. Subsequent virus production by human T-lymphoid C8166 cells, infected with viral particles derived from co-transfected 293T cells, is reduced by $>$ 75% compared to reference controls. As the observed effects are additive, in this model for virus spread, the total reduction of HIV particle formation by transient intracellular expression of the pseudoknot RNA aptamer amounts to $>$ 95%. Low-dose HIV infection of human T cells stably expressing the aptamer does not show any virus replication over a period of 35 days. These investigations therefore represent an example of an RNA aptamer selected against a viral enzyme target that shows powerful antiviral activity in HIV-1-permissive human T-lymphoid cell lines.

Endogenous expression of a high-affinity pseudoknot RNA aptamer suppresses replication of HIV-1. Chaloin L, Lehmann MJ, Sczakiel G, Restle T. Nucleic Acids Res 2002 Sep 15; 30 (18), pp. 4001–8.

HIV-1 reverse transcriptase-pseudoknot RNA aptamer interaction has a binding affinity in the low picomolar range coupled with high specificity. Kensch O, Connolly BA, Steinhoff HJ, McGregor A, Goody RS, Restle T. J Biol Chem 2000 Jun 16; 275 (24), pp. 18271–8.

The use of synthetic oligonucleotides as protein inhibitors and anticode drugs in cancer therapy: Accomplishments and limitations. Faria M, Ulrich H. Curr Cancer Drug Targets 2002 Dec; 2 (4), pp. 355–68.

Viral Infections: Human Immunodeficiecy Virus (HIV) Targeting
Bis-Tetraazamacrocycle Xylyl-Bicyclam/Metal-Binding Platform

Cellular/Molecular Mechanism of Action: Macrocycles can function as radioisotope delivery modalities. Macrocycles are rigid enough to provide strong metal binding sites that can stereoselectively orient functional groups while retaining sufficient flexibility to accommodate structural changes required for induced-fit recognition of biological targets.

Application: Radioisotope delivery whereby they function as a molecular platform for the design of metal-based therapeutic and diagnostic agents.

Dosage: Designed to directly establish a correlation between the specific biological activity (efficacy) of aptamer preparations and the corresponding pathological condition of interest.

Implementation: Evaluation of the recognition of the Zn^{+2} complex of the bis-tetraazamacrocycle xylyl-bicyclam, a potent anti-HIV agent, by the co-receptor CXCR4, a G-protein-coupled receptor used by HIV for membrane fusion and cell entry.

Synthetic Chemistry and Analysis: Nuclear magnetic resonance (NMR) studies show that the macrocycles of $[Zn^{2+}]_2$-xylyl-bicyclam perchlorate exist in aqueous solution as 2 major configurations, *trans*-I (nitrogen chirality R,S,R,S), and *trans*-III (S,S,R,R). Acetate addition induces major structural changes. X-ray crystallography shows that the acetate complex contains the unusual *cis*-V cyclam configuration (R,R,R,R and folded) with bidentate coordination of acetate to $[Zn^{2+}]$ plus 2nd-coordination-sphere double H-bond formation between diagonal NH protons on the opposite cyclam face and acetate carboxylate oxygens. Detailed 1D and 2D NMR studies show that the major configuration of $[Zn^{2+}]_2$-xylyl-bicyclam acetate in aqueous solution is *cis*-V/trans-I. Molecular modeling shows that an analogous *cis*-V site can be formed when $[Zn^{2+}]_2$-xylyl-bicyclam binds to CXCR4, involving the carboxylate groups of Asp262 ($[Zn^{2+}]$coordination) and Glu288 (double H-bonding). The 2nd cyclam can adopt the *trans*-I (or *trans*-III) configuration with $[Zn^{2+}]$ binding to Asp171.

Implementation: Evaluation of the recognition of $[Zn^{2+}]$ complex associated with bis-tetraazamacrocycle xylyl-bicyclam, (potent anti-HIV agent), by the co-receptor CXCR4 (G-protein-coupled receptor used by HIV for membrane fusion and cell entry).

Interpretation and Analysis: The observed molecular interactions are consistent with the known structure-activity relationships for bicyclam anti-HIV activity and receptor mutation. Recognition of the anti-HIV activity of xylyl-bicyclam complexes of other metal ions suggests that affinity for carboxylates, configurational flexibility, and kinetic factors may all play roles in receptor recognition. For example, Pd(II) cyclam complexes interact only weakly with axial ligands and are inflexible and inactive, whereas $[Co^{3+}]$ cyclams bind carboxylates strongly, are configurationally flexible, and yet have low activity. Results from preliminary investigations are anticipated to facilitate the future design of new generations of active macrocycles, including highly specific chemokine receptor antagonists.

Structure and dynamics of metallomacrocycles: Recognition of zinc xylyl-bicyclam by an HIV co-receptor. Liang X, Parkinson JA, Weishäupl M, Gould RO, Paisey SJ, Park HS, Hunter TM, Blindauer CA, Parsons S, Sadler PJ. J Am Chem Soc 2002 Aug 7; 124 (31), pp. 9105–12.

PARASITOLOGY

Onchocerca volvulus
Mazzotti Test (Diethylcarbamazine)

Cellular/Molecular Mechanism of Action: Induces microfilaremia.

Application: Diagnosis of *Onchocerca volvulus.*

Route of Administration: Dethylcarbamazine (oral), but a topical variation has been described.

Dosage: Diethylcarbamazine citrate (1.6% in Nivea lotion as a topical diagnostic test).

Implementation: Evaluate by comparison the diagnostic accuracy associated with results from skin biopsies for microfilariae, nodule examination, enzyme-linked immunosorbent assay (ELISA), and the Mazzotti test (dethylcarbamazine challenge).

Human Model: Children in 3 age groups in Guatemala.

Diagnosis of Onchocerca *volvulus* infection in Guatemalan children. Campbell CC, Figueroa H, Collins RC, Lujan R, Collins WE. Am J Trop Med Hyg 1983 Jul; 32 (4), pp. 760–3.

Onchocerciasis focus in Colombia: Follow-up study after 12 years. Ewert A, Corredor A, Lightner L, D'Alessandro A. Am J Trop Med Hyg 1979 May; 28 (3), pp. 486–90.

The use of a topical Mazzotti *test in the diagnosis of onchocerciasis***.** Kilian HD. Trop Med Parasitol 1988 Sep; 39 (3), pp. 235–8.

Trypanosoma Gambiense
Chlorambucil and Prednisolone

Application: Latent infection detection of Trypanosoma gambiense.

Considerations: Evaluation of the efficacy of chlorambucil and prednisolone for the diagnosis of Trypanosoma gambiense.

Interpretation and Analysis: Increased release of trypanosomes is observed within the peripheral blood following administration, thereby facilitating the diagnosis of latent trypanosomiasis.

Wuchereria Bancrofti/Dipetalonema Perstans
Daytime/Nighttime Diethylcarbamazine (DEC)

Application: Detection and characterization of *W. bancrofti* and *Dipetalonema perstans* microfilaremia.

Background Information: Sometimes a false-negative DEC provocative test is obtained with such findings emphasizing the importance of cytology and adjunct diagnostic methods for detecting conditions of amicrofilaremia.

Parasitic infections are common in the developing countries, but the cytologic diagnosis of such infections is infrequent or rare. Detection of microfilariae in pleural fluid in the absence of classic symptoms and signs of tropical pulmonary eosinophilia has been reported**.**

Considerations: Large-scale filariasis surveys in rural areas for microfilaremia, especially of periodic types such as *W. bancrofti,* are known to cause considerable administrative, technical, and social problems.

Implementation: Determination of the sensitivity and specificity of 2 diagnostic strategies: (i) daytime DEC provocative tests (blood smear and microfilter concentration analyses); and (ii) night-blood examination by blood smear and microfilter concentration analyses, especially for *W. bancrofti.*

Human Model: Population of 2 villages in the Malumfashi district of the Northern Nigerian savanna.

Human Model: Cases of *W. bancrofti* infestation (n = 2 with undetectable nocturnal microfilaremia despite Nuclepore filtration, thus suggesting the possible merits of cytology in the primary diagnosis of a filarial infection).

Interpretation and Analysis: Daytime DEC provocative tests are more efficient in terms of sensitivity and specificity, compared with some night-blood methods for *W. bancrofti* detection but less so for *Dipetalonema perstans* (the other blood microfilaria frequently found in some populations). A regression line can be calculated between night-blood survey results for *W. bancrofti* and the results from daytime DEC provocative test. Using such a regression line, it is possible to estimate *W. bancrofti* microfilarial prevalence for night surveys, using the DEC provocative test results of daytime surveys. This can be done with minimal, but known, loss of accuracy and imposes fewer administrative, technical, and social difficulties.

Peripheral microfilaremia is recognized in a high percentage of affected cases. Attention can be drawn to the need for a high index of suspicion on the part of the cytologist in the identification of parasitic organisms in material from high-risk groups to achieve an early diagnosis of such infections and the prompt institution of appropriate chemotherapy. This may obviate the more serious pathologic

changes of advanced disease, especially the disfigurement of chronic and late filariasis.

Evaluation of the diethylcarbamazine provocative test in the diagnosis of Wuchereria bancrofti infections in the Nigerian savanna and the effects on Dipetalonema *perstans.* Wijeyaratne PM, Singha P, Verma OP, Motha B. Trans R Soc Trop Med Hyg 1982; 76 (3), pp. 387–91.

Wuchereria Bancrofti Microfilaremia: Pacific Variant *Diethylcarbamazine (DEC)*

Application: Induction of microfilaremia in cases (n = 70) of subperiodic Pacific-variant *W. bancrofti* infection 1 hour following DEC administration.
Route of Administration: Diethylcarbamazine (oral).
Dosage: Diethylcarbamazine (5 mg/kg), while other references have described total doses of 100 mg, 150 mg, or 200 mg of DEC (e.g., administered at noon).
Implementation: Detection of alterations in microfilaremia in conditions of *W. bancrofti* infestations. Before DEC administration, blood samples of 60 microliters are obtained by finger-prick at 2-hour intervals for 24 hours. A blood sample is then taken just before each carrier case is administered 3 DEC doses orally at noon. After drug administration, blood samples are obtained at 45 and 60 seconds and at 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, and 120 minutes. Samples are then taken at 2-hour intervals for the next 12 hours and at 6-hour intervals for the next 7 days. Both *W. bancrofti* and *Brugia malayi* showed typical nocturnal periodicity.

Human Model: Cases classified as nocturnal microfilarial carriers (n = 3 *W. bancrofti;* n = 3 *B. malayi*) from mainland China (n = 6 total) selected to determine the earliest appearance and peak count of microfilariae following the administration of DEC at 12:00 noon.
Interpretation and Analysis: In contrast to the immediate DEC-induced increase in microfilaremia previously described in patients with nocturnal periodic filariasis, DEC has also been reported to fail to elicit such responses in cases with subperiodic bancrofti infections. In this context, 1 hour after oral DEC the number of circulating microfilariae may be reduced to about 8% of pretreatment values.

Case reports have described the initial detection of microfilaremia in the peripheral blood 45 seconds in a microfilaria carrier. The microfilaria peak counts can be appreciated 1–40 minutes after a noon DEC dose. The nocturnal cycle occurs during the 1st 2 days after DEC administration and becomes regular with a much lower peak count by the 3rd day after administration. Results from such surveys indicate that the provocative effect of DEC on microfilaria is very fast (1st appearance in the peripheral blood vessels could be under 45 seconds).

Bancroftian filariasis: Long-term effect of the DEC provocative day test on microfilaraemia. Simonsen PE, Meyrowitach DW, Makunde WH. Trans R Soc Trop Med Hyg 1997 May–Jun; 91 (3), pp. 290–3.

Determination of the earliest appearance and peak count of microfilariae of Wuchereria *bancrofti and Brugia malayi after taking a single dose of diethylcarbamazine at noon.* Fan PC. J Helminthol 1994 Dec; 68 (4), pp. 301–4.

15 Metabolism, Electrolytes, and Hypoxia

Anoxia/Aglycemia/Ischemia: γ-Glutamyl Transpeptidase/Glutamate Carboxypeptidase II/ Cystine Oxidation

Acivicin/N-Acylated γ-Glutamyl-Glutamate/ γ-Glutamyl-Glutamine/γ-Glutamyl-Glutamate/ β-Mercaptoethanol

Cellular/Molecular Mechanism of Action: Acivicin is a γ-glutamyl transpeptidase inhibitor.

Phosphonate and phosphinate analogues of N-acylated γ-glutamylglutamate inhibit glutamate carboxypeptidase II (GCP II). γ-glutamyl-glutamine and γ-glutamyl-glutamate are both γ-glutamyl transpeptidase substrates.

Background Information: Extracellular metabolism of the protective substance glutathione (γ-glutamyl-cysteinyl-glycine) may generate cysteine, glycine, several γ-glutamyl-containing dipeptides and possibly free glutamate, all of which could participate in neurotoxicity events.

Application: Potential utility of these compounds as diagnostic agents and probes to understand folate or antifolate poly-γ-glutamate metabolism.

Considerations: The comparison of the results to previously reported inhibitory studies of the same compounds toward folylpoly-γ-glutamyl synthetase (FPGS) and γ-glutamyl hydrolase (γ-GH) provides insight into structural and mechanistic features of each enzyme.

Implementation: Examine how blockage of γ-glutamyl transpeptidase, the key enzyme in glutathione degradation, influences the extracellular concentrations of glutathione, cysteine, and related metabolites during anoxia/aglycemia.

Animal Model: Rat (hippocampal slices).

Interpretation and Analysis: Phosphonate and phosphinate analogues of N-acylated γ-glutamyl-glutamate inhibit GCP II, reflected by IC_{50} values in the low nanomolar range.

The net efflux (i.e., increase in extracellular concentration) due to changes in release and/or uptake of cysteine, cysteine sulfinate, γ-glutamyl-glutamate, γ-glutamyl-glutamine, glutathione, γ-glutamyl-cysteine, and glutamate increases as a result of anoxia/aglycemia. These increases in net efflux of cysteine, cysteine sulfinate, γ-glutamyl-glutamate, and γ-glutamyl-glutamine are reduced or blocked by acivicin, an inhibitor of γ-glutamyl transpeptidase. In contrast, acivicin causes an increase in both basal and anoxia/aglycemia-induced net efflux of glutathione, whereas the basal and anoxia/aglycemia-induced efflux of glutamate is unchanged by acivicin exposure. The effect of acivicin on the efflux of γ-glutamyl-cysteine is similar to that of glutathione, although less pronounced. Addition of β-mercaptoethanol to the incubation medium during and after 30 minutes of anoxia/aglycemia decreases the net efflux of cysteine sulfinate specifically, indicating that the increase in cysteine sulfinate during anoxia/aglycemia may be partly derived from the spontaneous oxidation of cysteine. Such findings suggest that γ-glutamyl transpeptidase may be involved in the regulation of the extracellular concentrations of cysteine, several γ-glutamyl-containing dipeptides, and glutathione, but not glutamate, during ischemia.

Net efflux of cysteine, glutathione, and related metabolites from rat hippocampal slices during oxygen/glucose deprivation: Dependence on γ-glutamyl transpeptidase. Li X, Wallin C, Weber SG, Sandberg M. Brain Res 1999 Jan 2; 815 (1), pp. 81–8.

Apoptosis and Oncosis: Cardiac Myocardial Cells/ Apoptosis: Doxorubicin-Induced/Oncosis: Isoproterenol-Induced

[^{111}In]-Antimyosin Antibody/[^{99m}Tc]-Glucaric Acid

Background Information: Non-invasive imaging techniques would be highly desirable to differentiate oncotic cell death from apoptotic cell death.

Application: [^{99m}Tc]-glucaric acid can be applied to detect oncotic myocardial cell death. Conversely, radiolabeled antimyosin is capable of detecting both oncotic and apoptotic types of myocardial cell death.

Route of Administration: [^{111}In]-antimyosin and [^{99m}Tc]-glucaric acid (intravenous).

Implementation: Evaluation of the diagnostic efficacy of dual administration of [^{111}In]-antimyosin antibody and [^{99m}Tc]-glucaric acid in a manner that can facilitate the detection of and differentiation between doxorubicin-induced myocardial apoptosis and isoproterenol-induced myocardial oncosis. Scanning electron microscopy, DNA laddering, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining, [^{111}In]-antimyosin antibody, and [^{99m}Tc]-glucaric acid can be collectively utilized *in vitro* and *in vivo* to detect doxorubicin-induced apoptosis or isoproterenol-induced myocardial oncosis.

***Ex Vivo* Model:** Cultured rat H9C2 embryonic cardiocytes. In this context, myocardial cell apoptosis can be induced by exposure of H9C2 embryonic cardiocytes to serial dilutions of doxorubicin.

Animal Model: Rats (CD1 strain for *in vivo* investigations). Myocardial apoptosis can be induced *in vivo* in rats by doxorubicin administration. Related investigations can employ heat or subcutaneous isoproterenol administration to induce myocardial oncosis.

Interpretation and Analysis: Scanning electron microscopy, DNA laddering, and TUNEL staining of H9C2 cardiocytes treated with doxorubicin all show cell death by apoptosis. Rat hearts treated with doxorubicin (10 and 20 mg/kg) are DNA ladder-positive and are associated with significantly greater [^{111}In]-antimyosin antibody (mean ± SD, 0.1942 ± 0.0150% injected dose per gram [%ID/g] and 0.1825 ± 0.0238 %ID/g, respectively) than normal hearts (0.1154 ± 0.0270 %ID/g, $p < 0.05$). Increases in myocardial [^{99m}Tc]-glucaric acid activity cannot be observed in rat hearts after 6, 12, and 24 hours of doxorubicin injection (0.0311 ± 0.0066 %ID/g, 0.0356 ± 0.007 %ID/g, and 0.0368 ± 0.0047 %ID/g, respectively; control hearts, 0.0352 ± 0.0099 %ID/g; p = not significant). Alternatively, [^{99m}Tc]-glucaric acid uptake is significantly greater in isoproterenol-induced oncotic hearts (0.1256 ± 0.1023 %ID/g) than in controls ($p < 0.0001$). [^{99m}Tc]-glucaric acid is avid only for the oncotic myocardium. Antimyosin, on the other hand, is positive for both oncotic and apoptotic myocardium.

Indium 111 antimyosin and Tc-99m glucaric acid for non-invasive identification of oncotic and apoptotic myocardial necrosis. Khaw BA, Silva JD, Petrov A, Hartner W. J Nucl Cardiol 2002 Sep–Oct; 9 (5), pp. 471–81.

Apoptosis Phenomenon: Hepatic and Thymic *[^{99m}Tc]-Hydrazinonicotinamido (HYNIC)-Annexin V*

Cellular/Molecular Mechanism of Action: Annexin V, a 32-kDa endogenous human protein, has a high affinity for membrane-bound phosphatidylserine.

Background Information: Either inadequate or excessive apoptosis (programmed cell death) is associated with many diseases. A method to image apoptosis *in vivo*, rather than acquiring histologic tissue samples for evaluation, could assist with therapeutic decision making in these disorders. Programmed cell death is associated with a well-choreographed series of events resulting in the cessation of normal cell function and the ultimate disappearance of the cell. One component of apoptosis is signaling adjacent cells that a cell is committing suicide by externalizing phosphatidylserine to the outer leaflet of the cell membrane.

Application: [^{99m}Tc]-HYNIC-annexin V can be utilized for the *in vivo* detection and quantification of apoptosis phenomenon in tissues and organ systems. Dexamethasone can be applied to induce thymic apoptosis.

Route of Administration: [^{99m}Tc]-HYNIC-annexin V (intravenous).

Dosage: Dexamethasone (1–20 mg/kg) 5 hours prior to [^{99m}Tc]-HYNIC-annexin.

Semi-Synthetic Methods: Covalent coupling of annexin V with the bifunctional hydrazinonicotinamide reagent (HYNIC) for the preparation of [^{99m}Tc]-HYNIC-annexin V.

Implementation: Detection of apoptosis phenomenon in the liver and thymus. Biodistribution analysis of [^{99m}Tc]-HYNIC-annexin V can be performed (in rats) 10–180 minutes following intravenous injection. Delineation of the degree of apoptosis required for *in vivo* localization of [^{99m}Tc]-annexin V (mice) can be determined with the aid of dexamethasone administered 5 hours prior to [^{99m}Tc]-HYNIC-annexin V. The thymus is then excised 1 hour after radiolabeled HYNIC-annexin V injection; thymocytes isolated, incubated with Hoechst 33342 followed by propidium iodide, and analyzed on a fluorescence-activated cell sorter. Each sorted cell population is counted in a scintillation counter. Evaluation of [^{99m}Tc]-HYNIC-annexin V as a tracer for external radionuclide imaging of apoptotic cell death can then be performed by radionuclide imaging 1 hour following injection of anti-Fas IgG in Fas-defective mice (lpr/lpr mice) and wild-type mice.

Animal Model: Rat and mouse.

Interpretation and Analysis: Biodistribution analysis of [^{99m}Tc]-HYNIC-annexin V in rats reveals a blood clearance half-time of less than 10 minutes. The kidneys have the highest concentration of radioactivity at any particular time point. Studies in the mouse thymus have demonstrated a 40-fold increase in [^{99m}Tc]-HYNIC-annexin V concentration in apoptotic thymocytes compared with viable cell populations. A correlation value of $r = 0.78$ can be appreciated between radioactivity and flow cytometric and histologic evidence of apoptosis. Imaging studies in lpr/lpr and wild-type mice show a substantial increase in activity within the liver of wild-type mice treated with anti-Fas, while there is no significant change, irrespective of anti-Fas administration, in lpr/lpr mice. Excellent imaging of hepatic apoptosis can be obtained in wild-type mice 30 minutes after injection of [^{99m}Tc]-HYNIC-annexin V. Imaging results are consistent with histologic analysis.

Technetium-99m HYNIC-annexin V: A potential radiopharmaceutical for the *in vivo* detection of apoptosis. Ohtsuki K, Akashi K, Aoka Y, Blankenberg FG, Kopiwoda S, Tait JF, Strauss HW. Eur J Nucl Med 1999 Oct; 26 (10), pp. 1251–8.

Apoptosis: Neoplastic Disease/Gliosarcoma/ Lewis Lung Carcinoma *Cy Annexin V*

Cellular/Molecular Mechanism of Action: Annexin V is a 32-kDa endogenous human protein that has a high affinity for membrane-bound phosphatidylserine, which is expressed at higher concentrations in cells undergoing apoptosis phenomenon.

Background Information: Non-invasive imaging using radioactive annexin V is an emerging strategy for the *in vivo* assessment of cell death.

Application: Detection and characterization of apoptosis phenomenon, including the evalatuion of neoplastic disease response to therapeutic intervention.

Semi-Synthetic Methods: Preparation of active Cy-annexin (an equimolar fluorophore dye:protein ratio) and an inactive Cy-annexin probe ($>$ 2 dyes/mol protein) that does not bind to apoptic Jurkat T cells.

Implementation: Evaluate the capacity for active annexin V labeled with fluorophore Cy5.5 (Cy annexin) with binding avidity for apoptotic Jurkat T cells to function as a probe for imaging (tumor apoptosis using near-infrared fluorescence (NIRF). To detect the influence of chemotherapy on the development of apoptosis, (tumors can be imaged with and without cyclophosphamide treatment).

Neoplastic Disease Model: 9L gliosarcoma, constitutively expressing green fluorescent protein marker, and the CR8 variant of Lewis lung carcinoma, stably transfected to express DsRed2. The expression of transfected fluorescent protein provides an indication of tumor margins and a means of defining tumor-associated NIRF signal intensity with both tumor models.

Interpretation and Analysis: In both tumor models active Cy-annexin V tumor NIRF signal increases 2 to 3 times after the treatment. A tumor NIRF signal develops by 75 minutes after active Cy-annexin injection and remains positive for a 20-hour observation period. Application of inactive annexin V as a control using the CR8 carcinoma cell type results in a low non-specific signal. With the 9L gliomosarcoma model, active Cy-annexin V binds to both tumor cells (Cy-annexin V staining only) and endothelial cells (co-stained with Cy-annexin V and antibody to the endothelial marker CD31). Such observations demonstrate that active Cy-annexin can be used as a NIRF probe to image apoptosis from outside an intact living animal and may facilitate the development of non-radioactive methods of measuring the antiproliferative effects of cancer chemotherapeutic regimens.

Near-infrared fluorescent imaging of tumor apoptosis. Petrovsky A, Schellenberger E, Josephson L, Weissleder R, Bogdanov A Jr. Cancer Res 2003 Apr 15; 63 (8), pp. 1936–42.

Apoptosis: Neoplastic Disease State/ Chemotherapeutic-Induced *[^{99m}Tc]-Annexin V/[Cyclophosphamide]/ TUNEL Tissue Analysis*

Cellular/Molecular Mechanism of Action: Annexin V is a human protein that possesses a high affinity for phosphatidylserine.

Application: Annexin V can be labeled with [^{99m}Tc] to detect apoptosis *in vivo*.

Route of Administration: [^{99m}Tc]-annexin V (intravenous).
Semi-Synthetic Methods: Radiolabeling of annexin V with [^{99m}Tc] (specific activity, 3.0 MBq/μg protein).
Implementation: Determine the ability of [^{99m}Tc]-annexin V to detect apoptosis identified by alterations in biodistribution profiles within innoculated allogenic hepatoma lesions 20 hours after cyclophosphamide administration. Radioactivity within specific tissues is then quantified 6 hours after [^{99m}Tc]-annexin V administration. As a form of reference control, tumor uptake of [^{14}C]-iodoantipyrine can be employed as a marker of tumor blood flow. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) of tissue harvested at necropsy (animal model) can be utilized to validate detection of apoptosis within tumor tissue specimens.

Animal Model: Rat (inoculated with allogenic hepatoma cells [KDH-8] in the left calf muscle, followed 11 days later by randomized administration of a single dose of cyclophosphamide [150 mg/kg intraperitoneally]. Negative reference controls are not administered the chemotherapeutic agent).
Interpretation and Analysis: Cyclophosphamide treatment significantly increases tumor uptake (percentage activity of injected dose per gram of tissue after normalization to the animal's weight [%ID/g/kg]) of [^{99m}Tc]-annexin V (0.070 ± 0.007 %ID/g/kg for treated rats compared to 0.046 ± 0.009 %ID/g/kg for controls, $p < 0.001$). [^{14}C]-iodoantipyrine uptake is similar in the treated and untreated groups. The number of TUNEL-positive cells in the tumor is significantly larger in the treated rats (297.70 ± 50.34 cells/mm^2) than in the control rats (168.45 ± 23.60 cells/mm^2, $p < 0.001$). Tumor uptake of [^{99m}Tc]-annexin V correlates with the number of TUNEL-positive cells within tumor specimens ($r = 0.712$; $p < 0.001$). Tumor uptake of [^{99m}Tc]-annexin V is significantly increased by a single dose of cyclophosphamide treatment, and the increase is concordant with the number of TUNEL-positive cells in the tumor. Such observations suggest the utility of [^{99m}Tc]-annexin V as a noninvasive means to assess tumor response, although further testing, including clinical evaluation, is required.

Detection of apoptotic tumor response *in vivo* after a single dose of chemotherapy with [^{99m}Tc]-annexin V. Mochizuki T, Kuge Y, Zhao S, Tsukamoto E, Hosokawa M, Strauss HW, Blankenberg FG, Tait JF, Tamaki N. J Nucl Med 2003 Jan; 44 (1), pp. 92–7.

Apoptosis: Renal
[^{99m}Tc]-Hydrazinonicotinamido (HYNIC)-Annexin V

Background Information: [^{99m}Tc]-HYNIC-annexin V is a novel tracer for *in vivo* imaging of apoptosis.
Route of Administration: [^{99m}Tc]-HYNIC-annexin V (intravenous).
Dosage: [^{99m}Tc]-HYNIC-annexin V (250 MBq).
Implementation: Imaging is initiated with a transmission scan using a [^{57}Co]-flood source to obtain a map of the local thickness of the volunteer. [^{99m}Tc]-HYNIC-annexin V is then injected directly, followed by a 30-minute dynamic study. Whole-body scans are obtained at about 30 minutes, 3 hours, 6 hours, and 24 hours after injection. Organ uptake is determined after correction for background, scatter, and attenuation. The MIRDOSE3.1 program can be used to calculate organ-absorbed doses and effective dose. Signs of adverse effects can be detected by monitoring renal and liver function, hematology profiles, blood coagulation parameters, and vital signs (blood pressure, pulse, respiration rate, temperature, and electrocardiogram).

Human Model: Healthy male volunteers (n = 6).
Interpretation and Analyses: The kidneys accumulate 49.7 ± 8.1% of the injected dose (%ID) at 3 hours after injection; the liver, 13.1 ± 1.0 %ID; the red marrow, 9.2 ± 1.8 %ID; and the spleen, 4.6 ± 1.6 %ID. More than 90% of the blood activity is cleared with a half-life of 24 ± 3 minutes. The biologic half-life of the activity registered over the total body is long (69 ± 7 hours). Excretion of the activity is almost exclusively through the urine (22.5 ± 3.5 %ID at 24 hours), and hardly any activity is seen in the bowel or feces. Absorbed doses are approximately 196 ± 31 μGy/MBq for the kidneys, 41 ± 12 μGy/MBq for the spleen, 16.9 ± 1.3 μGy/MBq for the liver, and 8.4 ± 0.9 μGy/MBq for the red marrow. The effective dose is approximately 11.0 ± 0.8 μSv/MBq, or 2.8 ± 0.2 mSv for the average injected activity of 250 MBq. No adverse effects are usually observed. Therefore, [^{99m}Tc]-HYNIC-annexin V is a safe radiopharmaceutical, having a favorable biodistribution for imaging of apoptosis in the abdominal region as well as for the thoracic cavity at a radiation dose considered acceptable.
Instrumentation: A dual-head gamma camera can be used to acquire conjugate anterior and posterior views.

Safety, biodistribution, and dosimetry of [^{99m}Tc]-HYNIC-annexin V, a novel human recombinant annexin V for human application. Kemerink GJ, Liu X, Kieffer D, Ceyssens S, Mortelmans L, Verbruggen AM, Steinmetz ND, Vanderheyden JL, Green AM, Verbeke K. J Nucl Med 2003 Jun; 44 (6), pp. 947–52.

Beta-Oxidation: Hepatic Viability
p-[^{123}I]-Iodophenylenanthic Acid (IPEA)/ [$^{1\text{-}11}$C]-Octanoate

Cellular/Molecular Mechanism of Action: Octanoate and iodophenylenanthic acid are organic molecules that can be used to detect hepatic beta-oxidation capacity.
Background Information: Radiopharmaceuticals that reflect beta-oxidation in hepatocytes will provide useful information on the prognosis after surgery or on the efficacy of treatment, because beta-oxidation is the main pathway responsible for adenosine triphosphate in hepatocytes. The agent [$^{1\text{-}11}$C]-octanoate has been developed as a diagnostic agent for determining hepatic viability by means of positron emission tomography (PET).
Application: The agent *p*-[^{123}I]-iodophenylenanthic acid ([^{123}I]-IPEA) is a radiopharmaceutical that is potentially useful for the diagnosis of hepatic viability based on energy metabolism. Single photon emission computed tomography (SPECT) has the advantage of being more widely used than PET.
Route of Administration: *p*-[^{123}I]-iodophenylenanthic acid (intravenous).
Semi-Synthetic Methods: Production of 2 radioiodinated omega-(4-iodophenyl)-medium chain fatty acids, *p*-iodophenylvaleric acid (IPVA) and *p*-iodophenylenanthic acid (IPEA).
Implementation: Evaluation of IPVA and IPEA as candidate radiopharmaceuticals for the determination of hepatic viability. Utilizing SPECT methodologies, these agents can be applied to differentiate between conditions of hepatitis and normal liver function.

Animal Model: Rat (normal and hepatitis model).
Interpretation and Analysis: Metabolite *in vitro* and *in vivo* analyses and biodistribution studies in normal mice indicate that both compounds are actively taken up by the liver and metabolized by beta-oxidation. However, these studies also indicate that IPEA is more suitable as an imaging agent than IPVA [^{123}I]-IPEA. Hepatic [^{123}I-]IPEA time-activity curves reveal a 2-phase clearance of radioactivity in both normal and hepatitis model rats, but the clearance is delayed depending on the severity of hepatitis. Furthermore, the

clearance rate of the 1st phase correlates with adenosine triphosphate (ATP) levels in hepatocytes, which can be used as an index for energy production capacity in hepatocytes. In conclusion, IPEA is metabolized predominantly by beta-oxidation, and the clearance of IPEA from the liver is closely associated with the ATP concentration in the liver. Thus, [^{123}I]-IPEA is a potentially useful new radiopharmaceutical for diagnosis of hepatic viability as a function of energy metabolism.

Evaluation of radioiodinated medium chain fatty acids as new diagnostic agents for the determination of hepatic viability. Yamamura N, Magata Y, Konishi J, Saji H. Eur J Nucl Med 1999 Dec; 26 (12), pp. 1597–605.

Beta-Oxidation of Fatty Acids: Mitochondrial Disorders
Glucose/Pyruvate/Carnitine Loading/Phenylpropionic Acid Loading/Long-Chain Fat Loading

Cellular/Molecular Mechanism of Action: Glucose and pyruvate are precursors for the biochemical production of lactate.
Background Information: Since the discovery of muscle carnitine palmitoyltransferase deficiency in 1973, a dozen separate defects of mitochondrial fatty acid beta-oxidation in man have been identified. With the exception of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, which occurs with a frequency approaching 1:10,000 among Caucasians of Northern European origin, the other defects are quite rare. Collectively, however, they are common causes of disease resembling Reye's syndrome in early life, and some have a later and more chronic presentation with cardiomyopathy and skeletal muscle weakness. They also represent a small, but significant, proportion of cases of sudden and unexplained death within the 1st 2 years of life. Diagnosis of these disorders has become increasingly sophisticated, with the advent of new analytical technologies and an increased awareness of the appropriate clinical and laboratory investigations needed in order to evaluate potential defects of this pathway.
Diagnostic Application: Identification of mitochondrial "respiratory chain" disorders.
Implementation: Evaluation of mitochondrial disorders applying various molecular probes including pyruvate and glucose, in addition to performing provocative testing (e.g., carnitine loading, phenylpropionic acid loading, long-chain fat loading).
Interpretation and Analysis: Elevation in lactate concentrations following loading-dose administration of glucose and pyruvate compared to normal reference control subjects implies the presence of a biochemical defect in the assimilation of lactate at the level of the mitochondria (e.g., Krebs cycle). Application of these methodologies permits a specific diagnosis in the case of several, although not all, of the disorders of this pathway. Methods for the measurement of all of the enzymes of beta-oxidation are now available to enhance this diagnostic capability. There remain, however, many patients in whom clinical and laboratory signs point to a defect in beta-oxidation, but in whom no specific diagnosis has yet been made.
Laboratory Instrumentation: Conventional clinical pathology assay of serum lactate concentrations in addition to tandem fast atom bombardment-mass spectrometry and stable isotope dilution gas chromatography-mass spectrometry.

New developments in the diagnosis and investigation of mitochondrial fatty acid oxidation disorders. Coates PM. Eur J Pediatr 1994; 153 (7 Suppl 1), pp. S49–56.

Carbon Group Metabolization/Trans-Methylation, Trans-Sulfuration, Remethylation/ Hyperhomocysteinemia: Endstage Renal Failure/ Single-Carbon Group Transfer (5,10-Methylenetetrahydrofolate)/Hepatic Cytosol and Mitochondria
Carbon Dioxide Breath Tests/[$^{2}H_3$C-1-^{13}C]-Methionine/ [2,3,3-$^{2}H_3$] Serine

Cellular/Molecular Mechanism of Action: Collectively represent substrates that can be applied as "probes" to detect defects in hepatic biochemical function or capacity pertaining to the activity of specific enzyme systems involved in metabolic pathways.
Application: Estimation of *trans*-methylation, *trans*-sulfuration, and remethylation rates; and evaluation of carbon group transfer.
Route of Administration: Intravenous.
Implementation: Evaluation of whole-body *trans*-methylation, *trans*-sulfuration, and remethylation rates (e.g., [$^{2}H_3$C-1-^{13}C]-methionine]. Following administration, carbon radioisotope contained in exhaled air as carbon dioxide is then measured. Preparations of [2,3,3-$^{2}H_3$] serine can be applied to evaluate the transfer of a 1-carbon group from serine via 5,10-methylenetetrahydrofolate in human hepatocyte cytosol and mitochondria, respectively.
Animal Model: Ovine.
Interpretation and Analysis: The amount of carbon radioisotope contained in exhaled air is directly proportional to the rate the test molecular is metabolized *in vivo* following injection. Tissue-free methionine enrichments after infusion of universally labeled U-[^{13}C] methionine show the highest remethylation activity in postmortem investigation of jejunum, liver, and kidney tissue samples, but no such activity can be appreciated in muscle and brain preparations.
Instrumentation: Methods to quantitate 1-carbon acceptor metabolism pathways and folate metabolism have recently become available.

In vivo stable isotope measurements of methyl metabolism: Applications in pathophysiology and interventions. de Meer K, van den Akker JT, Smulders Y, Stam F, Stehouwer CD, Finglas P. Food Nutr Bull 2002 Sep; 23 (3 Suppl), pp. 113–9.

Carbonic Anhydrase (CA) Deficiency Syndromes/ (Genetic Disease of the Bone, Brain, and Kidney)
N-1-Tritylsulfenyl Histamine

Cellular/Molecular Mechanism of Action: Activation of carbonic anhydrase biochemical activity.
Application: Classes of CA activators can facilitate the development of drugs/diagnostic agents related to CA deficiency syndrome (genetic disease state affecting the bone, brain, and kidneys).
Chemistry: N-1-tritylsulfenyl histamine can be synthesized by reacting histamine (Hst) with tetrabromophthalic anhydride followed by protection of its imidazole moiety with tritylsulfenyl chloride. After hydrazinolysis, it affords a key intermediate, which is derivatized at the aminoethyl group in order to obtain new types of activators of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1). Reaction of the key intermediate with 4-chlorophenylsulfonylureido amino acids (cpu-AA) in the presence of carbodiimides affords, after deprotection of the imidazolic nitrogen atom, a series of compounds with the general formula cpu-AA-Hst (cpu, 4-$ClC_6H_4SO_2NHCO$).
Considerations: Some structurally related dipeptide derivatives with the general formula cpu-AA1-AA2-Hst (AA, AA1, and AA2 represent amino acyl moieties) can also be prepared by a strategy similar to that applied for the amino acyl compounds mentioned above.

Interpretation and Analysis: The new derivatives proved to be efficient activators of 3 CA isozymes. Best activity can be detected against hCA I and bCA IV, for which some of the new compounds show affinities in the 1–10 nM range (h, human; b, bovine isozymes). hCA II, on the other hand, is less prone to activation by the new derivatives, which possess affinities around 20–50 nM for this isozyme.

Carbonic anhydrase activators: Synthesis of high affinity isozymes I, II, and IV activators, derivatives of 4-(4-tosylureido-amino acyl)ethyl-1H-imidazole (histamine derivatives). Scozzafava A, Iorga B, Supuran CT. J Enzyme Inhib 2000; 15 (2), pp. 139–61.

Carboxylic Acid Metabolism
1-[^{14}C]-α-Ketoisovaleric Acid (KIV)

Cellular/Molecular Mechanism of Action: 1-[^{14}C]-α-ketoisovaleric acid is a keto analogue of valine and can function as a biochemical substrate for molecular mechanisms of carboxylic acid metabolism.

Background Information: The keto analogues of essential amino acids represent a promising therapeutic modality in hereditary and acquired disorders of nitrogen metabolism. The utilization of these substances in humans has been assayed primarily by nitrogen balance studies.

Application: Endogenous biochemical metabolism of KIV can be measured using the $^{14}CO_2$ breath excretion test. In this context, the agent can be applied as a tool for the evaluation of certain diet formulations.

Route of Administration: Oral.

Implementation: Assessement of *in vivo* decarboxylation of KIV as a function of exhaled $^{14}CO_2$ using a simple and accurate breath excretion test. The experimental protocol applied in such investigations entails the utilization of protein diets (5 g) supplemented with essential amino acids and KIV (in place of valine). Diets of 120 g protein/day are then reevaluated in normal volunteers and gastrectomy cases.

Human Model: Normal volunteers and cases affected by a spectrum of disease states (n = 6 diseased; gastrectomy, hepatic failure, renal failure, and myotonic dystrophy).

Interpretation and Analysis: In situations of low protein intake, 13–32% of ingested KIV undergoes rapid decarboxylation, and this proportion appears to correlate inversely with damage to organ systems containing the branched-chain keto acid dehydrogenase. With high protein intake, the proportion of decarboxylated substrate increases between 44 to 53%. Such findings confirm that the decarboxylation of KIV in man varies under different conditions of dietary intake and metabolic disease. The $^{14}CO_2$ breath excretion test is applicable to other related analyses of carboxylic acid metabolism in human subjects.

Decarboxylation of α-ketoisovaleric acid after oral administration in man. Epstein CM, Chawla RK, Wadsworth A, Rudman D. Am J Clin Nutr 1980 Sep; 33 (9), pp. 1968–74.

Cortisol Metabolism/11-β-HSD$_2$ Biochemical Activity (Renal)
Deuterium-Labeled Cortisol

Cellular/Molecular Mechanism of Action: Deuterium-labeled cortisol is an analog of endogenous cortisol.

Application: Deuterium-labeled cortisol can be used as a labeled marker molecule for accessing *in vivo* alterations in endogenous cortisol metabolism mediated by 11-β-HSD$_2$ activities based on urinary excretion of cortisol, cortisone, and their A-ring reduced metabolites.

Route of Administration: Deuterium-labeled cortisol (oral).

Implementation: Evaluation of cortisol metabolism as a function of measurements of deuterium-labeled cortisol and its deuterium-labeled metabolites. Sample evaluation involves the application of GC/MS analyses simultaneously with the detection of endogenous cortisol, cortisone, and their A-ring reduced metabolites.

Human Model: Normal cases without evidence of disease.

Interpretation and Analysis: The stable isotope approach offers unique advantages in assessing the appropriateness of measuring unconjugated and total (unconjugated + conjugated) cortisol, cortisone, and their A-ring reduced metabolites in urine as indices of renal 11-β-HSD$_2$ activity. Measurement of urinary unconjugated cortisol and cortisone has many advantages for assessing 11-β-HSD$_2$ activity.

Instrumentation: Integrated gas chromatography/mass spectrometry system.

The use of deuterium-labeled cortisol for *in vivo* evaluation of renal 11-β-HSD activity in man: Urinary excretion of cortisol, cortisone, and their A-ring reduced metabolites. Kasuya Y, Shibasaki H, Furuta T. Steroids 2000 Feb; 65 (2), pp. 89–97.

Diabetes: Charge Permselectivity of Protein
Captopril/Clonidine

Cellular/Molecular Mechanism of Action: Captopril (angiotensin-converting enzyme inhibitor).

Physiological Mechanism of Action: Clonidine (tricyclic antidepressant).

Application: Determination of the charge permselectivity of proteins in conditions of diabetes after systemic and renal hemodynamic changes induced by an intravenous injection of captopril or clonidine.

Route of Administration: Captopril (IV) or clonidine (IV).

Dosage: Captopril (25 mg/100 ml in 5 minutes), clonidine (150 μg/100 ml), or placebo (100 ml saline).

Considerations: Anionic immunoglobulin clearance (IgG4) has been compared with that of total immunoglobulins, (same size but are mostly cationic), and of albumin.

Implementation: Evaluation of charge permselectivity of proteins in conditions of diabetes following systemic and renal hemodynamic changes induced by captopril or clonidine. Glomerular filtration rate (GFR) and renal plasma flow (RPF) are measured before and during test evaluations.

Human Model: Cases without signs of clinical nephropathy or other disorders. Ten type I hypertensive diabetic patients (Group 1), 10 type 2 hypertensive diabetic patients (Group 2), 5 type 1 normotensive diabetic patients (Group 3), 10 subjects with essential hypertension (Group 4), and 7 normal volunteers (Group 5) are administered captopril (25 mg/100 ml in 5 minutes). Hypertensive diabetic patients (e.g., n = 12) can be evaluated utilizing clonidine (150 μg/100 ml) (Group 6) as a 2nd provocative test. Negative reference controls (Group 7) can be administered as a placebo (100 ml saline).

Interpretation and Analysis: A significant decrease in diastolic blood pressure can be observed in case group types (CGT) 1, 2, 4, and 6, together with an increase in the GFR and RPF values in CGT 1, 2, 3, and 4 only, as well as a decrease in renal vascular resistances in CGT 1, 2, 3, 4, and 6. Both clearances and fractional clearances of IgG4 and IgG decrease after captopril in CGT 1 and 2.2+. The decrease in IgG4 clearance can be correlated with renal vascular resistant CGT cases. A significant decrease in the anionic-cationic immunoglobulin ratio (IgG4/IgG) is observed in CGT 1, 2, and 3.

Selective decrement of anionic immunoglobulin clearance after induced renal hemodynamic changes in diabetic patients. Di Mario U, Bacci S, Morano S, Pugliese G, Pietravalle P, Andreani D, Morabito S, Simonetti B, Pierucci A. Am J Physiol 1992 Mar; 262 (3 Pt 2), pp. F381–8.

Diabetes Insipidus/Hypothalamus/Pituitary/Adrenal Axis Function/Desamino-D-AVP (DDAVP) Replacement Therapy Response
Corticotropin-Releasing Hormone (CRH)/Arginine Vasopressin (AVP)

Cellular/Molecular Mechanism of Action: Hypothalamic CRH promotes release of adenocorticotropic hormone (ACTH) from the pituitary, which in turn promotes the release of cortisol by the adrenal gland.
Application: Assessment of hypothalamus-pituitary-adrenal axis integrity and function.
Considerations: A strong relationship has been found between AVP and hypothalamus-pituitary-adrenal axis in humans.
Implementation: Evaluation of baseline and CRH-stimulated ACTH and F levels in patients with central diabetes insipidus (CDI), before and after replacement therapy with DDAVP. A standard CRH test is performed in all subjects involving the measurement of plasma ACTH and serum F before and every 15 minutes for 2 hours after the administration of 100 μg of human CRH preparations. In patients with CDI, CRH tests are repeated after 1 week of DDAVP administered at standard doses.
Interpretation and Analysis: Initially, ACTH and F levels are significantly higher in patients with CDI than in controls either at baseline (ACTH: 45.5 ± 4.8 vs 18.5 ± 3.3 ng/l, $p < 0.05$; F: 375.1 ± 55.7 vs 146.6 ± 19.4 μg/l, $p < 0.05$) or after CRH test considered as a peak (ACTH: 90.8 ± 14.4 vs 42.5 ± 7.4 ng/l, $p < 0.05$; F: 501.6 ± 65.7 vs 226.3 ± 25.6 μg/l, $p < 0.05$) and area-under-the-curve (AUC) values (ACTH: 3,997.0 ± 571.7 vs 2,136.0 ± 365.8 ng/l/120 min, $p < 0.05$; F: 31,489.0 ± 4,299.4 vs 14,854.5 ± 1,541.5 μg/l/120 min, $p < 0.05$). In patients with CDI, 1 week of replacement with DDAVP results in diminished ACTH concentrations (peak: 56.9 ± 9.3 ng/l; AUC: 2390.7 ± 480.7 ng/l/120 min) and F (peak: 310.3 ± 39.5 μg/l; AUC: 17,555.5 ± 2008.7 μg/l/120 min) responses to CRH to normal but do not significantly modify baseline hormone levels (ACTH: 29.6 ± 3.6 ng/l; F: 239.0 ± 32.3 μg/l). In this context, CDI is associated with increased baseline ACTH and F levels and increased responsiveness of ACTH and F to CRH administration. In addition, replacement treatment with DDAVP normalizes CRH-induced but not baseline ACTH and F secretion.

Hypothalamus-pituitary-adrenal axis in central diabetes insipidus: ACTH and cortisol responsiveness to CRH administration. Pivonello R, Faggiano A, Filippella M, Di Somma C, De Martino MC, Gaccione M, Lombardi G, Colao A. J Endocrinol Invest 2002 Dec; 25 (11), pp. 932–7.

Diabetes Mellitus: Erythrocyte Anionic Charge (RBCCh)/Diabetic Nephropathy/Microalbuminuria/Altered Glomerular Charge Selectivity
Lysine/[Urinary Glycosaminoglycan Excretion]

Application: Detection and evaluation of diabetic RBCCh and albuminuria.
Implementation: Evaluation of the anionic charge on the surface of erythrocytes and the sialic acid and acid glycosaminoglycans (GAGs) content within erythrocyte membrane structures.

Human Ex Vivo Model: Cases of insulin-dependent diabetes that have albumin excretion rates less than 300 mg/24 hours.
Interpretation and Analysis: Statistically significant reductions in RBCCh and GAGs content in erythrocyte ghosts can be appreciated. In view of the demonstration of a negative correlation between RBCCh and albuminuria after a lysine provocative test, these observations support the hypothesis that the onset of microalbuminuria in human diabetes is sustained by an alteration of glomerular charge and consequently of glomerular charge selectivity. Many insulin-dependent diabetic patients with albuminuria in the "not at risk range" for diabetic nephropathy display high urinary excretion rates of glycosaminoglycans. A lysine provocative test in these subjects reveals abnormal urinary excretion of albumin, unlike findings obtained in insulin-dependent diabetic patients with normal urinary excretion rates of glycosaminoglycans. Such findings support the hypothesis that high urinary excretion of glycosaminoglycans is a marker of glomerular involvement in diabetes mellitus.

Abnormal erythrocyte charge in diabetes mellitus. Link with microalbuminuria. Gambaro G, Baggio B, Cicerello E, Mastrosimone S, Marzaro G, Borsatti A, Crepaldi G. Diabetes 1988 Jun; 37 (6), pp. 745–8.

Diabetes Mellitus: Impaired Glucose Tolerance/Diabetes Mellitus: Non-Insulin-Dependent Diabetes Mellitus
Low-Osmolality Glucose/Glucose

Application: Screening agent for impaired glucose tolerance or non-insulin-dependent diabetes mellitus.
Route of Administration: Glucose (oral).
Dosage: Standard (1.38 mol/L) 75-g oral glucose tolerance test solution. Lower osmolar (0.62 mol/L) 50-g glucose solution.
Considerations: A 75-g oral glucose tolerance test beverage (gold standard for diagnosis of diabetes in accordance with criteria established by the World Health Organization) can be applied in combination with the collection of 1- and 2-hour blood glucose values.
Implementation: Evaluation of glucose regulation in response to the administration of a 75-g oral glucose tolerance test beverage or 50-g glucose solution. Blood samples are drawn at baseline and 30, 60, and 120 minutes after ingestion of each glucose solution.
Interpretation and Analysis: Both solutions were shown to have a high sensitivity and specificity for detecting diabetes and impaired glucose tolerance at 30 and 60 minutes.

The physiologic 50-g solution uses less glucose, is highly palatable, and is similar in sweetness and carbohydrate concentration commonly employed in the production of soft drink beverages. Studies need to be performed in small animal species to determine the feasibility of applying lower osmolality formulations of glucose as a screening technique for the diagnosis of impaired glucose tolerance or non-insulin-dependent diabetes mellitus. The ability to perform high-sensitivity and high-specificity screening of cases suffering from diabetes or impaired glucose tolerance with a single timed blood sample could make mass screening more practical.
Instrumentation: Glucometer.

Use of a physiologic oral glucose solution for screening for impaired glucose tolerance and non-insulin-dependent diabetes mellitus. Phillips WT, Tilson KS, Schwartz JG. Arch Pathol Lab Med 1996 Sep; 120 (9), pp. 842–6.

Diabetes Mellitus: Insulin Resistant/Growth Hormone: Pituitary Dwarfism/Acromegaly
Insulin

Cellular/Molecular Mechanism of Action: Promotes transport of glucose across cellular membranes into the cytosol.
Diagnostic Application: Pituitary-based (feline) acromegaly.
Route of Administration: Subcutaneous injection.
Dosage: Insulin (25 U/day/feline).

Interpretation and Analysis: Patients (feline) displaying insulin-resistant diabetes mellitus (persistent hyperglycemia) following administration of insulin at a dose equal to or greater than 25 U/day should be considered as possibly suffering from pituitary-based acromegaly. Additional clinical signs that support the diagnosis of acromegaly would include the presence of arthropathy and cardiomyopathy. Acromegaly patients will have excessively high systemic concentrations of growth hormone (GH) within the intravascular compartment. Further diagnostic pharmacology can be utilized to verify a diagnosis of acromegaly through the application of a glucose suppression test because glucose suppresses pituitary GH secretion (see glucose suppression test).
Laboratory Instrumentation: Laboratory facilities and instrumentation necessary for performing immunoassay procedures (RIA or ELISA).

Diabetes Mellitus: Residual Beta-Cell Secretory Capacity/Short-Term vs Long-Term Diabetes Mellitus/C-Peptide Response
Glucose/Tolbutamide/Glucagon

Application: Assessment of residual beta-cell secretory capacity in short-term (2 months–2 years) and long term (5–8 years) insulin-dependent diabetes by measuring serum C-peptide immunoreactivity.
Implementation: Evaluation of insulin-dependent conditions as a function of C-peptide responses to glucose, tolbutamide, and glucagon stimulatory tests.
Interpretation and Analysis: Minimal C-peptide secretion can be detected in only a small percentage of test cases following stimulatory tests in long-term diabetics (n = 1/7). A very high percentage of short-term diabetics will respond to at least 1 provocative test for beta-cell reserve (e.g., n = 7/7), although these responses are blunted. The greatest C-peptide responses occur after glucagon administration (mean increase 0.62 pmol/ml) in short-term responders. Patients that respond to 1 test do not necessarily respond to another stimulus. There is no correlation between basal C-peptide levels and the ability to provoke further C-peptide secretion by any of the 3 tests. C-peptide responses do not correlate with % hemoglobin A1c, mean fasting blood glucose levels, or mean blood glucose concentrations during oral glucose tolerance tests. Such observations suggest that stimulation tests are only useful in assessing endogenous beta-cell reserve in patients with diabetes of less than 5 years duration. In diabetics of longer duration there is little insulin reserve above basal levels.

A 2-year pilot trial of continuous subcutaneous insulin infusion vs intensive insulin therapy in patients with newly diagnosed type 1 diabetes (IMDIAB 8). Pozzilli P, Crinò A, Schiaffini R, Manfrini S, Fioriti E, Coppolino G, Pitocco D, Visalli N, Corbi S, Spera S, Suraci C, Cervoni M, Matteoli MC, Patera IP, Ghirlanda G. Diabetes Technol Ther 2003; 5 (6), pp. 965–74.

Electrolyte Membrane Transport Complexes/ Myocardial Na/K-ATPase
[^{125}I]-Digoxin/[^{125}I]-Digoxin-Iodohistamine (bis(O-Carboxymethyloxime))/Ouabain Displacement

Cellular/Molecular Mechanism of Action: Cardiac glycosides recognize and physically bind to membrane-associated myocardial Na/K-ATPase complexes.
Background Information: Cardiac glycosides have a high binding avidity for myocardial Na/K-ATPase complexes.
Implementation: Survey of radioiodinated digoxin (cardiac glycoside) derivatives as potential candidates for myocardial imaging with particular emphasis on non-invasive monitoring of cardiac glycoside therapy.
Interpretation and Analysis: One radioiodinated digoxin derivative, [^{125}I]-digoxin-iodohistamine(bis(O-carboxymethyloxime)) accumulates at relatively high levels in the myocardium and displays similar binding avidity for Na/K-ATPase as digoxin itself against ouabain displacement, as indicated by *in vivo* and *in vitro* studies. Based on these results, [^{123}I] labeling of digoxin-histamine(bis(O-carboxymethyloxime)) and imaging analyses in a dog demonstrate uptake of the agent within the myocardium.

High myocardial accumulation of radioiodinated digoxin derivative: A possible Na/K-ATPase imaging agent. Fujibayashi Y, Takemura Y, Matsumoto K, Wada K, Yonekura Y, Konishi J, Yokoyama A. J Nucl Med 1992 Apr; 33 (4), pp. 545–9.

Extrahepatic Metabolism/Vascular Perfusion: Hepatic Blood Flow/[Xenobiotic Metabolism]
Phenol Red/Lidocaine (Monoethylglycinexylidide [MEGX])

Background Information: Xenobiotic metabolism in extrahepatic tissues has been extensively studied *in vitro*, but it is difficult to estimate *in vivo* the share of xenobiotic transformation in extrahepatic tissues for lack of a suitable approach.
Application: Assessment of extrahepatic metabolism established by investigating the conversion of lidocaine to the metabolite MEGX.
Animal Model: Rat (rats were anesthetized with ethyl ether inhalation). In exposed livers, the hepatic liver artery is ligated, and the portal vein is clamped at its distal end. The left hepatic lobe is partly excised along its inferior margin, and a heparinized silicone catheter, diameter 0.2 cm, is inserted into the portal and left hepatic veins to allow recirculation of portal vein blood. Sham operations are performed in control group.
Interpretation and Analysis: Phenol red test shows that hepatic blood supply is absolutely blocked in model rats. At 30 minutes after establishing the portal-cavum bypass, the renal function and electrolytes do not change, but serum glucose decreases by 64.4 ± 30.4%; 30 minutes after intravenous administration of 1.0% lidocaine 2 mg/kg, serum MEGX in model rats is 32.0 ± 7.14% of that in the control group, which mostly exists in a free form and is not induced by phenobarbital pretreatment. The model is easy to establish and provides an *in vivo* method to study the extrahepatic metabolism of xenobiotics.

An *in vivo* rat model for assessment of extrahepatic metabolism. Ping H, Zhen-Fu C, Shao-Qing X, Ming L, Jian W, Guo-Qing Z, Lin Z, Lin-Fang L, Meng-Chao W. J Pharmacol Toxicol Methods 2001 May–Jun; 45 (3), pp. 181–5.

Gluconeogenesis: Hepatic Glucose Production (Glucose-6-Phosphatase Activity)/Abnormal Plasma Concentrations/Preterm Infants
Glucagon/(Tolerance Test)

Background Information: Developmental failures to adequately control postnatal blood glucose levels are common in the transition from fetal to infant life and can persist for many months. The standard method of functionally measuring hepatic glucose production and/or disordered glucose production is the response to a glucagon tolerance test.
Application: Detection and characterization of abnormalities in hepatic glucose production and glucose metabolism.

Implementation: Evaluation of an adapted version of the standard glucagon tolerance test currently used for children and adults for application in preterm infants.

Human Model: Consecutive cases of preterm infants (n = 79 preterm gestational age range 25–36 weeks; mean 32.2 weeks; mean birth weight 1.66 kg admitted to an intensive care unit that survived to time of discharge). At the time of discharge to home care, characteristics of the study group consist of adjusted mean gestational age 36.7 weeks, mean discharge weight 2.23 kg.

Interpretation and Analysis: Following the administration of a glucagon tolerance test, preterm infants display a maximal increase in plasma glucose of 1.39 ± 0.07 mmol/L (range 0–3.98 mmol/L for n = 78). An increase in plasma glucose of less than 4 mmol/L is considered abnormal in adults following administration of a fasting glucagon tolerance test. The responses of preterm infants and adults to glucagon are clearly different. The attenuated response to glucagon in the preterm infants is consistent with the low levels of hepatic glucose-6-phosphatase activity in premature infants, as glucose-6-phosphatase is the terminal step of the 2 main pathways of liver glucose production.

Developmental disorders of glucose metabolism in infants. Hume R, McGeechan A, Burchell A. Child Care Health Dev 2002 Sep; 28 Suppl 1, pp. 45–7.

Glucose Transport Protein (GluT)
4,6-(R)-O-(2′-Iodoethylidene)-α, β-D-Glucose

Cellular/Molecular Mechanism of Action: Mimics molecular glucose and is therefore subject to the same cellular transport mechanisms.

Application: Characterization of glucose transporter complexes. Characterization of glucose binding complexes and transport mechanisms.

Route of Administration: Intravenous.

Semi-Synthetic Methods: Preparation of 2 iodinated acetals of D-glucose, 4,6-(R)-O-(2′-iodoethylidene)-α, β-D-glucose (#1); and 4,6-(R)-O-(4′-iodobenzylidene)-α, β-D-glucose (#2).

Considerations: Analogs of acetal D-glucose derivatives are known to bind to the exofacial sites of the glucose transport protein (GluT).

Implementation: Assessment of the capacity of iodinated acetals #1 and #2 to interact with the glucose transporter. Radiolabeling of the glucose analogs with [^{123}I] can faciliate the detection and analysis of glucose transport proteins utilizing SPECT imaging analysis.

***Ex Vivo* Model:** Human erythrocytes (GluT1) and neonatal rat cardiomyocytes (GluT4).

Biological evaluation of 2 iodine-123-labeled D-glucose acetals prepared as glucose transporter radioligands. Brunet-Desruet MD, Ghezzi C, Morin C, Comet M, Fagret D. Nucl Med Biol 1998 Jul; 25 (5), pp. 473–80.

Hyperkalemic Periodic Paralysis (HYPP)
Potassium/Acetazolamide/Calcium Glucuronate/ Bicarbonate/Glucose (± Insulin)

Cellular/Molecular Mechanism of Action: Acetazolamide inhibits carbonic anhydrase (CA) activity. Pharmaceuticals within the thiazide class act directly on the kidney and increase the excretion of sodium chloride, potassium chloride, and water. At sufficient doses, pharmaceuticals within this class such as acetazolamide also promote bicarbonate excretion. Potassium excretion is achieved through a combination of enhanced excretion at the distal tubule, accented by drug-induced microvascular hyperperfussion at the level of the distal renal tubule.

Background Information: Hyperkalemic periodic paralysis is characterized by sporadic attacks of muscle tremors, weakness, and/or collapse, lasting for variable periods of time. Diagnosis is based on physical findings in association with hyperkalemia. In horses with HYPP, the regulation of ion transport through the sodium channels in the muscle cells occasionally fails, causing uncontrollable muscle twitching. Further investigations into molecular genetics reveal a mutation in the gene responsible for sodium and potassium regulation. The identification of this gene mutation is the basis for the blood test used to diagnose HYPP. Hyperkalemic periodic paralysis is inherited as an autosomal dominant trait.

Pharmacokinetics: The pharmacokinetics of intravascular and orally administered acetazolamide can be established in horses (e.g., n = 6) by the administration of 2 doses (4 mg/kg of body weight, IV; 8 mg/kg, per os) and blood samples collected at regular intervals before and after administration. Assay for acetazolamide concentrations can be determined by high-performance liquid chromatography. Following IV administration of acetazolamide, data analysis reveals a median mean residence time of 1.71 ± 0.90 hours and median total body clearance of 263 ± 38 ml/kg/hr. Median steady state volume of distribution is 433 ± 218 ml/kg. After oral administration, mean peak plasma concentration is 1.90 ± 1.09 μg/ml. Mean time to peak plasma concentration is 1.61 ± 1.24 hours. Median oral bioavailability is 25 ± 6%. Oral pharmacokinetic disposition of acetazolamide in horses is characterized by rapid absorption, low bioavailability, and slower elimination than observed initially after IV administration. Pharmacokinetic data can therefore facilitate estimation of appropriate dosages for acetazolamide use in horses with HYPP.

Diagnostic Application: Hyperkalemic periodic paralysis in the acute stages can be reversed by calcium gluconate, bicarbonate, glucose, and/or insulin. Hyperkalemic periodic paralysis can be induced or initiated by the administration of potassium electrolyte.

Route of Administration: Acetazolamide (e.g., orally.)

Dosage: Acetazolamide (2.2 mg/kg = equine).

Considerations: Administration can promote hypokalemia. Acetazolamide can also promote the development of hyperglycemia, in addition to elevations in triglycerides and cholesterol.

Interpretation and Analysis: During HYPP attacks, the intravenous administration of calcium gluconate, bicarbonate, and glucose results in a rapid reversal of clinical signs. Administration of potassium as an electrolyte solution can precipitate the condition in cases with a genetic predisposition.

HYPP—hyperkalemic periodic paralysis in horses. Zeilmann M. Tierarztl Prax 1993 Dec; 21 (6), pp. 524–7.

Pharmacokinetics of acetazolimide after intravenous and oral administration in horses. Alberts MK, Clarke CR, MacAllister CG, Homer LM. Am J Vet Res 2000 Aug; 61 (8), pp. 965–8.

Hyperkalemic Periodic Paralysis (HYPP): Equine
Potassium Chloride (KCl)

Cellular/Molecular Mechanism of Action: Stabilizes propagation of electrolyte-mediated electrical impulses across cell membranes. Normally, potassium (K^+) is maintained at a relatively higher concentration intracellularly in contrast to the extracellular environment. Additionally, sodium (Na^+) is usually in relatively higher concentration in the extracellular environment than in the intracellular milieu. During membrane depolarization, Na^+ conductance intracellularly increases, which is followed by increased extracellular K^+ conductance during the repolarization process. In this context,

administration of large amounts of K^+ serves to inhibit the processes of membrane depolarization/repolarization.

Diagnostic Application: Detection of cases suffering from HYPP syndrome. Positive diagnosis is recognized by a progressive increase in muscle weakness that can progress to collapse.

Route of Administration: Orally by nasogastric tube.

Dosage: KCl (88–160 gm/kg).

Considerations: False negative results can be observed when the lower dose range is administered.

Implementation: Administration should begin at the lower dose of approximately 40 g of KCl in water (6 liters). Blood samples are collected for measurement of KCl concentrations. Some patients with periodic hyperkalemic syndrome will not respond to the potassium chloride challenge test. In such instances, these cases can be diagnosed by increasing the dosage of potassium chloride in increments of 20 g. Only 1 challenge test should be performed within any given 24-hour period and horses should not be left unattended following KCl administration (2–4-hour period following dosage).

Interpretation and Analysis: Normal horses have a modest increase in plasma potassium concentrations but do not develop abnormal clinical signs even at the 180 mg/kg KCl dose level. Reversal of clinical signs following their induction by potassium chloride administration can be achieved with the subsequent administration of calcium chloride or calcium gluconate. Calcium gluconate can be administered at a dose of 0.2 to 0.4 ml/kg diluted to 1 to 2 liters in 5% dextrose and given intravenously. Response rate following calcium gluconate is rapid. Alternatively, dextrose 5% (D5W) at 4.4 to 6.6 ml/kg or $NaHCO_3$ at 1 mEq/kg can be administered rapidly by intravenous infusion.

Laboratory Instrumentation: Electron capture instrumentation is applied to measure concentrations of potassium cation.

Hypokalemic Periodic Paralysis (HYPP): Human *Glucose/Insulin*

Background Information: Some cases of hypokalemic periodic paralysis can present with signs of a sudden onset of flaccid paralysis. After exclusion of central nervous system involvement, marked hypokalemia implies a diagnosis of hypokalemic periodic paralysis, which can often be completely reversed with oral and parenteral potassium administration.

Application: Diagnosis of human hypokalemic paralysis.

Considerations: Pathogenetically, this syndrome is characterized by an excessive intracellular shift of potassium ions into myocytes. Therapeutic measures include potassium-sparing diuretics, beta blockers, acetazolamide, or diclofenamide. In less severe cases, oral potassium may be sufficient to reverse the symptoms.

Implementation: Administration of glucose and insulin to create hypokalemia and incipient paralysis of the limbs (provocative diagnostic test).

Interpretation and Analysis: Hypokalemia and incipient paralysis of the limbs induced by glucose and insulin confirms a diagnosis of hypokalemic periodic paralysis.

Sudden onset of paralysis in a twenty year-old male patient. Wallner M, Prischl FC, Pichler F, Kramar R. Wien Klin Wochenschr 1994; 106 (19), pp. 615–6.

Hyperthermia: Malignant Hypertheramia (MH) *Midazolam (Ineffective)/Caffeine/Halothane*

Cellular/Molecular Mechanism of Action: Midazolam (water-soluble benzodiazepine) potentiates the inhibitor effect of the neurotransmitter gamma amino butyrate (GABA) within the central nervous system (CNS). Investigations have demonstrated that the benzodiazopines possess binding avidity for GABA receptor sites.

Physiological Mechanism of Action: Raises the threshold for CNS toxicity for local anesthetics but has little effect on respiration and does not potentiate opioid-induced respiratory depression. Tends to produce amnesia and a state of tranquility/mild sedation.

Application: Premedicant for induction of anesthesia. Unfortunately, midazolam is a pharmaceutical that is not an effective agent for diagnostic screening of patients susceptible to the development of MH.

Dosage: Midazolam (ca. 0.5 μg/ml).

Considerations: Midazolam is applied to determine its influence on the resting tension of directly stimulated muscle biopsies. Interactions between midazolam and the 2 most commonly used MH diagnostic agents (halothane and caffeine) have also been examined.

Interpretation and Analysis: Midazolam, at maximum therapeutic concentrations (ca. 0.5 μg/ml), has no detectable effects on muscle contraction in control and MH preparations. However, midazolam does elicit a contracture from control and MH-positive preparations when used within a concentration range between 160 and 1,280 μg/ml. There are no significant differences between control and MH-positive patients in minimum concentration of midazolam causing contracture or the strength of contracture at the respective eliciting concentration. There appears to be no interaction between midazolam and either halothane or caffeine on the resting tension of the directly stimulated muscle twitch preparation.

Effects of midazolam on directly stimulated muscle biopsies from control and malignant hyperthermia positive patients. Fletcher JE, Rosenberg H, Hilf M. Can Anaesth Soc J 1984 Jul; 31 (4), pp. 377–81.

Hypoxia: Cardiovascular/Myocardial/Myocardial Oxygen Availability/Myocardial Intracellular Energy Level/Myocardial Mitochondrial Redox State *[^{99m}Tc]-Nitroimidazole*

Physiological Mechanism of Action: Nitroimidazole is preferentially internalized and retained by hypoxic cardiac myocardium.

Background Information: The agent, [^{99m}Tc]-labeled nitroimidazole is preferentially taken up and retained by hypoxic cardiac myocardium.

Application: Nitroimidazole is a candidate agent for the detection and characterization of myocardial hypoxia, including conditions such as myocardial ischemia.

Route of Administration: Nitroheterocycle (isolated perfused hearts but in a clinical scenario would be given intravascularly).

Implementation: Evaluation of the influence of O_2 or N_2 equilibrated cell-free medium perfused into hearts on the uptake of infused nitroheterocycle and its subsequent washout.

***Ex Vivo* Model:** Rat (isolated perfused hearts).

Interpretation and Analysis: Nitroheterocycle displays biphasic kinetic washout patterns in isolated perfused hearts (rat). For both uptake and washout, the early phase is very rapid, whereas the late phase is much slower. The amount of radioactivity retained after 40 minutes of clearance is about 2-fold greater in hypoxic hearts than in normoxic hearts. Cardiac myocytes and mitochondria isolated from rat heart also accumulate nitroheterocycle. Association of the compound with heart cells is inversely related to the level of available oxygen and is independent of intracellular energy level or mitochondrial redox state in the presence of oxygen. The results indicate that this [^{99m}Tc]-labeled nitroimidazole may serve as a sensitive marker of hypoxic myocardium.

The agent [^{99m}Tc]-labeled nitroimidazole is preferentially taken up and retained by hypoxic cardiac myocardium.

A novel [^{99m}]technetium-labeled nitroheterocycle capable of identification of hypoxia in heart. Rumsey WL, Cyr JE, Raju N, Narra RK. Biochem Biophys Res Commun 1993 Jun 30; 193 (3), pp. 1239–46.

Hypoxia/Infarction: Myocardial
[Antimyosin]-Poly(Ethylene Glycol) (PEG)/Liposomes/ PEG and Selective Antibody Content

Cellular/Molecular Mechanism of Action: Liposomes function as a physical barrier to dilution of pharmaceutical agents into biological fluids, prevent accelerated excretion or metabolism, and serve as a modality for selective delivery at "high" concentrations.
Background Information: Determination of the biodistribution and relative infarct accumulation of different liposome preparations in rabbits with experimental myocardial infarction have been previously described.
Application: Liposomes formulated with optimized concentrations of PEG and antimyosin immunoglobulin can function as a delivery format for the detection and characterization of experimental lesions of myocardial infarction.
Route of Administration: Intravenous.
Implementation: Delineation of the influence of such parameters as liposome size, presence or absence of PEG, and infarct-specific antimyosin antibody (AM) on *in vivo* liposome behavior.
Interpretation and Analysis: The variables of liposome size, PEG content, and infarct-specific antimyosin antibody all affect patterns of liposome biodistribution. Liposome size is the least significant variable. Statistical analysis reveals that of all variables, PEG coating expresses the strongest influence on liposome blood clearance, significantly ($p = 0.0001$) increasing the mean level of blood radioactivity in all conditions. Infarct accumulation depends upon the presence of both PEG ($p = 0.0013$) and antimyosin monoclonal antibody content ($p = 0.005$). The infarct-to-normal ratio is affected by the presence of antimyosin monoclonal antibody ($p = 0.0002$), but the extent of the effect depends also on the presence of PEG ($p = 0.01$). The 2 different mechanisms of slow accumulation via the impaired filtration (accumulation of PEG-liposomes) and specific binding of immunoliposomes with the exposed antigen (AM-liposomes) collectively contribute to the development of relatively high target-to-background ratios. Both mechanisms are supplementary (complementary) in scenarios of liposomes carrying PEG and AM simultaneously. Multiple strategies exist for futher optimizing PEG/AM liposome preparations as a delivery modality.

Poly(ethylene glycol)-coated anticardiac myosin immunoliposomes: Factors influencing targeted accumulation in the infarcted myocardium. Torchilin VP, Narula J, Halpern E, Khaw BA. Biochim Biophys Acta 1996 Feb 21; 1279 (1), pp. 75–83.

Hypoxia/Ischemia: Myocardial
Dithiosemicarbazone Derivatives (DTS)/[^{62}Cu or ^{64}CU]-DTS/[Tc]-ATSM(2)

Application: Dithiosemicarbazone derivatives radiolabeled with divalent copper (^{62}Cu, ^{64}Cu) have demonstrated potential value for detecting ischemic tissues. Development of analogous derivatives labeled with the more readily available [^{99m}Tc]-technetium radioisotope would be highly desirable.
Route of Administration: Intravascular.
Semi-Synthetic Methods: Radiolabeling of various synthesized DTS derivatives using a macromolecular [Sn^{2+}]-complex under an anaerobic condition at pH 3.4–4.5 in concert with ascorbate solution stabilization at pH 6.7–7.0. Characterization of [Tc]-DTS derivatives can be achieved applying various analytical methods (thin-layer chromatography [TLC], high-performance liquid chromatography [HPLC]).
Implementation: Evaluation of the ability of agents to detect regions of myocardial ischemia.
Animal Model: Mouse and rat (normal/healthy utilized in a vascular occlusion model: myocardial left anterior descending coronary artery).
Interpretation and Analysis: The dithiosemicarbazones [Tc]-ATSE, [Tc]-ATSM, and [Tc]-ATSM(2) have distinctive characteristics, with the latter preparations displaying high myocardium uptake in regions of ischemia in left anterior descending artery (LAD) rat myocardium models. The [Cu]-DTS mimetic agent [Tc]-ATSM(2) may have potential as an ischemia-damaged myocardium imaging agent.

The development of [^{99m}Tc]-analog of Cu-DTS as an agent for imaging hypoxia. Horiuchi K, Tsukamoto T, Saito M, Nakayama M, Fujibayashi Y, Saji H. Nucl Med Biol 2000 May; 27 (4), pp. 391–9.

Insulin (Islet Cell) Adenoma
Calcium

Application: Diagnosis of insulin-secreting tumors, including islet cell adenomas that remain undetectable applying preoperative morphology-based imaging modalities.
Route of Administration: Calcium test (selective intra-arterial catheterization).
Dosage: Calcium gluconate (4 mg/kg/hr) is infused over a 2-hour period of time.
Considerations: Calcium ion *in vitro* plays a fundamental role in regulating insulin secretion, but the influence of calcium excess on insulin release *in vivo* has not been clearly defined. The described test is not of diagnostic value for the stated purpose.
Implementation: Evaluate the efficacy of the intra-arterial calcium test for the purpose of localizing sources of hyperinsulinism that remain undetectable at preoperative morphologic studies (e.g., ultrasound, computed tomography, magnetic resonance imaging, endoscopic ultrasound, abdominal arteriography). The calcium test involves the measurement of insulin concentrations contained in hepatic venous blood after selective intra-arterial calcium stimulation.
Human Model: Cases of clinically proven endogenous hyperinsulinism due to tumorous insulin production (e.g., $n = 24$). Of these cases, approximately 30% ($n = 7/24$) will have negative morphologic findings ($n = 5/7$ females, $n = 2/7$ males; age range, 30–66 years; mean age, 47 years).
Interpretation and Analysis: Approximately 30% of affected cases will have negative results from morphology-based diagnostic methodologies (e.g., $n = 7/24$). Surgical intervention may lead to the detection of solitary insulinomas (e.g., $n = 6$ with a mean diameter, 0.73 cm) and nodular hyperplasia (e.g., $n = 1$). Calcium tests allow accurate localization of the pathologic source of insulin secretion (e.g., $n = 7/7$). In less than 50% of cases, results of arterial calcium stimulation with hepatic venous sampling (ASVS) may affect intraoperative management (e.g., $n = 3/7$). An increase in insulin concentration after stimulation in the hepatic artery is not observed, making hepatic metastases unlikely. Arterial calcium stimulation with hepatic venous sampling, which procedurally is simpler than transhepatic pancreatic venous sampling, results in effective localization of sources of hyperinsulinism not detected with preoperative morphologic studies.

Other clinical trials have reported mean calcium plasma levels can increase from 9.6 ± 0.4 to 11.6 ± 0.8 mg/100 ml. During calcium infusion, blood glucose and plasma insulin concentrations can

remain unchanged. Observations noted in these case-based studies suggest that calcium fails to stimulate basal insulin secretion even in cases of organic hyperinsulinism, and that calcium infusion is not helpful as a provocative test in the diagnosis of insulinoma.

Insulinoma and islet cell hyperplasia: Value of the calcium intra-arterial stimulation test when findings of other preoperative studies are negative. Pereira PL, Roche AJ, Maier GW, Huppert PE, Dammann F, Farnsworth CT, Duda SH, Claussen CD. Radiology 1998 Mar; 206 (3), pp. 703–9.

Ischemia: Brain Lesions/N-Methyl-D-Aspartate (NMDA) Receptor Activation

N-(1-Naphthyl)-N′-(3-[^{125}I]-Iodophenyl)-N′-Methylguanidine

Cellular/Molecular Mechanism of Action: N-(1-naphthyl)-N′-(3-iodophenyl)-N′-methylguanidine or CNS 1261 is a ligand that interacts with NMDA receptors following their activation.

Application: N-(1-naphthyl)-N′-(3-[^{125}I]-iodophenyl)-N′-methylguanidine or [^{125}I]-CNS 1261 can potentially function as a radioligand for imaging NMDA receptor activation.

Route of Administration: [^{125}I]-CNS 1261 (intravascular).

Semi-Synthetic Methods: Preparations of [^{125}I]-CNS 1261 can be produced by radioiodination of N-(1-naphthyl)-N′-(3-tributylstannylphenyl)-N′-methylguanidine using Na[^{125}I] and peracetic acid.

Implementation: Evaluation of the ability to detect regional areas of cerebral ischemia using [^{125}I]-CNS 1261.

Animal Model: Rat.

Interpretation and Analysis: Uptake of [^{125}I]-CNS 1261 *in vivo* reflects NMDA receptor distribution in normal rat brain, whereas in ischemic rat brain, uptake is markedly increased in areas of NMDA receptor activation. Radiolabeled CNS 1261 appears to be a good candidate for further development as a single photon emission computed tomography tracer in the investigation of NMDA receptor activation in cerebral ischemia.

Synthesis and binding characteristics of N-(1-naphthyl)-N′-(3-[^{125}I]-iodophenyl)-N′-methylguanidine ([^{125}I]-CNS 1261): A potential SPECT agent for imaging NMDA receptor activation. Owens J, Tebbutt AA, McGregor AL, Kodama K, Magar SS, Perlman ME, Robins DJ, Durant GJ, McCulloch J. Nucl Med Biol 2000 Aug; 27 (6), pp. 557–64.

Ischemia: Cardiovascular/Myocardial Ischemia/Perfusion-Metabolism Mismatch

[^{123}I]-(Rho-Iodophenyl)-3-R,S-Methylpentadecanoic Acid (BMIPP)/Non-Metabolized BMIPP Myocardial Backdiffusion/Long-Chain Fatty Acid BMIPP: Decreased Tissue Uptake

Background Information: [^{123}I]-(rho-iodophenyl)-3-R,S-methylpentadecanoic acid is a fatty acid analog that possesses the unique property of being able to detect perfusion-metabolism mismatch in conditions of ischemic heart disease. The mechanism by which the agent exactly functions remains uncertain.

Application: Detection and characterization of perfusion-metabolism mismatch in cases with ischemic heart disease applying single photon emission computed tomography (SPECT) imaging methodologies.

Route of Administration: [^{123}I]-(rho-iodophenyl)-3-R,S-methylpentadecanoic acid (intravascular).

Implementation: The agent BMIPP is injected into the left anterior descending artery (LAD), followed by the collection of blood samples from either the cardiac vein and abdominal aorta; or serial biopsy specimens are harvested from the LAD region for comparison to reference controls. The catabolites of BMIPP, including backdiffusion of non-metabolized BMIPP, are measured utilizing high-performance liquid chromatography (HPLC in efflux study). Thin-layer chromatography (TLC) techniques can be applied for the analytical evaluation of tissue specimens.

Animal Model: Canine (n = 11 dogs subjected to open-chest thoracotomy under anesthesia to facilitate a system developed for the release of all blood within the great cardiac vein outside without recirculation, and the LAD is occluded for 30 minutes after reperfusion; n = 6/11 dogs cardiac vein and abdominal aorta blood collection post-BMIPP injection, n = 5/11 dogs used for collection of serial biopsy specimens from the LAD region).

Interpretation and Analysis: The agent BMIPP is rapidly extracted from the plasma by the myocardium and the subsequent retention is unchanged. The early washout (8 minutes) of radioactivity significantly increases (51% ± 12% to 65% ± 7%; $p < 0.05$) with ischemia. The metabolites from the myocardium consist of a backdiffusion of non-metabolized BMIPP, alpha, intermediate, and fully oxidized metabolites. Among these metabolites, backdiffusion of non-metabolized BMIPP in blood significantly increases (27.9% ± 7.7% to 42.3% ± 8.1%; $p < 0.05$), especially in the early phase of ischemia. In tissue, radioactivity is concentrated in the triglyceride pool even in the early phase. In addition, BMIPP and alpha-oxidized metabolite significantly decreases in the early phase with ischemia (t = 1 minute after BMIPP injection, 25.9% ± 8.6% to 14.5% ± 2.1%, $p < 0.01$; t = 2 minutes, 8.9% ± 5.0% to 4.5% ± 1.7%, $p < 0.05$). Such findings demonstrate that backdiffusion of non-metabolized BMIPP from the myocardium increases and BMIPP (long-chain fatty acids) in tissue decreases with ischemia, suggesting backdiffusion of non-metabolized BMIPP might play an important role in myocardial perfusion-metabolism mismatch on SPECT images in cases of ischemic cardiac disease.

Myocardial metabolism of [^{123}I]-BMIPP in a canine model with ischemia: Implications of perfusion-metabolism mismatch on SPECT images in patients with ischemic heart disease. Hosokawa R, Nohara R, Fujibayashi Y, Okuda K, Ogino M, Hirai T, Fujita M, Tamaki N, Konishi J, Sasayama S. J Nucl Med 1999 Mar; 40 (3), pp. 471–8.

Ketosis without Hypoglycemia

Ketogenic Test

Application: Characterization of ketosis in cases without hypoglycemia.

Implementation: Evaluation of changes in serum free amino acids as a response to ketogenic provocative test. Following the initiation of the provocative test, plasma concentrations for glycemic amino acids (glycine, alanine, glutamine, glutamate) are quantitated.

Human Model: Children with a history of ketosis but without evidence of hypoglycemia (n = 17 tested; n = 3/17 displaying clinical signs).

Interpretation and Analysis: (i) Approximately 29% of cases respond to the test and develop hypoglycemia (n = 5/17 with n = 3/5 hypoglycemic prior to test). (ii) Hypoglycemic and non-hypoglycemic children differ greatly in the decrement of serum total gluconeogenic amino acids after completion of the test due to the relatively lower levels before provocation in the hypoglycemic group and the inversely higher levels in the non-hypoglycemic group. (iii) Gluconeogenic amino acids decreased sharply after administration of the provocative test reflected by alterations in the concentrations of glycine, alanine, glutamine, and glutamate.

Serum free amino acid depletion in ketotic children. Hayashi M, Konishi K, Kameoka A, Mino M. J Nutr Sci Vitaminol (Tokyo) 1981; 27 (5), pp. 449–54.

Lipemia/Fibrin and Fibrinolysis
Heparin (Sodium or Lithium Salt)

Cellular/Molecular Mechanism of Action: Stimulation of lipoprotein lipase activity.
Application: Heparin (bovine lung, porcine mucosal origin) can aid in determining if plasma opacity is due to hyperlipidemia.
Diagnostic Application #1: Detection of systemic lipemia and/or the temporary resolution of hyperlipemia in order to facilitate serum electrolyte analysis. Also applied in the therapeutic management of lipemia.
Diagnostic Application #2: Detection of fibrinolytic activity (FDP formation) and platelet consumption due to disseminated intravascular coagulation (DIC).
Route of Administration and Dosage: Intravenous or subcutaneous only.
Considerations: In man and some animal species, heparin becomes associated with the external surface membrane of red blood cell (RBC) populations, resulting in their premature removal from the intravascular compartment by the reticuloendothelial (RE) system. In general, heparin preparations of porcine intestinal mucosal origin tend to promote less anemia than extracts acquired from bovine lung. Occationally, heparin preparations can also promote thrombocytopenia involving mechanisms analogous to its ability to lower peripheral RBC levels. Heparin should never be administered intramuscularly due to the high probability of hematoma formation.
Laboratory Instrumentation: Serum is simply inspected visually when attempting to detect resolution of systemic hyperlipidemia. Detection of alterations in fibrin and FDP can be performed utilizing ELISA-based techniques.

Lipid Metabolism: Cardiovascular/Myocardial Ischemia vs Infarction
[^{123}I]-(Rho-Iodophenyl)-3-R,S-Methylpentadecanoic Acid (BMIPP)/Non-Radioactive Colored Microspheres

Cellular/Molecular Mechanism of Action: [^{123}I]-BMIPP is a radiolabeled lipid analog that is perceived by cells as an endogenous lipid molecule.
Background Information: The tracer [^{123}I]-BMIPP has been evaluated to determine its ability to function as a molecular probe for characterizing myocardial lipid metabolism. Studies in mice indicate that myocardial BMIPP uptake correlates with adenosine triphosphate (ATP) content. Details, however, of myocardial accumulation during episodes of ischemia from either infarct or ischemia are not well documented.
Application: [^{123}I]-BMIPP can be utilized to detect and characterize myocardial lipid metabolism that can potentially be used as a parameter to differentiate myocardial ischemia from myocardial infarction.
Route of Administration: [^{123}I]-BMIPP (intravascular).
Implementation: Evaluation of myocardial regional wall motion applying echocardiography in the short-axial view from the epicardium. Tissue blood flow can be calculated using non-radioactive colored microspheres. Changes in blood glucose levels, lipid levels, and lactate extraction can be examined in blood collected from the aorta and great cardiac vein (GCV). The ATP concentration and [^{123}I]-BMIPP count are determined by high-performance liquid chromatography and gamma counter measurements, respectively.

Animal Model: Canine (n = 16 adult mongrel dogs). The occluded left anterior descending artery (LAD) alone is reperfused to create an ischemic region, and the 1st diagonal branch of the LAD is kept occluded to make the infarct lesion.

Interpretation and Analysis: Two hours after reperfusion, blood flow decreases to 20% ± 5% in infarct regions and 64% ± 9% in ischemic areas ($p < 0.05$), despite comparable wall-motion reduction (32% ± 5% and 42% ± 12% in the infarct and ischemic areas, respectively). BMIPP content and ATP concentration display parallel reductions: 40% ± 7% and 75% ± 4% ($p < 0.05$) of BMIPP and 32% ± 9% and 69% ± 7% ($p < 0.05$) of ATP in infarct and ischemic regions, respectively. Non-esterified fatty acid extraction, defined as "flow × ([artery] − [GCV])," decreases to 87% ± 5.6% during occlusion and 75% ± 20.1% 2 hours after reperfusion compared to control values. BMIPP uptake correlates well with lipid metabolism and tissue ATP levels and may prove useful in differentiating myocardial infarction from ischemia in the acute phase of ischemic episodes.

Evaluation of myocardial viability with iodine-123-BMIPP in a canine model. Nohara R, Okuda K, Ogino M, Hosokawa R, Tamaki N, Konishi J, Fujibayashi Y, Yonekura Y, Fujita M, Sasayama S. J Nucl Med 1996 Aug; 37 (8), pp. 1403–7.

Lipoprotein Physiology: (Low-Density Lipoproteins [LDLs])/Atherosclerotic Lesion Pathogenesis: Altered Cell LDL Assimilation/Diabetes Mellitus and Hemodialysis
[^{125}I]-Labeled LDLs

Cellular/Molecular Mechanism of Action: [^{125}I]-labeled LDLs are radiolabeled analogs of LDLs. Low-density lipoproteins can function as a transport platform in the plasma and contain a relatively high amount of triglycerides and cholesterol compared to high-density lipoproteins.
Background Information: Patients on chronic hemodialysis treatment have an elevated risk of atherosclerosis disease, and dyslipidemia appears to be 1 of the major risk factors. However, most of these patients exhibit elevated serum triglycerides, whereas serum cholesterol and LDL cholesterol levels are in the normal range.
Application: Characterization of LDL metabolism in *in vitro* and *in vivo* environments.
Route of Administration: In animal (*in vivo*) models, [^{125}I]-labeled LDLs would usually be given intravenously.
Implementation: Evaluate the influence of hypertriglyceridemia condition of hemodialysis and diabetes mellitus on LDL metabolism. One approach to achieving this objective involves the isolation of LDLs from whole blood and separation into 5 subfractions by density gradient ultracentrifugation, followed by their characterization related to lipid/protein composition, degree of glycation, and oxidation. Uptake of [^{125}I]-labeled LDL is examined via LDL receptors of HepG2 cells and scavenger receptors of mouse peritoneal macrophages.

Human Model: Cases of hypertriglyceridemic diabetes; non-diabetic hemodialysis cases; and healthy reference controls (n = 30, n = 10/case group).
Interpretation and Analysis: Hemodialysis procedures promote elevations in serum triglycerides, but cholesterol levels are within normal ranges. Triglyceride enrichment occurs in the very low-density lipoprotein (VLDL) class and LDL class, and an accumulation of a highly atherogenic small dense LDL subfraction can be detected predominantly in cases of non-insulin-dependent diabetes mellitus. Low-density lipoprotein concentrations in responses to hemodialysis also contains elevated levels of lipid peroxidation products, which are at even higher levels in diabetic conditions. Alterations in composition, size, and configuration of LDL in response to hemodialysis in diabetic and non-diabetic conditions impairs LDL receptor-mediated degradation and enhances uptake of such modi-

fied LDL particles via non-saturable scavenger receptors. Diminished LDL receptor-mediated uptake of modified, triglyceride-rich, small dense LDL most likely leads to accumulation of these lipoproteins *in vivo*, favoring the development of atherosclerotic lesions. Future clinical studies must demonstrate whether patients will benefit from reducing these atherogenic particles by lipid-lowering intervention.

Abnormalities in lipoprotein metabolism in hemodialysis patients. Köniğer M, Quaschning T, Wanner C, Schollmeyer P, Krämer-Guth A. Kidney Int Suppl 1999 Jul; 71, pp. S248–50.

Metabolism: Glucose, Amino Acids, and Nucleic Acid/Neoplastic Disease Imaging: Monitoring Treatment Response/Glucose Metabolism/Amino Acid Metabolism/Nucleic Acid Metabolism

2′-Deoxy-5-[^{18}F]Fluorouridine/[^{18}F]-5-Fluorodeoxyuridine/L-Methyl-[^{14}C]Methionine/6-[^{3}H]Thymidine

Cellular/Molecular Mechanism of Action: Reflects neoplastic cell nucleic acid metabolism.
Application: Diagnostic imaging of neoplastic lesions.
Route of Administration: [^{18}F]-5-fluorodeoxyuridine.
Dosage: [^{18}F]-5-fluorodeoxyuridine.
Implementation: Evaluation of metabolic tracers for glucose, amino acid, and nucleic acid metabolism (2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}FDG), L-methyl-[^{14}C]methionine ([^{14}C]-Met), 6-[^{3}H]thymidine (^{3}H-Thd), and 2′-deoxy-5-[^{18}F]fluorouridine (^{18}FdUrd)], and the conventional radionuclide [^{67}Ga]-citrate can be used to assess the feasibility of monitoring tumor radiotherapy using a quadruple tracer technique. In this context, 2 combinations of 4 tracers (^{18}FDG or ^{18}FdUrd, [^{14}C]-Met, ^{3}H-Thd, and ^{67}Ga) can be compared in a time-course study after single-dose irradiation (20 Gy) and also used in a dose-dependency study performed 6 days after 5, 10, 15, or 20 Gy of irradiation.

Animal Model: Rat AH109A tumor model.
Interpretation and Analysis: Fluorine-18-FDG (^{18}F-FDG) shows a large change in uptake and a steady response to radiotherapy. Fluorodeoxyuridine shows a rapid decrease after radiotherapy, but the range of change in uptake is narrow. Gallium-67 cannot detect tumor response early after treatment but shows a marked change in uptake later. [$^{6\text{-}3}$H]Thd and [^{14}C]-Met show a rapid response to irradiation and a high sensitivity for monitoring radiotherapy, suggesting that they may be applicable for PET studies.

Tracer feasibility for monitoring tumor radiotherapy: A quadruple tracer study with fluorine-18-fluorodeoxyglucose or fluorine-18-fluorodeoxyuridine, L-[methyl-^{14}C]methionine, 6-[^{3}H]thymidine, and gallium-67. Kubota K, Ishiwata K, Kubota R, Yamada S, Tada M, Sato T, Ido T. J Nucl Med 1991 Nov; 32 (11), pp. 2118–23.

Metabolism Conditions: Multiple/Glucose: Ketogentic Hypoglycemia/Branched-Chain Ketoacid Decarboxylase: Leukocyte/Maple Syrup Urine Disease

α-Ketoisocaproic Acid (KIC)/α-Ketoisovaleric Acid (KIV)/Branched-Chain Amino Acids/Isocaloric High Protein/Ketogenic Diet

Cellular/Molecular Mechanism of Action: α-ketoisocaproic acid is a substrate for leukocyte branched-chain ketoacid decarboxylase.
Application: Detection and characterization of ketogenic hypoglycemic conditions.
Implementation: Evaluation of plasma amino acid concentrations during spontaneous episodes of hypoglycemia. Complementary studies include the evaluation of leukocyte branched-chain ketoacid decarboxylase biochemical activity applying KIC as a substrate probe.

Human Model: Cases of ketogenic hypoglycemia (e.g., n = 1 female 8 years old with mental deficiency and retarded motor and somatic development).
Interpretation and Analysis: Analysis of plasma amino acid concentrations during the onset of spontaneous hypoglycemia reveals increases in branched-chain amino acids (e.g., valine [4.1], leucine [7.8], and isoleucine [1.7 mg/100 ml]), while alanine decreases (1.2 mg/100 ml) and ketonuria can be detected. Measurement of branched-chain ketoacid decarboxylase in leukocytes can detect decreases of approximately 50% of normal for KIC as substrate, whereas values for KIV and α-keto-β-methylvaleric acid (MEVA) are normal. Values for fibroblast activities for all 3 substrates are usually within the normal range. Intermittent maple syrup urine disease can be excluded by oral loading tests with the branched-chain amino acids and with an isocaloric, high-protein diet. Impairment of oxydative decarboxylation of leucine, valine, and isoleucine secondary to increased ketogenesis may play an etiological role in ketotic hypoglycemia, because reports have observed, by gaschromatographic analysis, increases in the urinary excretion of KIV (5.5 μmol/h), KIC (29.4), and MEVA (47.9) after a provocative test with an isocaloric ketogenic diet for 36 hours. The significance of branched-chain hyperaminoacidemia and branched chain α-ketoaciduria has been described.

Intermittent branched-chain ketoacidurie in ketotic hypoglycemia: Investigations to localize the biochemical defect (author's transl). Held KR, Sternowsky HJ, Singh S, Plettner C, Grüttner R. Monatsschr Kinderheilkd 1976 Feb; 124 (2), pp. 59–65

Mitosis/Proliferation: Neoplastic Disease/Deoxyribonucleic Acid (DNA) Synthesis/Thymidine Kinase Expression

5-Iodo-4′-Thio-2′-Deoxyuridine (ITdU)/5-Iodo-4′-Thio-2′-Deoxyuridine (IUdR)

Cellular/Molecular Mechanism of Action: 5-iodo-4′-thio-2′-deoxyuridine is a metabolically stable nucleoside that can function as a substrate for cellular thymidine kinase biochemical activity.
Application: Radioiodinated forms of ITdU and IUdR can be applied to detect elevations of DNA synthesis in cell populations that positively express thymidine kinase activity. In this capacity, both ITdU and IUdR can serve as indicator molecules for measuring alterations in cellular proliferation especially relevant to the characterization of neoplastic disease states.
Route of Administration: Most frequently, ITdU or IUdR would be administered intravascularly. Theoretically, it may be possible to effectively image lesions if the agents are administered intrathecally for central nervous system (CNS) tumors, by direct injection into tumors, or into the arterial network supplying a suspected neoplastic lesion. Future investigation remain to be conducted to determine the feasibility, effectiveness, and safety of such applications.
Semi-Synthetic Methods: Radiolabeling of ITdU and 5-iodo-1-(4-thio-beta-D-arabinofuranosyl)uracil (ITAU) with [^{125}I] utilizing a destannylation reaction of the trimethylstannyl precursor of each nucleoside. The products can be isolated in high yields and with a > 99% level of radiochemical purity.
Implementation: Evaluation of ITdU and ITAU for their ability to detect and characterize alterations in cellular proliferation as a function of DNA synthesis.

***Ex Vivo* Model:** Thymidine kinase-expressing L-M cells rather than a thymidine kinase-deficient mutant L-M (TK^-) cells.

Interpretation and Analysis: [^{125}I]-ITdU is effectively phosphorylated by cytosolic nucleoside kinases and specifically incorporated into a thymidine kinase-expressing L-M cell rather than a thymidine kinase-deficient mutant L-M (TK^-) cell. In addition, *in vitro* cell metabolism studies employing [^{125}I]-ITdU reveal that [^{125}I]-ITdU is effectively and specifically incorporated into a DNA fraction (> 90% at 60 minutes). Therefore, [^{125}I]-ITdU appears to be an effective DNA synthesis marker such as IUdR. In contrast, [^{125}I]-ITAU is neither remarkably phosphorylated by cytosolic nucleoside kinases nor notably incorporated into an L-M cell rather than an L-M (TK^-) cell. [^{125}I]-ITdU and [^{125}I]-ITAU show a higher resistance to phosphorolytic cleavage by recombinant thymidine phosphorylase than does [^{125}I]-IUdR. Furthermore, biodistribution of [^{125}I]-ITdU and [^{125}I]-ITAU display better *in vivo* stability of radioiodination than do [^{125}I]-IuUR preparations. [^{125}I]-ITdU also displays a significantly higher uptake in proliferating organs (thymus, spleen, small intestine, and bone) than in non-proliferating organs (brain, muscle, liver, and lung), compared to [^{125}I]-IUdR, at 18 hours postinjection. As indicated by *in vitro* studies, [^{125}I]-ITAU does not show any significant uptake in proliferating organs. Therefore, radioiodinated ITdU is potentially useful as a proliferation-imaging agent, and further studies should be performed to clarify the usefulness of this compound as a single photon emission computed tomography tumor-imaging agent.

Rationale of 5-[^{125}I]-iodo-4′-thio-2′-deoxyuridine as a potential iodinated proliferation marker. Toyohara J, Hayashi A, Sato M, Tanaka H, Haraguchi K, Yoshimura Y, Yonekura Y, Fujibayashi Y. J Nucl Med 2002 Sep; 43 (9), pp. 1218–26.

Mitosis/Proliferation Rate of Cell Populations/ Neoplastic Lesions: Potential Application
3′-Deoxy-3′-[^{18}F]Fluorothymidine

Cellular/Molecular Mechanism of Action: Thymidine is rapidly assimilated by proliferating cell populations.

Application: 3′-deoxy-3′-[^{18}F]fluorothymidine has been designed as an agent for the detection of alterations in *in vivo* cellular proliferation as a function of thymidine metabolism utilizing positron emission tomography imaging techniques.

Route of Administration: Variable but most often intravascular.

Semi-Synthetic Methods: A reliable radiosynthesis of 3′-deoxy-3′-[^{18}F]fluorothymidine ([^{18}F]-FLT) has been developed based on [^{18}F]10 mCi (370 MBq) of radiochemically pure [^{18}F]1 Ci/μmol (37 GBq/μmol) at end of synthesis (EOS) within 100 minutes and in 13% radiochemical yield (end of bombardment [EOB]; 7% EOS).

Implementation: Evaluation of neoplastic lesions that display relatively rapid rates of proliferation/growth.

Interpretation and Analysis: Detection of increased radioactivity at a specific anatomical location corresponds to a region with a relatively high rate of cellular proliferation.

Radiosynthesis of 3′-deoxy-3′-[^{18}F]fluorothymidine: [^{18}F]FLT for imaging of cellular proliferation *in vivo*. Grierson JR, Shields AF. Nucl Med Biol 2000 Feb; 27 (2), pp. 143–56.

Nitric Oxide Production Potential/Gastroenteritis/ Renal Failure/Septic Conditions
Guanidino-Labeled L-Arginine/[Nitrite/Nitrate or Citrulline Metabolites]

Cellular/Molecular Mechanism of Action: Substrate for nitric oxide synthase.

Background Information: Nitric oxide is an important mediator of both physiological and pathophysiological processes. Nitric oxide is produced during direct conversion of arginine to citrulline. Nitric oxide is rapidly metabolized, mainly to nitrite/nitrate, and finally excreted as urinary nitrate. For that reason, plasma and urinary nitrite/nitrate have been measured frequently as indicators of nitric oxide production, but it is becoming clear that these methods only give qualitative data. More recently, stable isotope methods have been introduced for quantitative measurement of nitric oxide production.

Application: Detection and characterization of nitric oxide synthase biochemical activity.

Implementation: Different stable isotope methods are used to measure whole-body nitric oxide production *in vivo*. These methods are all based on infusion of guanidino-labeled L-arginine and subsequent measurement of labeled products (e.g., nitrite/nitrate or citrulline).

Interpretation and Analysis: Nitric oxide synthesis in healthy individuals is found to be in the range of 0.2–1.0 μmol/kg/hour (only 0.5–1% of arginine production). In diseased states, nitric oxide synthesis may be either decreased or increased. Increased nitric oxide synthesis has been observed in gastroenteritis patients and in some animal models of sepsis. In patients with renal failure, however, both increased and decreased nitric oxide production have been reported. Nitric oxide production is not changed in familial hypercholesterolemia patients and after typhoid vaccination. Therefore, application of stable isotopes to measure whole-body nitric oxide production *in vivo* is the most accurate method to study quantitative changes in the nitric oxide production rate. This technique is easy to perform in both healthy and diseased individuals, requiring infusion of stable isotopes for only a few hours and blood sampling.

Isotopic investigation of nitric oxide metabolism in disease. Luiking YC, Deutz NE. Curr Opin Clin Nutr Metab Care 2003 Jan; 6 (1), pp. 103–8.

Oxidation Potential in Tissues/Central Nervous System: Amyloid Beta Deposition/Free Radical Formation/Alzheimer's Disease
N-Tert-Butyl-α-Phenylnitrone/Oxidation-Activated Fluorogenic Probes

Cellular/Molecular Mechanism of Action: N-tert-butyl-α-phenylnitrone functions as a free radical spin trap.

Background Information: Amyloid beta, the primary constituent of senile plaques in Alzheimer's disease, is hypothesized to cause neuronal damage and cognitive failure, but the mechanisms are unknown.

Application: Fluorogenic compounds can be used *in vivo* to determine the degree of association between amyloid beta deposits and free radical production. The compound N-tert-butyl-α-phenylnitrone can be utilized to validate the effectiveness and accuracy of tracers and probes designed to detect anatomical regions of elevated oxidation potential.

Implementation: Evaluation of microenvironments with elevated levels of oxidative potential utilizing compounds that are actively fluorescent that can serve as *in vivo* infrared laser imaging agents.

As a validation procedure, N-tert-butyl-α-phenylnitrone (a free radical spin trap) can be systemically administered to determine the accuracy of oxidation-activated fluorogenic probes. Collectively, these agents can be utilized to delineate if a direct association exists between amyloid beta deposits and free radical production.

Animal Model: Mouse (*in vivo* transgenic Alzheimer's disease).

Human Model: Brain tissue from cases of Alzheimer's disease (*ex vivo*).

Interpretation and Analysis: Fluorescence can be appreciated in dense core plaques, but not diffuse plaques, as determined by subsequent addition of thioflavine S and immunohistochemistry analysis for amyloid beta. Systemic administration of N-tert-butyl-α-phenylnitrone greatly reduces the detection of probes activated by tissue oxidative processes. It is therefore believed that a subset of amyloid plaques produces free radical species in living Alzheimer's models and in human Alzheimer tissue sections. Antioxidant therapy neutralizes these highly reactive molecules and may therefore be of therapeutic value in Alzheimer's disease.

Instrumentation: Multiphoton imaging.

In vivo imaging of reactive oxygen species specifically associated with thioflavine S-positive amyloid plaques by multiphoton microscopy. McLellan ME, Kajdasz ST, Hyman BT, Bacskai BJ. J Neurosci 2003 Mar 15; 23 (6), pp. 2212–7.

Oxidation/Reduction Potential in Tissues: Intracellular Production and Extracellular Release of Reactive Oxygen Species/Myocardium Metabolism
Dihydroethidium/Dityrosine/Protein Kinase Inhibitors/Chelerythrine/PP101/PP149

Cellular/Molecular Mechanism of Action: Dihydroethidium functions as a fluorescent probe; chelerythrine is a protein kinase C (PKC) inhibitor; PP101 is a PKC delta inhibitor; and PP149 is a PKC-epsilon inhibitor.

Background Information: Protein kinase C and reactive oxygen species (ROS) are known to have a role in anesthetic preconditioning (APC). Cardiac preconditioning by triggers other than volatile anesthetics, such as opioids or brief ischemia, is known to be isoform selective, but the isoform required for APC is not known.

Application: Detection and characterization of intracellular ROS production.

Implementation: Identify the PKC isoform that is involved in APC and elucidate the relative positions of PKC activation and ROS formation in the APC signaling cascade. One approach to addressing this objective can involve the evaluation of the influence of sevoflurane APC in heart tissue prior to the initiation of ischemic reperfusion injury in the absence or presence of the non-specific PKC inhibitor chelerythrine, the PKC-delta inhibitor PP101, or the PKC-epsilon inhibitor PP149. Spectrofluorometry and the fluorescent probes dihydroethidium can be used to measure intracellular ROS, while assay of effluent dityrosine is used to measure extracellular ROS release.

Animal Model: Isolated guinea pig hearts subjected to 30 minutes of ischemia and 120 minutes of reperfusion.

Interpretation and Analysis: Previous sevoflurane exposure protects heart tissues against ischemia-reperfusion injury, as previously described. Chelerythrine or PP149 abolishes such protection, but PP101 does not. Reactive oxygen species formation is observed during sevoflurane exposure and is not altered by any of the PKC inhibitors. Anesthetic preconditioning is mediated by PKC-epsilon but not by PKC-delta. Furthermore, PKC activation probably occurs downstream of ROS generation in the APC signaling cascade.

Reactive oxygen species precede the epsilon isoform of protein kinase C in the anesthetic preconditioning signaling cascade. Novalija E, Kevin LG, Camara AK, Bosnjak ZJ, Kampine JP, Stowe DF. Anesthesiology 2003 Aug; 99 (2), pp. 421–8.

Oxygen Concentration (Oximetry)/Neoplastic vs Normal Tissues
Octa-n-Butoxy-Substituted Naphthalocyanine

Application: Detection and characterization of neoplastic cell types.

Semi-Synthetic Methods: The probe is synthesized as a needle-shaped microcrystalline particulate. The particulate shows a single-line electron paramagnetic resonance (EPR) spectrum that is highly exchange-narrowed with a line-width of 210 mG. The EPR line-width is sensitive to molecular oxygen, showing a linear relationship between the line-width and concentration of oxygen (pO_2) with a sensitivity of 8.5 mG/mm Hg.

Implementation: Evaluation of a variety of physicochemical and biological properties of LiNc-BuO particulates to determine the suitability of the probe for *in vivo* oximetry.

Interpretation and Analysis: The probe is unaffected by biological oxidoreductants, stable in tissues for several months, and can be successfully internalized in cells. The probe has been applied to monitor changes in concentration of oxygen in the normal muscle and RIF-1 tumor tissue of mice as a function of tumor growth. The data showed a rapid decrease in the tumor pO_2 with increases in tumor volume. Human arterial smooth muscle cells, upon internalization of the LiNc-BuO probe, show a marked oxygen gradient across the cell membrane. In summary, the newly synthesized octa-n-butoxy derivative of lithium naphthalocyanine has unique properties that are useful for determining oxygen concentration in chemical and biological systems by EPR spectroscopy and also for magnetic tagging of cells.

Instrumentation: Electron paramagnetic resonance spectroscopy.

Novel particulate spin probe for targeted determination of oxygen in cells and tissues. Pandian RP, Parinandi NL, Ilangovan G, Zweier JL, Kuppusamy P. Free Radic Biol Med 2003 Nov 1; 35 (9), pp. 1138–48.

Oxygen Tension Indicator
Hemoglobin (Hb)

Cellular/Molecular Mechanism of Action: Iron ions contained within Hb fractions bind molecular oxygen.

Application: Exogenous molecular Hb is an effective indicator of relative local oxygen tension in magnetic resonance (MR) microscopy studies performed *in vivo*.

Implementation: Injection of Hb to delineate its ability to permeate through relatively dense neural tissues.

Experimental Model: Flies.

Analysis and Interpretation: Exogenous molecular Hb is more sensitive than other agents for application in MRI oximetry methods, and therefore can be used at higher resolutions and in specimens with no blood oxygen level-dependent (BOLD) effects.

Hemoglobin is able to permeate through relatively dense neural tissue but does not detectably disrupt normal neurophysiology. Large changes in signal intensity (40–50%) can be recognized when external O_2 levels are manipulated artificially from 0% to 21% with hemoglobin injections. Oxygen-dependent contrast changes produced by exogenous Hb can be detected by T_2-weighted imaging analysis and can be roughly calibrated if necessary. Therefore, it may be feasible to apply hemoglobin as a contrast agent technique that may be useful for functional MRI (fMRI) studies of metabolism at minute resolution levels (e.g., 10s of microns).

In vivo oxygen detection using exogenous hemoglobin as a contrast agent in magnetic resonance microscopy. Sun PZ, Schoening ZB, Jasanoff A. Magn Reson Med 2003 Apr; 49 (4), pp. 609–14

Superoxide/Anoxia/Reoxygenation/Cerebral Cortex Ischemia-Reperfusion

2-Methyl-6-[p-Methoxyphenyl]-3,7-Dihydroimidazo [1,2-α]Pyrazin-3-One/[Chemiluminescence Probe]

Application: Detection and characterization of superoxide formation, reperfusion injury, and anoxia/ischemia.

Route of Administration: Intravascular.

Implementation: Evaluation of the exact time profile of superoxide generation during anoxia-reoxygenation and ischemia-reperfusion using chemiluminescence techniques with a probe specific for superoxide (e.g., 2-methyl-6-[*p*-methoxyphenyl]-3,7-dihydroimidazo[1,2-α]pyrazin-3-one or MCLA).

MCLA solutions are superfused on the cortex throughout the experimental protocol, and MCLA chemiluminescence is measured using a newly developed photon counting system. Reflectance at 398 nm is simultaneously measured to compensate for hemodynamic artifacts resulting from cerebral blood volume changes. A 90-second anoxia can be induced by the inhalation of 100% nitrogen followed by a 40-minute reoxygenation. In phase II studies, superoxide dismutase (SOD) is continuously superfused and anoxia-reoxygenation is performed in the same manner. In phase II studies, a 15-minute forebrain ischemia is induced by the occlusion of the bilateral common carotid arteries with systemic hypotension (systolic blood pressure less than 50 mm Hg) followed by a 30-minute reperfusion.

Animal Model: Feline cerebral cortex (anoxia-reoxygenation and ischemia-reperfusion; phase I studies n = 19 animals; phase II studies n = 6 animals; phase III studies n = 8 animals).

Interpretation and Analysis: Phase #1 studies: Chemiluminescence decreases during a period of anoxia ($p < 0.01$) and then exceeds baseline levels at 15 and 20 minutes after reoxygenation ($p < 0.05$). Phase II studies: Chemiluminescence decreases during a period of anoxia ($p < 0.05$) but does not exceed baseline levels during the reoxygenation period, indicating that an increase in superoxide production is the main cause of the chemiluminescence increase**.** In phase III studies chemiluminescence decreases during periods of ischemia ($p < 0.01$) and then increases at 20 and 25 minutes after reperfusion ($p < 0.05$). Such findings indicate that superoxide generation decreases during anoxia and ischemia and then increases within 20 minutes after reoxygenation or reperfusion.

In vivo measurement of superoxide in the cerebral cortex during anoxia-reoxygenation and ischemia-reperfusion. Yamaguchi K, Uematsu D, Itoh Y, Watanabe S, Fukuuchi Y. Keio J Med 2002 Dec; 51 (4), pp. 201–7.

Superoxide Anion Production: Myocardium/ Reactive Oxygen Species/K^+/Adenosine Triphosphate (ATP) Channels: Mitochondrial/ Infarct

Dihydroethidium (Fluorescent Probe)/ 5-Hydroxydecanoate (5-HD = K^+/ATP Channel Blocker)/N-Acetylcysteine and N-2-Mercaptopropionyl Glycine (2-MPG) Reactive Oxygen Species (ROS) Scavengers/Triphenyltetrazolium Superoxide Indicator

Cellular/Molecular Mechanism of Action: Dihydroethidium emits fluorescent color when exposed to certain wavelengths of light energy. 5-hydroxydecanoate inhibits the cellular function of membrane-associated K^+/ATP channels. N-acetylcysteine and 2-MPG function as ROS scavengers. Triphenyltetrazolium detects superoxide anion production.

Background Information: It is unknown if the opening of mitochondrial K^+/ATP channels is a trigger or an end effector of anesthetic-induced preconditioning.

Application: Assess superoxide anion production.

Implementation: Determination if opening of mitochondrial K^+/ATP channels *in vivo* triggers isoflurane-induced preconditioning by generating ROS. One approach to addressing this objective can involve the application of the fluorescent probe dihydroethidium to assess superoxide anion production during administration of 5-HD or the ROS scavengers N-acetylcysteine or 2-MPG in the presence or absence of 1.0 minimum alveolar concentration (MAC) isoflurane. Myocardial infarct size and superoxide anion production can be measured using triphenyltetrazolium staining and confocal fluorescence microscopy, respectively.

Animal Model: Rabbits (pentobarbital anesthetized) subjected to a 30-minute coronary artery occlusion followed by a 3-hour period of reperfusion. Rabbits were randomly assigned to receive a vehicle (0.9% saline) or the selective mitochondrial K^+/ATP channel blocker 5-HD alone 10 minutes before or immediately after a 30-minute exposure to 1.0 MAC isoflurane.

Interpretation and Analysis: Isoflurane ($p < 0.05$) decreases infarct size to 19 ± 3% (mean ± standard error of measurement [SEM]) of the left ventricular area at risk as compared to the control (38 ± 4%). 5-HD administered before but not after isoflurane abolishes this beneficial effect (37 ± 4% as compared to 24 ± 3%). 5-HD alone has no effect on infarct size (42 ± 3%). Isoflurane increases fluorescence intensity. Pretreatment with N-acetylcysteine, 2-MPG, or 5-HD before isoflurane abolishes increases in fluorescence, but administration of 5-HD after isoflurane only partially attenuates increases in fluorescence produced by volatile anesthetic agents (e.g., isoflurane). Therefore, mitochondrial K^+/ATP channel opening acts as a trigger for isoflurane-induced preconditioning by generating ROS *in vivo.*

Instrumentation: Confocal fluorescence microscopy.

Mitochondrial adenosine triphosphate-regulated potassium channel opening acts as a trigger for isoflurane-induced preconditioning by generating reactive oxygen species. Tanaka K, Weihrauch D, Ludwig LM, Kersten JR, Pagel PS, Warltier DC. Anesthesiology 2003 Apr; 98 (4), pp. 935–43.

3 CONTRAST AND IMAGE-ENHANCING AGENTS

16 Fluorescent and Pigment Dye Reagents

Biochemical Markers

Enzyme-Activated Near-Infrared (NIR) Fluorescent Magnetic Nanoparticles/[Indocyanine Dye Cy5.5]

Background Information: A number of quantitative 3-dimensional tomographic NIR fluorescence imaging techniques have recently been developed and combined with magnetic resonance imaging (MRI) to yield highly detailed anatomic and molecular information in living organisms.

Application: Can be utilized as magnetic nanoparticle-based MR contrast agents that have NIR fluorescence (NIRF) capabilities that are activated by certain biological enzymes.

Route of Administration: Subcutaneous, but other prototypes could potentially be given intravascularly.

Semi-Synthetic Methods: Probes are prepared by the conjugation of arginyl peptides to cross-linked iron oxide amine (amino-CLIO), either by a disulfide linkage or a thioether linker, followed by the attachment of the indocyanine dye Cy5.5. The NIRF of disulfide-linked conjugate is activated by dithiothreitol (DTT), while the NIRF of thioether-linked conjugate is activated by trypsin. Fluorescent quenching of the attached fluorochrome occurs in part due to interaction with iron oxide, as evident by the activation of fluorescence with DTT in the presence of nanoparticles that have less than 1 dye molecule attached per particle.

Implementation: Evaluate the potential for enzyme-activated NIR fluorescent magnetic nanoparticles to detect specific biochemical entities expressed by specific cell populations.

Interpretation and Analysis: Following subcutaneous injection of the probe, axillary and brachial lymph nodes become darkened on MR images and easily delineated by NIRF imaging. The probes may provide the basis for a new class of so-called smart nanoparticles, capable of pinpointing their position through their magnetic properties, while providing information on their environment by optical imaging techniques.

Near-infrared fluorescent nanoparticles as combined MR/optical imaging probes. Josephson L, Kircher MF, Mahmood U, Tang Y, Weissleder R. Bioconjug Chem 2002 May–Jun; 13 (3), pp. 554–60.

CARDIOVASCULAR SYSTEM

Angiogenesis: Neoplastic Disease/Tumor Dimethylarginine Dimethylaminohydrolase (DDAH) Overexpression

NC100150/Hoechst 33342

Cellular/Molecular Mechanism of Action: The special orientation of NC100150 becomes altered within a magnetic field. Alternatively, Hoechst 33342 emits fluorescent color when exposed to light energy.

Background Information: Intracellular factors that regulate nitric oxide (NO) synthesis represent important targets in tumor progression. Overexpression of DDAH, which metabolizes the endogenous inhibitors of NO synthesis asymmetric dimethylarginine and N-monomethyl-L-arginine, results in C6 gliomas with enhanced growth rate compared with wild types.

Application: NC100150 can be utilized as an intravascular blood pool contrast agent that is detectable by magnetic resonance imaging (MRI). Hoechst 33342 can be used as a fluorescent perfusion-marker dye that can be measured by flourescent detection systems.

Route of Administration: Utilization of NC100150 and Hoechst 33342 for neoplastic lesion angiogenesis requires that these be delivered intravascularly.

Implementation: Evaluation of the *in vitro* effects of DDAH on tumor vascular morphogenesis by measurement of the transverse relaxation rates R_2* and R^2 in (i) clone D27 gliomas overexpressing DDAH; (ii) C6 wild-type gliomas using intrinsic susceptibility MRI sensitive to changes in endogenous deoxyhemoglobin; (iii) evaluation of CN100150 as an intravascular blood pool contrast agent for susceptibility contrast-enhanced MRI; and (iv) comparison of results with fluorescence microscopy utilized to assess tumor uptake of Hoechst 33342 (perfusion marker system).

Interpretation and Analysis: The baseline R_2* is significantly faster in the D27 tumors, consistent with a greater vascular development ($p < 0.02$, ANOVA). There is no significant difference between the response of the 2 tumor types to hypercapnia (5% CO_2/95% air) used as a probe for vascular maturation, or hyperoxia (5% CO_2/95% O_2) used as a probe for vascular function. NC100150 increases the R_2* and R_2 rates of both tumor types and demonstrates a significantly larger blood volume in the D27 tumors ($p < 0.02$, ANOVA). This correlates with a significantly greater uptake of Hoechst 33342 in D27 tumors compared to C6 wild-type tumors ($p < 0.02$, ANOVA). Despite increased tumor blood volume, the Delta R_2*/Delta R_2 ratio, an index of microvessel size, shows that the capillaries in the 2 tumor types are of a similar caliber. Such observations demonstrate the potential of susceptibility MRI-derived quantitative end points to non-invasively assess tumor angiogenesis. Application of intravascular blood pool contrast agents such as NC100150 for such purposes appears very promising. Overexpression of DDAH results in increased *in vivo* neovascularization of C6 gliomas. The lack of significant difference in hypercapnic/hyperoxic response between the C6 and D27 tumors and the similar vessel caliber is also consistent with a role for DDAH in the initial stages of vasculogenesis.

Instrumentation: MRI and fluorescence microscopy instrumentation.

Effects of overexpression of dimethylarginine dimethylaminohydrolase on tumor angiogenesis assessed by susceptibility magnetic resonance imaging. Kostourou V, Robinson SP, Whitley GS, Griffiths JR. Cancer Res 2003 Aug 15; 63 (16), pp. 4960–6.

Blood Volume Estimation/Intravascular Compartment

Fluorescein Isothiocyanate-Labeled Hydroxyethyl Starch/Radiolabeled Red Blood Cells/Radiolabeled Serum Albumin

Cellular/Molecular Mechanism of Action: Labeled albumin, red blood cells, and hydroxyethyl starch can be applied to measure approximate intravascular blood volume.

Application: Estimation of approximate blood volume.
Route of Administration: Intravascular.
Semi-Synthetic Methods: The proprietary brand of hydroxyethyl starch (Elohaes) can be conveniently labeled with fluorescein isothiocyanate.
Implementation: Evaluation of blood volume measurements in suspected cases of polycythemia utilizing labeled albumin, red blood cells, and hydroxyethyl starch (Elohaes). Dilution of labeled hydroxyethyl starch (Elohaes) *in vivo* is used for measuring blood volume, and the results are then compared with values obtained using radiolabeled albumin and radiolabeled red cells.

Human Model: Cases of suspected polycythemia (e.g., n = 17; n = 8 undergoing major surgery and/or receiving intensive postoperative care).
Interpretation and Analysis: The elimination of labeled starch follows the same progress as that of the parent compound, indicating that *in vivo* the fluorescent tag is stable and does not significantly alter the biological properties of the molecule. The volume of distribution of labeled starch is 2.5 mL/kg lower than that for labeled albumin ($p = 0.05$). Blood volume, measured from the dilution of fluorescent starch, is lower (4.9 mL/kg) than that measured with albumin ($p = 0.048$) but higher (6.61 mL/kg) than that measured with red blood cells ($p = 0.0007$). This latter difference may be even smaller at marginally higher doses of the fluorescent starch. Hydroxyethyl starch appears to provide a valid alternative to labeled red cells as a means of calculating blood volume. Labeling of starch with a fluorescent marker makes the assay procedure more sensitive and infinitely easier. The dose required is not high enough to affect hemodynamic parameters.

Measuring blood volume with fluorescent-labeled hydroxyethyl starch. Thomas E, Jones G, de Souza P, Wardrop C, Wusteman F. Crit Care Med 2000 Mar; 28 (3), pp. 627–31.

Capillary Blood Flow/Multiphoton *In Vivo* Imaging
Semiconductor Nanocrystal Fluorescent Markers/Cadmium Selenide-Zinc Sulfide Quantum Dots

Cellular/Molecular Mechanism of Action: Cadmium selenide-zinc sulfide quantum dots modalities emit fluorescent light energy.
Application: Cadmium selenide-zinc sulfide quantum dots can be applied as a fluorescent marker system for evaluation of various physiological parameters.
Implementation: Semiconductor nanocrystals (quantum dots) can be utilized as fluorescent marker agents for application miltiphoton microscopy, enabling the *in vivo* acquisition of multicolor images. Examples in this regard include water-soluble cadmium selenide-zinc sulfide quantum dots because these fluorescent probes have 2-photon action cross sections as high as 47,000 Goeppert-Mayer units, which is by far the largest of any label used in multiphoton microscopy.

Animal Model: Mouse.
Interpretation and Analysis: It is possible to visualize quantum dots dynamically through the skin of living mice, in capillaries hundreds of micrometers deep. Evidence of blinking (fluorescence intermittency) cannot be appreciated in solution on nanosecond to millisecond time scales.
Instrumentation: Multiphoton microscopy.

Water-soluble quantum dots for multiphoton fluorescence imaging *in vivo*. Larson DR, Zipfel WR, Williams RM, Clark SW, Bruchez MP, Wise FW, Webb WW. Science 2003 May 30; 300 (5624), pp. 1434–6.

Capillary Permeability/Blood Flow and Arterial Input Function/Neoplastic Disease (Adenocarcinoma)
Indocyanine Green (ICG)/Methylene Blue (MB)

Cellular/Molecular Mechanism of Action: Indocyanine green and MB are "passive" dye-based marker molecules.
Application: Indocyanine green and MB can be utilized as aqueous soluble molecular marker systems for the evaluation of various physiological processes.
Implementation: Evaluation of the ability for ICG and MB to differentiate between normal and diseased tissues. Following administration of ICG and MB, measurements are conducted with a combined frequency-domain and steady state optical technique that facilitate rapid measurement of tissue absorption in the 650–1,000-nm spectral region. Tumors can also be imaged by use of contrast-enhanced magnetic resonance imaging (MRI) and co-registered with the location of the optical probe. The absolute concentrations of contrast agent, oxyhemoglobin, deoxyhemoglobin, and water are measured simultaneously each second for approximately 10 minutes.

Animal Model: Rat (adenocarcinoma).
Interpretation and Analysis: The differing tissue uptake kinetics of ICG and MB in late-stage tumors arise from differences in their effective molecular weights. Indocyanine green, because of its binding to plasma proteins, behaves as a macromolecular contrast agent with a low degree of vascular permeability (low passive diffusion across endothelial barriers). A compartmental model describing ICG dynamics is used to quantify physiologic parameters related to capillary permeability. In contrast, MB behaves as a small-molecular-weight contrast agent that leaks rapidly from the vasculature into the extravascular, extracellular space and is sensitive to blood flow and the arterial input.

In vivo quantification of optical contrast agent dynamics in rat tumors by use of diffuse optical spectroscopy with magnetic resonance imaging co-registration. Cuccia DJ, Bevilacqua F, Durkin AJ, Merritt S, Tromberg BJ, Gulsen G, Yu H, Wang J, Nalcioglu O. Appl Opt 2003 Jun 1; 42 (16), pp. 2940–50.

Cardiac Output: Cardiography/Cardiac and Intravascular Filling/Output
Lithium Chloride/Lithium Dilution Cardiac Output (LiDCO)

Cellular/Molecular Mechanism of Action: Lithium ion can be applied as a "passive" elemental tracer agent.
Background Information: Lithium dilution cardiac output was primarily developed as a simple calibration for the PulseCO trade mark (LiDCO, London, UK) continuous arterial waveform analysis monitor.
Application: Utilized in minimally-invasive techniques designed to measure cardiac output where lithium is used as a passive tracer element that is detected in dilution-based analysis procedures.
Route of Administration: Lithium chloride (intravenous).
Implementation: Evaluation of cardiac output employing a small dose of lithium chloride injected as an intravenous bolus. Cardiac output is derived from the dilution curve generated by a lithium-sensitive electrode attached to the arterial line.
Interpretation and Analysis: The technique is quick, simple, and requires only an arterial line and central or peripheral venous access. Studies in humans and animals have shown good agreement compared with results obtained with other techniques, and the efficacy of LiDCO in pediatric patients has also been proven. Compared with thermodilution, lithium dilution shows closer agreement in

clinical studies with electromagnetic flow measurement. PulseCO is a beat-to-beat cardiac output monitor that calculates stroke volume from the arterial pressure waveform using an autocorrelation algorithm. The algorithm is not dependent on waveform morphology, but instead it calculates nominal stroke volume from a pressure-volume transform of the entire waveform. The nominal stroke volume is converted to actual stroke volume by calibration of the algorithm with LiDCO instrumentation. Initial studies indicate good fidelity, and results from centers in the United States and the United Kingdom have been very beneficial. The PulseCO trademark monitor incorporates software for interpretation of the hemodynamic data generated and provides a real-time analysis of arterial pressure variations (i.e., stroke volume variation, pulse pressure variation, and systolic pressure variation) as theoretical guides to intravascular and cardiac filling.

Instrumentation: Lithium-sensitive electrode attached to the arterial line.

Lithium dilution measurement of cardiac output and arterial pulse waveform analysis: An indicator dilution calibrated beat-by-beat system for continuous estimation of cardiac output. Jonas MM, Tanser SJ. Curr Opin Crit Care 2002 Jun; 8 (3), pp. 257–61.

Cardiovascular: Volume Estimation/Intravascular (Blood) Volume/Extravascular Volume
Indocyanine Green (ICG) (COLD System)/Methylene Blue (MB)/Dual Thermal-Dye Double Indicator Technique

Cellular/Molecular Mechanism of Action: Indocyanine green and MB are molecules that can function as passive marker molecules within body fluid compartments (e.g., intravascular).

Physiological Mechanism of Action: Methylene blue apparently induces acute vasoconstrictive and positive inotropic effects during episodes of septic shock.

Application: Indocyanine green and MB can be used as detectable molecular markers in biological systems to evaluate a variety of physiological processes.

Route of Administration: Methylene blue (intravenous).

Dosage: Bolus dose of MB (3 mg/kg over 10 minutes).

Implementation: Assessment of the effects of inhibition of guanylate cyclase, an enzyme involved in sepsis-related vascular and myocardial dysfunctions, on hemodynamic variables including blood volume and pulmonary vascular permeability during septic shock.

A fiber-optic catheter is inserted to determine blood and extravascular volumes applying the thermal-dye double indicator technique, using ICG (COLD system) in combination with MB. COLD-derived variables are recorded before MB and 20 minutes, 1 hour, and 2 hours after the end of MB infusion.

Human Model: Septic shock associated with persistent hypotension despite conventional treatment including fluid loading, vasopressors, and inotropes (n = 15 cases). Standard hemodynamic and oxygen-derived variables; total, intrathoracic, systolic, and diastolic cardiac blood volumes; extravascular lung water; plasma osmolarity; and lactate and protein concentrations are recorded.

Interpretation and Analysis: Mean arterial and pulmonary artery pressures, systemic and pulmonary vascular resistances, and left ventricular stroke work index increase, and blood lactate transiently decreases after MB ($p < 0.05$). Other variables recorded are unchanged during a 2-hour period following MB infusion. Such findings demonstrate the acute vasoconstrictive and positive inotropic effects of MB during septic shock. These effects are not associated with changes in blood volume, myocardial diastolic function, or pulmonary vascular permeability assessed by extravascular lung water.

Does methylene blue administration to septic shock patients affect vascular permeability and blood volume? Donati A, Conti G, Loggi S, Münch C, Coltrinari R, Pelaia P, Pietropaoli P, Preiser JC. Crit Care Med 2002 Oct; 30 (10), pp. 2271–7.

Dermal Microvasculature Perfusion/Delineation of Perfused Capillary to Total Capillary Ratio/Dermal Nutritional Capillaries
Sodium Fluorescein

Application: Delination of the nature of temporal variation in capillary numbers, and determination of the proportion of perfused to total nutritional capillaries in normal skin.

Implementation: Using *in vivo* microscopy, the temporal behavior of the number of visible capillaries in the skin of the dorsum of the foot is observed over time periods varying from 5 minutes to 55 days. Capillary perfusion is then studied by comparing capillary numbers before and after intravenous injection of sodium fluorescein.

Human Model: Healthy subjects.

Interpretation and Analysis: The mean percent difference in the number of visible capillaries over a mean period of 25.3 days is 5.5%. The percentage ratio of perfused to total capillaries is 54.2%. There appears to be little quantitative change in capillary numbers over periods of up to 50 days, and under physiological conditions, about half of the nutritional capillaries of the skin are not perfused.

Study of temporal and perfusion physiology of skin capillaries in the dorsum of the foot. Lamah M, Mortimer PS, Dormandy JA. J Vasc Res 2001 Jan–Feb; 38 (1), pp. 59–63.

Dermal Microvasculature Perfusion/Ulcers of Dermal Structures/ Dermal Microvascular Disease (Diabetes-Associated Foot Lesions)/Prognosis and Prediction of Outcome
Sodium Fluorescein

Background Information: Fluorescein dermofluorometry can be used to relate the uptake of fluorescein in the skin to blood flow.

Application: The dye wash-in time constant parameters are inversely proportional to local dermal blood flow.

Implementation: Evaluation of fluorescein dermofluorometry for the detection and characterization of dermal ulcers. Following intravenous injection of sodium fluorescein, changes in the fluorescein signal with time are continuously measured at the plantar surface of the foot. Both the initial slope of the signal and the wash-in time constant are then calculated. In order to establish a correlation between image profiles and prognosis for foot ulcer lesions, outcomes are monitored for a variable period of time following initial evaluation (e.g., n = 4- to 14-month follow-up period).

Human Model: Cases of cutaneous diabetic foot ulcers (n = 4 groups: non-diabetic control patients, diabetic control patients, diabetic patients with chronic foot ulcers, and diabetic patients with acute foot ulcers).

Interpretation and Analysis: Significant differences in the wash-in time constant can be appreciated between diabetic and non-diabetic cases and between diabetic subjects with and without foot ulcers. In cases with foot ulcers, a relatively large percent may not display an early washout in the dermofluorometer signal and later require amputation (e.g., n = 2/8 or 25%). The fluorescein wash-in time constant demonstrates better correlation with the presence of

diabetic microvascular disease than does the initial slope of the signal. Differences in the wash-in time constants of non-diabetic and diabetic subjects support the hemodynamic hypothesis for the development of microvascular disease. The indication of early washout of the fluorescein signal may also be useful in the prediction of ulcer healing.

Fluorescein dermofluorometry for the assessment of diabetic microvascular disease. Oh DK, Jones RN, Marshall W, Magin RL. Skin Res Technol 2001 May; 7 (2), pp. 105–11.

Endothelial Cell Adhesion: Colon Carcinoma/ Intravital Observation of Cancer Cell/ Microcirculation Interactions/Hepatic Metastasis Assessment
CalceinAM

Background Information: Organ-specific tumor cell adhesion within the microcirculation of host organs is an important step in metastatic behavior. Circulating tumor cells have to adhere within the microcirculatory vessels, quickly stabilize their adhesion, and probably leave the circulation to avoid toxic effects of hydrodynamic shear forces of circulating blood.
Application: Intravital observation of colon carcinoma cell adhesion within the hepatic microcirculation.
Implementation: CalceinAM is used to label colon carcinoma cell populations (e.g., HT-29 [human] and CC531 [rat]) that are then injected intra-arterially as single-cell suspensions. Intravital fluorescence microscopy is then applied to detect adhesive interactions between circulating tumor cells and endothelial surfaces within the hepatic microcirculation. These interactions are then analyzed regarding their time course and site location within the vascular tree.
Animal Model: Rats (Sprague-Dawley).
Interpretation and Analysis: Autofluorescence of liver parenchyma is sufficient for distinction of hepatic sinusoids. Intravital microscopy is capable of differentiating early events in adhesion formation within hepatic sinosoids, adhesion stabilization, and extravasation of tumor cells into the liver parenchyma. Tumor cell adhesion occurs almost exclusively within sinusoidal capillaries; however, the diameter of these vessels is usually larger than that of the tumor cells. Colon carcinoma cells rapidly migrate into the liver parenchyma after successful adhesion within the sinusoids. In contrast to common end point assays of the metastatic cascade, this *in vivo* model allows characterization of metastatic colon carcinoma cell adhesion within the liver microcirculation as a specific step during the formation of hematogenous metastasis and underlying mechanisms.
Instrumentation: Intravital fluorescence microscopy.

An intravital model to monitor steps of metastatic tumor cell adhesion within the hepatic microcirculation. Haier J, Korb T, Hotz B, Spiegel HU, Senninger N. J Gastrointest Surg 2003 May–Jun; 7 (4), pp. 507–14; discussion 514–5.

Endothelium Cell Adhesion/Platelet Endothelium Interactions/[Retinal Microvascular Pathology]
Carboxyfluorescein Diacetate Succinimidyl Ester/Platelets (Fluorescent-Labeled)

Cellular/Molecular Mechanism of Action: Carboxyfluorescein diacetate succinimidyl ester interacts with light and emits fluorescent color. In this context, it can function as a fluorescent marker molecule suitable for "tracing" the migration of different cell types.
Application: Visualization of *in vivo* platelet behavior in the retinal microcirculation. The methodology allows for quantitative evaluation of platelet dynamics and platelet-endothelial interactions in retinal pathologic conditions.
Route of Administration: Intravascular.
Considerations: Represents the development of a new method for evaluation of the *in vivo* dynamics of platelets in the retinal microcirculation and quantitative investigation of platelet-endothelial interactions.
Implementation: Isolated platelet samples are labeled *in vitro* with carboxyfluorescein diacetate succinimidyl ester. After intravenous administration, platelet behavior in the retinal microcirculation can be evaluated with a scanning laser ophthalmoscope. Images are recorded on S-VHS videotape and analyzed with a computer-assisted image analysis system. Interactions between platelets and retinal microvascular endothelium can also be investigated employing lipopolysaccharide-stimulated endothelium or platelets activated with thrombin.
Animal Model: Rat retina.
Interpretation and Analysis: Fluorescent platelets are recognized as distinct dots in the retinal microcirculation and can be traced frame by frame. The velocity of platelets in the retinal arteries, capillaries, and veins is approximately 26.1 $\pm$ 6.4, 1.6 $\pm$ 0.4, and 19.9 $\pm$ 8.2 mm/sec, respectively. In control rats, even activated platelets show minimal interaction with retinal endothelial cells. In contrast, stimulated retinal endothelium displays active platelet-endothelial interactions, with many platelets observed rolling and adhering along the major retinal veins. The interactions between platelets and stimulated endothelial cells are substantially inhibited with the injection of P-selectin monoclonal antibody.

In vivo evaluation of platelet-endothelial interactions in retinal microcirculation of rats. Tsujikawa A, Kiryu J, Nonaka A, Yamashiro K, Nishiwaki H, Tojo SJ, Ogura Y, Honda Y. Invest Ophthalmol Vis Sci 1999 Nov; 40 (12), pp. 2918–24.

Extracellular Fluid Space Distribution Parameters/ Interstitial Fluid Compartment/Water Diffusion Coefficients
Gallamine/Inulin

Cellular/Molecular Mechanism of Action: Gallamine and inulin per se do not exert any biochemical or physiological mechanism of action. The mechanism whereby they function as diagnostic agents is based on their molecular weight relative to normal pathological processes of capillary permeability and the capacity for them to be easily detected in biological fluid samples.
Application: Gallamine and inulin can be detected at diluted concentrations within biological fluids were they can function as passive marker molecules.
Route of Administration: Gallamine and inulin (simultaneous intravenous injection).
Implementation: Simultaneous evaluation of the kinetics of inulin and gallamine. The distribution of both compounds in extracellular fluid space can be characterized by a 3-compartment model in which the mean central compartment blood volume of 1.37 liters is essentially identical to expected values.
Animal Model: Canine (anesthetized).
Interpretation and Analysis: The 2 peripheral compartments of the model appear to represent rapid and slow equilibrating interstitial fluid compartments. A mammillary model structure can be selected in which intercompartmental clearance corresponds to trans-

capillary exchange. Previous studies indicate that inulin and smaller hydrophyllic molecules diffuse across capillary walls at rates proportional to their respective free water diffusion coefficients. For the ratio of the transcapillary permeability coefficients of inulin and gallamine to equal their free water diffusion coefficient ratio of 5.34 ± 0.02 (± standard deviation [SD]), it appears that the sum of blood flow to the fast and slow interstitial fluid compartments is less than cardiac output. When this assumption is made, blood flow to fast equilibrating interstitial fluid is estimated to be 39% of cardiac output, in agreement with previous measurements of splanchnic blood flow. This supports the hypothesis that the fast equilibrating interstitial fluid space is supplied by porous splanchnic capillaries that lack a continuous investment of basement membrane.

Heterogeneity of interstitial fluid space demonstrated by simultaneous kinetic analysis of the distribution and elimination of inulin and gallamine. Henthorn TK, Avram MJ, Frederiksen MC, Atkinson AJ Jr. J Pharmacol Exp Ther 1982 Aug; 222 (2), pp. 389–94.

Myocardial Infarct Characterization/Necrosis/Areas of Risk
[^{87}Rb]/Evans Blue Dye/Triphenyl Tetrazolium Chloride/ Glibenclaminde/Pinacidil/Bumetanide

Cellular/Molecular Mechanism of Action: (i) Rubidium ion (Rb^+) is a K^+ tracer element; (ii) glibenclamide is a blocker of adenosine triposphate (ATP)-sensitive K^+ channels; (iii) pinacidil is a K^+(ATP) opener; (iv) bumetanide is a $K^+/Na^+/2Cl^-$ co-transporter inhibitor.

Application: [^{87}Rb] can be applied in magnetic resonance imaging (MRI) procedures to identify and characterize myocardial infarcts. Evans blue and triphenyl tetrazolium chloride staining can be applied to delineate areas at risk (AAR) and areas of necrosis, respectively.

Dosage: Glibenclamide (3 μM); pinacidil (10 μM); bumetanide (10 μM).

Implementation: Delineation of the effect of drugs that can modulate passive permeability of K^+ into cardiomyocytes in normal and reperfusion-damaged cardiac muscle. Rubidium ion (Rb^+) is used as a K^+ tracer and [^{87}Rb]-MRI as a detection method. Preparations are incubated in buffer containing either (i) glibenclamide; (ii) pinacidil; (iii) bumetanide; or (iv) no drug (control). Upon reperfusion 3-dimensional [^{87}Rb] MR images are acquired to obtain kinetic profiles for Rb^+ uptake and its distribution. Areas at risk and areas of necrosis are determined by Evans blue and triphenyl tetrazolium chloride staining, respectively.

Animal Model: Swine (left anterior descending artery [LAD] of isolated pig hearts perfused with Krebs-Henseleit buffer [KHB] was occluded for 2 hours and subsequently reperfused for 2 hours with KHB containing 4.7 mM RbCl instead of KCl).

Interpretation and Analysis: The kinetics remain monoexponential in the affected anterior wall and the uptake rates are approximately 32, 36, 37, and 21% of that in the posterior wall in the control, Glib, Pin, and Bum groups, respectively. Infarct sizes determined histologically as a percentage of total ventricular (left + right) mass (14–22%) correspond to sizes of areas with 20 to 40% of maximal Rb image intensity [I(Rb)(max), 15–22%], except for values observed with pinacidil (12.5 vs 21%). The sizes of areas with 20 to 50% of I(Rb)(max) (30–36%) closely correlate with those of AAR determined histologically (31–33%). Lactate dehydrogenase release does not differ in any of the preparations. Therefore, (i) reperfusion damage quickly inhibits Rb^+ uptake; (ii) Rb^+ uptake in normal and reperfused tissue does not significantly depend on K(ATP) or the $K^+/Na^+/2Cl^-$ co-transporter; and (iii) areas with 20–40% of maximal image intensity correspond to infarct areas.

The effects of drugs modulating K^+ transport on Rb^+ uptake and distribution in pig hearts following regional ischemia: [^{87}Rb] MRI study. Kupriyanov VV, Xiang B, Sun J, Jilkina O. NMR Biomed 2002 Aug; 15 (5), pp. 348–55.

Myocardium/Superoxide Anion Production/ Mitochondrial K^+/Adenosine Triposphate (ATP) Channels/Infarct
Fluorescent Probe/ K^+/ATP Channel Blocker/Vitality Stain/Dihydroethidium (Fluorescent Probe)/ 5-Hydroxydecanoate (5-HD = K^+/ATP Channel Blocker)/ Triphenyltetrazolium (Myocardial Infarct)/ N-Acetylcysteine and N-2-Mercaptopropionyl Glycine (ROS Scavengers).

Cellular/Molecular Mechanism of Action: Dihydroethidium emits fluorescent color when exposed to certain wavelengths of light energy. 5-hydroxydecanoate inhibits the cellular function of membrane-associated K^+/ATP channels.

Background Information: It is unknown if the opening of mitochondrial ATP-regulated potassium (K^+/ATP) channels is a trigger or an end effector of anesthetic-induced preconditioning.

Application: Assess superoxide anion production.

Implementation: Determination of whether opening of mitochondrial K^+/ATP channels *in vivo* triggers isoflurane-induced preconditioning by generating reactive oxygen species (ROS). To address this objective, the fluorescent probe dihydroethidium can be used to assess superoxide anion production during the administration of 5-HD or the ROS scavengers N-acetylcysteine or N-2-mercaptopropionyl glycine (2-MPG) in the presence or absence of 1.0 minimum alveolar concentration (MAC) isoflurane. Myocardial infarct size and superoxide anion production can be measured using triphenyltetrazolium staining and confocal fluorescence microscopy, respectively.

Animal Model: Rabbit (pentobarbital anesthetized) subjected to a 30-minute coronary artery occlusion followed by a 3-hour period of reperfusion. Rabbits were randomly assigned to receive a vehicle (0.9% saline) or the selective mitochondrial K^+/ATP channel blocker 5-HD alone 10 minutes before or immediately after a 30-minute exposure to 1.0 MAC isoflurane.

Interpretation and Analysis: Isoflurane ($p < 0.05$) decreases infarct size to 19 ± 3% (mean ± standard error of measurement [SEM]) of the left ventricular area at risk as compared to the control (38 ± 4%). 5-HD administered before but not after isoflurane abolishes this beneficial effect (37 ± 4% as compared to 24 ± 3%). 5-HD alone has no effect on infarct size (42 ± 3%). Isoflurane increases fluorescence intensity. Pretreatment with N-acetylcysteine, 2-MPG, or 5-HD before isoflurane abolishes increases in fluorescence, but administration of 5-HD after isoflurane only partially attenuates increases in fluorescence produced by volatile anesthetic agents (e.g., isoflurane). Therefore, mitochondrial K^+/ATP channel opening acts as a trigger for isoflurane-induced preconditioning by generating ROS *in vivo.*

Instrumentation: Confocal fluorescence microscopy.

Mitochondrial adenosine triphosphate-regulated potassium channel opening acts as a trigger for isoflurane-induced preconditioning by generating reactive oxygen species. Tanaka K, Weihrauch D, Ludwig LM, Kersten JR, Pagel PS, Warltier DC. Anesthesiology 2003 Apr; 98 (4), pp. 935–43.

Reperfusion Injury/Leukocyte Rolling Phenomenon Following Ischemic Insult
Acridine Orange

Cellular/Molecular Mechanism of Action: Acridine orange emits a fluorescent color when exposed to certain wavelengths of light energy.

Background Information: Recent reports have shown that ischemic preconditioning induces strong protection against retinal damage subsequent to prolonged ischemia and that this protection is mediated by mechanisms involving the adenosine A1 receptor.

Application: Evaluate quantitatively the effects of ischemic preconditioning on leukocyte-mediated reperfusion injury after transient retinal ischemia and define the role of the adenosine A1 receptor in these effects.

Implementation: The adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) is administered intramuscularly immediately after ischemic preconditioning. Leukocyte behavior in the retina after 60 minutes of ischemia can be evaluated *in vivo* with acridine orange digital fluorography.

Animal Model: Rats (male) subjected to transient retinal ischemia induced by temporary ligation of the optic nerve. Ischemic preconditioning (5 minutes of ischemia) is induced 24 hours before 60 minutes of ischemia.

Interpretation and Analysis: Ischemic preconditioning inhibits leukocyte rolling. The maximum number of rolling leukocytes is reduced to 3.0% at 12 hours after reperfusion ($p < 0.01$). Subsequent leukocyte accumulation is also decreased with ischemic preconditioning. The maximum number of accumulated leukocytes is reduced to 22.6% at 24 hours after reperfusion ($p < 0.01$). These inhibitory effects are suppressed by the administration of DPCPX ($p < 0.0001$). The numbers of rolling leukocytes at 12 hours after reperfusion and accumulated leukocytes at 24 hours after reperfusion are approximately 102.7% (ns) and 83.4% ($p < 0.01$), respectively, compared with numbers without ischemic preconditioning. Ischemic preconditioning, therefore, has an inhibitory effect on leukocyte rolling and subsequent leukocyte accumulation during retinal ischemia-reperfusion injury. Furthermore, the adenosine A1 receptor may play an important role in these inhibitory effects.

Instrumentation: Digital fluorography.

Inhibitory effect of ischemic preconditioning on leukocyte participation in retinal ischemia-reperfusion injury. Nonaka A, Kiryu J, Tsujikawa A, Yamashiro K, Nishijima K, Miyamoto K, Nishiwaki H, Honda Y, Ogura Y. Invest Ophthalmol Vis Sci 2001 Sep; 42 (10), pp. 2380–5.

Stem Cell Retention, Engraftment, and Migration
Iron Fluorophore Particle (IFP)/Mesenchymal Stem Cells (MSCs)

Cellular/Molecular Mechanism of Action: Stem cells have the potential to differentiate into a multitude of different cell types within tissue/organ environments.

Background Information: Delivery and tracking of endomyocardial stem cells are limited by the inability to image transplanted cells non-invasively in the beating heart.

Application: Mesenchymal stem cells can be labeled with IFP to facilitate *in vivo* magnetic resonance imaging (MRI) to determine the immediate and long-term localization of these cell types in order to assess their retention, engraftment, and migration within tissues/organs.

Route of Administration: Mesenchymal stem cells (variable including percutaneous).

Implementation: Short-term incubation of MSCs with IFP results in efficient dose-dependent labeling. Postlabeling, cells remain viable for multiple passages and retain *in vitro* proliferation and differentiation capacities. Labeled mesenchymal stem cells (10^4–10^6 cells/150 μL) are then injected percutaneously into normal and freshly infarcted myocardium. Serial cardiac MRI (1.5T) is then performed post–myocardial infarction. Magnetic resonance imaging contrast properties are then measured both *in vivo* and *in vitro* for cells embedded in agar.

Animal Model: Swine (mesenchymal stem cells and porcine myocardium [normal & infarcted]). Serial infarction of the myocardium can be induced on days 4, 8, and 21 (e.g., n = 1, 3, and 1 animals, respectively).

Interpretation and Analysis: Iron fluorophore particle labeling of MSCs imparts useful MRI contrast, enabling ready detection in the beating heart on a conventional cardiac MR scanner after transplantation into normal and infarcted myocardium. The dual-labeled MSCs can be identified at locations corresponding to injection sites, both *ex vivo* using fluorescence microscopy and *in vivo* using susceptibility contrast on MRI. This technology may permit effective *in vivo* study of stem cell retention, engraftment, and migration.

Serial cardiac magnetic resonance imaging of injected mesenchymal stem cells. Hill JM, Dick AJ, Raman VK, Thompson RB, Yu ZX, Hinds KA, Pessanha BS, Guttman MA, Varney TR, Martin BJ, Dunbar CE, McVeigh ER, Lederman RJ. Circulation 2003 Aug 26; 108 (8), pp. 1009–14. Epub 2003 Aug 11.

Vascular: Thrombus Detection
Near-Infrared (NIR) Hirudin Conjugated Probe

Cellular/Molecular Mechanism of Action: Hirudin anticoagulant has a relatively strong binding avidity for thrombin and functions as an inhibitor of most biological properties associated with this endogenous enzyme fraction.

Background Information: Thrombin, a serine protease, plays an important role in thrombosis as well as other cellular and developmental processes.

Application: Can be utilized as a thrombin-activatable molecular probe for the detection of thrombin activity at sites of vascular thrombus formation.

Semi-Synthetic Methods: The thrombin probe consists of an NIR fluorochrome attached to a delivery vehicle via a thrombin-specific oligopeptide substrate.

Implementation: Evaluation of the ability of an NIR fluorochrome-hirudin conjugate to detect thrombus formation in both *in vitro* and *in vivo* environments.

Animal Model: Murine (acute thrombosis model).

Interpretation and Analysis: In human blood, endogenous thrombin biochemical (enzymatically) activates the thrombin probe and increases the fluorescence signal by 18-fold ($p = 0.008$). Hirudin, a specific thrombin inhibitor, suppresses probe activation by 82% ($p = 0.007$). Imaging of *in vivo* thrombin activity up to 12 hours using the systemic thrombin probe reveals that focal NIR fluorescence signal enhancement can be rapidly detected within acute and subacute thrombi. In contrast, no thrombosis signal enhancement is seen in similar experiments with a control NIR fluorochrome. Thrombin activity can be imaged *in vivo* by using a novel thrombin-activatable and thrombin-specific NIR molecular probe. The thrombin probe could enhance the understanding of the role of thrombin in thrombogenesis and other homeostatic and pathological conditions.

Vascular: Vascular Permeability (Retinal)/ Minimally-Invasive Diagnostic Strategy [Diabetes]
Sodium Fluorescein

Background Information: Diabetic retinopathy is the leading cause of blindness in working-age individuals in the United States. Breakdown of the blood-retinal barrier is 1 of the earliest events in the progression of diabetic retinopathy. Ideally, therapeutic measures would be directed at this early stage, but there are few sensitive, quantitative methods to assess the retinal vascular barrier *in vivo.*
Application: Quantitative assessment of the retinal vascular barrier.
Implementation: Utilization of 2 fluorescent tracers in combination with fluorescence microangiography to quantitatively assess retinal vascular endothelium barrier integrity (± infusion of a hyperosmolar solution of 1.6 M mannitol for 5 minutes).
Animal Model: Rat (Long-Evans strain).
Interpretation and Analysis: Permeability × surface area/flow (PS/F) describing the retinal vasculature is 0.086 ± 0.031 (n = 13, avg. ± standard deviation). Based on estimates of flow and surface area, estimated permeability of sodium fluorescein is approximately 1.2×10^{-5} cm/s. Infusion of a hyperosmolar mannitol solution significantly increases PS/F within individual veins and significantly increases flow-weighted PS/F from 0.073 ± 0.028 to 0.16 ± 0.034 (n = 3). Quantitative *in vivo* assessment of retinal vasculature can be delineated applying an adapted indicator dilution technique. Dual-tracer fluorescence angiography appears to be a sensitive indicator for detecting increases in the blood-retinal barrier produced by hyperosmolar mannitol. The methodology may be a promising new minimally-invasive strategy that may be adapted to quantitatively monitor retinal vascular permeability.
Instrumentation: Fluorescence microangiography.

Retinal vascular permeability determined by dual-tracer fluorescence angiography. Russ PK, Gaylord GM, Haselton FR. Ann Biomed Eng 2001 Aug; 29 (8), pp. 638–47.

Vascular Blood/Red Blood Cell (RBC) Flow Characteristics
Fluorescent-Labeled RBCs

Considerations: Whole-organ studies in cat skeletal muscle have shown that increases in venous resistance associated with reduced arterial pressures are nearly abolished when the muscle is perfused with a non-aggregating RBC suspension.
Implementation: Delineation of the influence RBC aggregation has on *in vivo* vascular flow patterns and determination if such events lead to blunted RBC velocity profiles at reduced shear rates. In this manner, fluorescent-labeled RBCs applied in tracer quantities can be used to obtain velocity profiles in venous microvessels.
Interpretation and Analysis: Velocity profiles in venous microvessels (45–75 μm) of rat spinotrapezius muscle have centerline velocities between 0.3 and 14 mm/s (pseudoshear rates 3–120/s) under normal (non-aggregating) conditions and following induction of RBC aggregation with dextran 500. Profiles are nearly parabolic (Poiseuille flow) over this flow rate range in the absence of aggregation. When aggregation is present, profiles are parabolic at high shear rates and become significantly blunted at pseudoshear rates of 40/s and below. These results indicate a possible mechanism for increased venous resistance at reduced flows.
Instrumentation: Video system equipped with a gated image intensifier.

Effect of erythrocyte aggregation on velocity profiles in venules. Bishop JJ, Nance PR, Popel AS, Intaglietta M, Johnson PC. Am J Physiol Heart Circ Physiol 2001 Jan; 280 (1), pp. H222–36.

Vascular Blood Flow/Capillary Circulation/Perfusion
Fluorescein Isothiocyanate (FITC)-Labeled Dextran

Cellular/Molecular Mechanism of Action: Fluorescein isothiocyanate can be used as a light-emitting molecular marker system.
Application: Fluorescein isothiocyanate can be used as a light-emitting molecular marker in biological systems. Dextran labeled with fluorescein isothiocyanate can be utilized as an *in vivo* signal/marker molecular system for the evaluation of the acute microhemodynamic effects of various stimuli (e.g., static and alternating magnetic fields delivered at threshold levels in muscle capillary mirocirculation beds).
Route of Administration: Fluoroscein isothiocyanate-dextran solution (intravascular: e.g., vena cava).
Implementation: Evaluation of muscle capillary integrity in response to various stimuli (e.g., electromagnetic field exposure). The skin in a tibialis anterior is circularly removed with 1.5-mm diameter sufficient to faciliate intravital-microscopic recording of capillary blood velocity in the tibialis anterior muscle. Fluorescein isothiocyanate-labeled dextran (MW 150 kDa) can be used as an *in vivo* fluorescent plasma marker of muscle capillaries. Following a bolus injection of FITC-dextran solution into the caudal vein, the peak blood velocity in the muscle capillaries is measured prior to, during, and following exposure to static magnetic fields (SMF) or 50-Hz electromagnetic fields (EMF). The whole body of experimental animals is then placed on an observation stage of a fluorescence microscope and exposed to static magnetic fields (SMF = 0.3, 1, and 10 mT) or 50-Hz EMF (0.3 and 1 mT) for 10 minutes with the electromagnet not energized during sham exposures.
Animal Model: Mouse (anesthetized with pentobarbital and exposed to static and alternating magnetic fields with exposures performed at threshold levels).
Interpretation and Analysis: During and following exposure to SMF of 10 mT, peak blood velocity significantly increases compared to sham exposure. After the withdrawal of SMF and 50-Hz EMF of 1 mT, significant similar effects on the blood velocity develop or are enhanced. These findings suggest that a field intensity of 1 mT might be considered as a threshold level for enhancing muscle microcirculation under pentobarbital-induced hypnosis.
Instrumentation: Utilization of (i) an electromagnet specifically designed to deliver static magnetic fields (SMF = 0.3, 1, and 10 mT); (ii) 50-Hz EMF (0.3 and 1 mT) instrumentation (fluorescence epi-illumination system); and (iii) a fluorescence microscope.

Acute effects of whole-body exposure to static magnetic fields and 50-Hz electromagnetic fields on muscle microcirculation in anesthetized mice. Xu S, Okano H, Ohkubo C. Bioelectrochemistry 2001 Jan; 53 (1), pp. 127–35.

Vascular Blood Flow and Perfusion: Hepatic Blood Flow/[Xenobiotic Metabolism]/Extrahepatic Metabolism
Phenol Red/Lidocaine (Monoethylglycinexylidide [MEGX])

Background Information: Xenobiotic metabolism in extrahepatic tissues has been extensively studied *in vitro,* but it is difficult to estimate in *in vivo* environments the share of xenobiotic transformation in extrahepatic tissues for lack of a suitable approach.
Application: Assessment of extrahepatic metabolism investigated using the conversion of lidocaine to MEGX.

Animal Model: Rat (rats were anesthetized with ethyl ether inhalation). In exposed livers, the hepatic artery is ligated, and the portal vein clamped at its distal end. The left hepatic lobe is partly excised along its inferior margin, and a heparinized silicone catheter, diameter 0.2 cm, is inserted into the portal and left hepatic veins to allow the recirculation of portal vein blood. A sham operation is performed in control groups.

Interpretation and Analysis: Phenol red tests show that hepatic blood supply is absolutely blocked in modified rats. At 30 minutes after establishing the portal-cavum bypass, renal function and electrolytes do not change, but serum glucose decreases by 64.4 ± 30.4%; 30 minutes after intravenous administration of 1.0% lidocaine 2 mg/kg, serum MEGX in model rats is 32.0 ± 7.14% of that in the control group, which mostly exists in a free form that is not induced by phenobarbital pretreatment. The model is easy to establish and provides an *in vivo* method to study the extrahepatic metabolism of xenobiotics.

An *in vivo* rat model for assessment of extrahepatic metabolism. Ping H, Zhen-Fu C, Shao-Qing X, Ming L, Jian W, Guo-Qing Z, Lin Z, Lin-Fang L, Meng-Chao W. J Pharmacol Toxicol Methods, 2001 May–Jun; 45 (3), pp. 181–5.

Vascular Blood Flow Characterization/Aorta/Renal Artery and Vein/Portal Vein/Vena Cava
Indocyanine Green (ICG)/SH U 508 Microbubbles (Levovist)

Cellular/Molecular Mechanism of Action: Indocyanine green and microbubbles can be infused into the intravascular compartment where they dispurse throughout the blood volume.

Application: Indocyanine green and microbubbles can be applied to characterize blood flow through vascular structures.

Route of Administration: Indocyanine green and microbubbles are both administered intravascularly.

Dosage: Indocyanine green (1.25 mg/mL at 0.1 mL/kg); SH U 508 A (300 mg/mL at 0.1 mL/kg).

Considerations: SH U 508 A microbubbles (Levovist) can be detected by Doppler ultrasound.

Implementation: Evaluation of blood flow within the vena cava, aorta, renal artery, renal vein, and portal vein. The kinetics of ICG can be determined by measuring absorbance values using a photodiode, while SH U 508 A microbubbles (Levovist) are detected using Doppler ultrasound.

Animal Model: Rabbit (n = 5).

Interpretation and Analysis: Peak signal intensity is observed immediately after injection of SH U 508 A, followed by a biphasic decay profile. The rates of biphasic decay are similar in all vessels. A 2nd peak of the signal that represents microbubble recirculation is observed within the vena cava. The circulation and recirculation times of the microbubbles after injection of SH U 508 A are similar to that of ICG. These findings suggest that the majority of SH U 508 A microbubbles circulate through the body similar to blood flow, and without retention, in microvasculature structures.

In vivo kinetics of microbubbles of SH U 508 A (Levovist): Comparison with indocyanine green in rabbits. Uchimoto R, Niwa K, Eguchi H, Kamiyama N, Mine Y, Miyazawa T, Brautigam M. Ultrasound Med Biol 1999 Nov; 25 (9), pp. 1365–70.

Vascular Extravasation and Permeability
Evans Blue Dye

Cellular/Molecular Mechanism of Action: Evans blue dye is a pigment-based reagent that can be detected visually within the visible light range.

Background Information: Oxidative stress appears to be relevant to pathogenesis of asthma-like conditions.

Application: Evans blue dye is a pigment-based reagent that can be used as a visual marker or indicator molecule because of its compatability with biological systems. In this context, it is often applied as a dilution marker system within the intravascular compartment for measuring total intravascular compartment size and extrasation of plasma proteins and hematological cell types into the extravascular tissue space.

Implementation: Application of Evans blue dye to evaluate extravasation into bronchoalvealar lavage fluid following antigen-induced pulmonary responses in sensitived rats and to determine the effectiveness of the antioxidant N-acetylcysteine (oral, 1 mmol/kg/day for 7 days before challenge).

Animal Model: Rat (Brown Norway strain).

Interpretation and Analysis: N-acetylcysteine does not reduce the immediate bronchospasms that follow aerosol antigen exposure but does prevent airway hyperreactivity to 5-hydroxytryptamine at 24 hours after antigen challenge, in addition to reducing eosinophil numbers (from 0.178 ± 0.038 in the absence of N-acetylcysteine to 0.064 ± 0.020 × 10^6 cells/ml in the presence of N-acetylcysteine; $p < 0.05$) and decreasing Evans blue dye extravasation in bronchoalveolar lavage fluid. Taurine levels in bronchoalveolar lavage fluid from antigen-challenged rats are higher than control values but treatment with N-acetylcysteine fails to further increase these augmented levels. N-acetylcysteine appears to be beneficially effective when applied *in vivo* in models of experimental asthma and serves to validate related findings pertaining to other models of lung injury.

Effectiveness of oral N-acetylcysteine in a rat experimental model of asthma. Blesa S, Cortijo J, Martinez-Losa M, Mata M, Seda E, Santangelo F, Morcillo EJ. Pharmacol Res 2002 Feb; 45 (2), pp. 135–40.

Vascular Extravasation and Permeability/ Endothelial Injury: Viability/Integrity
Evans Blue Dye Staining

Cellular/Molecular Mechanism of Action: Evans blue dye is a pigment-based reagent that becomes differentially associated with viable tissues compared to compromised cell populations (e.g., vascular endothelium).

Physiological Mechanism of Action: Tumor necrosis factor soluble receptor (TNFsr) has been shown *in vitro* and *in vivo* to neutralize TNF-mediated biological activity.

Background Information: Tumor necrosis factor-alpha (TNF-α) is expressed locally in arteries at sites of balloon injury. *In vitro* studies have shown that TNF inhibits cell cycle progression and induces apoptosis in endothelial cells.

Application: Evans blue dye staining can be applied to evaluate viable *ex vivo* preparations of vascular structures for integrity of endothelial surfaces.

Implementation: Evaluation of the ability to accelerate endothelial recovery following angioplasty by inhibiting TNF utilizing Evans blue dye as a molecular detection modality. Given this objective, treatment groups are administered TNFsr for comparison to reference controls that receive IgG (intraperitoneal injection). Deendothelializing balloon injuries are then created, and the resulting induced lesions evaluated 1 week later for evidence of reendothelialization (Evans blue dye staining) and after 2 weeks to evaluate reendothelialization and endothelial function.

Animal Model: Rat (Sprague-Dawley strain).

Interpretation and Analysis: At both time points, blockade of TNF biological activity with TNFsr results in increased reendothe-

lialization, as measured as absolute area and percent area reendothelialized. Tumor necrosis factor soluble receptor also accelerates functional endothelial recovery that manifests as an increase in nitric oxide production. Neointimal thickening can also be appreciated as being inhibited. Blockade of TNF *in vivo* accelerates functional endothelial recovery after barotraumatic deendothelializing injury. Locally expressed TNF therefore acts to inhibit functional endothelial recovery after angioplasty and transient blockade of TNF may improve the long-term success of angioplasty.

Evans Blue Staining: The reagent facilitates detection of a demarcation between viable endothelial cell populations and subendothelial collagen.

In vivo blockade of tumor necrosis factor-alpha accelerates functional endothelial recovery after balloon angioplasty. Krasinski K, Spyridopoulos I, Kearney M, Losordo DW. Circulation 2001 Oct 9; 104 (15), pp. 1754–6.

Vascular Extravasation and Permeability: Anaphylaxis
Evans Blue Dye/Passive Cutaneous Anaphylaxis Test

Cellular/Molecular Mechanism of Action: Immunoglobulin (IgE) promotes mast cell degranulation in normal individuals.
Diagnostic Application: Detection of immune hypersensitivity conditions.
Implementation: Dilutions of serum potentially containing IgE immunoglobulin are injected intradermally over a grid-like pattern. Purified antigen is then administered by intravenous injection. Simultaneous injection of Evans blue dye markedly increases the sensitivity of visually detecting regions of positive response. In this context, Evans blue dye "leaks" across capillary structures into the surrounding intracellular matrix due to the reaction of vascular capillary endothelium to histamine released from activated mast cell populations.
Interpretation and Analysis: A striking blue discoloration at the site of injection supports a diagnosis of a hypersensitivity reaction.

Vascular Perfusion: Dermal Microvasculature/ Delineation of Perfused to Total Capillary Ratio
Sodium Fluorescein

Application: Delination of the nature of temporal variation in capillary numbers, and determination of the proportion of perfused to total nutritional capillaries in normal skin.
Implementation: Using *in vivo* microscopy, the temporal behavior of the number of visible capillaries in the skin of the dorsum of the foot is observed over periods of time varying from 5 minutes to 55 days. Capillary perfusion is then studied by comparing capillary numbers before and after intravenous injection of sodium fluorescein.
Human Model: Healthy subjects.
Interpretation and Analysis: The mean percent difference in the number of visible capillaries over a mean period of 25.3 days is 5.5%. The percentage ratio of perfused to total capillaries is 54.2%. There appears to be little quantitative change in capillary numbers over periods of up to 50 days, and under physiological conditions, about half of the nutritional capillaries of the skin are not perfused.

Study of temporal and perfusion physiology of skin capillaries in the dorsum of the foot. Lamah M, Mortimer PS, Dormandy JA. J Vasc Res 2001 Jan–Feb; 38 (1), pp. 59–63.

CELLULAR: GENERAL

Vitality Staining of Living Cells
Trypan Blue Dye Exclusion

Cellular/Molecular Mechanism of Action: Penetrates the cell membrane and accumulates within the cytoplasm of non-viable cell populations.
Application: Stain identification of non-viable cell populations.
Route of Administration: Topical *in vitro* bathing.
Implementation: Trypan blue is incubated with cell populations.
Interpretation and Analysis: Non-viable cell populations are positively stained with Trypan blue pigment dye reagent.

Vitality Staining of Living Cells: Biochemical Viability
3-[4,5-Dimethylthiazol-2-yl]-2,5-Diphenyltetrazolium Bromide (MTT Vitality Stain)

Cellular/Molecular Mechanism of Action: Viable cell populations have the necessary biochemical components in their cytoplasm to convert MTT reagent to navy blue furazone crystals.
Application: Assessment of cell viability.
Route of Administration: Topical *in vitro* bathing.
Formulation: The vitality staining reagent MTT is prepared as a solution dispersed in serum-free and pH indicator-free growth media (e.g., 60 ml/300 ml as MTT/RPMI 1640).
Implementation: MTT vitality staining reagent (e.g., 375 μl/well of a 48-chamber plate) is incubated with viable cell populations at 37°C over a 3- to 4-hour period. Cells (e.g., tissue culture monolayers) stained with MTT reagent are then destained with isopropyl alcohol (200 μl) at 25°C for 25 minutes with the aid of gently horizontal agitation. The resulting blue/purple supernatant is harvested by pipette and transferred to a 96-well microtiter plate for measurement of spectrophometric absorbance (550 nm).
Interpretation and Analysis: Values obtained for optical density at 550 nm are directly proportional to the extent of cell viability/ proliferation.

Vitality Staining of Living Cells: Micronucleus Assessment
Acridine Orange/Giemsa/New Methylene Blue

Background Information: The micronucleus test has been widely used as an *in vivo* cytogenetic test. It employs 2 different kinds of supravital staining methods that use either new methylene blue (N) and Giemsa (G) or acridine orange (AO).
Implementation: The methylene blue/acridine orange-staining method involves 3 steps: (i) combination of the target tissue or target cells with an equivalent volume of 0.5% solution of new methylene blue (new methylene blue-staining step); (ii) immediate smear of the mixture, followed by treatment with methanol for 10 minutes for fixation and removal of new methylene blue and drying (referred to as fixed-decolorized specimens); and (iii) staining with 0.007% solution of acridine orange for 3 minutes, followed by washing with Sorensen's buffer (pH 6.8) and covering of specimens before observation (acridine orange-staining step). To examine whether the new methylene blue/acridine orange-staining method is useful for the micronucleus test, comparisons can be made between new methylene blue-, new methylene blue/acridine orange-, and acridine orange-stained specimens prepared supravitally from peripheral blood of rats with and without treatment of cyclophosphamide.

Interpretation and Analysis: Results indicate that N/acridine orange-stained specimens can be supravitally observed after long-term storage with the same coloration and comparable frequencies of micronucleated reticulocytes with a positive response as acridine orange-stained specimens, if the staining process is temporarily stopped before acridine orange staining (as fixed-decolorized specimens) or if the acridine orange-staining step is repeated. The results also show that separate reticulocyte types are supravitally stained in a similar fashion to N-stained specimens but not to acridine orange-stained specimens, indicative of the preservation of the supravital feature of new methylene blue staining. Taken together these results suggest that the new methylene blue/acridine orange-staining procedure could offer an additional useful staining tool for the micronucleus test.

A staining procedure for micronucleus test using new methylene blue and acridine orange: Specimens that are supravitally stained with possible long-term storage. Sugihara T, Sawada S, Hakura A, Hori Y, Uchida K, Sagami F. Mutat Res 2000 Oct 31; 470 (2), pp. 103–8.

CENTRAL NERVOUS SYSTEM AND SPECIAL SENSES

Amyloid: Beta-Sheet Proteins/Amyloid Plaques: Abeta Peptide
Thioflavin Derivatives/Neurofibrillary Tangles: Tau Protein

Cellular/Molecular Mechanism of Action: Thioflavin-T (ThT) derivatives possess binding avidity for beta-sheet proteins deposited in amyloid plaques (abeta peptide) and neurofibrillary tangles (tau protein).
Background Information: The beta-sheet proteins deposited *in vivo* in amyloid plaques (abeta peptide) or neurofibrillary tangles (tau protein) represent a target for the development of biological markers for Alzheimer's disease (AD).
Application: Detection and characterization of beta-sheet proteins deposited in amyloid plaques (abeta peptide) and neurofibrillary tangles (tau protein) similar to those associated with the pathogenesis of AD.
Route of Administration: [Intravenous.] Anticipated that such reagents would be given intravascularly if applied in a clinical environment.
Semi-Synthetic Methods: Production of ThT derivatives and the carbon-11 labeled compound [N-methyl-^{11}C]6-Me-BTA-1.
Implementation: Characterization of the entry into and clearance of [N-methyl-^{11}C]6-Me-BTA-1 from brain tissues.
Animal Model: Mouse (Swiss-Webster).
Interpretation and Analysis: These compounds lack the positively charged quaternary heterocyclic nitrogen of ThT and are therefore uncharged at physiological pH. They are 600-fold more lipophilic than ThT. Derivatives of ThT bind to abeta(1-40) fibrils with higher affinity (Ki = 20.2 nM) than ThT (Ki = 890 nM). The uncharged ThT derivatives stain both plaques and neurofibrillary tangles in postmortem AD brain, showing some preference for plaque staining.

The compound [N-methyl-^{11}C]6-Me-BTA-1 enters the brain at levels comparable to commonly used neuroreceptor imaging agents (0.223 %ID-kg/g or 7.61 %ID/g at 2 minutes postinjection) and shows good clearance of free and non-specifically bound radioactivity in normal rodent brain tissue (brain clearance $T_{1/2}$ = 20 minutes). The combination of relatively high affinity for amyloid, specificity for staining plaques and neurofibrillary tangles in postmortem AD brain, and good brain entry and clearance makes [N-methyl-^{11}C]6-Me-BTA-1 a promising candidate as an *in vivo* positron emission tomography (PET) beta-sheet imaging agent.

Uncharged thioflavin-T derivatives bind to amyloid beta protein with high affinity and readily enter the brain. Klunk WE, Wang Y, Huang GF, Debnath ML, Holt DP, Mathis CA. Life Sci 2001 Aug 17; 69 (13), pp. 1471–84.

Amyloid (Alzheimer's Disease [AD])/Abeta(1-40) Fibrils/Senile Plaques (SPs) and Neurofibrillary Tangles (NFTs)
2-(1-(6-[(2-[^{18}F]Fluoroethyl)-(Methyl)Amino]-2-Naphthyl)Ethylidene)Malononitrile ([^{18}F]FDDNP)

Cellular/Molecular Mechanism of Action: The agent [^{18}F]FDDNP is a hydrophobic dual-function semi-synthetic compound that is fluorescent emitting and possesses binding avidity for certain types of molecular amyloid.
Background Information: Senile plaques and NFTs are hallmark pathological changes that accompany the neurodegeneration involved in AD. Deposits of beta-amyloid (abeta) peptide are a major constituent of SPs.
Application: The hydrophobic, fluorescent molecular-imaging probe [^{18}F]FDDNP can cross the blood-brain barrier and can be utilized to determine the location and deposition load of SPs and NFTs *in vivo* in cases of AD.
Route of Administration: Intravascular.
Implementation: Determination of the binding avidity of FDDNP and its analog, 1-(6-[(2-[^{18}F]fluoroethyl)(methyl)amino] naphthalen-2-yl)ethanone ([^{18}F]FENE), for synthetic fibrils of abeta(1-40) applying fluorimetric and radioactive binding assays.
Interpretation and Analysis: Both FDDNP and FENE bind to kinetically distinguishable binding sites (n = 2 sites) on abeta(1-40) fibrils. Fluorescence titrations yield apparent K_d values of 0.12 and 0.16 nm for high-affinity binding sites for FDDNP and FENE, respectively, and apparent K_d values of 1.86 and 71.2 nm for low-affinity binding sites. Traditional radioactive binding assays produce apparent K_d values in the low nanomolar range. The presence of 2 kinetically distinguishable binding sites for FDDNP and FENE suggests multiple binding sites for SPs and identifies properties that will allow for the structural optimization of this family of probes for *in vivo* use. The high-affinity binding of the probes to multiple binding sites on fibrils is consistent with results obtained with digital autoradiography, immunohistochemistry, and confocal fluorescence microscopy using human brain specimens of AD cases.

Binding characteristics of radiofluorinated 6-dialkylamino-2-naphthylethylidene derivatives as positron emission tomography imaging probes for beta-amyloid plaques in Alzheimer's disease. Agdeppa ED, Kepe V, Liu J, Flores-Torres S, Satyamurthy N, Petric A, Cole GM, Small GW, Huang SC, Barrio JR. J Neurosci 2001 Dec 15; 21 (24), p. RC189.

Amyloid Beta Protein: Extracellular/Abnormal Tau Protein/Abnormal Synuclein Proteins/ [Alzheimer's Disease (AD)]
[Trans/Trans]-1-Bromo-2,5-bis-(3-Hydroxycarbonyl-4-Hydroxy)Styrylbenzene/Thioflavin S

Cellular/Molecular Mechanism of Action: Congo red-derived fluorescent probe [*trans*/*trans*]-1-bromo-2,5-*bis*-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (BSB) binds to molecular amyloid.
Background Information: The novel Congo red-derived fluorescent probe BSB binds to amyloid plaques of postmortem AD brains and *in vivo* in transgenic mouse brains.
Application: Prototype imaging agent for detecting and characterizing amyloid deposits/plaques (e.g., AD).
Implementation: Evaluation of the ability for BSB to probe postmortem tissues from patients with various neurodegenerative

diseases containing diagnostic lesions. In this context, the agent can be utilized to characterize fibrillar intra- or extracellular lesions and compare properties with standard histochemical dyes such as thioflavin S and immunohistochemical stains specific for the same lesion type.

Interpretation and Analysis: The agent BSB binds not only to extracellular amyloid beta protein but also many intracellular lesions composed of abnormal tau and synuclein proteins, and suggests that radioiodinated BSB derivatives or related ligands may be useful *in vivo* imaging agents to monitor diverse types of amyloid deposition.

The fluorescent Congo red derivative, (trans, trans)-1-bromo-2,5-*bis*-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (BSB), labels diverse beta-pleated sheet structures in postmortem human neurodegenerative disease brains. Schmidt ML, Schuck T, Sheridan S, Kung MP, Kung H, Zhuang ZP, Bergeron C, Lamarche JS, Skovronsky D, Giasson BI, Lee VM, Trojanowski JQ. Am J Pathol 2001 Sep; 159 (3), pp. 937–43.

Amyloid Beta Protein Detection/Senile Plaques and Neurofibrillary Tangles/Alzheimer's Disease (AD) *Chrysamine-G (CG)/[Congo Red Analog]/[^{14}C]-CG*

Cellular/Molecular Mechanism of Action: Chrysamine-G is a carboxylic acid analogue of Congo red, a histologic dye that stains amyloid protein deposits.

Background Information: CG binds to the beta-amyloid protein of AD *in vitro* and partitions into the brain of normal mice.

Interpretation and Analysis: The agent [^{14}C]-CG possesses increased binding to homogenates of several regions of AD brain tissue compared to control brain tissue. The total binding of CG to AD brain tissue is approximately 2- to 3-fold that of control brain. The cerebellum can serve as an internal standard for each brain because CG binding to cerebellum does not differ between AD and control. The binding of [^{14}C]-CG correlates with numbers of senile plaques and neurofibrillary tangles. Based on the nature of such findings, CG could be used to stain cerebrovascular amyloid in tissue sections. These results suggest that CG may prove useful as an *in vivo* probe of amyloid deposition in AD.

Chrysamine-G binding to Alzheimer and control brain: Autopsy study of a new amyloid probe. Klunk WE, Debnath ML, Pettegrew JW. Neurobiol Aging 1995 Jul–Aug; 16 (4), pp. 541–8.

Amyloid Deposits: [Alzheimer's Disease–Related]/ Differential Stain Recognition: Non-Fibrillar vs Fibrillar Amyloid/Transthyretin: Amyloidic Protein *X-34/Congo Red/Thioflavin S*

Cellular/Molecular Mechanism of Action: The agents Congo red, thioflavin S, and X-34 possess physical binding avidity for molecular amyloid.

Background Information: Transgenic *Caenorhabditis elegans* animals can be engineered to express high levels of the human beta-amyloid peptide (abeta). Histochemistry of fixed tissue from these animals reveals deposits reactive with the amyloid-specific dyes Congo Red and thioflavin S.

Application: Alzheimer's disease.

Implementation: Discovery of the potential for X-34 to bind to amyloid deposits in *Caenorhabditis elegans* applying immunoelectron microscopy as a validation methodology.

Interpretation and Analysis: Immunoelectron microscopy can detect intracellular immunoreactive deposits with classic amyloid fibrillar ultrastructure. These deposits can be visualized in living animals using the newly developed, intensively fluorescent, amyloid-specific dye X-34. Utilizing this amyloid staining dye *in vivo* allows monitoring of amyloid deposition in individual animals over time. The specificity of X-34 staining is demonstrated by examining transgenic animals expressing high levels of a non-fibrillar beta peptide variant, the beta single-chain dimer. These animals have deposits that are immunoreactive with antibeta antibodies but do not have X-34 positive-staining deposits or deposits with a fibrillar ultrastructure. Visualization of putative amyloid deposits resulting from accumulation of human transthyretin, another amyloidic protein can also be achieved *in vivo* using X-34 dye reagent. *In vivo* amyloid staining with X-34 may be a useful tool for monitoring antiamyloid treatments in real time or screening for genetic alterations that affect amyloid formation.

Visualization of fibrillar amyloid deposits in living, transgenic Caenorhabditis elegans animals using the sensitive amyloid dye, X-34. Link CD, Johnson CJ, Fonte V, Paupard M, Hall DH, Styren S, Mathis CA, Klunk WE. Neurobiol Aging 2001 Mar–Apr; 22 (2), pp. 217–26.

Amyloid (Abeta) Fibrils/Alzheimer's Disease (AD): Senile Plaques and Neurofibrillary Tangles *Methoxy-X04 [Congo Red Derivative]/Chrysamine-G*

Cellular/Molecular Mechanism of Action: Similar to Congo red, methoxy-X04 is a fluorescent dye that possesses binding avidity for molecular amyloid beta fibrils.

Background Information: The identification of amyloid deposits in living AD patients is important for both early diagnosis and for monitoring the efficacy of newly developed antiamyloid therapies.

Application: Visual fluorescent detection and characterization of amyloid beta fibrils contained within plaques, tangles, and cerebrovascular amyloid deposits.

Route of Administration: Methoxy-X04 (IV = 5–10 mg/kg; IP = 10 mg/kg in mice).

Dosage: Methoxy-X04 (intravenous, intraperitoneal in mice).

Semi-Synthetic Methods: Methoxy-X04 is a derivative of Congo red and Chrysamine-G that contains no acid groups and is therefore smaller and much more lipophilic than Congo red or Chrysamine-G.

Implementation: Evaluation of the *in vivo* ability of methoxy-X04 to detect individual plaques that can be non-invasively detected applying multiphoton microscopy.

Animal Model: Mouse (PSI/APP).

Interpretation and Analysis: Methoxy-X04 retains *in vitro* binding affinity for amyloid beta (abeta) fibrils (Ki = 26.8 nM) very similar to that of Chrysamine-G (Ki = 25.3 nM). The fluorescent properties of methoxy-X04 facilitate successful staining of plaques, tangles, and cerebrovascular amyloid in postmortem sections of AD brain with good specificity. In an *in vivo* environment (mice), multiphoton microscopy can be applied to obtain high-resolution (1 μm) fluorescent images of the brains. Under such conditions, it is possible to detect individual plaques that can be distinguished within 30 to 60 minutes after a single intravenous injection (e.g., PSI/APP mouse brain). A single intraperitoneal methoxy-X04 injection at 10 mg/kg also produces high-contrast images of plaques and cerebrovascular amyloid (e.g., PSI/APP mouse brain). Complementary quantitative studies using tracer doses of carbon-11-labeled methoxy-X04 show that it enters rat brain in amounts that suggest it is a viable candidate as a positron emission tomography (PET) amyloid-imaging agent for *in vivo* application in human studies.

Instrumentation: Multiphoton microscopy.

Imaging abeta plaques in living transgenic mice with multiphoton microscopy and methoxy-X04, a systemically administered Congo red derivative. Klunk WE, Bacskai BJ, Mathis CA, Kajdasz ST, McLellan ME, Frosch MP,

Debnath ML, Holt DP, Wang Y, Hyman BT. J Neuropathol Exp Neurol 2002 Sep; 61 (9), pp. 797–805.

Amyloid Plaques (Abeta)/Alzheimer's Disease (AD)
Stilbene Derivatives

Cellular/Molecular Mechanism of Action: Stilbene derivatives recognize and physically bind to amyloid plaques analogous to those associated with AD.
Application: [^{11}C]4, N-[^{11}C]methylamino-4′-hydroxystilbene may be useful as a positron emission tomography (PET) imaging agent for mapping abeta plaques in the brain of patients with AD.
Route of Administration: Intravenous.
Semi-Synthetic Methods: Synthesis of a series of stilbene derivatives can be successfully achieved by performing a simple Wadsworth-Emmons reaction between diethyl (4-nitrobenzyl)phosphonate and 4-methoxybenzaldehyde. The [^{11}C]-labeled 4-N-methylamino-4′-hydroxystilbene, [^{11}C]4, is prepared by [^{11}C] methylation of 4-amino-4′-hydroxystilbene.
Interpretation and Analysis: 4-N,N-dimethylamino-4′-methyoxy and the corresponding 4-N-monomethylamino-, 4′-hydroxy stilbenes show good binding affinities toward abeta aggregates *in vitro* [K(i) $<$ 10 nM]. The [^{11}C]4 displays a moderate lipophilicity (log p = 2.36) and shows very good brain penetration and washout from normal rat brain following intravenous injection. *In vitro* autoradiography of transgenic AD mouse brain sections shows a high specific labeling of beta-amyloid plaques, whereas control sections show no binding.

^{11}C-labeled stilbene derivatives as abeta-aggregate-specific PET imaging agents for Alzheimer's disease. Ono M, Wilson A, Nobrega J, Westaway D, Verhoeff P, Zhuang ZP, Kung MP, Kung HF. Nucl Med Biol 2003 Aug; 30 (6), pp. 565–71.

Amyloid Plaques: Dense Beta Deposit Oxidative Properties/Alzheimer's Disease (AD)
Oxidation-Activated Fluorogenic Probes/N-Tert-Butyl-α-Phenylnitrone

Cellular/Molecular Mechanism of Action: *N*-tert-butyl-α-phenylnitrone functions as a free radical spin trap.
Background Information: Amyloid beta, the primary constituent of senile plaques in AD, is hypothesized to cause neuronal damage and cognitive failure, but the mechanisms are unknown.
Application: Fluorogenic compounds can be used *in vivo* to determine the degree of association between amyloid beta deposits and free radical production. The compound *N*-tert-butyl-α-phenylnitrone can be applied as a means for validating the effectiveness and accuracy of tracers and probes designed to detect anatomical regions of elevated oxidation potential.
Implementation: Utilization of compounds that are actively fluorescent following exposure to conditions with elevated oxidative potential for the purpose of infrared laser imaging of anatomical structures. As a validation procedure, *N*-tert-butyl-α-phenylnitrone (a free radical spin trap) can be systemically administered to determine the accuracy of oxidation-activated fluorogenic probes. Collectively, these agents can be applied to delineate if a direct association exists between amyloid beta deposits and free radical production.
Animal Model: Mouse (*in vivo* transgenic AD).
Human Model: Brain tissue from cases of AD (*ex vivo*).
Interpretation and Analysis: Fluorescence can be appreciated in dense core plaques, but not diffuse plaques, as determined by subsequent addition of thioflavin S and immunohistochemistry analysis for amyloid beta. Systemic administration of *N*-tert-butyl-α-phenylnitrone greatly reduces the detection of probes activated by tissue oxidative processes. It is therefore believed that a subset of amyloid plaques produce free radical species in living Alzheimer's models and in human Alzheimer tissue sections. Antioxidant therapy neutralizes these highly reactive molecules and may therefore be of therapeutic value in AD.
Instrumentation: Multiphoton imaging.

In vivo imaging of reactive oxygen species specifically associated with thioflavin S-positive amyloid plaques by multiphoton microscopy. McLellan ME, Kajdasz ST, Hyman BT, Bacskai BJ. J Neurosci 2003 Mar 15; 23 (6), pp. 2212–7.

Choriocapillaris: Choroid Circulation/Angiogram Imaging
Liposomes: Heat Sensitive/Carboxyfluorescein (Encapsulated)/[Laser-Targeted Angiography (LTA)]

Background Information: To obtain high-quality angiograms of the rat choriocapillaris with continuous LTA, for the purpose of assessing the choroidal circulation system.
Application: Characterization of choroidal circulation using angiogram imaging techniques.
Route of Administration: Heat-sensitive liposomes (intravascular).
Implementation: Evaluation of a carboxyfluorescein encapsulated in heat-sensitive liposomes injected intravenously to characterize the choroidal circulatory system. Local release of encapsulated carboxyfluorescein is induced by applying a continuous heat beam emitted by a diode laser (810 nm) of variable power. Video angiograms are recorded with excitation illumination provided by argon laser (488 and 514 nm) to observe highly selective images of the choriocapillaris.
Animal Model: Rat.
Interpretation and Analysis: Three distinct phases (filling, plateau, and draining) can be observed in fluorescent images of choriocapillaris by applying the diode laser continuously. In the plateau phase, a lobe-shaped area of choriocapillaris peripheral to the laser site can be illuminated, and this finite area does not change in size with continuous laser application to the same location. When laser power is increased, a larger area of choriocapillaris can be illuminated in the plateau phase. The filling and draining phases reveal the flow patterns within the choriocapillaris lobules that fill from a central spot and drain along a peripheral ring. Choriocapillaris (rat) is divided into independent functional units and the choroidal circulation is segmental under normal conditions. In LTA, the diode laser warms up a choroidal artery and releases fluorescein that flows downstream to an area of choriocapillaris fed by the same artery. Laser-targeted angiography appears to be a powerful method for analyzing choroidal circulation *in vivo.*
Instrumentation: Modified slit lamp that incorporates 2 kinds of lasers (argon and diode).

In vivo analysis of choroidal circulation by continuous laser-targeted angiography in the rat. Hirata Y, Nishiwaki H, Miura S, Ieki Y, Kiryu J, Honda Y. Invest Ophthalmol Vis Sci 2003 Jul; 44 (7), pp. 3103–9.

Corticospinal Neuron Trophic Dependencies/Response to Pathological Lesions and Therapeutic Agents
Fast Blue

Application: Evaluation of the response of corticospinal neurons (CSN) of the sensory motor cortex to lesions and therapeutic drugs.
Implementation: Before induction of lesions, CSN are retrogradely labeled with Fast blue (FB). A stereotaxic cut lesion through

the entire internal capsule (ICL) is used to axotomize CSN. The extent of axotomy is determined by application of a control tracer.

Animal Model: Rat and mouse (lesion model for the induction of death of analogous populations of CSN in rodent species).

Interpretation and Analysis: In both species, FB-labeled CSN are localized in 3 major areas: (i) the sensory motor cortex; (ii) the supplementary motor and medial prefrontal cortex; and (iii) the somatosensory cortex. The ICL does not lead to complete axotomy of CSN of the rat and mouse somatosensory cortex. In rats, ICL results in complete axotomy of CSN of the sensory motor cortex and incomplete axotomy of the caudal portion of the supplementary motor and medial prefrontal cortex. In mice, the area of axotomized CSN extends significantly further frontally. In both species, axotomy-induced death of CSN is observed in the center of the sensory motor cortex. This lesion model is useful for investigations of the response of CSN of the sensory motor cortex to pathological lesion and therapeutic drugs.

An axotomy model for the induction of death of rat and mouse corticospinal neurons *in vivo*. Bonatz H, Rohrig S, Mestres P, Meyer M, Giehl KM. J Neurosci Methods 2000 Jul 31; 100 (1–2), pp. 105–15.

Cranial Trauma: Cerebrospinal Fuid (CSF) Rhinorrhea
Fluorescein Dye

Background Information: Cerebrospinal fluid rhinorrhea typically results from trauma to the skull base, producing leaks through either the cribiform plate region or the sphenoid bone. Traditional approaches to the repair of such leaks include a frontal craniotomy or external ethmoidectomy. An endoscopic approach through the nose has also proven to be successful.

Application: CSF flow tracing.

Implementation: Evaluation of traumatic CSF rhinorrhea and development of a meningocele in the region of the cribiform plate using fluorescein dye to demonstrate the site of CSF leakage.

Interpretation and Analysis: Visualization of the defect with fluorescein dye can faciliate repair of the defect via a transnasal endoscopic approach. Given this condition a variety of complementary diagnostic procedures (radiographic assessment) and approaches for repair and postoperative care can be employed.

Endoscopic repair of traumatic CSF rhinorrhea in a pediatric patient. Wetmore RF, Duhaime AC, Klausner RD. Int J Pediatr Otorhinolaryngol 1996 Jul; 36 (2), pp. 109–15.

Reconstruction of the lacrimal drainage system. Holt JE, Holt GR. Arch Otolaryngol 1984 Apr; 110 (4), pp. 211–20.

Epilepsy/Spatiotemporal Patterns of Spontaneous Epileptiform Activity
RH414 (Fluorescent Voltage-Sensitive Dye)/Bicuculline

Application: RH414 functions as a fluorescent voltage-sensitive dye and the GABA(A) receptor antagonist bicuculline can be applied to induce acute spontaneous epileptoform discharges.

Implementation: Characterization of spatiotemporal patterns of *in vivo* spontaneous epileptiform activity in the cerebral cortex. The surface of the cerebral cortex is exposed under anesthesia and stained with a fluorescent voltage-sensitive dye, RH414, and optical recording techniques applied for the detection of transmembrane voltage changes. Acute spontaneous epileptiform discharges can be induced by application of bicuculline [GABA(A) receptor antagonist]. Changes in the intensity of fluorescence from the cerebral cortex are recorded using a photodiode system.

Animal Model: Rat cerebral cortex.

Interpretation and Analysis: Spontaneous epileptiform discharge recording can be acquired in a manner that facilitates the construction of initiation-site maps. Initiation sites are neither unique nor randomly located but instead exhibit a multimodal distribution pattern. The incidence of epileptiform discharges is different between the initiation sites, and some sites show dominance in the induction of spontaneous epileptiform discharges.

Instrumentation: Optic fiber photodiode system (464-channel).

Optical analysis of acute spontaneous epileptiform discharges in the *in vivo* rat cerebral cortex. Miyakawa N, Yazawa I, Sasaki S, Momose-Sato Y, Sato K. Neuroimage 2003 Mar; 18 (3), pp. 622–32.

Neoplasia (Gliosarcoma)
Hoechst 33258 Dye/Rhodaminated Long-Circulating Dextran-Coated Iron Oxide (LCDIO)/Green Fluorescent Protein

Cellular/Molecular Mechanism of Action: Hoechst 33258 dye can be applied as an intravital endothelial stain. Rhodaminated long-circulating dextran-coated iron oxide (LCDIO) particles can preferentially localize intracellularly within certain malignant neoplastic cell types (e.g., gliosarcoma).

Application: Detection and characterization of central nervous system neoplastic lesions (e.g., gliosarcoma).

Route of Administration: Intravascular.

Implementation: Determination of the distribution of LCDIO preparation in tumors. Accumulation of LCDIO in tissue sections is evaluated using multichannel fluorescence microscopy in combination with rhodaminated LCDIO, green fluorescent protein functioning as a tumor marker, and Hoechst 33258 dye serving as an intravital endothelial stain. Uptake into tumor cells is corroborated with results from immunohistochemical and cell culture uptake analyses. The effect of intratumoral LCDIO uptake on magnetic resonance imaging (MRI) signal intensity is evaluated with a 1.5-T superconducting magnet.

Animal Model: Rodent gliosarcoma.

Interpretation and Analysis: Tumoral accumulation of LCDIO is $0.11 \pm 0.06\%$ of the injected dose per gram of tissue in brain tumors and is sufficient for detection using MR imaging. In tumor sections, LCDIO preferentially localizes in tumor cells ($49.0 \pm 4.6\%$) but is also taken up by macrophages in tumors ($21.0 \pm 3.1\%$) and by endothelial cells in the areas of active angiogenesis ($6.5 \pm 1.4\%$). In cell culture, LCDIO uptake strongly correlates with growth rate of tumor cell lines. Tumoral LCDIO accumulation is not negligible and helps explain MRI signal intensity changes observed in clinical trials. Microscopically, LCDIO accumulates predominantly in tumor cells and tumor-associated macrophages. Uptake into tumor cells appears to be directly proportional to cellular proliferation rate.

Instrumentation: Magnetic resonance imaging signal intensity evaluated with a 1.5-T superconducting magnet.

Tumoral distribution of long-circulating dextran-coated iron oxide nanoparticles in a rodent model. Moore A, Marecos E, Bogdanov A Jr, Weissleder R. Radiology 2000 Feb; 214 (2), pp. 568–74.

Neuroendocrine Neoplasia/Somatostatin Receptor Expression
Indotricarbocyanine-Octreotate/(Cyanine Dye: Somatostatin Conjugate)

Cellular/Molecular Mechanism of Action: Octreotate is a biologically functional analog of somatostatin.

Application: Contrast agent for the optical *in vivo* imaging of neoplastic lesions that express somatostatin receptors at relatively high densities to the extent that they can be visualized using endoscopy/videoendoscopy.
Route of Administration: Indotricarbocyanine-octreotate (intravenous).
Implementation: Detection and characterization of neuroendocrine neoplastic lesions utilizing indotricarbocyanine-octreotate as a selective optical imaging agent.

Animal Model: Mouse (xenographs of human neuroendocrine neoplastic cell types).
Interpretation and Analysis: During *in vivo* imaging procedures, indotricarbocyanine-octreotate preferentially accumulates in tumors (murine xenografts). The level of fluorescence for tumors rapidly increases and is often more than 3-fold higher than that of normal tissue between 3 and 24 hours after administration. The indotricarbocyanine-octreotate targeting conjugate is also selectively internalized by primary human neuroendocrine tumor cells. Approaches to imaging neuroendocrine neoplastic lesions that combine the specificity of ligand/receptor interaction in concert with near-infrared fluorescence detection represent a diagnostic platform that may be applied in various other fields of clinical oncology.

Receptor-targeted optical imaging of tumors with near-infrared fluorescent ligands. Becker A, Hessenius C, Licha K, Ebert B, Sukowski U, Semmler W, Wiedenmann B, Grotzinger C. Nat Biotechnol 2001 Apr; 19 (4), pp. 327–31.

Stem Cell Migration/[Parkinson's Disease Research]/[Hemopoeitic]
Gadolinium Rhodamine Dextran (GRID)

Cellular/Molecular Mechanism of Action: Rhodamine emits light energy while gadolinium functions as an element that can be selectively detected by magnetic resonance imaging (MRI).
Background Information: The ability to track stem cell transplants in the brain by *in vivo* neuroimaging will improve current understanding about how these cells mediate functional recovery after neural transplantation. One major challenge for the development and refinement of stem cell transplantation is to map the spatial distribution and rate of *in situ* migration.
Application: Gadolinium rhodamine dextran is a bifunctional contrast agent that can emit light energy and be detected in a magnetic field (MRI). These attributes allow it to be applied as a tracer or marker molecule for profiling the migration of transplanted stem cell populations within ischemia-damaged rat hippocampus by MRI.
Route of Administration: Intravenous.
Semi-Synthetic Methods: Conjugation of rhodamine to dextran applying cross-linking strategies. Addition of gadolinium to dextran can potentially be achieved by covalent bond formation or through the application of chelation groups.
Implementation: Assessment of stem cell migration applying *in vitro* labeling of populations with the bifunctional contrast agent GRID prior to transplantation. Detection *in vivo* is achieved applying both MRI and fluorescence microscopy, or with PKH26, visible exclusively under fluorescence microscopy. At different time points following engraftment, brains can be evaluated by both histology analyses and *ex vivo* MRI as validation procedures for research purposes.
Interpretation and Analysis: Transplanted stem cells can be identified by MRI only if prelabeled with GRID, whereas fluorescence microscopy can detect transplanted cells using either labeling reagent. Distribution of GRID-labeled stem cells established by the application of MRI corresponds to results observed using fluorescence microscopy. Stem cells and their migration can therefore be reliably identified applying GRID-enhanced MRI methodologies.

Tracking transplanted stem cell migration using bifunctional, contrast agent-enhanced, magnetic resonance imaging. Modo M, Cash D, Mellodew K, Williams SC, Fraser SE, Meade TJ, Price J, Hodges H. Neuroimage 2002 Oct; 17 (2), pp. 803–11.

Thalamus/Nucleus Rotundus (Crossed-Tectal Projection Tracing)/Thalamic Tectorotundal Pathway Development (Embryonic)
1,1′-Dioctadecyl-3,3,3′,3′-Tetramethylinodocarbocyanine/Cholera Toxin B Subunit

Application: Mapping of axons within the central nervous system.
Implementation: Evaluation of the potential to trace crossed-tectal projections to the nucleus rotundus of the thalamus (tectorotundal pathways). Molecular tracers are injected into the optic tectum under 3 experimental conditions (carbocyanine postfix, carbocyanine *in vivo,* and cholera toxin B subunit *in vivo*) and the anterograde transport of the nucleus rotundus monitored and compared. In the carbocyanine postfix method, small crystals of carbocyanine dye were inserted into the tectum of paraformaldehyde-fixed embryos. A 6-month postinsertion period is required to label the crossed tectorotundal pathway. In the carbocyanine *in vivo* method, a small volume of carbocyanine dye solution is injected into the tectum of living embryos. Similarly, in the cholera toxin B subunit *in vivo* method, a small volume of cholera toxin B subunit solution is injected into the tectum of living embryos.

Animal Model: Avian (paraformaldehyde-fixed and living chick embryos).
Interpretation and Analysis: Tectal neurons do not begin to innervate the ipsilateral nucleus rotundus until embryonic day 9 and the contralateral nucleus rotundus until embryonic day 17. This slow progression of labeling through the crossed-tectal projection results in significant contrast of labeling between the ipsilateral and contralateral nuclei rotundus. In the carbocyanine *in vivo* method, an 8- to 12-hour survival period is sufficient for *in vivo* labeling of the tectorotundal pathway. By embryonic day 8, labeled axons terminate in the ipsilateral nucleus rotundus and the crossed tectorotundal projection can be 1st detected by embryonic day 10. Applying the cholera toxin B subunit *in vivo* method, heavily labeled axons can be found to innervate bilaterally the nucleus rotundus by embryonic day 8 after an initial 6- to 10-hour survival period. This appears to be the earliest time frame for detecting crossed-tectorotundal projection, compared with that of both the postfix and *in vivo* methods of carbocyanine dye. Based on the differences in the detectability of the crossed-tectorotundal projection between the postfix and *in vivo* methods, the present data suggest that the former method is of limited purpose for labeling tectal collaterals during embryogenesis. Moreover, given the rapid transport rate and absence of photobleaching, which is often seen when using carbocyanine dye, the cholera toxin B subunit *in vivo* method appears to be the tracer of choice for investigating embryonic pathways.

Tracing developing pathways in the brain: A comparison of carbocyanine dyes and cholera toxin b subunit. Wu CC, Russell RM, Nguyen RT, Karten HJ. Neuroscience 2003; 117 (4), pp. 831–45.

GASTROINTESTINAL SYSTEM

Mucosal Integrity and Permeability/Bacterial Translocation: Postoperative Hepatic Resection Conditions
Phenolsulfonphthalein (PSP)

Application: One application includes its use in the gastrointestinal system to determine mucosal integrity/permeability. The peroral PSP test appears to be a possible predictor of bacterial translocation after hepatic resection surgery.
Route of Administration: Phenolsulfonphthalein (peroral).
Dosage: Phenolsulfonphthalein (30 mg).
Implementation: Evaluate perioperative changes in intestinal PSP permeability relevant to conditions of hepatic resection. In this context, peroral PSP tests can be scheduled prior to surgery and then on postoperative days 3, 7, and 14, followed by urine collection over a 24-hour period following surgical intervention. Urinary PSP can be measured applying a colorimetric assay. The correlation between the preoperative PSP value and various clinical variables, such as perioperative changes in urinary PSP excretion, and the relationship between the postoperative PSP value and postoperative complications are then determined.

Human Model: Cases of hepatic resection ($n = 50$; $n = 16$ postoperative complications; $n = 10/16$ sepsis).
Interpretation and Analysis: Preoperative urinary PSP excretion increases in a manner proportional to the degree of liver dysfunction. In contrast, urinary PSP excretion does not significantly change during the perioperative period. However, urinary PSP excretion on postoperative day 3 in patients with postoperative infectious complications (27.3%) is significantly greater than that in those without infectious complications (17.4%; $p < 0.05$). Furthermore, PSP excretion on postoperative day 3 in those with infectious complications is also significantly greater as compared to the preoperative level. Cases with a urinary PSP excretion level on postoperative day 3 of greater than 25% exhibit infectious complications more frequently than patients with a level under 25% (60% vs 10.3%, respectively; $p < 0.05$). The peroral PSP test is thus suggested to be a possible predictor of bacterial translocation after hepatic resection.

Significance of the peroral phenolsulfonphthalein test in hepatic resection: A possible predictor of bacterial translocation. Shimada M, Takenaka K, Fujiwara Y, Gion T, Hasegawa H, Shirabe K, Sugimachi K. Hepatogastroenterology 1998 Mar–Apr; 45 (20), pp. 483–7.

Mucosal Integrity and Permeability Tendency/ Hepatic Cirrhosis/Gastrointestinal Surgery
Phenolsufonphthalein (PSP)

Cellular/Molecular Mechanism of Action: Phenolsufonphthalein is a passive marker molecule that is absorbed into the systemic intravascular compartment from the gastrointestinal tract much more extensively in conditions of mucosal damage/injury.
Background Information: The oral PSP absorption test can function as a simple and non-invasive method for the assessment of either the extent of permeability or damage associated with the integrity of the gastrointestinal mucosa.
Application: Assessment of gastrointestinal tract mucosa permeability.
Route of Administration: Phenolsufonphthalein (per os).
Dosage: Phenolsufonphthalein (30 mg).
Implementation: Evaluation of the permeability of the gastrointestinal mucosa in conditions of liver cirrhosis and postoperative gastrointestinal surgery cases utilizing oral phenolsufonphthalein and subsequent measurement of PSP urine concentrations (e.g., urinary recovery rate).
Interpretation and Analysis: Urinary PSP excretion in conditions of liver cirrhosis ($n = 8$; $28.8 \pm 6.0\%$) is significantly higher than in cases subjected to vascular surgery ($n = 8$; $10.0 \pm 1.7\%$) ($p < 0.01$). Such findings reflect the presence of increased gastrointestinal mucosa permeability in conditions of liver cirrhosis. The urinary PSP excretion rates in patients who undergo a total gastrectomy with Roux-en-Y reconstruction are $17.3 \pm 1.7\%$ ($n = 5$ and significantly higher than values observed in controls; $p < 0.05$), while the same rates in cases subjected to a partial gastrectomy ($n = 10$) or colectomy ($n = 10$) are approximately $10.2 \pm 1.8\%$ or $10.6 \pm 0.7\%$, respectively. Parameters of this nature suggest that the intestinal mucosa is damaged by total resection of the stomach. The oral PSP absorption test, which is non-invasive, simple, and inexpensive, is thus considered to be useful for assessing the degree of damage to or the permeability of the gastrointestinal mucosa under various conditions.

Assessing the permeability of the gastrointestinal mucosa after oral administration of phenolsulfonphthalein. Toh Y, Korenaga D, Maekawa S, Matsumata T, Muto Y, Ikeda T, Sugimachi K. Hepatogastroenterology 1997 Jul–Aug; 44 (16), pp. 1147–51.

Mucosal Pathology/Erosions/Histopathological Imaging (*In Vivo*)
Cresyl Violet

Cellular/Molecular Mechanism of Action: Cresyl violet functions as a fluorophore.
Application: Cresyl violet can function as a dye reagent that is compatable in viable biological systems.
Implementation: Confocal laser scanning microscope analysis of *ex vivo* esophageal and gastric mucosa preparations. A series of images is recorded for different levels of cresyl violet concentrations ranging from 0.1% to 10% with staining performed over a time period of 2 minutes after mucolysis with acetylcysteine.

Animal Model: Swine (esophageal and gastric mucosa).
Interpretation and Analysis: Staining with 2% cresyl violet can facilitate the identification of nuclei and cell membranes. Lower concentrations are associated with a lack of homogeneous dye absorption, particularly with regard to the gastric mucosa. Cresyl violet is a clinically usable fluorophore for confocal laser scanning microscopy. Its use in combination with an endoluminally applicable system has the potential applicability for *in vivo* histopathology analysis.

Cresyl violet as a fluorophore in confocal laser scanning microscopy for future *in vivo* histopathology. George M, Meining A. Endoscopy 2003 Jul; 35 (7), pp. 585–9.

Pancreatic Exocrine Insufficiency/ [Renal Failure: Anuria]
Pancreolauryl Test

Cellular/Molecular Mechanism of Action: Pancreolauryl is a substrate molecule that is proteolytically degraded by pancreatic enzymes within the lumen of the intestinal tract. Fluoroscein is subsequently liberated, absorbed across the surface of the intestinal mucosa and excreted in the urine.
Application: Although patients with chronic renal failure have a high incidence of chronic pancreatic disease, the condition is frequently overlooked. Pancreolauryl can be applied to detect and characterize alterations in exocrine pancreatic function in conditions of anuria.
Route of Administration: Pancreolauryl (oral).

Implementation: Modification of the pancreolauryl test as an indirect pancreatic function test for application in cases of anuria.

Interpretation and Analysis: The test permits good discrimination between patients with chronic pancreatic disease and those with a normal pancreas when serum levels of fluorescein are measured 10 hours following administration with a standard meal. The sensitivity at this time interval is 80%, while the level of specificity is approximately 83%. Pancreolauryl test with serum measurements provides a simple, non-invasive, and reliable diagnostic test for chronic pancreatic disease in conditions of simultaneous anuria and chronic renal failure.

Diagnostic value of indirect pancreatic function test in serum of anuric patients with chronic renal failure. Lerch MM, Nolte I, Riehl J, Gladziwa U, Mann H, Sieberth HG, Matern S. Scand J Clin Lab Invest 1994 May; 54 (3), pp. 247–50.

Zollinger-Ellison Syndrome: Gastrinoma Recurrent
Methylene Blue/Secretin

Application: Identification of gastrinoma (e.g., 6 mm) in the duodenum to facilitate surgical excision.

Route of Administration: Selective intra-arterial delivery.

Implementation: Evaluation of Zollinger-Ellison syndrome when other localizing modalities fail to detect a tumor (e.g., ultrasonography, computed tomography, magnetic resonance imaging, duodenal endoscopy, endoscopic ultrasonography, intraoperative endoscopic transillumination of the duodenum). In addition, postoperatively, a negative secretin provocative test result may also be appreciated.

In difficult cases presenting with this type of profile, a novel method can be utilized that involves selective arterial injection of secretin with hepatic venous gastrin sampling to identify the main vessel feeding a gastrinoma lesion. An angiographic catheter is then positioned within the lumen of the identified artery. At laparotomy, methylene blue is injected through this catheter to selectively stain the gastrinoma, thereby facilitating its identification.

Human Model: Cases of persistent Zollinger-Ellison syndrome (n = 1 female, 40 years old) despite excision of a 4-cm duodenal gastrinoma. Localizing studies including ultrasonography, computed tomography, magnetic resonance imaging, duodenal endoscopy, endoscopic ultrasonography, and intraoperative endoscopic transillumination of the duodenum failed to detect the presence or location of a tumor.

Interpretation and Analysis: Selective intra-arterial methylene blue injection can enhance intraoperative detection of small gastrinomas and may improve the rate of curative resection in the Zollinger-Ellison syndrome. Further evaluation of this novel localizing technique is warranted.

Endocrine tumors of the pancreas. Meko JB, Norton JA. Curr Opin Gen Surg 1994, pp. 186–94.

Selective intra-arterial methylene blue injection: A novel method of localizing gastrinoma. Ko TC, Flisak M, Prinz RA. Gastroenterology 1992 Mar; 102 (3), pp. 1062–4.

GENETIC

Gene Therapy Evaluation: Antisense Oligonucleotides/Neoplastic Disease: Leukemia Nucleoli Targeting
Fluorescein-Labeled Phosphorothioate-Triple Helix-Forming Oligonucleotide (PS-TFO)

Cellular/Molecular Mechanism of Action: Oligonucelotide sequences can be designed that recognize and bind to specific gene/messenger RNA (mRNA) sequences.

Physiological Mechanism of Action: Selective inhibition of the transcription/translation of specific genes.

Background Information: The c-myc gene is frequently deregulated and overexpressed in human cancers, and strategies designed to inhibit c-myc expression in cancer cells may have considerable therapeutic value.

Application: Characterize the antigene and antiproliferative activity of a triple helix-forming oligonucleotide (TFO) targeted to a homopurine-homopyrimidine sequence in the P2 promoter of the c-myc gene. Because c-myc is overexpressed in certain neoplastic cell types, genetic targeting modalities could function as effective diagnostic platforms.

Route of Administration: Potentially variable (intravenous, intralesional).

Semi-Synthetic Methods: Triple helix-forming oligonucleotide can be synthesized with phosphorothioate (PS) internucleotide linkages to confer resistance to intra- and extracellular nucleases. The resulting preparation can be conjugated with fluorescein as a tracer agent to identify selective accumulation within certain tissues and subcellular organelles.

Implementation: Delineation of the affinity of fluorescein-labeled PS-TFO.

Interpretation and Analysis: Resistance to intra- and extracellular nucleases is a property that is required for the design of oligonucleotides intended for *in vivo* testing and therapeutic applications.

Phosphorothioate-modified triple helix-forming oligonucleotide forms triplex DNA complexes with affinity and specificity comparable with that of the corresponding phosphodiester TFO, as delineated by gel mobility shift and footprinting assays. Fluorescence microscopy and polyacrylamide gel analysis reveal that fluorescein-labeled PS-TFO accumulates in the nuclei of CEM leukemia cells and remains intact for duration of at least 72 hours. Incubation of CEM cells with PS-TFO reduces c-myc RNA and protein levels. A single exposure of leukemia cells to the PS-TFO is sufficient to induce dose-dependent growth inhibitory effects. Growth inhibition correlates with accumulation of cells in S phase and with induction of cell death by apoptosis. Preparations of PS-TFO are also effective in other leukemia and lymphoma cell lines. Control oligonucleotides have minimal effects in all assays. These data indicate that c-myc-targeted PS-TFO is an effective antigene and antiproliferative agent, with potential for testing *in vivo* as a novel approach to cancer therapy.

Antigene and antiproliferative effects of a c-myc-targeting phosphorothioate triple helix-forming oligonucleotide in human leukemia cells. McGuffie EM, Pacheco D, Carbone GM, Catapano CV. Cancer Res 2000 Jul 15; 60 (14), pp. 3790–9.

HEPATIC SYSTEM

Bile Acid Transport/Visual Detection and Monitoring
Lysyl Fluorescein Conjugated Bile Acid Analogs (LFCBAA)

Background Information: The distribution and metabolism of lysyl fluorescein conjugated bile acid analogues closely parallels the same parameters for their natural counterparts.

Application: Lysyl fluorescein conjugated bile acid analogues can be applied as molecular probes for visualizing bile acid transport within hepatic tissues.

Route of Administration: Intravenous or retrograde injection into the biliary tree.

Semi-Synthetic Methods: Conjugation of lysyl fluorescein to bile acid analogues.

Implementation: Physiological concentrations of the primary bile acid analogue cholyllysyl fluoroscein (CLF) and of the secondary bile acid analogue lithocholyllysyl fluorescein (LLF) are administered, followed by the collection of serial liver biopsies at fixed timed intervals. Both compounds are also injected retrogradely into the biliary tree. Frozen sections are then examined by fluorescence microscopy.

Animal Model: Rat (Wistar).

Interpretation and Analysis: Both CLF and LLF are rapidly taken up from sinusoidal blood but differ significantly in their hepatic handling. Cholyllysyl fluoroscein is rapidly transported into the bile, whereas LLF transport is slower and produces significantly more bile duct fluorescence. Clearance of LLF involves a lobular gradient with the last remaining bile acid being confined (largely zone 3). Both compounds are avidly taken up by cholangiocytes after intravenous or retrograde injection into the biliary tree. Visualization of lysyl fluorescein conjugated bile acid analogues by fluorescence microscopy may yield further information regarding hepatobiliary bile acid localization during studies of physiological and pathological mechanisms involved in the transport of bile acids. The presence of both compounds within cholangiocytes strongly suggests that they may undergo some degree of chole-hepatic recirculation.

Instrumentation: Fluorescence microscopy.

Visualization of the transport of primary and secondary bile acids across liver tissue in rats: *In vivo* study with fluorescent bile acids. Milkiewicz P, Mills CO, Hubscher SG, Cardenas R, Cardenas T, Williams A, Elias E. J Hepatol 2001 Jan; 34 (1), pp. 4–10.

Biliary Excretion Capacity
Sulfobromophthalein (BSP)/Indocyanine Green (ICG)

Route of Administration: Sulfobromophthalein and ICG are given as a single-dose IV injection.

Dosage: Sulfobromophthalein (5 mg/kg); ICG (1.5 mg/kg).

Implementation: Evaluation of hepatic viability as a function of BSP and ICG plasma disappearances. The BSP is given at a dose of 5 mg/kg of body weight, while ICG is given at a dose of 1.5 mg/kg of body weight. The half-life, disappearance rate (percent per minute), and clearance (ml/min/kg) are determined during the initial phase of plasma dye elimination.

Animal Model: Feline.

Interpretation and Analysis: The half-life for BSP is 2.2 ± 0.7 minutes, and for ICG, 3.8 ± 0.9 minutes. The disappearance rate for BSP is 34.7 ± 10.1, and for ICG, 19.2 ± 4.5. The clearance for BSP is 26.3 ± 23.1 and for ICG, 8.6 ± 4.1. The 30-minute percentage retention for BSP is 0.6 ± 0.8% and for ICG, 7.3 ± 2.9%. In the cat, ICG is not cleared as rapidly from the plasma compared to BSP plasma clearance rates. There are no significant differences between males and females for these values. For both BSP and ICG, a significantly shorter half-life, faster disappearance rate, and lower 30-minute percentage retention is observed in the cat, compared with values observed in the dog given similar dye dosages. Clearance curves for BSP and ICG in the cat are similar to those in the dog, indicating that the 2 species may have similar volumes of dye distribution and excretory mechanisms. An assay of a 30-minute plasma level following a 5 mg/kg dose of BSP in normal cats is often too low for measurement, whereas measurement of a 30-minute plasma concentration of ICG following a 1.5 mg/kg dose is adequate. For the 30-minute dye retention test in the cat, ICG may be a preferred diagnostic agent.

Comparison of sulfobromophthalein and indocyanine green clearances in the cat. Center SA, Bunch SE, Baldwin BH, Hornbuckle WE, Tennant BC. Am J Vet Res 1983 Apr; 44 (4), pp. 727–30.

Hepatic Perfusion/Pharmaceutical Modification of Hepatic Blood Flow
Indocyanine Green (ICG)

Cellular/Molecular Mechanism of Action: Indocyanine green is removed from the intravascular compartment by the liver when the reagent enters the hepatic sinusoids and is then excreted into the bile.

Application: Indocyanine green is a hepatic function test marker molecule that is not metabolized by the liver but rather only excreted in the bile. The *in vivo* pharmacokinetic profile of ICG at steady state can be applied to detect pharmaceuticals that alter hepatic blood flow in a manner that can change hepatic blood clearance parameters.

Route of Administration: Indocyanine green (intravenous at a constant infusion rate).

Dosage: Indocyanine green (3.78 mg/kg bolus and constant rate infusion of 10,100 μg/kg/hr is administered until a steady state concentration of Css = 10 μg/ml is achieved at 20, 25, and 30 minutes).

Implementation: Evaluation of the influence of diltiazem (DTZ) on the hepatic blood clearance of ICG as a function of its pharmacokinetic profile at steady state. Following development of a steady state concentration of ICG of 10 μg/ml and a subsequent 90-minute washout period, an intravenous bolus injection (108, 430, 860, and 1,720 μg/kg) and a constant-rate infusion (108, 433, 866, and 1,730 μg/kg/hr) of test pharmaceutical (e.g., DTZ) is introduced into the right femoral vein for the purpose of achieving steady state plasma concentrations (e.g., Css = 50, 200, 400, and 800 ng/ml DTZ) that are confirmed at 60, 70, and 80 minutes. During the steady state phase for the test pharmaceutical (e.g., DTZ), the intravenous bolus injection and the constant-rate infusion of ICG into the left femoral vein are made and also the steady state plasma concentration of ICG measured at 20, 25, and 30 minutes. Plasma concentrations of DTZ and ICG are determined by HPLC analysis.

Animal Model: Rat (left femoral vein injection site).

Interpretation and Analysis: At steady state, the hepatic blood clearance of ICG is derived from values for ICG plasma concentration and the blood-to-plasma concentration ratio (RB). An example of a pharmaceutical that does not influence ICG hepatic clearance is DTZ. Pretreatment with various doses of DTZ does not influence plasma concentrations, RB, and plasma free fraction (fp) of ICG, so the hepatic blood clearance of ICG is independent of DTZ concentration. The hepatic blood clearance of ICG can possibly be affected by both hepatic blood flow and hepatic intrinsic clearance, but there is no change in the hepatic blood clearance of ICG between the control and DTZ-pretreated rats. Diltiazem therefore may not influence hepatic blood flow.

Instrumentation: High-performance liquid chromatography analytical systems.

No effect of diltiazem on the hepatic clearance of indocyanine green in the rats. Joo EH, Lee YB. Arch Pharm Res 1998 Aug; 21 (4), pp. 411–7.

Hepatocellular Function
Indocyanine Green (ICG) Dye

Cellular/Molecular Mechanism of Action: Indocyanine green is removed from the vascular compartment by the function of normal hepatocytes.

Background Information: Although depression in hepatocellular function occurs early after trauma and severe hemorrhage and persists despite fluid resuscitation, it remains unknown whether reactive oxygen species (ROS) play any role in the initiation of hepatocellular depression and damage under those conditions.

Application: Evaluation of hepatic function.

Implementation: Evaluation of the hypothesis that the administration of an ROS scavenger at the beginning of resuscitation attenuates organ injury after severe shock. Male Sprague-Dawley rats following laparotomy are bled and then maintained at a mean arterial pressure of 40 mm Hg until 40% of the maximal bleed-out volume is returned in the form of Ringer's lactate (RL). Animals are then resuscitated with 4 times the volume of maximal bleed-out with RL over a 60-minute period. The ROS scavenger 2-mercaptopropionyl glycine (30 mg/kg) or vehicle is then administered intravenously as a bolus at the beginning of resuscitation. At 2 hours after the completion of crystalloid resuscitation or the equivalent interval after sham operation, cardiac index is measured using a dye-dilution technique. Hepatocellular function, that is, the maximum velocity of ICG clearance (Vmax) and the efficiency of the active transport (Km), can be determined using an *in vivo* hemoreflectometer. Serum levels of tumor necrosis factor-alpha (TNF-α) and alanine aminotransferase can be determined using ELISA and colorimetric methodologies, respectively.

Animal Model: Male Sprague-Dawley rats (275–325 g) subjected to laparotomy (i.e., induction of soft tissue trauma).

Interpretation and Analysis: At 2 hours following trauma, hemorrhage, and resuscitation, cardiac index and hepatocellular function are markedly depressed with concomitant increases in serum levels of TNF-α and alanine aminotransferase ($p < 0.05$). Administration of 2-mercaptopropionyl glycine, however, restores depressed cardiac and hepatic function and markedly attenuates liver enzyme release and serum levels of TNF-α ($p < 0.05$). Reactive oxygen species therefore play a role in the induction of depressed organ function after severe hemorrhagic shock. Adjuncts that attenuate the detrimental effects of ROS may be useful for improving the depressed cardiac and hepatocellular functions after trauma, hemorrhage, and resuscitation.

Critical role of oxygen radicals in the initiation of hepatic depression after trauma hemorrhage. Jarrar D, Wang P, Cioffi WG, Bland KI, Chaudry IH. J Trauma 2000 Nov; 49 (5), pp. 879–85.

Hepatotoxic Injury: Cadmium
Bromosulfophthalein (BSP)

Cellular/Molecular Mechanism of Action: Bromosulfophthalein is removed from the intravascular compartment through the normal functions of liver hepatocytes and the biliary system.

Background Information: Elimination of BSP via the biliary system following intravenous injection requires dissociation from albumin in plasma, translocation across the sinusoidal membrane, conjugation with glutathione within the hepatocyte, translocation across the bile canalicular membrane, and excretion in bile.

Application: Bromosulfophthalein is a relatively non-toxic organic anion used as an *in vivo* indicator of liver performance.

Implementation: Characterization of the *in vivo* hepatotoxicant effects of cadmium (Cd) on BSP kinetics in isolated perfused rat liver (IPRL) to determine the association between liver toxicity and BSP kinetics. After a 30-minute period for acclimation to the IPRL system, livers are dosed with cadmium acetate in the presence of 0.25% bovine serum albumin, sufficient to achieve initial concentrations of 10 and 100 μM. Sixty minutes after Cd dosing, the IPRL system is dosed with BSP to give an initial concentration of 150 μM and the elimination kinetics of BSP from the perfusion medium monitored. Cadmium concentrations in livers at the end of the experiments are 60 ± 4 and 680 ± 210 μmol/kg for the 10 and 100 μM doses, respectively.

Animal Model: Rat (Fisher strain, n = 344 males; isolated perfused liver preparations).

Interpretation and Analysis: Exposure to 10 μM Cd for 60 minutes results in a reduction in bile flow, no significant effect on lactate dehydrogenase (LDH) leakage, and slight effects on BSP clearance. Similar studies following exposure to 100 μM Cd show dramatic decreases in bile flow with complete cholestasis 60 minutes after Cd addition. Total leakage of LDH into perfusion medium is less than 10%, indicating that Cd affects bile production well before the liver shows significant signs of necrosis. Clearance of BSP from the perfusion medium is dramatically reduced. Taken together, the data indicate that Cd has a significant effect on the kinetics of BSP in the IPRL and the dominant effects are mediated through Cd-mediated cholestatic effects.

Effect of cadmium on bromosulfophthalein kinetics in the isolated perfused rat liver system. Soto A, Foy BD, Frazier JM. Toxicol Sci 2002 Oct; 69 (2), pp. 460–9.

Metabolism: Hepatic CYP450 Enzyme Biochemical Activity
Indocyanine Green (ICG)/Lidocaine/Cimetidine

Cellular/Molecular Mechanism of Action: Indocyanine green functions as a global marker of function, while lidocaine and its metabolite, MEGX, can serve as specific markers of the CYP450 enzyme system. Cimetidine functions as a CYP450 enzyme system inhibitor.

Application: Indocyanine green functions as a global marker of function, while lidocaine and its metabolite, MEGX, can serve as specific markers of CYP450 enzyme activity.

Route of Administration: Infusion of ICG and lidocaine via the portal vein after a 40-minute stabilization period until steady state concentrations are reached in the venous outflow.

Dosage: Cimetidine (80 mg/kg daily for 3 days); ICG and lidocaine are infused after a 40-minute stabilization period into the portal vein until steady state concentrations are reached in the venous outflow.

Considerations: Endotoxin administration and cecal ligation and puncture produce significant hepatocellular dysfunction when studied *in vivo.* Specific factors that develop *in vivo* after endotoxin administration and cecal ligation and puncture, such as alterations in liver blood flow, circulating mediators, and hypoxia, can alter hepatic function.

Implementation: Delineation of hepatic clearance and CYP450 function during fabricated conditions of endotoxemia. Indocyanine green (control, n = 6; endotoxin, n = 5) or lidocaine (control, n = 8; endotoxin, n = 8) is infused into livers of control and endotoxin-exposed rat populations. Cimetidine (CYP450 enzyme system inhibitor) can be employed as a reference control (e.g., n = 6 rats).

Animal Model: Rats exposed to endotoxin (*Escherichia coli* lipopolysaccharide at 45 mg/kg intraperitoneally) followed by a 6-hour monitoring period and subsequent isolation of an *in situ* perfused liver preparation. Livers are perfused via the portal vein using a single-pass system with a balanced salt solution 6 hours after receiving either endotoxin or saline or 24 hours after receiving the last dose of cimetidine.

Interpretation and Analysis: Total hepatic clearance and intrinsic hepatic clearance for ICG and lidocaine are unchanged in the livers obtained from endotoxin-treated rats. The animal model can adequately detect CYP450 inhibition because cimetidine-treated rats have significantly lower initial MEGX concentrations (0.63 ± 0.03 mg/L) compared with control (0.77 ± 0.03 mg/L) and endotoxin-treated (0.74 ± 0.04 mg/L) rats. Septic livers have significantly higher initial hepatic oxygen consumption levels (HVO_2) than do control livers (45 ± 3 μL/min/g vs 82 ± 9 μL/min/g). The HVO_2 remains higher in septic livers, significantly increases throughout the

course of observation, and demonstrates that livers remain viable and functional. These data indicate that there is no detectable hepatocellular dysfunction after endotoxin shock using ICG, lidocaine, and MEGX in the isolated perfused liver; therefore the dysfunction reported from *in vivo* studies may be reversible when the liver is removed from such insults.

Effect of endotoxin shock on the clearance of lidocaine and indocyanine green in the perfused rat liver. McKindley DS, Chichester C, Raymond R. Shock 1999 Dec; 12 (6), pp. 468–72.

Sinusoidal Endothelial and Hepatocyte Cell Function/[Experimental Model of Acute and Chronic Liver Failure]
Indocyanine Green (ICG)/Hyaluronic Acid

Cellular/Molecular Mechanism of Action: Indocyanine green and hyaluronic acid are removed from the peripheral circulation by the normal function of hepatocytes and sinusoidal endothelial cells.
Background Information: Experimental models of liver transplantation use normal recipients, although most patients undergoing liver transplantation suffer from acute or chronic liver failure.
Application: Indocyanine green and hyaluronic acid uptake can be utilized to evaluate the *in vivo* function of hepatocytes and sinusoidal endothelial cells.
Implementation: Evaluation of the outcome of orthotopic liver transplantation in compromised hosts. Hepatic injury/dysfunction is evaluated by monitoring enzymes, bilirubin, ammonia levels, prothrombin, thrombin time, and cytokines. The *in vivo* function of hepatocytes and sinusoidal endothelial cells can be evaluated applying ICG and hyaluronic acid uptake.
Animal Model: Rat (D-galactosamine-induced acute liver failure or chronic liver failure secondary to common bile duct ligation). Transplantation performed in normal, acute, and chronic liver failure rats at different time points using either freshly harvested or cold-preserved syngeneic livers.
Interpretation and Analysis: Survival with fresh grafts decreases significantly when transplants are performed 48 hours after the induction of acute liver failure. Rats with acute liver failure almost invariably do not survive transplantation with grafts stored for 12 or 24 hours, but in chronic failure survival is more than 80%. Survival of acute liver failure rats receiving grafts preserved for 6 hours is 16.6% compared with 83.3% observed with fresh grafts transplanted at the same time point after D-galactosamine exposure. Elevated tumor necrosis factor-alpha and interleukin-1beta levels as well as impaired sinusoidal endothelial cell function can be detected in acute liver failure rats with grafts preserved for 6 hours. Speculation therefore suggests that preoperative status and different host factors have a significant effect on outcome and graft function after liver transplantation in rats.

Graft function and survival depend primarily on host factors in compromised recipient models of orthotopic liver transplantation in the rat. Wang L, Roayaie S, Basile J, Florman S, Liu J, Tarcsafalvi A, Boros P, Miller CM. Transplantation 2001 Jan 15; 71 (1), pp. 14–20.

HEMATOLOGY

Cell/Endothelial Adhesion (Colon Carcinoma)/ Intravital Observation of Cancer Cell/ Microcirculation Interactions
CalceinAM

Background Information: Organ-specific tumor cell adhesion within the microcirculation of host organs is an important step in the metastatic cascade. Circulating tumor cells have to adhere within the microcirculatory vessels, quickly stabilize their adhesion, and probably leave the circulation to avoid toxic effects of hydrodynamic shear forces of circulating blood.
Application: Intravital observation of colon carcinoma cell adhesion within the hepatic microcirculation.
Implementation: Characterization of neoplastic cell metastatic phenomenon. CalceinAM can be used to label colon carcinoma cell populations (e.g., HT-29 [human] and CC531 [rat]) that are then injected intra-arterially as single-cell suspensions. Intravital fluorescence microscopy is then applied to detect adhesive interactions between circulating tumor cells and endothelial surfaces within the hepatic microcirculation. These interactions are then analyzed pertaining to their time course and site localization within the vascular tree.
Animal Model: Rats (Sprague-Dawley).
Interpretation and Analysis: Autofluorescence of liver parenchyma is sufficient for distinction of hepatic sinusoids. Intravital microscopy is capable of differentiating early events in adhesion formation within hepatic sinosoids, adhesion stabilization, and extravasation of the tumor cells into the liver parenchyma. Tumor cell adhesion occurs almost exclusively within sinusoidal capillaries; however, the diameter of these vessels is usually larger than that of the tumor cells, leaving remaining lumen of the capillaries available for perfusion. Colon carcinoma cells rapidly migrate into the liver parenchyma after successful adhesion within the sinusoids. In contrast to common end point assays of the metastatic cascade, this *in vivo* model allows investigations of metastatic colon carcinoma cell adhesion within the liver microcirculation as specific steps during the formation of hematogenous metastasis and their underlying mechanisms.
Instrumentation: Intravital fluorescence microscopy.

An intravital model to monitor steps of metastatic tumor cell adhesion within the hepatic microcirculation. Haier J, Korb T, Hotz B, Spiegel HU, Senninger N. J Gastrointest Surg 2003 May–Jun; 7 (4), pp. 507–14; discussion 514–5.

Endothelium Cell Adhesion/Platelet Endothelium Interactions/[Retinal Microvascular Pathology]
Carboxyfluorescein Diacetate Succinimidyl Ester/Platelets (Fluorescent-Labeled)/Platelets (Fluorescein-Labeled)

Cellular/Molecular Mechanism of Action: Carboxyfluorescein diacetate succinimidyl ester interacts with light and functions as a fluorescent marker molecule suitable for "tracing" the migration of different cell types.
Application: Visualization of *in vivo* platelet behavior in the retinal microcirculation. The methodologies utilizing carboxyfluorescein diacetate succinimidyl ester allow for quantitative evaluation of platelet dynamics and platelet-endothelial interactions in conditions of retinal pathology.
Route of Administration: Intravascular.
Considerations: Represents the development of a new method for evaluating the *in vivo* dynamics of platelets in the retinal microcirculation and quantitative investigation of platelet-endothelial interactions.
Implementation: Isolated platelet samples are labeled with carboxyfluorescein diacetate succinimidyl ester. After intravenous administration, platelet behavior in the retinal microcirculation can be evaluated with a scanning laser ophthalmoscope. Images are recorded on S-VHS videotape and analyzed with a computer-assisted image analysis system. The platelet-endothelial interactions in the retinal microcirculation can also be investigated employing lipopolysaccharide-stimulated endothelium or platelets activated with thrombin.

Animal Model: Rat retina.

Interpretation and Analysis: Fluorescent platelets are recognized as distinct dots in the retinal microcirculation and can be traced frame by frame. The velocity of platelets in the retinal arteries, capillaries, and veins is approximately 26.1 ± 6.4, 1.6 ±0.4, and 19.9 ± 8.2 mm/sec, respectively. In control rats, even activated platelets show minimal interaction with retinal endothelial cells. In contrast, stimulated retinal endothelium shows active platelet-endothelial interactions, with many platelets observed rolling and adhering along the major retinal veins. The interactions between platelets and stimulated endothelial cells are substantially inhibited by the injection of P-selectin monoclonal antibody.

In vivo evaluation of platelet-endothelial interactions in retinal microcirculation of rats. Tsujikawa A, Kiryu J, Nonaka A, Yamashiro K, Nishiwaki H, Tojo SJ, Ogura Y, Honda Y. Invest Ophthalmol Vis Sci 1999 Nov; 40 (12), pp. 2918–24.

Hematopoietic Stem Progenitor Cell Tracking
PKH Dyes/PKH2/PKH26/PKH67

Cellular/Molecular Mechanism of Action: PKH dyes can be utilized as marker molecules where they irreversibly bind to cell membrane structures. The lipophilic dyes PKH26 and PKH67 have red and green fluorescent properties, respectively.

Background Information: The process of hematopoietic stem and progenitor cell (HSPC) seeding in recipient bone marrow (BM) early after transplantation has not been fully characterized.

Application: PKH dyes serve as marker molecules that can be utilized to track the *in vivo* migration of cell populations in biological systems.

The PKH dyes were initially developed to provide appropriate probes for *in vitro* and *in vivo* cell tracking. It has been reported for many cell types that PKH binds irreversibly to the cell membrane without significantly affecting cell growth. Thus these probes provide an opportunity for long-term cell monitoring and the identification of cells of interest among a heterogeneous cell population. An important feature is that upon cell division, the probe is partitioned equally between each daughter cell, making it possible to quantify fluorescence by flow cytometry. In this scenario, the flow cytometric study of PKH67 characteristics shows that this probe does not affect the main cell functions such as viability or proliferation. Moreover, the intracellular distribution of PKH67 is demonstrated by following its kinetics of internalization by confocal microscopy. Therefore, PKH67 is a probe suitable for dynamic analysis of cell proliferation as well as the study of intracellular localization and membrane recycling mechanisms.

There are no significant phototoxic effects of PKH2 and PKH26 dyes on cell viability and growth when using dye concentrations up to 5 μM. However, when stained cells are exposed to excitation light, cell viability decreases dramatically, showing the phototoxicity of PKH2 and PKH26 dyes. More than 60% of cells stained with 5 μM PKH26 die after 5 minutes of continuous light exposure. The phototoxic effect is more extensive in cells stained with higher concentrations of the dyes.

Implementation: *In vivo* tracking of HSPCs labeled with PKH dyes facilitated by utilizing an optical window surgically implanted into the femur (e.g., murine model) in a manner that can recognize transplanted cell clusters within recipient BM.

Interpretation and Analysis: In the first 24 hours after intravenous injection, 86 ± 6% of the cells seed in clusters ($p < 0.001$ vs scattered cells) in the endosteal surfaces of the epiphyses. The primary clusters are formed by concomitant seeding of 6 to 10 cells over an area of approximately 70 μm, and secondarily injected cells do not join the already existing clusters but form new clusters. Major antigen-disparate HSPCs participate in formation of primary clusters, and T lymphocytes are also incorporated. After 4–5 days, some cellular clusters can be observed in the more central regions of the BM, where the brightness of PKH fluorescence decreases, indicating cellular division. These later cellular aggregates are classified as secondary clusters, assuming that the mechanisms of migration in the BM may be different from those of primary seeding. Some clusters remain in the periphery of the BM and retain bright fluorescence, indicating cellular quiescence. The number of brightly fluorescent cells in the clusters decreases exponentially to 2 to 3 cells after 24 days ($p < 0.001$). Hematopoietic niche is a functional unit of the BM stromal microenvironment that hosts seeding of a number of transplanted cells, which form a cluster. This may be the site where auxiliary non-HSPC cells, such as T lymphocytes, act in support of HSPC engraftment.

Innocuousness and intracellular distribution of PKH67: A fluorescent probe for cell proliferation assessment. Rousselle C, Barbier M, Comte VV, Alcouffe C, Clement-Lacroix J, Chancel G, Ronot X. In Vitro Cell Dev Biol Anim 2001 Nov–Dec; 37 (10), pp. 646–55.

Phototoxicity of the fluorescent membrane dyes PKH2 and PKH26 on the human hematopoietic KG1a progenitor cell line. Oh DJ, Lee GM, Francis K, Palsson BO. Cytometry 1999 Aug 1; 36 (4), pp. 312–8.

Transplanted hematopoietic cells seed in clusters in recipient bone marrow *in vivo*. Askenasy N, Zorina T, Farkas DL, Shalit I. Stem Cells 2002; 20 (4), pp. 301–10.

IMMUNOLOGY

Immunity/Antigen Delivery to Nodes and Processing
[Fluorescein Isothiocyanate (FITC)]-H-2K(b) CTL Epitope (SIINFEKL)/pH-Sensitive Liposomes

Cellular/Molecular Mechanism of Action: Fluorescein isothiocyanate is a molecule that emits fluorescent color when excited by certain wavelengths of light energy. Liposomes that are described as being pH sensitive provide a modality for protecting their internal contents from metabolic degradation or accelerated elimination but also have the property of degrading upon exposure to environments within a specific range of hydrogen ion concentration. Fluorescein isothiocyanate-conjugated H-2K(b) CTL epitope (SIINFEKL) is perceived by the immune system as a foreign antigen.

Application: Fluorescein isothiocyanate can be applied as a fluorescent tracer molecule in various biological systems. Fluorescein isothiocyanate-conjugated H-2K(b) CTL epitope (SIINFEKL) can be used as a "labeled" antigen for the purpose of tracing the fate of immunogens within the immune system. Liposomes that have been designed to possess pH-sensitive properties release their internal contents through membrane lysis when exposed to environments within a specific pH range.

Interpretation and Analysis: At 3 hours following immunization, fluorescence can initially be detected in lymph node structures, and its intensity reaches a peak value in superficial inguinal lymph node at 9 hours. Traces of fluorescence can be detected within the spleen even with prolonged monitoring for up to 24 hours. These results strongly suggest that the presentation of CTL-peptide antigen vehicled by pH-sensitive liposomes exclusively occurs within the lymph nodes. In mice immunized with the H-2K(b) CTL epitope encapsulated pH-sensitive liposomes, peptide-specific CTL response can be detected at day 3. The response is strongly augmented by a 2nd immunization and persists up to 45 days or longer. Therefore, liposomes formulated to be pH sensitive can function as potential

adjuvants of peptide antigens and are useful for the *in vivo* induction of antigen-specific CTLsv.

Investigation of antigen delivery route *in vivo* and imune-boosting effects mediated by pH-sensitive liposomes encapsulated with K(b)-restricted CTL epitope. Lee KY, Chun E, Seong BL. Biochem Biophys Res Commun 2002 Apr 5; 292 (3), pp. 682–8.

Lymph Nodes/Guide for Sentinel Lymphadenectomy (Inguinal)/Epidermoid Carcinoma: Anal Canal
Patent Blue V Dye/[^{99m}Tc]-Dextran 500

Implementation: Evaluation of sentinel lymph node structures applying a combination of preoperative lymphoscintigraphy with [^{99m}Tc]-dextran 500 injected around the tumor and intraoperative detection of the sentinel node with a gamma probe. Patent blue V dye can also be injected at the periphery of the tumor to facilitate direct identification of the blue-stained lymph nodes. After removal, the sentinel nodes are studied by hematoxylin and eosin staining for immunohistochemistry to detect pancytokeratins (antigen A1 and A3).

Human Model: Cases of epidermoid carcinoma of the anal canal without clinical evidence of inguinal involvement (n = 14).

Interpretation and Analysis: Detection and removal of sentinel lymph nodes is possible in the vast majority of cases (100% reported). There is no correlation between tumor size and pattern of lymphatic drainage to the groin. Tumors located in the midline of the anal canal give rise to bilateral sentinel nodes in most cases (e.g., n = 8/9; n = 23 sentinel lymph nodes removed). Some cases (n = 1/8) have a node identified as positive for metastatic carcinoma on immunohistochemical staining. Surgical complications are minimal. The standardized technique is safe and highly effective in sampling inguinal sentinel lymph nodes in carcinoma of the anal canal. It has also proved useful as an approach for detecting micrometastatic deposits in clinically normal nodes. Early experience with the technique suggests that the sentinel lymph node procedure may have a role in guiding a more selective approach for patients with anal cancer. Additional studies in a larger patient population are warranted to determine the sensitivity and specificity of this method.

Sentinel lymph node identification and sampling in women with early breast cancer using [^{99m}Tc]-labeled dextran 500 and patent blue V dye. Xavier NL, Amaral BB, Cerski CT, Fuchs SC, Spiro BL, Oliveira OL, Menke CH, Biazús JV, Cavalheiro JA, Schwartsmann G. Nucl Med Commun 2001 Oct; 22 (10), pp. 1109–17.

Sentinel lymph node procedure in patients with epidermoid carcinoma of the anal canal: Early experience. Damin DC, Rosito MA, Gus P, Spiro BL, Amaral BB, Meurer L, Cartel A, Schwartsmann G. Dis Colon Rectum 2003 Aug; 46 (8), pp. 1032–7.

Lymph Nodes: Guide for Sentinel Lymphadenectomy (Melanoma)
Isosulfan Blue Dye/[^{99m}Tc]-Human Serum Albumin ([^{99m}Tc]-HSA)/[^{99m}Tc]-Sulfur Colloid ([^{99m}Tc]-SC)

Cellular/Molecular Mechanism of Action: Isosulfan blue dye serves as a fluid pigment-based marker system. [^{99m}Tc]-human serum albumin and [^{99m}Tc]-SC are phagocytized by reticuloendothelial cell populations residing within peripheral lymph nodes.

Background Information: Radiopharmaceutical agents appear to improve the accuracy of sentinel node (SN) identification in patients with early-stage melanoma, but the optimal radiopharmaceutical agent and its timing from injection until surgery still remains controversial.

Application: Isosulfan blue dye can be used to validate the localization of radiobiopharmaceutical agents within anatomical structures (e.g., lymph nodes).

Route of Administration: Isosulfan blue dye, [^{99m}Tc]-SC, and [^{99m}Tc]-HSA can be administered intraoperatively into lymphatic vessels.

Implementation: Evaluation of intraoperative lymphatic mapping with radiopharmaceutical-directed sentinel lymphadenectomy (LM/SL) procedures (e.g., n = 3). In this regard, cutaneous lymphoscintigraphy can be performed before LM/SL utilizing 1 of 3 techniques including (i) [^{99m}Tc]-HSA injected at least 24 hours before LM/SL (n = 124 patients); (ii) filtered [^{99m}Tc]-SC injected no more than 4 hours before LM/SL (same-day SC, n = 95 patients); or (iii) [^{99m}Tc]-SC injected at least 18 hours before LM/SL (prior-day SC, 28 patients). At the time of LM/SL, isosulfan blue dye is injected alone (SC groups) or with a 2nd dose of HSA (HSA group). A handheld gamma probe is then used to identify radioactive (hot) counts of blue-stained and non-stained nodes, and the *in vivo* and *ex vivo* node-to-background count ratios of the nodes compared.

Human Model: Melanoma (n = 247 classified as American Joint Committee on Cancer stage I or II).

Interpretation and Analysis: Preoperative LS can identify a large percentage of drainage basins (e.g., n = 299 in 247 cases). Lymphatic mapping with radiopharmaceutical-directed sentinel lymphadenectomy (LM/SL) is capable of identifying at least 1 sentinal lymph node in a large percentage of cases. Specific examples in this regard include (i) 119 (98%) of 121 basins using same-day SC; (ii) 142 (97%) of 146 basins using HSA; and (iii) 32 (100%) of 32 basins using prior-day SC. There is no difference ($p = 0.62$) in the accuracy rate between the 3 techniques (total of 463 sentinel nodes identified in 247 cases). Same-day SC yields higher intraoperative node-to-background count ratios than do either of the other techniques ($p < 0.0001$). Same-day SC also has the greatest relative change in radioactivity between the blue sentinel node and the postexcision basin ($p < 0.0001$), and the highest rate of SNs that are both blue and hot (*in vivo* or *ex vivo* ratio ≥ 2, $p = 0.05$). Therefore, LS and LM/SL performed on the same day with a single injection of filtered [^{99m}Tc]-SC serves as the most useful method for probe-directed LM/SL. The technique demonstrates the highest *in vivo* and *ex vivo* count ratios, falloff of radioactivity between the excised nodes and postexcision basin, and concordance between blue dye and hot nodes. It should be recommended as the method of choice for probe-directed LM/SL.

Standardized probe-directed sentinel node dissection in melanoma. Essner R, Bostick PJ, Glass EC, Foshag LJ, Haigh PI, Wang HJ, Morton DL. Surgery 2000 Jan; 127 (1), pp. 26–31.

MEMBRANE CHARACTERIZATION

Liposome Membrane Characterization: Stability/Optimization of Properties
ANS Fluorescent Probe/DPH Fluorescent Probe

Cellular/Molecular Mechanism of Action: Fluorescent probes emit detectable light energy when excited by specific wavelengths of radiant energy.

Application: Fluorescent probes can be applied as visible molecular markers for assessing various *in vitro* and *in vivo* properties associated with liposome preparations.

Implementation: Evaluation of the systematic physicochemical characteristics of 2 branched chain polymers (based on a poly-L-lysine backbone) with the general formula poly[Lys-(DL-Ala_m-X_i)], where X = Orn (OAK) or N-acetyl-Glu (Ac-EAK) and m congruent with 3, using surface pressure and fluorescence polarization methods.

Experimental findings are compared with those of the linear poly(L-Lys) from which OAK and Ac-EAK are derived. Complementary investigations can involve the delineation of interactions between these polymers with phospholipid bilayers (neutral or negatively charged) with vesicles labeled with 2 fluorescent probes: ANS and DPH.

Interpretation and Analysis: The 2 polymers show a moderate surface activity, able to form stable monomolecular layers at the air-water interface. Poly(L-Lys), the most hydrophilic, has the lowest surface activity. Results indicate that the polymers are able to accommodate in their internal structure, mainly through electrostatic interactions, a certain amount of ANS marker molecules, but fluorescence increases with the ANS-polypeptide complexes are so low that its influence in subsequent polarization measurements can be discarded. After interaction with liposomes, polymers induce an increase in the polarization of the probes, thus indicating a rigidification of the bilayers. Electrostatic forces seem to be very important in this interaction; cationic polymers are clearly more active, with PG-containing liposomes, than Ac-EAK. Moreover, in assays, poly(L-Lys) behaves as the more active compound. This fact is probably due to its major ability to form alpha-helical structures that can insert readily into bilayers. Such findings indicate that the polymeric structures studied can be used as carriers for biologically active molecules, because their interactions with bilayers remain soft and have a positive effect on the stability of membranes.

Physicochemical characterization of branched chain polymeric polypeptide carriers based on a poly-lysine backbone. Nagy IB, Hudecz F, Alsina MA, Reig F. Biopolymers 2003 Nov; 70 (3), pp. 323–35.

Macrophage/Mononuclear/Neutrophil/Phagocytic Function
Fluorescent Polymethacrylic Nanoparticles

Cellular/Molecular Mechanism of Action: Fluorescent polymethacrylic nanoparticles are recognized by and intracellularly assimilated by mononuclear cells and, to a lesser extent, neutrophils.

Application: Quantitative evaluation of phagocytosis by human mononuclear and polymorphonuclear cells. One possible application of fluorescent-labled nanoparticles is the simultaneous characterization of cell surface antigens and cell phagocytic activity.

Route of Administration: Intravascular.

Semi-Synthetic Methods: Preparation of nanoparticles involves emulsion copolymerization of methacrylic monomers combined with delineation of their physicochemical properties.

Implementation: Evaluation of fluorescent polymethacrylic nanoparticles (0.3 micron) as a flow cytometric reagent in the quantitative evaluation of phagocytosis by human mononuclear and polymorphonuclear cells. In this context, nanoparticles coupled with a fluorescent agent (ethidium bromide) can be used in flow cytometric assays to study opsonin-independent phagocytosis by human neutrophils (PMNs) and human monocytes. Phagocytosis of nanospheres by monocytes is determined by flow cytometry based on fluorescence distribution, while ingestion is visualized by scanning and transmission electron microscopy.

Instrumentation: Flow cytometry and scanning and transmission electron microscopy.

Flow cytometric quantitative evaluation of phagocytosis by human mononuclear and polymorphonuclear cells using fluorescent nanoparticles. Rolland A, Merdrignac G, Gouranton J, Bourel D, Le Verge R, Genetet B. J Immunol Methods 1987 Feb 11; 96 (2), pp. 185–93.

Membrane Permeable Marker Molecules: Non-Radioactive Tracer
Cyanine Fluorochromes (Aqueous Soluble)/Auto-Quenching Enzyme-Sensitive Near-Infrared (NIR) Fluorescent Probe

Cellular/Molecular Mechanism of Action: Cyanine fluorochromes emit fluorescent light energy in the NIR range.

Background Information: Recently, NIR fluorescence light has been applied to image various biological events *in vivo,* because it penetrates tissue more efficiently than light in the visible spectrum.

Application: Cyanine fluorochromes emit light energy in the NIR range, and this property facilitates their use as tracers or marker molecule systems in living biological systems.

Semi-Synthetic Methods: Synthesis of water-soluble NIR cyanine fluorochromes (n = 4). Each fluorochrome is designed with a monoreactive carboxyl group that can be utilized for labeling purposes. In addition, they possess superior chemical stability and optical properties. An autoquenched enzyme-sensitive NIR fluorescence probe can be designed and synthesized to address the problem of fluorescence quenching when multiple fluorochromes are conjugated to single macromolecules.

Interpretation and Analysis: Certain water-soluble NIR cyanine fluorochromes possess superior chemical stability and optical properties that permit exterior *in vivo* detection. Attachment of multiple fluorochromes to a single macromolecule can result in fluorescence quenching.

Novel near-infrared cyanine fluorochromes: Synthesis, properties, and bioconjugation. Lin Y, Weissleder R, Tung CH. Bioconjug Chem 2002 May–Jun; 13 (3), pp. 605–10.

Membrane Pore Size: Permeation Properties/ Cardiovascular: Myocardial Cells
Ethidium Bromide (EB)

Cellular/Molecular Mechanism of Action: Ethidium bromide selectively binds to nucleic acids, thereby allowing it to function as a passive biological marker molecule. Ethidium bromide also possesses fluorescent properties when exposed to ultraviolet light energy.

Background Information: Strong electric pulses produce reversible or irreversible membrane breakdown (electroporation).

Application: Detection of alterations in membrane pore size.

Implementation: Analyze the permeation properties of minute pores caused by hyperpolarization or lysophosphatidylcholine (LPC) by comparing the amount of charge carried by irregular inward currents (I^{hi}) with changes in EB fluorescence in isolated rabbit ventricular myocytes.

Technique: Forty-second negative pulses from a holding potential of −20 mV induce (I^{hi}), whose conductance increases with hyperpolarization.

Interpretation and Analysis: Mean conductance (G^{hi}) is 63.6 ± 9.9 pS/pF (mean ± standard error of measurement [SEM], n = 9) at −160 mV. Ethidium bromide fluorescence increases during voltage pulses in parallel with the time integral of I^{hi} (Q^{hi}), with the magnitude of increases in nuclear EB fluorescence being 5.3 times greater than in the cytoplasm at −160 mV. Similar hyperpolarization-induced parallel increases in I^{hi} and EB fluorescence are also obtained in Na^+-free, N-methyl-D-glucamine (NMDG) solution. LPC (10 μM) induces large (101.2 ± 21.2 pS pF(−1), n = 16), rapid (rise times, 1–10 ms) I^{hi} with slow relaxation rates at −80 mV that

reflect increases in G^{hi} to 94.3 ± 24.8 pS pF(−1) (n = 8) at 6 minutes. Plots of EB fluorescence vs Q^{hi} are well fitted by a common Hill's equation with a Hill coefficient of 0.97. Taken together, findings indicate that hyperpolarization and LPC produces pores that have the same filter properties for the permeation of small ions, including ethidium^{+}, and that I^{hi} (carried in part by Ca^{2+} generated by membrane breakdown) is capable of supplying sufficient ions to evoke abnormal excitation and contraction in cardiac myocytes.

Hyperpolarization and lysophosphatidylcholine induce inward currents and ethidium fluorescence in rabbit ventricular myocytes. Song YM, Ochi R. J Physiol 2002 Dec 1; 545 (Pt 2), pp. 463–73.

Membrane Protein-Lipid Interactions
N-[10-(1-Pyrenyl)Decanoyl]Sphingomyelin

Cellular/Molecular Mechanism of Action: The agent N-[10-(1-pyrenyl)decanoyl]sphingomyelin is a fluorescent sphingomyelin derivative.

Background Information: Selective enzymatic hydrolysis, lipid compositional analyses, and fluorescence studies have been carried out on acetylcholine receptor (AChR)-rich membranes from Torpedinidae to investigate the topology of sphingomyelin (SM) in the native membrane and its relationship with the AChR protein. Controlled sphingomyelinase hydrolysis of native membranes has shown that SM is predominantly (approximately 60%) localized in the outer half of the lipid bilayer. Differences have also been observed in the distribution of SM fatty acid molecular species in the 2 bilayer leaflets.

Application: Characterization of membrane protein-lipid interactions.

Implementation: A fluorescent SM derivative (N-[10-(1-pyrenyl)decanoyl]sphingomyelin; Py-SM) can be used to study protein-lipid interactions in the AChR-rich membrane and in affinity-purified Torpedinidae AChR reconstituted in liposomes made from Torpedinidae electrocyte lipid extracts. The efficiency of Förster resonance energy transfer (FRET) from protein to the pyrenyl-labeled lipid as a function of acceptor surface density can be used to estimate distances and topography of the SM derivative relative to the protein. The dynamics of the lipid acyl chains can be explored by measuring the thermal dependence of Py-SM excimer formation, sensitive to the fluidity of the membrane.

Interpretation and Analysis: Differences can observed in the concentration dependence of excimer/monomer pyrenyl fluorescence when measured by direct excitation of the probe relative to FRET conditions, indicating differences in the intermolecular collisional frequency of the fluorophores between bulk and protein-vicinal lipid environments, respectively. Py-SM exhibits a moderate selectivity for the protein-vicinal lipid domain, with a calculated relative affinity K_r approximately 0.55. Upon sphingomyelinase digestion of the membrane, FRET efficiency increases by about 50%, indicating that the resulting pyrenyl-ceramide species have higher affinity for the protein than the parental SM derivative.

Sphingomyelin composition and physical asymmetries in native acetylcholine receptor-rich membranes. Bonini IC, Antollini SS, Gutiérrez-Merino C, Barrantes FJ. Eur Biophys J 2002 Oct; 31 (6), pp. 417–27.

Metabolism: Intracellular Reactive Oxygen Species (ROS)/ROS Production: Intracellular
Dihydroethidium

Cellular/Molecular Mechanism of Action: Dihydroethidium functions as a fluorescent probe; chelerythrine is a protein kinase C (PKC) inhibitor; PP101 is a PKC delta inhibitor; and PP149 is a PKC-epsilon inhibitor.

Background Information: Protein kinase C and ROS are known to have a role in anesthetic preconditioning (APC). Cardiac preconditioning by triggers other than volatile anesthetics, such as opioids or brief ischemia, is known to be isoform selective, but the isoform required for APC is not known.

Application: Detection and characterization of intracellular ROS production.

Implementation: Identify the PKC isoform that is involved in APC and elucidate the relative positions of PKC activation and ROS formation in the APC signaling cascade. One approach to addressing this objective can involve the evaluation of the influence of sevoflurane APC in heart tissue prior to the initiation of ischemic reperfusion injury in the absence or presence of the non-specific PKC inhibitor chelerythrine, the PKC-delta inhibitor PP101, or the PKC-epsilon inhibitor PP149. Spectrofluorometry and the fluorescent probe dihydroethidium can be used to measure intracellular ROS, while assay of effluent dityrosine is used to measure extracellular ROS release.

Animal Model: Isolated guinea pig hearts subjected to 30 minutes of ischemia and 120 minutes of reperfusion.

Interpretation and Analysis: Previous sevoflurane exposure protects heart tissues against ischemia-reperfusion injury, as previously described. Chelerythrine or PP149 abolishes such protection, but PP101 does not. Reactive oxygen species formation is observed during sevoflurane exposure and is not altered by any of the PKC inhibitors. Anesthetic preconditioning is mediated by PKC-epsilon but not by PKC-delta. Furthermore, PKC activation probably occurs downstream of ROS generation in the APC signaling cascade.

Reactive oxygen species precede the epsilon isoform of protein kinase C in the anesthetic preconditioning signaling cascade. Novalija E, Kevin LG, Camara AK, Bosnjak ZJ, Kampine JP, Stowe DF. Anesthesiology 2003 Aug; 99 (2), pp. 421–8.

Microsphere Release Evaluation and Characterization
Brilliant Blue Microspheres/Tumor Necrosis Factor-Alpha (TNF-α) Microspheres/Poly(D,L-Lactic-Co-Glycolic Acid) Multiphase Microspheres

Cellular/Molecular Mechanism of Action: Brilliant blue functions as a dye marker reagent that is compatible with biological systems.

Application: Brilliant blue can be applied to evaluate the rate of release of compounds incorporated into microsphere preparations.

Route of Administration: Intravascularly or within individual body cavities.

Semi-Synthetic Methods: The microspheres can be prepared using an anhydrous multiple emulsion solvent evaporation method.

Implementation: Intraperitoneal delivery to evaluate rate of release.

Animal Model: Murine (intraperitoneal).

Interpretation and Analysis: Multiphase microspheres containing brilliant blue exhibit a 3-phase release profile *in vitro* and display a significantly lower level of dye released during the initial phase compared to conventional matrix-type microspheres. Slow release of the dye is observed during the 2nd phase, which is followed by a disintegration of the polymer wall during the 3rd phase of the release process. *In vitro* dissolution profiles of TNF-α are calculated by compensation for the simultaneous degradation of the protein in the dissolution medium. The initial burst release of TNF-α is

significantly reduced with the multiphase microspheres. The 3-phase release profile, as seen with the dye, is not observed for the microspheres containing the TNF-α. The rate of release of protein from microspheres is determined *in vivo* by analyzing the residual level of TNF-α remaining in the particles following intraperitoneal administration of the microspheres to mice. The release of protein from microspheres *in vivo* is significantly faster than predicted from the results of the *in vitro* studies. The absence of an initial burst release of TNF-α from multiphase microspheres is recognized by the observation of a significant reduction in plasma TNF-α levels when compared to matrix-type microspheres and a solution of the protein. The controlled release property of multiphase microspheres is expected to overcome adverse reactions due to dose dumping that can occur following local TNF-α administration.

In vitro and *in vivo* release properties of brilliant blue and tumor necrosis factor-alpha (TNF-α) from poly(D,L-lactic-co-glycolic acid) multiphase microspheres. Iwata M, Nakamura Y, McGinity JW. J Microencapsul 1999 Nov–Dec; 16 (6), pp. 777–92.

MUSCULOSKELETAL SYSTEM

Bone Growth Marker
Tetracycline

Cellular/Molecular Mechanism of Action: Tetracycline becomes associated with and incorporated into hydroxyapatite crystals within the osseous bony matrix.
Diagnostic Application: Detection and measurement of new osseous growth; has also reportedly been successful in the correction of certain equine angular limb deformities if administered in the early days of life shortly after parturition.
Route of Administration and Dosage: Oral or intravenous.
Considerations: Can cause severe diarrhea in equine patients due to disruption of the normal balance of gastrointestinal bacterial flora that can occasionally lead or predispose to development of life-threatening salmonellosis.
Interpretation and Analysis: Measurement of the osseous segment deposited following tetracycline administration is an indication of the extent of new bone growth.

Bioassayable growth hormone activity in blood from healthy individuals and acromegalic patients. Thorngren KG, Hallengren B. Acta Endocrinol (Copenh) 1986 Jan; 111 (1), pp. 3–9.

Effects of unilateral arterial infusion of GH and IGF-I on tibial longitudinal bone growth in hypophysectomized rats. Nilsson A, Isgaard J, Lindahl A, Peterson L, Isaksson O. Calcif Tissue Int 1987 Feb; 40 (2), pp. 91–6.

Bone Physiology and Growth/Aluminum ($Al(OH)_3$) Body Stores/Overload/Toxicity/Hemodialysis/ Hematopoeitin/Iron Absorption: Iron Deficiency
Tetracycline/Desferrioxamine/Deferoxamine/[^{59}Fe]

Cellular/Molecular Mechanism of Action: Tetracycline is incorporated into bone hydroxyapatite deposits. Desferrioxamine and deferoxamine are metallic ion chelating agents. Desferrioxamine can induce elevations in basal plasma aluminum concentrations. Tetracycline is incorporated into bone hydroxyapatite deposits.
Application: Desferrioxamine can be applied to characterize body aluminum stores while tetracycline can be used as a marker for profiling bone physiology and bone deposition parameters.
Implementation: Evaluation of the influence of hemofiltration (HF) and hemodialysis (HD) in connection with the risk of aluminum overload and renal osteodystrophy by analysis of double bone biopsies after double tetracycline labeling and use of the desferrioxamine test.

Human Model: Cases managed by hemofiltration (n = 12 HF) and hemodialysis (n = 15 HD). No cases recieving 1-α-OH-D administration. No cases have florid osteomalacia with increased osteoid thickness, and n = 1 with traces of stainable aluminum.
Interpretation and Analysis: Aluminum concentrations are low (< 0.6 μmol/l) in both the dialysate and the substitution fluid. The duration of treatment (about 2 years) and the cumulative doses of $Al(OH)_3$ and $CaCO_3$ are comparable in the 2 groups. The aluminum balance during an HF run can range from −22 to +1.8 μmol/l, the balance being positive only when the plasma aluminum is less than 0.5 μmol/l. Basal plasma aluminum and its increase induced by desferrioxamine are comparable in the 2 groups. Bone aluminum content is also comparable but is about 10 times higher than in non-uremic controls (e.g., n = 7). Bone aluminum content and plasma aluminum increase after desferrioxamine can be correlated to $Al(OH)_3$ cumulative dose. The mineralization front is decreased in most cases of hemofiltration (e.g., n = 8/12) and hemodialysis cases (n = 9/14) to the extent that differences cannot be appreciated between each management scheme. The predominant histological bone picture of the patients is osteitis fibrosa, which is present in a large percent of hemofiltration and hemodialysis cases (e.g., n = 10/12 HF and n = 13/15 HD, respectively). Mean osteoclast count and active resorption surface are comparable in the 2 groups, but is increased 5–10 times the mean of the controls.

Comparative evaluation of bone aluminum content and bone histology in patients on chronic hemodialysis and hemofiltration. Sebert JL, Fournier A, Leflon P, Fohrer P, de Frémont JF, Morinière P, Galy C, Marie A, Demontis R, Boudailliez B, et al. Nephron 1986; 42 (1), pp. 34–40.

Bone Physiology and Growth: Hydroxyapatite (HA) Deposition/Osteoblastic Activity/Neoplastic Disease: Osteosarcoma/Atherosclerosis
Near-Infrared (NIR) Light-Emitting Probes/Fluorescent Bisphosphonate Derivatives

Background Information: In vertebrates, the development and integrity of the skeleton requires HA deposition by osteoblasts. Sites of osteoblastic activity have most commonly been imaged *in vivo* using gamma-emitting radioisotopes. The scan times required are long, and the resultant radioscintigraphic images suffer from relatively low resolution. One of the advantages of the application of NIR fluorescence light in imaging modalities for various *in vivo* biological processes is related to its ability to penetrate tissues more efficiently than light in the visible spectrum.
Application: Hydroxyapatite deposition as a marker of, or a participant in, processes as diverse as cancer and atherosclerosis.
Semi-Synthetic Methods: Production of an NIR fluorescent bisphosphonate derivative.
Implementation: Evaluation of the ability of NIR fluorescent bisphosphonate derivatives to physically bind to hydroxyapatite deposits.
Interpretation and Analysis: Near-infrared light-based probes like fluorescent bisphosphonate derivatives posess an ability to bind to hydroxyapatite under *in vitro* conditions, in addition to recognizing osteoblastic activity in living animals. Such biotechnology can be applied to study skeletal development, osteoblastic metastasis, coronary atherosclerosis, and other disease states.

In vivo near-infrared fluorescence imaging of osteoblastic activity. Zaheer A, Lenkinski RE, Mahmood A, Jones AG, Cantley LC, Frangioni JV. Nat Biotechnol 2001 Dec; 19 (12), pp. 1148–54.

NEOPLASTIC DISEASE STATES

Neoplasia/Mammary Carcinoma
Indocyanine Green (ICG)

Cellular/Molecular Mechanism of Action: Indocyanine green (extrinsic contrast agent) functions as a non-radioactive tracer molecule.
Route of Administration: Indocyanine green (bolus injection).
Implementation: Evaluation of the uptake of a non-targeted contrast agent by breast tumors using a continuous-wave diffuse optical tomography apparatus (detection of the tracer molecule on the surface of tumor-bearing breast structures [coronal geometry]). A 2-compartment model can be used to analyze the pharmacokinetics of ICG and preprocessing of data, in addition to diffuse optical tomography used for imaging.

Human Model: Clinical scenarios with 3 different pathologies.
Interpretation and Analysis: Localization and delineation of tumor lesions can be achieved in good agreement with prior information. Moreover, different dynamic features can be observed for differing pathologies. Malignant cases exhibit slower rate constants (uptake and outflow) compared to healthy tissue. Such observations provide further evidence that *in vivo* pharmacokinetics of ICG in breast tumors may be a useful diagnostic tool for differentiation (classification) of benign and malignant pathologies.
Instrumentation: The instrument operates in the near-infrared spectral window and employs 16 sources and 16 detectors to collect light in parallel.

In vivo continuous-wave optical breast imaging enhanced with indocyanine green. Intes X, Ripoll J, Chen Y, Nioka S, Yodh AG, Chance B. Med Phys 2003 Jun; 30 (6), pp. 1039–47.

Neoplasia/Mammary Carcinoma: Estrogen Receptors
[Rhenium-N_2S_2]-Z-Aminotamoxifen/[Rhenium- N_2S_2]-E-Aminotamoxifen/[^{99m}Tc-N_2S_2]-Z-Aminotamoxifen/[^{99m}Tc -N_2S_2]-E-Aminotamoxifen

Cellular/Molecular Mechanism of Action: Aminotamoxifen recognizes and binds to membrane-associated estrogen receptor complexes.
Application: Imaging agent for the detection and characterization of breast cancer lesions. Traditionally, tamoxifen is used as a estrogen antagonist chemotherapeutic agent.
Route of Administration: Intravascular.
Semi-Synthetic Methods: The bifunctional chelator agent is conjugated to Z- and E-aminotamoxifens through an amide linker. Both technetium-99m [^{99m}Tc] and rhenium can be bound to N_2S_2 by chelation.
Implementation: Detection and characterization of mammary carcinoma lesions (*in vivo*).
Interpretation and Analysis: For the Z-isomer, chelation with rhenium in the presence of sodium acetate yields a mixture of 2 isomers, anti and syn, in a 1:1 ratio and in the presence of hydroxide results in only the anti isomer. Both the Z- and E-tamoxifen conjugates can be chelated with technetium-99m at tracer levels, yielding a single isomer product, which is assigned as anti based on chromatographic comparison to the rhenium complexes. Radiochemical yields are consistently greater than 80%, with Sep-Pak column purification yielding a final product with $>$ 99% radiochemical purity and no residual starting material. Both *in vitro* and *in vivo* biological evaluation of the tamoxifen chelates indicates very limited estrogen receptor binding.

Single isomer technetium-99m tamoxifen conjugates. Hunter DH, Luyt LG. Bioconjug Chem 2000 Mar–Apr; 11 (2), pp. 175–81.

Neoplasia/Mammary Carcinoma Explants
Glucamine-Substituted Cyanine/Gluosamine-Substituted Cyanine

Background Information: One of the advantages of near-infrared (NIR) fluorescence light for application in imaging modalities for various *in vivo* biological processes is related to its ability to penetrate tissues more efficiently than light in the visible spectrum.
Application: Glucamine and gluosamine-substituted cyanine are dyes structurally related to indocyanine green (ICG) that can be applied as contrast-enhancing optical imaging agents for the detection of neoplastic lesions (e.g., human mammary carcinoma explants in rats) utilizing NIR modalities.
Route of Administration: Intravenous.
Semi-Synthetic Methods: Production of a group of glucamine and gluosamine-substituted cyanine dyes structurally related to ICG.
Implementation: Characterization of glucamine and gluosamine-substituted cyanine dyes structurally related to ICG for their potential to function as contrast agents for biomedical optical imaging modalities. Derivatives with the highest hydrophilicity are evaluated for their efflux from tumor and normal tissue by monitoring with intensity-modulated diffuse optical spectroscopy after intravenous injection.

Animal Model: Rat (tumor-bearing).
Interpretation and Analysis: Glucamine and gluosamine-substituted cyanine dyes exhibit increased hydrophilicity and less plasma protein binding ($<$ 50%) compared to ICG. Accordingly, they also have different pharmacokinetic properties. Measurable enhancement in fluorescence quantum yields (7–15%) can be detected in a physiological environment compared to ICG. The derivative 5a has the highest level of hydrophilicity. In comparison with ICG, derivative 5a exhibits considerably enhanced tissue-efflux half-life (73 minutes vs $<$ 10 minutes for ICG in tumor tissue) and a 2-fold higher initial tissue absorption coefficient compared to ICG, and it generates an elevated tumor-to-tissue concentration gradient up to 1 hour after injection. In conclusion, compounds such as 5a are promising contrast agents for optical imaging and can facilitate highly sensitive and specific detection of breast cancer or other malignancies by utilizing mechanisms similar to contrast-enhanced magnetic resonance imaging or computerized tomography.

Hydrophilic cyanine dyes as contrast agents for near-infrared tumor imaging: Synthesis, photophysical properties, and spectroscopic *in vivo* characterization. Licha K, Riefke B, Ntziachristos V, Becker A, Chance B, Semmler W. Photochem Photobiol 2000 Sep; 72 (3), pp. 392–8.

Neoplasia/Pancreatic Adenocarcinoma
Fluorescein-Neurotensin [Arg(8), Arg(9), and Ile(12)]/[^{111}In]-Diethylenetriamine Pentaacetic Acid (DTPA)-Neurotensin [Arg(8), Arg(9), and Ile(12)]

Cellular/Molecular Mechanism of Action: Native neurotensin (NT) is a tridecapeptide that binds to neurotensin receptor (NTR) and induces tumor growth.
Background Information: The prevalence of NTR in several human tumors makes it an attractive target for the delivery of cytotoxic drugs and imaging agents. Unfortunately, NT has a short plasma half-life, which hinders its use for *in vivo* biomedical applications.

Numerous reports suggest that Arg(8), Arg(9), and Tyr(11)-Ile(12) amide bonds are particularly susceptible to degradation by proteolytic enzymes.

Application: Detection of NTR-positive human adenocarcinoma.

Semi-Synthetic Methods: Substitution of Arg(8), Arg(9), and Ile(12) amino acids with the corresponding commercially available mimics. Preparations can be conjugated to DTPA for subsequent radiolabeling to produce [^{111}In]-DTPA or alternatively conjugated to fluorescein dye.

Animal Model: Mouse (severe combined immunodeficient [SCID] strain bearing NTR-positive human adenocarcinoma [HT29] xenografts).

Interpretation and Analysis: The surrogate amino acids are amenable to standard 9-fluorenylmethyl chloroformate (Fmoc) peptide synthesis strategy, and the resulting compounds are stable in biological media for $>$ 4 hours and bind to NTR with high affinity. Conjugation of DTPA to the synthetic peptides and subsequent labeling with [^{111}In]-DTPA for nuclear imaging or fluorescein for optical imaging does not diminish NTR binding avidity. Evalution of the *in vivo* biodistribution of a representative [^{111}In]-DTPA-NT peptide analogue in HT29-bearing SCID mice reveals that the compound is primarily retained in tumor tissue (2.2% ID/g) and the kidneys (4.8% ID/g) at 4 hours postinjection. Co-injection of cold NT and the radiolabeled NT peptide analogue inhibits tumor but not kidney uptake, and demonstrates that retention of the radiolabeled compound in tumor tissue is mediated by NTR specific uptake while it accumulates in the kidneys by a non-specific mechanism. These findings show that NT peptide analogues are robust and can deliver imaging agents to NTR-positive tumors such as pancreatic cancer.

Novel bioactive and stable neurotensin peptide analogues capable of delivering radiopharmaceuticals and molecular beacons to tumors. Achilefu S, Srinivasan A, Schmidt MA, Jimenez HN, Bugaj JE, Erion JL. J Med Chem 2003 Jul 17; 46 (15), pp. 3403–11.

Neoplasia/Skin Cancer: Melanoma/Non-Invasive Transcutaneous Detection
Fluorescein-Isothiocyanate-Labeled Antimelanoma IgG/Texas Red–Labeled Dextran

Cellular/Molecular Mechanism of Action: Fluorescein-isothiocyanate and Texas Red emit fluorescent color when exposed to light energy of a specific wavelength.

Application: Fluorescein-isothiocyanate and Texas Red can be used as visual color marker or tracer molecules in biological systems. Fluorescein-isothiocyanate-labeled antimelanoma and Texas Red–labeled dextran can be applied for the transcutaneous detection of melanoma lesions.

Route of Administration: Variable.

Semi-Synthetic Methods: Covalent conjugation and cross-linking conjugation strategies for the semi-synthetic production of fluorescein-isothiocyanate-labeled antimelanoma IgG and Texas Red–labeled dextran.

Implementation: Determination of the relative effectiveness of 3 different fluorescein-isothiocyanate-labeled antimelanoma antibodies to selectively localize within melanoma lesions. Detection is achieved *in vivo* by applying a single-channel confocal imaging system.

Animal Model: Mice (athymic BALB/c strain) with human melanoma cells (n = 3 cell lines) cultured from human skin implanted intradermally into the haunch skin of anesthetized mice and allowed to grow to a maximum size of 2 mm in diameter.

Interpretation and Analysis: Fiber-optic confocal imaging performed following intravenous administration of fluorescently labeled antibodies and Texas Red-dextran facilitates the *in vivo* detection of melanoma and surrounding blood vessels in athymic mice. Using non-invasive techniques, the overall *in vivo* melanoma detection rate for tumors within 0.2 mm of the skin surface is 84% (27 of 32 tumors). Normal cultured human skin cells have little or no fluorescence after administration of the fluorescein-isothiocyanate-labeled antibodies and tumors are not labeled by an isotype control antibody. Dual-channel *in vivo* imaging of implanted melanoma tumors and surrounding dermal vasculature reveals increased blood vessel density at the melanoma site. Detection *in vivo* of melanoma tumors up to 0.2 mm below the skin surface by fiber-optic confocal imaging can be confirmed by conventional immunoperoxidase histology.

Instrumentation: Single-channel confocal imaging system.

In vivo detection of small subsurface melanomas in athymic mice using noninvasive fiber optic confocal imaging. Anikijenko P, Vo LT, Murr ER, Carrasco J, McLaren WJ, Chen Q, Thomas SG, Delaney PM, King RG. J Invest Dermatol 2001 Dec; 117 (6), pp. 1442–8.

Neoplasia/Skin Cancer: Trans-Cutaneous Lesion Detection
Light-Emitting Probes/Firefly Luciferase-Tagged Cells/ Fluorescent Protein-Tagged Cells

Cellular/Molecular Mechanism of Action: Firefly luciferase or fluorescent proteins emit color (e.g., red to infrared range $\geq$ 600 nm) when exposed to a specific substrate.

Background Information: *In vivo* imaging of cells tagged with light-emitting probes, such as firefly luciferase or fluorescent proteins, is a powerful technology that enables a wide range of biological studies in small research animals. Reporters with emission in the red to infrared ($>$ 600 nm) are preferred due to the low absorption in tissue at these wavelengths. One of the advantages of the application of near-infrared (NIR) fluorescence light in imaging modalities for various *in vivo* biological processes is related to its ability to penetrate tissues more efficiently than light in the visible spectrum.

Application: Light-emitting probes can be used as visible markers for detecting cell migration and localization in a manner that can be utilized for *in vivo* lesion detection (e.g., neoplastic disease).

Interpretation and Analysis: Reporter molecules that emit light in the red to infrared range ($>$ 600 nm) are preferred due to their low absorption in tissue at these wavelengths. Modeling of photon diffusion through tissue indicates that bioluminescent cell counts as low as a few hundred can be detected subcutaneously, while approximately 10^6 cells are required to detect signals at approximately 2 cm depth in tissue. Signal-to-noise estimates show that cooled back-thinned integrating charged-coupled devices (CCDs) are preferred to image-intensified CCDs for this application, mainly due to their high quantum efficiency (approximately 85%) at wavelengths $>$ 600 nm where tissue absorption is low.

Instrumentation: Cooled back-thinned integrating CCDs are preferred to image-intensified CCDs.

In vivo imaging of light-emitting probes. Rice BW, Cable MD, Nelson MB. J Biomed Opt 2001 Oct; 6 (4), pp. 432–40.

Neoplasia/Skin Cancer Differentiation/Basal Cell Carcinoma (BCC)/Squamous Cell Carcinoma (SCC)/Precancerous Lesions/Benign Lesions
Nitrogen/Dye Laser (410 nm)

Cellular/Molecular Mechanism of Action: Induced fluorescence of neoplastic tissues.

Application: Laser-induced fluorescence spectroscopy for *in vivo* diagnosis of non-melanoma skin cancers in a manner that can accurately differentiate normal tissues from BCC, SCC, precancerous lesions, and benign lesions based on the interpretation of emissions profiles.
Implementation: Cases are classified as having either skin types I, II, or III and biopsy samples from abnormal tissues collected by biopsy for evaluation. Each analysis measurement is designated as being either normal, BCC, SCC, precancerous, or benign. The total emission photon count is used as a discriminating index. A threshold value is calculated to separate normal tissue indices from indices of cancer tissues. The classification accuracy of each data point is determined using the threshold value.

Human Model: Cases with either skin types I, II, or III (n = 49 total; n = 279 analyses on normal and abnormal tissues).
Interpretation and Analysis: Applying the described categorization scheme, cancers can be classified 93%, 89%, and 78% correctly in cases with skin types I, II, and III, respectively. Normal tissues can be classified 93%, 88%, and 50% correctly in cases with skin types I, II, and III, respectively. Using the same threshold, precancerous spectra can be classified 78% and 100% correctly in skin types I and III, respectively. Benign lesions can be classified 100%, 46%, and 27% correctly in cases with skin types I, II, and III, respectively. Therefore, *in vivo* laser-induced fluorescence spectroscopy at 410 nm excitation and using the intensity of emission signal is an effective approach for detecting BCC, SCC, and actinic keratosis, especially in patients with light-colored skin.
Instrumentation: Nitrogen/dye laser tuned at 410 nm, an optical multichannel analyzer, and a fiber-optic probe for excitation of tissue and collection of fluorescence emission.

Laser-induced fluorescence spectroscopy for *in vivo* diagnosis of non-melanoma skin cancers. Panjehpour M, Julius CE, Phan MN, Vo-Dinh T, Overholt S. Lasers Surg Med 2002; 31 (5), pp. 367–73.

Neoplasia/Squamous Cell Carcinoma
Fluoroscein Isothiocyanate (FITC)-CD3 Conjugates

Cellular/Molecular Mechanism of Action: Fractions of CD3 recognize and physically bind to unique membrane-associated complexes expressed on the exterior surface membrane of certain neoplastic cell types. Fluorescein isothiocyanate emits color when exposed to certain wavelengths of light energy.
Application: Fluorescein isothiocyanate can be used as a light-emitting molecular marker system for non-invasive detection and characerization of neoplastic disease states.
Route of Administration: Intravenous, intramuscular, or intralesional (variable).
Semi-Synthetic Methods: Covalent conjugation of the fluorescent agent FITC to the CD3 marker fraction.
Implementation: Intratongue injection of FITC-conjugated CD3 marker for the identification of neoplastic lesions. Detection is performed by excitation of marker molecules with an argon laser (488 nm) and the resulting fluorescence signal measured as a function of time. Biopsies are taken from each mouse and excised tissue histologically evaluated.

Animal Model: Mouse (BALB/c strain; n = 14 mice; n = 7 controls). Seven mice receive injections of a squamous cell carcinoma cell line into the tongue.
Interpretation and Analysis: Analysis of clearance times revealed a 2nd order exponential decay for both groups with a slower pace of signal clearance for sick mice.
Instrumentation: Argon laser (488 nm) and the fluorescence signal measurement.

Laser activated fluorescence measurements and morphological features: An *in vivo* study of clearance time of fluorescein isothiocyanate tagged cell markers. Gannot I, Gannot G, Garashi A, Gandjbakhche A, Buchner A, Keisari Y. J Biomed Opt 2002 Jan; 7 (1), pp. 14–9.

Neoplasia/Squamous Cell Carcinoma/ Differentiation: Oral Premalignant vs Malignant
5-Aminolevulinic Acid (ALA)-Induced Protoporphyrin IX (PPIX) Fluorescence

Background Information: Studies of 5-aminolevulinic acid-induced PPIX fluorescence have displayed sensitivity levels of 95 to 100% for the diagnosis of oral cancer lesions, but the specificity is only about 50 to 60%.
Application: Quantifying PPIX fluorescence imaging for specific diagnostic detection of early oral lesions (differentiation of premalignant from malignant disease states).
Implementation: Fluorescence endoscopy and imaging are performed on cases of suspected premalignant or malignant oral cavity lesions in conjunction with the collection of biopsy samples for histological analysis (e.g., n = 70) harvested from tissue sites following imaging procedures. The red-to-blue and red-to-green intensity ratios are calculated from fluorescent images for correlation with histology analysis.

Human Model: Cases with known or suspected premalignant or malignant oral cavity lesions (e.g., n = 28).
Interpretation and Analysis: Suspicious lesions display bright reddish fluorescence, while normal mucosal structures exhibit blue color background in the fluorescence images. The red-to-blue and red-to-green intensity ratios of malignant tissues are larger than those of benign tissues. Combining the 2 ratio diagnostic algorithms yields a sensitivity and specificity of 95% and 97%, respectively, exceeding each diagnostic algorithm alone for discriminating malignant tissue from benign tissue. Quantifying PPIX fluorescence endoscopic images combined with ratio diagnostic algorithms has the potential to significantly improve the *in vivo* non-invasive diagnosis of oral cavity lesions.
Instrumentation: Fluorescence endoscopy.

Detection of squamous cell carcinomas and precancerous lesions in the oral cavity by quantification of 5-aminolevulinic acid-induced fluorescence endoscopic images. Zheng W, Soo KC, Sivanandan R, Olivo M. Lasers Surg Med 2002; 31 (3), pp. 151–7.

Neoplasia/Squamous Cell Carcinoma/ Differentiation: Premalignant vs Malignant Oral Lesions
5-Aminolevulinic Acid (ALA)-Induced Protoporphyrin IX (PPIX) Fluorescence

Cellular/Molecular Mechanism of Action: 5-aminolevulinic acid is capable of promoting fluorescence from PPIX compounds.
Background Information: 5-aminolevulinic acid induces fluorescence of PPIX that provides a sensitivity level of 95 to 100% for oral cancer diagnosis with a corresponding specificity of only approximately 50–60%.
Application: Differentiation of premalignant from malignant forms of oral cancer applying PPIX fluorescence endoscopy and fluorescence image quantification.
Implementation: Characterization of oral premalignant and malignant lesions applying PPIX fluorescence endoscopy and fluorescence image quantification. To improve diagnostic specificity, ALA-mediated digitized fluorescence endoscopic imaging systems have been designed that enable on-line image acquisition, analysis,

and fluorescence quantification for early detection of oral cavity neoplasms.

Human Model: Cases of suspected premalignant and malignant lesions of the oral cavity (e.g., n = 16).

Interpretation and Analysis: Head and neck clinical trials have shown that the red-to-blue intensity ratio of malignant tissue is larger than that of benign tissue. By applying the intensity ratio as a diagnostic algorithm, both high specificity and sensitivity can be achieved for discriminating malignant tissue from benign tissue, and the capability of classifying different histopathological stages of oral lesions can also be demonstrated. Red fluorescence intensity distribution in lesion areas could also be obtained to better understand PPIX tissue accumulations. Initial results indicate that the digitized endoscopic imaging system combined with the fluorescence image quantification method and the ratio diagnostic algorithm has the potential of significantly improving the non-invasive *in vivo* diagnosis of early oral neoplasms.

Detection of neoplasms in the oral cavity by digitized endoscopic imaging of 5-aminolevulinic acid-induced protoporphyrin IX fluorescence. Zheng W, Soo KC, Sivanandan R, Olivo M. Int J Oncol 2002 Oct; 21 (4), pp. 763–8.

Neoplasia/Urinary Neoplasia/Bladder Cancer
5-Aminolevulinic Acid (ALA)/5-Aminolevulinic Acid Methyl Ester (AME)

Cellular/Molecular Mechanism of Action: 5-aminolevulinic acid and AME derivative emit fluorescent light energy. Certain neoplastic cell populations preferentially accumulate these agents intracellularly.

Background Information: The use of ALA and its esters to induce endogenous porphyrins for the purpose of detecting epithelial cancers is being studied extensively in many centers around the world. One major challenge is the development of techniques for preparation of efficacious formulations for the purpose of cancer detection.

Application: 5-aminolevulinic acid and AME can be used as fluorescent imaging agents for the photodynamic detection of neoplastic lesions on "exterior" dermal or mucosal surfaces such as cancers of the urinary bladder.

Implementation: Compare ALA with AME derivative in terms of PPIX fluorescence intensity applying *in vitro* and *in vivo* systems of bladder carcinoma. Complementary studies involve comparison evaluations of intravenous and topical application modes for drug administration in an *in vivo* system with RT112 xenografts. An endoscopy system is used to obtain macroscopic fluorescence images. The macroscopic images are further analyzed for fluorescence intensity distribution.

Animal Model: Murine (tumor-bearing mice with RT112 xenographs of human bladder cancer cell types).

Interpretation and Analysis: For intravenous administration, over all time points studied (1, 3, 6 hours), AME-PPIX fluorescence is lower than ALA-PPIX fluorescence and is cleared at a faster rate than the ALA-PPIX when administered intravenously. Topical application with 2 different polymers, Gantrez and Polyvinyl pyrrolidone (PVP), which are fast-releasing polymers, is comparable for inducing PPIX fluorescence. Topical AME-PPIX fluorescence is comparable to ALA-PPIX fluorescence. The agent AME also appears to be applicable as a viable diagnostic agent.

Instrumentation: Karl Storz fluorescence endoscopy system.

A study of 5-aminolevulinic acid and its methyl ester used in *in vitro* and *in vivo* systems of human bladder cancer. Manivasager V, Heng PW, Hao J, Zheng W, Soo KC, Olivo M. Int J Oncol 2003 Feb; 22 (2), pp. 313–8.

Neoplasia: Antineoplastic Property Correlation with Biodistribution/Delivery Characterization
[Fluoroscein Isothiocyanate (FITC)]-Glycol-Chitosan Nanoaggregates

Application: Fluoroscein isothiocyanate can be applied as a fluorescent tracer molecular for characterizing a variety of physiological processes or delineating the distrubution of delivery modalities (e.g., [FITC]-glycol-chitosan nanoaggregates).

Route of Administration: Intravenous (e.g., rat tail vein).

Semi-Synthetic Methods: Conjugation of fluoroscein isothiocyanate to glycol-chitosan-doxorubicin (GC-DOX) results in the production of nanoaggregates with a diameter of about 250 nm in aqueous media. To increase the applicability of such preparations, GC-DOX nanoaggregates can be produced that contain an acid-sensitive spacer moiety. Preparations of GC-DOX spontaneously form micelle-like nanoaggregates in aqueous media. Glycol-chitosan-doxorubicin nanoaggregates have a narrow and unimodal size distribution, and their hydrodynamic diameter as measured by dynamic light scattering ranges from 250 to 300 nm. A loading content of doxorubicin into GC-DOX nanoaggregates as high as 38%, with 97% loading efficiency, can be achieved using a physical entrapment method.

Implementation: Characterization of the *in vivo* biodistribution of GC-DOX nanoaggregates (doxorubicin-loaded GC-DOX nanoaggregates or DOX/GC-DOX) facilitated by conjugation to FITC. Observations can be applied to determine if these parameters correlate with the antineoplastic properties exerted by conjugated preparations.

Animal Model: Rat (tumor-bearing animals developed by inoculating tumor cells into the region of the back).

Interpretation and Analysis: Nanoaggregates of FTC-GC are distributed mainly within kidney, tumor, and the liver with very scarce amounts observed in other tissues/organ systems. They maintain high levels for 8 days and their distribution within neoplastic tissues increases gradually. Chitosan nanoaggregates appear to accumulate passively in tumor tissue due to the enhanced permeability and retention (EPR) effect. Tumor growth is suppressed over a 10-day period.

Biodistribution and antitumor efficacy of doxorubicin-loaded glycol-chitosan nanoaggregates by EPR effect. Son YJ, Jang JS, Cho YW, Chung H, Park RW, Kwon IC, Kim IS, Park JY, Seo SB, Park CR, Jeong SY. J Control Release 2003 Aug 28; 91 (1–2), pp. 135–45.

Neoplasia: Colon Cancer/Optical Lesion Imaging
Indotricarbocyanine (ITCC)/ITCC-Transferrin (Tf)/ ITCC-Human Serum Albumin (HAS)

Cellular/Molecular Mechanism of Action: Indotricarbocyanine is a dye that can serve as a detectable marker molecule in biological systems.

Background Information: Macromolecules accumulate in solid tumors and can thus be used as carriers for the delivery of attached contrast agents to tumors.

Application: Indotricarbocyanine can be applied as a contrast agent for the optical imaging of neoplastic lesions.

Route of Administration: Intravascular.

Semi-Synthetic Methods: Production of serum protein-dye conjugates consisting of Tf or HSA covalently linked to ITCC derivative.

Implementation: Evaluation of properties associated with the semi-synthetic ITCC-HSA and ITCC-Tf conjugates pertaining to their individual (i) photophysical characteristics; (ii) ability to bind

in vitro to tumor cells; and (iii) *in vivo* potential to detect/characterize experimental tumors.

Model: Human HT29 colon cancer cell types propagated *in vitro* in a tissue culture environment and HT29 tumor-bearing nude mice.

Interpretation and Analysis: In contrast to ITCC-HSA, ITCC-Tf displays *in vitro* receptor-mediated uptake by HT29 human colon cancer cells. After intravenous injection, ITCC-HAS and ITCC-Tf both induce increased *in vivo* fluorescence of tumors. After 24 hours the contrast between tumor and normal tissue is significantly higher for ITCC-Tf than for HAS-ITCC-HSA. Dye-induced fluorescence can be found to be predominantly located in perinecrotic areas of tumor lesions. Furthermore, ITCC-Tf produces fluorescence of viable tumor cells, whereas ITCC-HAS fluorescence can be detected along connective tissue. Therefore, ITCC-labeled Tf and HSA can serve as macromolecular contrast agents for the optical imaging of tumors, with ITCC-Tf showing higher efficiency.

Macromolecular contrast agents for optical imaging of tumors: Comparison of indotricarbocyanine-labeled human serum albumin and transferrin. Becker A, Riefke B, Ebert B, Sukowski U, Rinneberg H, Semmler W, Licha K. Photochem Photobiol 2000 Aug; 72 (2), pp. 234–41.

Neoplasia: Colon Carcinoma: Endothelium Cell Adhesion/Intravital Observation of Cancer Cell/ Microcirculation Interactions
CalceinAM

Background Information: Organ-specific tumor cell adhesion within the microcirculation of host organs is an important step in the metastatic cascade. Circulating tumor cells have to adhere within the microcirculatory vessels, and then must quickly stabilize their adhesion, followed by exiting the circulation to avoid the toxic effects of hydrodynamic shear forces of circulating blood.

Application: Intravital observation of colon carcinoma cell adhesion within the hepatic microcirculation.

Implementation: CalceinAM can be used to label colon carcinoma cell populations (e.g., HT-29 [human] and CC531 [rat]) that are then injected intra-arterially as single-cell suspensions. Intravital fluorescence microscopy is then applied to detect adhesive interactions between circulating tumor cells and endothelial surfaces within the hepatic microcirculation. These interactions are then analyzed regarding their time course and site localization within the vascular tree.

Animal Model: Rats (Sprague-Dawley).

Interpretation and Analysis: Autofluorescence of liver parenchyma is sufficient for the distinction of hepatic sinusoids. Intravital microscopy is capable of differentiating early events of adhesion formation within hepatic sinosoids, adhesion stabilization, and extravasation of tumor cells into the liver parenchyma. Tumor cell adhesion occurs almost exclusively within sinusoidal capillaries; however, the diameter of these vessels is usually larger than that of the tumor cells leaving the lumen of these perfused capillaries. Colon carcinoma cells rapidly migrate into the liver parenchyma after successful adhesion within the sinusoids. In contrast to common end point assays of the metastatic cascade, this *in vivo* model allows investigations of metastatic colon carcinoma cell adhesion within the liver microcirculation as specific steps during the formation of hematogenous metastasis and their underlying mechanisms.

Instrumentation: Intravital fluorescence microscopy.

An intravital model to monitor steps of metastatic tumor cell adhesion within the hepatic microcirculation. Haier J, Korb T, Hotz B, Spiegel HU, Senninger N. J Gastrointest Surg 2003 May–Jun; 7 (4), pp. 507–14; discussion 514–5.

Neoplasia: Colorectal Cancer/Gastric Cancer
[Indocyanine Green (ICG)]-Anti-MUC1 Mucin

Cellular/Molecular Mechanism of Action: Monoclonal antibody (IgG) with selective binding properties directed at MUC1 mucin recognizes and physically binds to these complexes expressed on the exterior surface membrane of certain neoplastic cell types.

Background Information: Videoendoscopy has not significantly advanced diagnostic accuracy beyond that attainable by conventional fiberscopy, with respect to microcarcinomas of the digestive tract. Theoretically, it is possible to selectively label such lesions with agents that can be detected by videoendoscope. Digital processing of the images could facilitate more accurate endoscopic diagnosis of microcarcinomas.

Application: [Indocyanine green]-anti-MUC1 mucin can be utilized to detect and characterize conditions of gastric and colorectal cancer lesions when used in combination with direct visualization using videoendoscopy methodologies.

Route of Administration: [Indocyanine green]-anti-MUC1 mucin (intravenous).

Implementation: Evaluation of the ability of [ICG]-anti-MUC1 mucin to selectively recognize and bind *in vivo* to gastric and colorectal cancer cell types. Validation of findings can be established by immunohistochemistry analysis using labeled secondary antibody.

Animal Model: Murine (nude mouse with human gastric or colorectal cancer cell types).

Interpretation and Analysis: [Indocyanine green]-anti-MUC1 mucin preferentially accumulates within gastric and colorectal cancer explants in nude mice, which can be appreciated on gross inspection and by examination of histochemical analyses. Therefore, antibody labeled with an ICG derivative may be clinically useful in detecting gastrointestinal microcarcinoma by videoendoscopy.

Instrumentation: Videoendoscopy.

Vital immunostaining of human gastric and colorectal cancers grafted into nude mice: A preclinical assessment of a potential adjunct to videoendoscopy. Kusaka Y, Ito S, Muguruma N, Tadatsu M, Bando T, Ii K, Irimura T, Shibamura S. J Gastroenterol 2000; 35 (10), pp. 748–52.

Neoplasia: Folate Receptors (KB Tumors)/ Nasopharyngeal Epidermoid Carcinoma/ Fibrosarcoma
Near-Infrared (NIR) Folate Receptor (FR) Probe

Background Information: Fluorescence optical imaging technologies are currently being developed to image specific molecular targets *in vivo.* Detection technologies range from those providing microscopic detail to whole-body imaging systems with potential clinical uses. A number of target-specific NIR imaging probes have recently been developed to image receptors, antigens, and enzymes.

Implementation: Evaluation of the capacity of an NIR folate receptor (FR)-targeted imaging probe to improve the detection of FR-positive cancers. Speculation suggests that modification of the folate molecule will retain receptor affinity *in vivo,* despite the bulkier NIR fluorochrome, NIR2 (em = 682 nm).

Interpretation and Analysis: Cellular uptake of the NIR conjugates is significantly higher in FR-positive nasopharyngeal epidermoid carcinoma, KB cells, compared to FR-negative human fibrosarcoma, HT1080 cells. When tumors are implanted *in vivo,* equal-sized KB tumors show a 2.4-fold higher signal intensity compared to HT1080 tumors (24 hours). The maximum signal-to-background ratio (3-fold) is observed at 24 hours in KB tumor. Injection of the unmodified NIR2 fluorochrome does not result in persistent contrast increases under similar conditions. Furthermore, tumor

enhancement with the NIR2-folate probe persists over 48 hours and is inhibited *in vivo* by the administration of unlabeled folate. These results indicate that folate-modified NIR fluorochrome conjugate can be used for improved detection of FR-positive tumors.

Enhanced tumor detection using a folate receptor-targeted near-infrared fluorochrome conjugate. Moon WK, Lin Y, O'Loughlin T, Tang Y, Kim DE, Weissleder R, Tung CH. Bioconjug Chem 2003 May–Jun; 14 (3), pp. 539–45.

Neoplasia: Gastric Carcinoma
Green Fluorescent Protein (GFP)

Cellular/Molecular Mechanism of Action: Retroviral agents expressing the GFP gene can insert their genome into certain mammalian cell populations. Cells that have been transfected by such retroviral agents express the GFP gene and emit a green color when exposed to certain wavelengths of light.
Application: Visualization of metastatic patterns and profiles.
Route of Administration: Intraperitoneal (other routes possible).
Implementation: Identification of potential regional and distance sites of metastasis. One approach to achieving this objective entails the injection of GFP retroviral supernatants intraperitoneally (i.p.) from day 4 to day 10 after implantation of gastric cancer cells. Tumor and metastasis fluorescence are then visualized every other week utilizing fluorescence optics via a laparotomy on the tumor-bearing animals.
Animal Model: Mouse (i.p. implanted with viable human gastric carcinoma cell types).
Interpretation and Analysis: At 2 weeks after retroviral GFP delivery, GFP-expressing tumor cells can be observed in gonadal fat, greater omentum, and intestine, indicating that these primary i.p. growing tumors are efficiently transduced by the GFP gene and can be visualized by its expression. At 2nd and 3rd laparotomies, GFP-expressing tumor cells can be observed spreading to lymph nodes in the mesentery and other regional sites. At the 4th laparotomy, widespread tumor growth is visualized by GFP expression, inducing liver metastasis. Normal tissues can rarely be found to be transduced by the GFP retrovirus. Thus, reporter gene transduction of the primary tumor can enable detection of its subsequent metastasis. This gene therapy model could be applied to primary tumors before resection or other treatment to have a fluorescent early detection system for metastasis and recurrence.

In vivo tumor delivery of the green fluorescent protein gene to report future occurrence of metastasis. Hasegawa S, Yang M, Chishima T, Miyagi Y, Shimada H, Moossa AR, Hoffman RM. Cancer Gene Ther 2000 Oct; 7 (10), pp. 1336–40.

Neoplasia: Gastric Carcinoma/Intestinal Microcarcinomas
Indocyanine Green (ICG)-Labeled Anti-MUC1 Mucin IgG

Cellular/Molecular Mechanism of Action: Anti-MUC1 mucin IgG possesses binding avidity for complexes expressed on the exterior surface membrane of human gastric carcinoma and carcinoma of the colon.
Application: Labeling of microcarcinomas to a degree sufficient for accurate and sensitive detection by videoendoscopy.
Considerations: Videoendoscopy has not significantly advanced diagnostic accuracy beyond that attainable by conventional fiberscopy, with respect to microcarcinomas of the digestive tract.
Semi-Synthetic Methods: Conjugation of ICG to anti-MUC1 mucin immunoglobulin producing a conjugated product that has binding avidity for complexes expressed on the exterior surface membrane of intestinal carcinoma cell types.
Implementation: Detection of foci of gastric carcinoma lesions in the form of intestinal microcarcinomas.
Animal Model: Nude mice transplanted with human gastric cancer or colorectal cancer.
Interpretation and Analysis: Anti-MUC1 mucin antibody binds *in vivo* to human gastric cancer or colorectal cancer transplants in nude mice that can be confirmed by performing immunohistochemistry analysis with a secondary antibody. The antibody labeled with an ICG derivative may therefore be clinically useful in detecting gastrointestinal microcarcinoma by videoendoscopy.
Instrumentation: Videoendoscopy.

Vital immunostaining of human gastric and colorectal cancers grafted into nude mice: A preclinical assessment of a potential adjunct to videoendoscopy. Kusaka Y, Ito S, Muguruma N, Tadatsu M, Bando T, Ii K, Irimura T, Shibamura S. J Gastroenterol 2000; 35 (10), pp. 748–52.

Neoplasia: General/Optical Imaging: Endoscopy and Videoendoscopy
[Bis-Propanoic Acid Cyanine]-IgG

Cellular/Molecular Mechanism of Action: Receptor-specific small peptide molecule that selectively recognizes and physically binds to complexes expressed on the exterior surface membrane of certain neoplastic cell types.
Application: Potential *in vivo* optical imaging of neoplastic lesions using endoscopy/videoendoscopy.
Route of Administration: [*Bis*-propanoic acid cyanine]-IgG (intravenous).
Semi-Synthetic Methods: Conjugation of the near-infrared (NIR) dyes indocyanine green and its derivatized analogue, *bis*-propanoic acid cyanine dye (cypate), to a biological peptide molecule.
Implementation: Evaluation of the ability of an NIR dye-peptide conjugate to recognize tumor-targeting efficacy.
Animal Model: Rat (neoplastic cell type CA20948 that expresses the corresponding biological protein receptor complex).
Interpretation and Analysis: Tumor retention of 2 non-tumor-specific dyes, indocyanine green and its derivatized analogue, *bis*-propanoic acid cyanine dye (cypate), is negligible. In contrast, the receptor-specific peptide-cypate conjugate (cypate) is retained within CA20948 tumors, with an excellent tumor-to-normal-tissue ratio (e.g., n = 6 rats). Optical detection of tumors with a receptor-targeted fluorescent contrast agent has been demonstrated. This result represents a new direction in cancer diagnosis and patient management.
Instrumentation: A simple continuous-wave optical imaging system, consisting of an NIR laser diode, a cooled charged-coupled device (CCD) camera, and an interference filter.

Novel receptor-targeted fluorescent contrast agents for *in vivo* tumor imaging. Achilefu S, Dorshow RB, Bugaj JE, Rajagopalan R. Invest Radiol 2000 Aug; 35 (8), pp. 479–85.

Neoplasia: General/Selective Detection
HAT-DO1 Fluorescent Agents

Cellular/Molecular Mechanism of Action: Represents a fluorescent molecular system that can be covalently conjugated to a selective "targeting" agent for the detection of specific biological entities without the simultaneous delivery of photodynamic properties.
Application: Sensor (tracer) molecule that facilitates the detection of "targeting" modalities designed to selectively interact with a specific biological/pathological foci or entities.

Synthesis and Design Considerations: Various heterodimers linked by several types of "spacers" between a chlorine derivative and its Mn or Cu complex are synthesized. One representative agent referred to as HAT-DO1 has a molecular formula of m-phthalyl-([13, 17-*bis*-propanoic acid-3-ethenyl-8-formylethylidene-7-hydroxy-2, 7,12,18-tetramethyl-porphyrinate]-manganese (III))-[3′-ethenyl-8′-formylethylidene-7′-hydroxy-2′,7′,12′,18′-te tramethyl-porphine-13′,17′-bispropanoyl aspartic acid]-bishydrazone.

Tumor-localizing fluorescent diagnostic agents without phototoxicity. Takemura T, Nakajima S, Sakata I. Photochem Photobiol 1994 Mar; 59 (3), pp. 366–70.

Neoplasia: General/Optical Imaging
Indocyanine Green (ICG)/Bis-Propanoic Acid Cyanine Dye (Cypate)

Cellular/Molecular Mechanism of Action: Indocyanine green and *bis*-propanoic acid cyanine dye (cypate) function as dye marker reagents that are activated when exposed to light energy in the near-infrared (NIR) range. Tumor receptor ligands recognize and physically bind selectively to complexes associated with the exterior surface membrane of cancer cells.
Background Information: Recently, NIR fluorescence light has been applied to image various biological events *in vivo,* because it penetrates tissue more efficiently than light in the visible spectrum.
Application: Indocyanine green and *bis*-propanoic acid cyanine dye reagents can be applied as visible marker molecules for application in optical imaging modalities. In this context, they can be conjugated to small peptides for specific tumor-associated receptor complexes.
Route of Administration: Intravenous.
Semi-Synthetic Methods: Covalent conjugation of an NIR dye to a biologically active peptide that recognizes and binds to tumor-associated receptor complexes.
Implementation: Evaluation of the ability of a semi-synthetic NIR dye-peptide conjugate for its ability to selectively recognize and physically bind to tumor lesions. Detection can be achieved with a simple continuous-wave optical imaging system.

Animal Model: Rat tumor model (CA20948) known to express receptors for a specific peptide (e.g., n = 6).
Interpretation and Analysis: Tumor retention of 2 non-tumor-specific dyes, ICG and its derivatized analogue, *bis*-propanoic acid cyanine dye (cypate), is negligible. In contrast, the receptor-specific peptide-cypate conjugate (cypate) is retained by CA20948 tumors, producing an excellent tumor-to-normal-tissue ratio.
Instrumentation: A simple continuous-wave optical imaging system, consisting of an NIR laser diode, a cooled charged-coupled device (CCD) camera, and an interference filter.

Novel receptor-targeted fluorescent contrast agents for *in vivo* tumor imaging. Achilefu S, Dorshow RB, Bugaj JE, Rajagopalan R. Invest Radiol 2000 Aug; 35 (8), pp. 479–85.

Neoplasia: General (Human): Near-Infrared (NIR) Fluorescence/Molecular/Physiological Marker System: NIR Fluorescence
IRDye78

Cellular/Molecular Mechanism of Action: IRDye78 is a tetra-sulfonated heptamethine indocyanine that can emit NIR fluorescence.
Background Information: The detection of human malignancies by NIR fluorescence usually requires the conjugation of a cancer-specific ligand to NIR fluorophores that have optimal photoproperties and pharmacokinetics. IRDye78, a tetra-sulfonated heptamethine indocyanine NIR fluorophore, meets most of the criteria for an *in vivo* imaging agent and is available as an N-hydroxysuccinimide ester for conjugation to low-molecular-weight ligands. However, IRDye78 has a high charge-to-mass ratio, complicating purification of conjugates. It also has a potentially labile linkage between the fluorophore and ligand.
Application: IRDye78 can be used as a fluorophore marker molecular in biological systems to assess a variety of physiological processes.
Route of Administration: IRDye78 (variable).
Dosage: IRDye78 (variable).
Semi-Synthetic Methods: Purification of IRDye78 can be performed with an ion-pairing purification strategy for IRDye78 that utilizes a standard C18 column under neutral conditions, thus preserving the stability of fluorophore, ligand, and conjugate. By employing parallel evaporative light scatter and absorbance detectors, all reactants and products can be identified, and conjugate purity maximized. During the purification process, reversible and irreversible conversions of IRDye78 can occur, but strategies for preserving conjugate stability have been established. The robust nature of these methodologies has been validated using 7 ligands, spanning several classes of small molecules and peptides (neutral, charged, and/or hydrophobic), and serves to confirm that IRDye78 conjugates so purified retain bioactivity and permit NIR fluorescence imaging of a specific target.

IRDye78 conjugates for near-infrared fluorescence imaging. Zaheer A, Wheat TE, Frangioni JV. Mol Imaging 2002 Oct; 1 (4), pp. 354–64.

Neoplasia: Glioma
Luciferase Transfected Glioma/Antisense Nucleotide Radiopharmaceuticals/[^{125}I]PNA:Antitransferrin Receptor Conjugate/[^{125}I]PNA Avid/Biotin Monoclonal Antibody Conjugate

Application: Reference model for the development of antisense radiobiopharmaceutical imaging agents for the *in vivo* detection and characterization of neoplastic disease affecting the central nervous system.
Route of Administration: [^{125}I]PNA antisense conjugates are administered intravenously.
Dosage: [^{125}I]PNA conjugate.
Background Information: Antisense radiopharmaceuticals can be used *in vivo* to image gene expression in the brain, if it is possible to facilitate the transport of these polar molecules across the blood-brain barrier.
Semi-Synthetic Methods: Production of an antisense imaging agent comprised of an iodinated peptide nucleic acid (PNA) conjugated to a monoclonal antibody to the rat transferrin receptor utilizing avidin-biotin technology. The PNA is a 16-mer antisense to the sequence around the methionine initiation codon of the luciferase messenger RNA (mRNA).
Implementation: Detection *in vivo* of glioma neoplastic cell types utilizing a luciferase expression plasmid. Frozen sectioning of the brain for film autoradiography is then performed 2 hours following intravenous injection of [^{125}I]PNA conjugates.

Animal Model: Rat glioma cells (C6 type) are permanently transfected with a luciferase expression plasmid for development of rat experimental brain tumor model.
Interpretation and Analysis: The expression of the luciferase transgene in the tumors *in vivo* can be confirmed by measurement of luciferase enzyme activity in the tumor extract. No image of the

luciferase gene expression can be obtained after the administration of either the unconjugated antiluciferase PNA or a PNA conjugate that is an antisense strand to the mRNA of a viral transcript. In contrast, tumors can be successfully imaged with [^{125}I]PNA that is an antisense strand to the luciferase sequence and conjugated to the targeting antibody. In conclusion, these studies demonstrate gene expression in the brain *in vivo* can be imaged with antisense radiopharmaceuticals that are conjugated to a brain drug-targeting system.

Antisense imaging of gene expression in the brain *in vivo.* Shi N, Boado RJ, Pardridge WM. Proc Natl Acad Sci U S A. 2000 Dec 19;97(26):14709–14.

Neoplasia: Glioma
EtNBA Fluorescent Dye/Nile Blue Dyes

Cellular/Molecular Mechanism of Action: EtNBA emits a fluorescent color when exposed to certain wavelengths of light energy.
Background Information: Nile blue dyes have been shown to have affinity for tumor tissue compared to surrounding normal tissue and to be relatively non-toxic.
Application: EtNBA can function as a fluorescent dye marker/signal molecular that can be used to detect and characterize neoplastic lesions (e.g., glioma cell types).
Dosage: EtNBA (2.5–5.0 mg/kg).
Implementation: Evaluate the ability of EtNBA, as a lipophilic, fluorescent benzophenoxazine dye, to image subcutaneous and intracranial U-87 glioma implants. Tumors are illuminated with a 632.8-nm diffuse beam from a helium-neon laser. The resulting video image is processed using an image processor to give real-time pseudocolor and enhanced black-and-white images.
Animal Model: Mouse (expressing U87-MG glioma solid tumor implants).
Interpretation and Analysis: Following subcutaneous injection of EtNBA dye at doses of 2.5 to 5.0 mg/kg bw, a gradual increase in the fluorescent signal can be observed from tumors that peaks 1–3 hours postinjection with variable selectivity (typically 4:1) for tumor to normal surrounding tissues, thereby permitting clear tumor demarcation. The reagent EtNBA is a safe and effective photodiagnostic agent when applied *in vivo* and is capable of demarcating U87-MG solid tumors on a real-time basis (2.5–5.0 mg/kg) for 1 to 3 hours following administration.
Instrumentation: Imaging system for detecting fluorescence consisting of a silicon-intensified target (SIT) video camera fitted with a zoom microscope-magnifying lens complemented by an image processor to give real-time pseudocolor and enhanced black-and-white images.

Fluorescent imaging in a glioma model *in vivo.* Nikas DC, Foley JW, Black PM. Lasers Surg Med 2001; 29 (1), pp. 11–7.

Neoplasia: Gliosarcoma/Lewis Lung Carcinoma/Apoptosis/Cell Death
Cy-Annexin V Conjugate

Background Information: Non-invasive imaging using radioactive annexin V is an emerging strategy for the *in vivo* assessment of cell death (apoptosis).
Application: Detection and characterization of *in vivo* tumor apoptosis (cell death) phenomenon.
Semi-Synthetic Methods: Preparation of active Cy-annexin (an equimolar dye:protein ratio) that possesses binding avidity for apoptotic Jurkat T cells complemented by the production of an inactive Cy-annexin probe (> 2 dyes/mol protein) that does not.
Implementation: Evaluation of the capacity for annexin V labeled with the fluorophore Cy5.5 (Cy) to serve as a probe for imaging of tumor apoptosis using near-infrared fluorescence (NIRF). In this context, active Cy-annexin conjugates can be applied to image 9L gliosarcoma, constitutively expressing green fluorescent protein marker, and the CR8 variant of Lewis lung carcinoma, stably transfected to express DsRed2.
Interpretation and Analysis: The expression of transfected fluorescent protein provides an indication of tumor margins and a means of defining tumor-associated NIRF signal intensity with both tumor models. Tumors can be imaged with and without cyclophosphamide treatment. In both tumor models active Cy-annexin V tumor NIRF signals increase 2–3 times after cyclophosphamide treatment. Tumor NIRF signals develop by 75 minutes after active Cy-annexin injection and remain detectable for a 20-hour observation period. Inactive annexin V can be used as a control in the CR8 carcinoma experiments and results in a low non-specific signal. With the 9L gliosarcoma model, active Cy-annexin V binds to both tumor cells (Cy-annexin V staining only) and endothelial cells (co-stained with Cy-annexin V and antibody to the endothelial marker CD31). Such findings demonstrate that active Cy-annexin can be used as a NIRF probe to image apoptosis from outside an intact living animal and may provide a non-radioactive method of measuring the antiproliferative effects of cancer chemotherapeutic regimens.

Near-infrared fluorescent imaging of tumor apoptosis. Petrovsky A, Schellenberger E, Josephson L, Weissleder R, Bogdanov A Jr. Cancer Res 2003 Apr 15; 63 (8), pp. 1936–42.

Neoplasia: Leukemia Nucleoli Targeting/Gene Therapy Evaluation Model: Antisense Oligonucleotides
Fluorescein-Labeled Phosphorothioate-Triple Helix-Forming Oligonucleotide (PS-TFO)

Cellular/Molecular Mechanism of Action: Oligonucelotide sequences can be designed that recognize and bind to specific gene/messenger RNA (mRNA) sequences.
Physiological Mechanism of Action: Selective inhibition of the transcription/translation of specific genes.
Background Information: The c-myc gene is frequently deregulated and overexpressed in human cancers, and strategies designed to inhibit c-myc expression in cancer cells may have considerable therapeutic value.
Application: Gene (oligonucleotide)-directed detection of neoplastic cell types (e.g., leukemias).
Route of Administration: Potentially variable (intravenous, intralesional).
Semi-Synthetic Methods: Triple helix-forming oligonucleotide (TFO) can be synthesized with phosphorothioate (PS) internucleotide linkages to confer resistance to intra- and extracellular nucleases. The resulting preparation can be conjugated with fluorescein as a tracer agent to identify selective accumulation within certain tissues and subcellular organelles.
Implementation: Characterization of the antigene and antiproliferative activity of a TFO targeted to a homopurine-homopyrimidine sequence in the P2 promoter of the c-myc gene. Delineation of the affinity of fluorescein-labeled PS-TFO.
Interpretation and Analysis: Resistance to intra- and extracellular nucleases is a property that is required for the design of oligonucleotides intended for *in vivo* testing and therapeutic applications.

Phosphorothioate-modified triple helix-forming oligonucleotide forms triplex DNA complexes with affinity and specificity compara-

ble with that of the corresponding phosphodiester TFO, as delineated by gel mobility shift and footprinting assays. Fluorescence microscopy and polyacrylamide gel analysis reveal that fluorescein-labeled PS-TFO accumulates in the nuclei of CEM leukemia cells and remains intact for the duration of at least 72 hours. Incubation of CEM cells with PS-TFO reduces c-myc RNA and protein levels. A single exposure of leukemia cells to the PS-TFO is sufficient to induce dose-dependent growth inhibitory effects. Growth inhibition correlates with accumulation of cells in S phase and with induction of cell death by apoptosis. Preparations of PS-TFO are also effective in other leukemia and lymphoma cell lines. Control oligonucleotides have minimal effects in all assays. These data indicate that c-myc-targeted PS-TFO is an effective antigene and antiproliferative agent, with potential for *in vivo* testing as a novel approach to cancer therapy and possibly diagnosis.

Antigene and antiproliferative effects of a c-myc-targeting phosphorothioate-triple helix-forming oligonucleotide in human leukemia cells. McGuffie EM, Pacheco D, Carbone GM, Catapano CV. Cancer Res 2000 Jul 15; 60 (14), pp. 3790–9.

Neoplasia: Lymph Nodes (Sentinel Mapping)/ Adenocarcinoma Colorectal Cancer/Mammary Carcinoma/Melanoma
Isosulfan Blue Dye

Cellular/Molecular Mechanism of Action: Isosulfan blue is a pigment-based dye reagent.
Background Information: Sentinel lymph node (SLN) biopsy is a widely accepted method for staging breast cancer and melanoma, and it has also been applied as a means of improving the staging in colorectal cancer. However, lymphatic mapping in colorectal cancer has been plagued by studies demonstrating high false-negative rates. Poor accuracy may be due to bulky tumor mass or complete replacement of lymph nodes by tumor lesions.
Application: Evaluation of SLNs in conditions of neoplastic disease (e.g., mammary carcinoma, melanoma, colorectal cancer).
Route of Administration: Isosulfan blue dye (intralesional injection).
Dosage: Isosulfan blue dye (1 ml volumetric).
Implementation: Evaluate possible mechanisms for high false-negative rates after SLN biopsy in colorectal cancer. One approach to characterizing the efficacy of isosulfan blue dye can involve the intraoperative injection of isosulfan blue dye into colorectal adenocarcinoma lesions for the purpose of lymphatic mapping using either an *in vivo* or *ex vivo* experimental model. Routine pathological evaluations are then performed. Sentinel nodes are examined by hematoxylin and eosin stains, and if they are negative they are subsequently evaluated by cytokeratin immunohistochemistry. The patient's age, operation type, tumor stage, tumor diameter, method of SLN detection, presence of palpable nodes, and pathological description of nodes completely replaced by tumor are recorded.

Human Model: Cases undergoing colorectal cancer resection (e.g., n = 50; mean age 62.8 years, 50% men) and lymphatic mapping (e.g., n = 51 procedures).
Interpretation and Analysis: Right- and left-sided colorectal resections are almost equally distributed (48% vs 42%). Sentinel lymph nodes can be successfully identified in a large percentage of cases (e.g., n = 47/51 or 92%). The mean number of SLNs obtained from each specimen is approximately 1.5 (range, 1–5). Routine pathological evaluations demonstrate lymph node metastasis in less than half of affected cases (e.g., n = 20/47 or 43%) that have SLN identified. The SLN is positive for metastasis in about half of these cases (e.g., n = 10/20). Approximately half of cases with metastisis have a negative SLN, resulting in a false-negative rate of 50% (e.g., n = 10/20 cases). The rate of false negatives is significantly higher in patients undergoing left-sided procedures vs right-sided procedures. Differences among gender, tumor stage, tumor diameter, method of SLN detection, presence of palpable nodes, and pathological description of nodes completely replaced by tumor are not associated with a higher false-negative rate.

Does tumor burden limit the accuracy of lymphatic mapping and sentinel lymph node biopsy in colorectal cancer? Broderick-Villa G, Ko A, O'Connell TX, Guenther JM, Danial T, DiFronzo LA. Cancer J 2002 Nov–Dec; 8 (6), pp. 445–50.

Neoplasia: Lymphoma/Mammary Carcinoma
Fluorescein-Somatostatin Receptor-Avid Peptide/ Fluorescein-Bombesin/Carbocyanine-Somatostatin Receptor-Avid Peptide/Carbocyanine-Bombesin

Cellular/Molecular Mechanism of Action: Somatostatin receptor-avid peptides and bombesin recognize and possess binding avidity for somatostatin receptor complexes.
Background Information: Site-specific delivery of drugs and imaging agents to tumors protects normal tissues from the cytotoxic effects of drugs and enhances the diagnostic contrast between normal and pathologic tissues. One approach to achieving selectivity is to target overexpressed receptors on the membranes of tumor cells and to visualize the tumors by a non-invasive optical imaging method.
Application: Detection and characterization of neoplastic lesions that express increased densities of membrane-associated somatostatin receptor complexes.
Semi-Synthetic Methods: Covalent conjugation of fluorescein and carbocyanine dyes to somatostatin and bombesin receptor-avid peptides. Complementary investigation can involve the preparation of potential dual-imaging probes consisting of a bioactive peptide for tumor targeting, a biocompatible dye for optical imaging, and a radioactive or paramagnetic metal chelator for scintigraphic or magnetic resonance imaging of tumors.
Implementation: Delineation of the receptor binding affinities of fluorescein and carbocyanine dyes conjugated to somatostatin and bombesin receptor-avid peptides.

Animal Model: Rats bearing somatostatin- and bombesin-positive tumors.
Interpretation and Analysis: Carbocyanine derivatives of somatostatin and bombesin analogues retain high binding for their respective receptors. Further evaluation of representative molecules in somatostatin- and bombesin-positive tumors show selective uptake of the agents by the tumor cells. Unlike carbocyanine derivatives, receptor binding of fluorescein-somatostatin peptide conjugates is highly sensitive to the type of linker and the site of fluorescein attachment on the non-receptor binding region of the peptide. In general, the presence of flexible linkers disrupts binding affinity, possibly due to the interaction of the linker's thiourea group with the peptide's cyclic disulfide bond. While the receptor binding affinity of the dual probes is not dependent on the type of chelating group examined, it is affected by the relative positions of fluorescein and chelator on the lysine linker. For somatostatin compounds, best results are obtained when the chelator is on the alpha-amino lysine linker and fluorescein is on the epsilon-amino group. In contrast, conjugation of the chelator to epsilon- and fluorescein to the alpha-amino lysine linker of bombesin peptides results in high receptor binding. These findings indicate that despite their small size, conjugation of dyes to truncated somatostatin and bombesin peptide analogues results in the generation of promising diagnostic agents that retain high *in vitro* receptor binding activity. These contrast agents

can selectively and specifically localize in receptor-positive tumors in rat models.

Synthesis, *in vitro* receptor binding, and *in vivo* evaluation of fluorescein and carbocyanine peptide-based optical contrast agents. Achilefu S, Jimenez HN, Dorshow RB, Bugaj JE, Webb EG, Wilhelm RR, Rajagopalan R, Johler J, Erion JL. J Med Chem 2002 May 9; 45 (10), pp. 2003–15.

Neoplasia: Mammography/Mammary Adenocarcinoma/Phantom Calibration *NIR96010 (Infrared Contrast Agent)*

Cellular/Molecular Mechanism of Action: NIR96010 is a molecular agent that can emit light energy in the infrared range (755 nm).

Background Information: Near-infrared (NIR) optical mammography without contrast has a low specificity. The application of optical contrast medium may improve the performance. The concentration-dependent detectability of a new NIR contrast medium can be determined with a prototype optical breast scanner.

Application: The NIR contrast agent NIR96010 is a semi-synthesized, hydrophilic contrast agent that can be applied for NIR mammography imaging.

Route of Administration: NIR96010 (intravenous).

Dosage: NIR96010 (2 μmol/kg).

Implementation: Delineation of the potential for NIR96010 to function as an *in vivo* imaging agent for the detection of neoplastic lesions (e.g., mammary adneocarcinoma). One step in this evaluation process can involve determining the concentration-dependent contrast resolution for tissue phantoms (e.g., whole milk powder and gelatin). A central part of the phantoms measuring 2×2 cm^2 without contrast is replaced with phantom material containing 1 μmol/L to 25 nmol/L NIR96010. The composite phantoms are measured with a prototype NIR breast scanner with lasers. Intensity profiles and standard deviations of the transmission signal in areas with and without contrast are determined by linear fit procedures. Signal-to-noise ratios and spatial resolution as a function of contrast concentration are subsequently established. Near-infrared imaging is then performed before and after intravenous NIR96010 injection.

Animal Model: Murine (severe combined immunodeficient [SCID] mice, n = 5, bearing tumor lesions [MX1 breast adenocarcinoma = diameter of 5 to 10 mm]).

Interpretation and Analysis: Spectrometry analysis reveals an absorption maximum of 755 nm for the contrast agent. No spectral shifts occur in protein-containing solutions. Signal-to-noise ratio in the transmission intensity profiles ranges from 1.1 at 25 nmol/L compared to 28 at 1 μmol/L. At concentrations < 40 nmol/L, no differentiation from background is evident. The transitional area between the contrast-free edge of the phantom and the central contrast-containing part appear in the profiles as a steep increase with a width of 4.2 ± 1.8 mm. Experimental tumors can be detected in non-enhanced images as well as contrast-enhanced images, with better delineation after contrast administration. In postcontrast absorption profiles, a 44.1 ± 11.3% greater absorption increase is seen in tumor tissue compared with normal tissue. The laser wavelength λ1 of the prototype laser mammography device is not situated at maximum absorption of the contrast agent NIR96010 but instead on the descending shoulder of the absorption spectrum profile. This implies a 20% signal loss for contrast detection. Despite the non-ideal measurement conditions, *in vitro* concentrations as low as 40 nmol/L are detectable. All tumors *in vivo* were detectable in color-coded non-enhanced scans as well as in contrast-enhanced scans, with better delineation after contrast administration.

Instrumentation: Near-infrared breast scanner with lasers (e.g., $\lambda 1 = 785$ nm and $\lambda 2 = 850$ nm wavelength).

Contrast-enhanced near-infrared laser mammography with a prototype breast scanner: Feasibility study with tissue phantoms and preliminary results of imaging experimental tumors. Boehm T, Hochmuth A, Malich A, Reichenbach JR, Fleck M, Kaiser WA. Invest Radiol 2001 Oct; 36 (10), pp. 573–81.

Neoplasia Angiogenesis/Tumor Dimethylarginine Dimethylaminohydrolase (DDAH) Overexpression *NC100150/Hoechst 33342*

Cellular/Molecular Mechanism of Action: The spacial orientation of NC100150 becomes altered within a magnetic field. Alternatively, Hoechst 33342 emits fluorescent color when exposed to light energy.

Background Information: Intracellular factors that regulate nitric oxide (NO) synthesis represent important targets in tumor progression. Overexpression of DDAH, which metabolizes the endogenous inhibitors of NO synthesis asymmetric dimethylarginine and N-monomethyl-L-arginine, results in C6 gliomas with enhanced growth rate compared with wild type populations.

Application: NC100150 can be utilized as an intravascular blood pool contrast agent that is detectable by magnetic resonance imaging (MRI). Hoechst 33342 can be used as a fluorescent perfusion-marker dye that can be measured by flourescent detection systems.

Route of Administration: Utilization of NC100150 and Hoechst 33342 for neoplastic lesion angiogenesis requires that these be delivered intravascularly.

Implementation: Evaluation of the *in vitro* effects of DDAH on tumor vascular morphogenesis by measurement of the transverse relaxation rates R_2* and R^2 in (i) clone D27 gliomas overexpressing DDAH; (ii) C6 wild-type gliomas using intrinsic susceptibility MRI sensitive to changes in endogenous deoxyhemoglobin; (iii) evaluation of CN100150 as an intravascular blood pool contrast agent for susceptibility contrast-enhanced MRI; and (iv) comparison of results with fluorescence microscopy utilized to assess tumor uptake of Hoechst 33342 (perfusion marker system).

Interpretation and Analysis: The baseline R_2* is significantly faster in the D27 tumors, consistent with a greater vascular development ($p < 0.02$, ANOVA). There is no significant difference between the response of the 2 tumor types to hypercapnia (5% CO_2/95% air), used as a probe for vascular maturation, or hyperoxia (5% CO_2/95% O_2), used as a probe for vascular function. NC100150 increases the R_2* and R_2 rates of both tumor types and demonstrates a significantly larger blood volume in the D27 tumors ($p < 0.02$, ANOVA). This correlates with a significantly greater uptake of Hoechst 33342 in D27 tumors compared to C6 wild-type tumors ($p < 0.02$, ANOVA). Despite increased tumor blood volume, the Delta R_2*/Delta R_2 ratio, an index of microvessel size, shows that the capillaries in the 2 tumor types are of a similar caliber. Such observations demonstrate the potential of susceptibility MRI-derived quantitative end points to non-invasively assess tumor angiogenesis. Application of intravascular blood pool contrast agents such as NC100150 for such purposes appears very promising. Overexpression of DDAH results in increased *in vivo* neovascularization of C6 gliomas. The lack of significant difference in hypercapnic/hyperoxic response between the C6 and D27 tumors and the similar vessel caliber are also consistent with a role for DDAH in the initial stages of vasculogenesis.

Instrumentation: Magnetic resonance imaging and fluorescence microscopy instrumentation.

Effects of overexpression of dimethylarginine dimethylaminohydrolase on tumor angiogenesis assessed by susceptibility magnetic resonance imaging.

Kostourou V, Robinson SP, Whitley GS, Griffiths JR. Cancer Res 2003 Aug 15; 63 (16), pp. 4960–6.

Neoplasia Chemosensitivity
Near-Infrared Fluorescent (NIRF) Annexin V

Background Information: A rapid and accurate assessment of the antitumor efficacy of new therapeutic drugs could speed up drug discovery and improve clinical decision making.
Implementation: Based on the hypothesis that most effective antitumor agents induce apoptosis, the NIRF annexin V can be used for optical sensing of tumor environments. To demonstrate probe specificity, both active (i.e., apoptosis-recognizing) and inactive forms of annexin V have been developed that have very similar properties (to account for non-specific tumor accumulation).

Animal Model: Murine (nude mice bearing a cyclophosphamide [CPA], chemosensitive [LLC], and a chemoresistant LLC [CR-LLC]).
Interpretation and Analysis: After injection with active annexin V, the tumor-annexin V ratio (TAR; tumor NIRF/background NIRF) for untreated mice is 1.22 ± 0.34 for LLC and 1.43 ± 0.53 for CR-LLC ($n = 4$). The LLC of CPA-treated mice has significant elevations of TAR (2.56 ± 0.29, $p = 0.001$, $n = 4$), but only moderate increases are obtained for CR-LLC (TAR $= 1.89 \pm 0.19$, $p = 0.183$). The *in vivo* measurements correlated well with terminal deoxyribosyl transferase-mediated dUTP nick end labeling indexes. When inactive Cy-annexin V is used, with or without CPA treatment and in both CCL and CR-CCL tumors, tumor NIRF values range from 0.91 to 1.17 (i.e., tumor equal to background). Therefore, active Cy-annexin V and surface reflectance fluorescence imaging provide a non-radioactive, semi-quantitative method of determining chemosensitivity in LLC xenografts. The method may be used to image pharmacologic responses in other animal models and, potentially, may permit the clinical imaging of apoptosis with non-invasive or minimally-invasive instrumentation.

Optical imaging of apoptosis as a biomarker of tumor response to chemotherapy. Schellenberger EA, Bogdanov A Jr, Petrovsky A, Ntziachristos V, Weissleder R, Josephson L. Neoplasia 2003 May–Jun; 5 (3), pp. 187–92.

SPECIAL SENSES

Ophthalmic: Choroidal Microvasculature Structures/Estimation of Microvasculature Blood Flow Velocity/Leukocyte Dynamics in the Choroidal Microcirculation
Indocyanine Green (ICG) Dye-Stained Leukocytes

Cellular/Molecular Mechanism of Action: Indocyanine green is a dye marker molecule that can be used *in vivo* as a detection (tracer) molecule.
Application: Visualization of leukocytes moving through the choroidal vessels of pigmented animals in a manner that enables the evaluation of leukocyte dynamics in the choroidal microcirculation.
Route of Administration: Intravascular.
Implementation: Leukocytes are collected by centrifugal separation of autologous blood and then stained with ICG dye. The ICG-stained leukocyte fluid is then injected intravenously, and the fundus image obtained with a scanning laser ophthalmoscope. Images are recorded on videotape and analyzed with a personal computer–based image analysis system.

Animal Model: Pigmented rabbits and monkeys.
Interpretation and Analysis: In pigmented rabbits, fluorescent leukocytes moving in the choroidal circulation are clearly visible for a period of more than 1 hour. In monkeys, distinct fluorescent dots are seen moving approximately 50–200 μm in the foveal avascular zone for more than 30 minutes after injection of ICG-stained leukocyte fluid. Dim fluorescent dots can be seen moving in the fundus. Although the movement of the dim dots is difficult to trace, they seem to be moving in the choroidal vessels. In rabbits, the mean flow velocity of leukocytes moving without plugging is 0.48 ± 0.14 mm/sec in the peripheral choriocapillaris. In the monkeys, the mean flow velocity of distinct fluorescent leukocytes without plugging is 2.45 ± 0.48 mm/sec in the posterior choroid. In pigmented rabbits and monkeys, this method allows visualization of leukocytes passing through the choroidal vessels and provides a new way to investigate, non-invasively and *in vivo,* leukocyte dynamics in the choroidal microcirculation.
Instrumentation: Scanning laser ophthalmoscope.

Evaluation of leukocyte dynamics in choroidal circulation with indocyanine green-stained leukocytes. Takasu I, Shiraga F, Okanouchi T, Tsuchida Y, Ohtsuki H. Invest Ophthalmol Vis Sci 2000 Sep; 41 (10), pp. 2844–8.

Ophthalmic: Retinal Angiography/Choroid/Retinal Pigment Epithelium/[Premortem Diagnostic Agent]
Rose Bengal/Aluminum Phthalocyanine Tetrasulfonate (CASPc)/Chlorin e6/Photosensitizers (Xanthene, Phthalocyanine, Chlorin)

Application: Localization and site of action of photosensitizers in the eye may be important for photodynamic therapy for fundus disorders but remain poorly understood for most agents.
Route of Administration: Rose bengal (intravenous), CASPc (intravenous), chlorin e6 (intravenous).
Dosage: Rose bengal (40 mg/kg), CASPc (5 mg/kg), or chlorin e6 (2 mg/kg). Digital fundus fluorescence angiography: rose bengal (2–4 mg/kg), CASPc (2 mg/kg), and chlorin e6 (2 mg/kg).
Implementation: Evaluate and characterize the intraocular localization of xanthene, phthalocyanine, and chlorin photosensitizers utilizing fluorescence microscopy and digital fundus fluorescence angiography. In this context, rose bengal, CASPc, or chlorin e6 are injected intravenously. The eyes are then enucleated and examined by means of fluorescence microscopy 5, 20, 60, and 120 minutes and 24 hours after dye injection. *In vivo* digital fundus fluorescence angiography can be performed with rose bengal, CASPc, or chlorin e6.

Animal Model: Rabbit (albino).
Interpretation and Analysis: Pathologically, all agents displayed moderate fluorescence from the choroid and retinal pigment epithelium 5 minutes after dye injection. Mild fluorescence detected from the photoreceptor outer segments at 5 minutes is increased at 20 minutes. Angiographic studies with use of rose bengal, CASPc, and chlorin e6 reveal differences in the pattern and rate of photosensitizer accumulation. Rose bengal, CASPc, and chlorin e6 accumulate rapidly in the choroid and retinal pigment epithelium and less rapidly in the outer retina. Differences in ocular localization of the photosensitizers can be demonstrated. The significance of these findings for potential photodynamic therapy with these agents requires further investigation. Potentially the agents xanthene, phthalocyanine, and chlorin photosensitizers could be utilized in concert with fluorescence microscopy in premortem diagnostic procedures.
Instrumentation: Fluorescence microscopy.

Localization of rose bengal, aluminum phthalocyanine tetrasulfonate, and chlorin e6 in the rabbit eye. Haimovici R, Ciulla TA, Miller JW, Hasan T, Flotte TJ, Kenney AG, Schomacker KT, Gragoudas ES. Retina 2002 Feb; 22 (1), pp. 65–74.

Ophthalmology/Ocular Integrity Assessment Following Cataract and Macular Hole Surgical Procedures
Indocyanine Green (ICG)

Cellular/Molecular Mechanism of Action: Indocyanine green emits fluorescent color when excited by certain wavelengths of light energy.
Application: Measurement of the elimination kinetics within the fluid compartments of the ocular globe after intraocular surgical operations using ICG staining and a monitoring indicator.
Route of Administration: Ocular (topical).
Implementation: Intraocular fluorescence of ICG can be determined using the ICG angiographic mode of a scanning laser ophthalmoscope (*in vivo*) and fluorescence microscopy (*in vitro*) after circular curvilinear capsulorhexis with ICG staining during cataract surgery and internal limiting membrane (ILM) peeling with ICG staining during macular hole surgery.

Human Model: White cataract (n = 7 cases and n = 9 eyes) and idiopathic macular holes (n = 14 cases; n = 14 eyes).
Interpretation and Analysis: Scanning laser ophthalmoscopy can reveal fluorescence in the anterior segment in cases with cataracts on the 1st postoperative day, and fluorescence persists for a mean of 6.0 ± 2.2 days. Scanning laser ophthalmoscopy also reveals fluorescence in the posterior pole of patients with macular holes, and it remains for a mean ± standard deviation of 2.7 ± 1.4 months postoperatively. Fluorescence microscopy shows fluorescence of the entire tissues, suggesting that ICG stains not only the surface of membranes but also penetrates these structures. In both operations, visual outcomes are not significantly different from results obtained without ICG. Because entire tissues are stained, the differences in ICG kinetics may also be caused by factors other than differences in stainability, such as the environment surrounding tissue or molecular structural differences between the lens capsule and the ILM. Although complete disappearance of fluorescence and good functional recovery can be appreciated, the longer resident time of the dye after macular hole surgery may suggest a potential risk to intraocular tissues.
Instrumentation: Fluorescence microscopy (*in vitro*) and scanning laser ophthalmoscope (*in vivo*).

Kinetics of indocyanine green dye after intraocular surgeries using indocyanine green staining. Horiguchi M, Nagata S, Yamamoto N, Kojima Y, Shimada Y. Arch Ophthalmol 2003 Mar; 121 (3), pp. 327–31.

Ophthalmology: Retinal Microglial Cell Postnatal Development
Fluorogold (FG)/4-[4-Didecylaminostyryl]-N-Methylpyridinium Iodide (4Di-10ASP)

Cellular/Molecular Mechanism of Action: Fluorogold and 4Di-10ASP function as fluorescent dye reagents.
Application: Characterization of retinal microglial cell populations and their development during the postnatal period.
Route of Administration: Injected into the superior colliculus (SC) at postnatal day 0.
Implementation: Fluorogold or 4Di-10ASP are injected into the SC at postnatal day 0. Retinas are examined to quantify the microglial cells from P5 to adulthood. In addition, the reaction of microglia to optic nerve crush can be studied in adult animals.

Animal Model: Mouse (postnatal day 0 [P0] to adulthood).
Interpretation and Analysis: Fluorogold or 4Di-10ASP are transported retrogradely to the retinal ganglion cell (RGC) soma when either dye is injected into the SC at P0. Some of the labeled RGCs die due to natural apoptosis during this stage of development and are phagocytosed by microglial cells that move to the site of RGC death and become labeled with the same dye. Both dye reagents label RGCs in the contralateral retina and a few RGCs in the retina ipsilateral to the injected superior colliculus (SC). The density of labeled RGCs decreases by 22% between P5 and P7. During this phase, microglial cells become visible as they ingest fluorescent detritus of the dying RGCs. Microglial cells are evenly distributed across the entire retinal surface and migrate to the outer plexiform layer. Migrating microglia consecutively alter their morphology from the amoeboid to the ramified form. In terms of intracellular storage of the dyes, resident microglial cells retain the fluorescent dye 4Di-10ASP over a period of 12 months. Conversely, FG is completely transferred from the RGCs and microglial cells to intramural cells (pericytes) of the retinal capillaries after 10 months. This results in delineation of the entire intraretinal vascular network. Finally, resident retinal microglial cells are also activated by injury to the adult optic nerve and phagocytosed degenerating neurons. Retinal microglial cells can be monitored with vital fluorescent dyes while they migrate across the retina and establish their intraretinal network. It is possible to label microglia with lipophilic dyes, and they remain labeled for an extended period of time. In addition, intramural pericytes can be labeled by slow release of FG from RGCs and microglial cells. Such observations suggest that ingested fluorescent dyes having different properties can be used *in vivo* to study different cell populations of the retina.

Migration of phagocytotic cells and development of the murine intraretinal microglial network: An *in vivo* study using fluorescent dyes. Bodeutsch N, Thanos S. Glia 2000 Oct; 32 (1), pp. 91–101.

Ophthalmology: Retinal Neuron Visualization
Retrogradely Transportable Fluorescent Dyes

Cellular/Molecular Mechanism of Action: Fluorescent reagents emit color when exposed to certain wavelengths of light energy.
Background Information: Posttraumatic death of mature retinal neurons occurs in glaucoma and after optic nerve injury.
Application: Characterization of dynamic processes associated with cell death applying analysis methods that monitor changes over time.
Implementation: Coupling the development of retrograde transported fluorescent dyes with modification of conventional epifluorescence microscopy to manipulate and visualize *in vivo* rat retinal neurons.
Interpretation and Analysis: Retinal conditions can be effectively monitored, including those associated with glaucoma or optic nerve transection, and for evaluation of neuroprotective strategies.

In vivo FM: Using conventional fluorescence microscopy to monitor retinal neuronal death *in vivo.* Thanos S, Indorf L, Naskar R. Trends Neurosci 2002 Sep; 25 (9), pp. 441–4.

Ophthalmic: Retinal Vascular Permeability/ Minimally-Invasive Diagnostic Strategy/Diabetic Retinopathy
Sodium Fluorescein

Background Information: Diabetic retinopathy is the leading cause of blindness in working-age individuals in the United States. Breakdown of the blood-retinal barrier is 1 of the earliest events in the progression of diabetic retinopathy. Ideally, therapeutic measures

would be directed at this early stage, but there are few sensitive, quantitative methods to assess the retinal vascular barrier *in vivo.*

Application: Quantitative assessment of the retinal vascular barrier.

Implementation: Utilization of 2 fluorescent tracers in combination with fluorescence microangiography to quantitatively assess retinal vascular barriers (± infusion of a hyperosmolar solution of 1.6 M mannitol for 5 minutes).

Animal Model: Rat (Long-Evans strain).

Interpretation and Analysis: Permeability × surface area/flow (PS/F) describing the retinal vasculature is 0.086 ± 0.031 (n = 13, avg. ± standard deviation). Based on estimates of flow and surface area, permeability of sodium fluorescein is approximately 1.2×10^{-5} cm/s. Infusion of a hyperosmolar mannitol solution significantly increases PS/F within individual veins and significantly increases flow-weighted PS/F from 0.073 ± 0.028 to 0.16 ± 0.034 (n = 3). Quantitative *in vivo* assessment of retinal vasculature can be delineated applying an adapted indicator dilution technique. Dual-tracer fluorescence angiography appears to be a sensitive indicator for detecting increases in blood-retinal barrier permeability produced by hyperosmolar mannitol. The methodology may be a promising new minimally-invasive strategy that may be adapted to quantitatively track retinal vascular permeability.

Instrumentation: Fluorescence microangiography.

Retinal vascular permeability determined by dual-tracer fluorescence angiography. Russ PK, Gaylord GM, Haselton FR. Ann Biomed Eng 2001 Aug; 29 (8), pp. 638–47.

Ophthalmology/Visual Pathway Staining (*In Vivo* Antegrade Staining)/Retinal Ganglion Staining (*In Vivo* Antegrade Staining)/Vitreoretinal Surgery: Intraoperative Probe
Indocyanine Green (ICG)

Cellular/Molecular Mechanism of Action: Indocyanine green emits fluorescent color when excited by certain wavelengths of light energy.

Background Information: It has been shown that the application of ICG over the retinal surface is followed by prolonged staining of the optic disc.

Application: Indocyanine green can function as a bidirectional axonal tracer that can be used *in vivo* to stain the visual pathway and retinal ganglion. Such properties may be of value as an intraoperative diagnostic pharmaceutical for vitreoretinal surgery and as a tool in performing ophthalmic investigative research.

Implementation: Evaluation of ICG (i) anterograde diffusion after injection into the vitreous; and (ii) retrograde diffusion after microinjection into the lateral geniculate nucleus (e.g., discovery of the extent of ICG diffusion along the optic tract).

Animal Model: Rabbit (vitreous ICG injection) and rats (lateral geniculate nucleus injection).

Interpretation and Analysis: Anterograde and retrograde diffusion occurs along axons at a rate of about 2 mm per hour following ICG injection. Anterograde staining of the visual pathway persists for several weeks. After injection into the lateral geniculate nucleus, fluorescent retinal ganglion cells can be visualized for at least 7 days in conscious rats. Microscopic examination reveals evidence of retrograde-labeled retinas and the detection of ICG vesicles inside the axons, cytoplasm, and dendrites of retinal ganglion cells. Evidence of toxic effects can usually not be detected applying optical microscopy. Injection of ICG into normal vitreous results in long-term staining of the visual pathway. *In vivo* counting of ICG-labeled retinal ganglion cells in rats can be performed for several days after injection. Indocyanine green is therefore potentially of interest for use in experimental neurophysiological studies. It is speculated that in humans, the epiretinal application of ICG results in prolonged staining of the visual pathway. Additional studies of long-term cytotoxic effects of ICG on neural cells are warranted before recommending its use in humans as an intraoperative tool for vitreoretinal surgery.

Instrumentation: Conventional infrared photography.

Axon-tracing properties of indocyanine green. Paques M, Genevois O, Regnier A, Tadayoni R, Sercombe R, Gaudric A, Vicaut E. Arch Ophthalmol 2003 Mar; 121 (3), pp. 367–70.

PATHOLOGY: GENERAL

Lesion Detection
Fluorophore-Antibody Conjugate/Fluorescein Isothiocyanate (FITC)

Cellular/Molecular Mechanism of Action: Fluorescein isothiocyanate emits fluorescent color when exposed to light of certain wavelengths.

Application: Molecular marker (tracer) system that can be applied in biological systems to assess various physiological processes.

Implementation: Evaluation of the capacity of immunoglobulin conjugates to interact with antigens expressed by the diseased tissue or neoplastic lesions, resulting in fluorescent labeling of cell-membrane associated antigen that can be visualized applying optical detection systems. Three-dimensional images can be reconstructed corresponding to the location of a fluorophore (FITC) in tongue tissue. Measurements are performed with the fluorophore embedded at various simulated depths. The simulations are performed with agarose-based gel slabs applied to the tongue as tissue-like phantoms. Reconstructed fluorophore locations agree well with actual observed measurements.

Animal Model: Mouse (tongue imaging with a marker system at various simulated depths).

Interpretation and Analysis: By combining an optical detection system with a reconstruction algorithm developed on the basis of the random-walk model, it is possible to determine the position of the fluorophore (e.g., diseased target cells) in tissue structures.

Instrumentation: Optical detection system.

In vivo quantitative 3-dimensional localization of tumor labeled with exogenous specific fluorescence markers. Gannot I, Garashi A, Gannot G, Chernomordik V, Gandjbakhche A. Appl Opt 2003 Jun 1; 42 (16), pp. 3073–80.

PHARMACEUTICAL DELIVERY

Antineoplastic Property Correlation with Biodistribution/Delivery Characterization
[Fluoroscein Isothiocyanate (FITC)]-Glycol-Chitosan Nanoaggregates

Application: Fluoroscein isothiocyanate can be applied as a fluorescent tracer molecule for characterizing a variety of physiological processes or delineating the distribution of delivery modalities (e.g., [FITC]-glycol-chitosan nanoaggregates).

Route of Administration: Intravenous (e.g., rat tail vein).

Semi-Synthetic Methods: Conjugation of fluoroscein isothiocyanate to glycol-chitosan-doxorubicin (GC-DOX) results in the production of nanoaggregates with a diameter of about 250 nm in aqueous media. To increase the applicability of such preparations,

GC-DOX nanoaggregates can be produced that contain an acid-sensitive spacer moiety. Preparations of GC-DOX spontaneously form micelle-like nanoaggregates in aqueous media. Glycol-chitosan-doxorubicin nanoaggregates have a narrow and unimodal size distribution, and their hydrodynamic diameter as measured by dynamic light scattering ranges from between 250 to 300 nm. A loading content of doxorubicin in GC-DOX nanoaggregates can be as high as 38% with a 97% loading efficiency when using a physical entrapment method.

Implementation: Characterization of the *in vivo* biodistribution of GC-DOX nanoaggregates (doxorubicin-loaded GC-DOX nanoaggregates or DOX/GC-DOX) facilitated by conjugation to FITC. Observations can be applied to determine if these parameters correlate with the antineoplastic properties exerted by conjugated preparations.

Animal Model: Rat (tumor-bearing animals developed by inoculating tumor cells into the region of the back).

Interpretation and Analysis: Nanoaggregates of FITC-GC are distributed mainly within the kidney, tumor, and liver with very scarce amounts observed in other tissues/organ systems. They maintain high levels for 8 days and their distribution within neoplastic tissues increases gradually. Chitosan nanoaggregates appear to accumulate passively in tumor tissue due to a enhanced permeability and retention (EPR) effect. Tumor growth is suppressed over a 10-day period.

Biodistribution and antitumor efficacy of doxorubicin-loaded glycol-chitosan nanoaggregates by EPR effect. Son YJ, Jang JS, Cho YW, Chung H, Park RW, Kwon IC, Kim IS, Park JY, Seo SB, Park CR, Jeong SY. J Control Release 2003 Aug 28; 91 (1–2), pp. 135–45.

Gene Therapy Assessment: Antisense Oligonucleotides/Neoplastic Disease: Leukemia Nucleoli Targeting
Fluorescein-Labeled Phosphorothioate-Triple Helix-Forming Oligonucleotide (PS-TFO)

Cellular/Molecular Mechanism of Action: Oligonucelotide sequences can be designed that recognize and bind to specific gene/messenger RNA (mRNA) sequences.

Physiological Mechanism of Action: Selective inhibition of the transcription/translation of specific genes.

Background Information: The c-myc gene is frequently deregulated and overexpressed in human cancers, and strategies designed to inhibit c-myc expression in cancer cells may have considerable therapeutic value.

Application: Characterize the antigene and antiproliferative activity of a triple helix-forming oligonucleotide (TFO) targeted to a homopurine-homopyrimidine sequence in the P2 promoter of the c-myc gene.

Route of Administration: Potentially variable (intravenous, intralesional).

Semi-Synthetic Methods: Triple helix-forming oligonucleotide can be synthesized with phosphorothioate (PS) internucleotide linkages to confer resistance to intra- and extracellular nucleases. The resulting preparation can be conjugated with fluorescein as a tracer agent to identify selective accumulation within certain tissues and subcellular organelles.

Implementation: Delineation of the binding affinity of fluorescein-labeled PS-TFO for target molecules.

Interpretation and Analysis: Resistance to intra- and extracellular nucleases is a property that is required for the design of oligonucleotides intended for *in vivo* testing and therapeutic applications.

Phosphorothioate-modified triple helix-forming oligonucleotides form triplex DNA complexes with affinity and specificity comparable with that of the corresponding phosphodiester TFO, as delineated by gel mobility shift and footprinting assays. Fluorescence microscopy and polyacrylamide gel analysis reveal that fluorescein-labeled PS-TFO accumulates in the nuclei of CEM leukemia cells and remains intact for a duration of at least 72 hours. Incubation of CEM cells with PS-TFO reduces c-myc RNA and protein levels. A single exposure of leukemia cells to the PS-TFO is sufficient to induce dose-dependent growth inhibitory effects. Growth inhibition correlates with accumulation of cells in S phase and with induction of cell death by apoptosis. Preparations of PS-TFO are also effective in other leukemia and lymphoma cell lines. Control oligonucleotides have minimal effects in all assays. These data indicate that c-myc-targeted PS-TFO is an effective antigene and antiproliferative agent, with potential for testing *in vivo* as a novel approach to cancer therapy.

Antigene and antiproliferative effects of a c-myc-targeting phosphorothioate-triple helix-forming oligonucleotide in human leukemia cells. McGuffie EM, Pacheco D, Carbone GM, Catapano CV. Cancer Res 2000 Jul 15; 60 (14), pp. 3790–9.

Mammary Carcinoma: Potential Delivery Platform/ Central Nervous System Glioma
T3,4BCPC

Cellular/Molecular Mechanism of Action: The exact mechanism that facilitates the selective uptake of T3,4BCPC by certain neoplastic cell types has not been precisely established.

Application: Potential application as a detectable delivery modality that selectively recognizes certain neoplastic cell types (e.g., glioma, mammary carcinoma).

Route of Administration: T3,4BCPC (intravenous).

Dosage: T3,4BCPC (5.0 mg/kg body weight; murine fibrosarcoma model).

Semi-Synthetic Methods: The new water-soluble photosensitizer 5,10,15,20-tetrakis[3,4-bis(carboxymethyleneoxy)-phenyl] chlorin (T3,4BCPC) can be prepared, characterized, and labeled with [^{99m}Tc] radionuclide.

Implementation: Determination of biodistribution studies in mouse and rat tumor models for determining the comparative properties of the tumor-seeking radiopharmaceuticals [$^{99m}Tc^{V}$]-dimercaptosuccinic acid (DMSA), [^{201}Thallous]-chloride (TlCl), and [^{99m}Tc]-citrate using a gamma camera computer system. In the 2nd phase of evaluation, *in vivo* efficacy of the compound is determined by establishing its potential as photodynamic therapeutic agent achieved by the conduction of a preclinical photodynamic therapy (PDT) study performed in fibrosarcoma-bearing mice (postinjection of T3,4BCPC).

Animal Model: Murine (C_3 H/J virus-induced spontaneous mammary tumors).

Animal Model: Murine (fibrosarcoma-bearing).

Animal Model: Rats (Wistar strain).

Animal Model: Rat (N-nitrosomethylurea [NMU]-induced mammary tumors].

Animal Model: Rat (transplanted C_6-glioma).

Interpretation and Analysis: Accumulation of administered activities within the liver, kidney, bladder, and large intestine at 4 hours postinjection indicates that the labeled ligand is largely eliminated through renal excretion and partly through the hepatobiliary system. In NMU-induced rat mammary tumors, the labeled ligand

shows a 5-fold tumor to muscle (T/M) ratio compared to [$^{99m}Tc^{V}$]-DMSA (3-fold) and [^{201}Tl]Cl (3-fold). In the case of C_3 H/J virus-induced spontaneous mammary tumors, differences are not marked. However, in the transplanted rat C_6-glioma, the T/M ratio of the labeled compound is appreciably higher (4-fold) than that noted with [$^{99m}Tc^{V}$]-DMSA (2-fold), [^{201}Tl]Cl (3-fold), and [^{99m}Tc]-citrate (more than 3-fold). Therefore, radiolabeled T3,4BCPC may have potential for the detection of different cancer cell types. Photodynamic therapy applications of T3,4BCPC in fibrosarcoma-bearing mice evaluated following delivery of a laser dose of 20 mW for 60 seconds reveal 80% destruction of tumor mass. The molecule may therefore be useful for PDT of cancer. The labeled agent could also be useful in monitoring the progression/regression of tumors before, during, and after chemotherapy, radiation therapy, or PDT.

Preparation and biological evaluation of the new chlorin photosensitizer T3,4BCPC for detection and treatment of tumors. Murugesan S, Shetty SJ, Srivastava TS, Samuel AM, Noronha OP. J Photochem Photobiol B 2002 Aug; 68 (1), pp. 33–8.

Polypeptide Delivery: Insulin/Sustained *In Vivo* Peptide Release: Insulin
Microspheres: Inherently Fluorescent

Cellular/Molecular Mechanism of Action: Microspheres can function as a delivery platform that prevents degradation, delays the rate of elimination, controls systemic release, and/or facilitates selective delivery in a manner that minimizes innocent organ/tissue exposure and enhances concentrations at desired target regions.
Application: Facilitation of selective or protracted release of biologically active peptide molecules.
Route of Administration: Intravenous.
Semi-Synthetic Methods: Inherently fluorescent microspheres composed of a fluorescent polyanhydride, poly(*p*-(carboxyethylformamido) benzoic anhydride) (PCEFB), and poly(lactide-co-glycolide) (PLGA) can be prepared using the water-in-oil-in-water (w/o/w) emulsion solvent evaporation technique. Characterization of microsphere blends can be achieved applying combinations of size exclusion gel permeation chromatography (GPC), infrared spectrometry (IR), fluorometry, and scanning electron microscopy (SEM) analyses.
Implementation: Evaluation of the effect of the PCEFB/PLGA feed ratio and composition of the oil phase on insulin entrapment and microsphere diameter.
Interpretation and Analysis: Insulin entrapment efficiency increases with PCEFB content and acetone percent in the oil phase. Microsphere diameter decreases as acetone is added into the oil phase. Although slight degradation of PCEFB during the fabrication process can be identified by GPC and IR, PCEFB/PLGA microspheres can still be clearly visualized by either confocal laser scanning microscopy (CLSM) or fluorescent microscopy in a manner that makes it possible to directly detect *in vivo* microspheres by fluorometry without the need for fluorescent dye-labeling techniques. The surface of PCEFB/PLGA (1:2) microspheres is smooth, while PCEFB/PLGA (2:1) microspheres have rough and uneven surfaces. Sustained release of insulin from the microspheres can be achieved for approximately 4 days.

Bioadhesive fluorescent microspheres as visible carriers for local delivery of drugs. I: Preparation and characterization of insulin-loaded PCEFB/PLGA microspheres. Jiang HL, Zhu KJ. J Microencapsul 2002 Jul–Aug; 19 (4), pp. 451–61.

Protection of Fluorescent Probe: Delivery Modification
Probes Encapsulated by Biologically Localized Embedding (PEBBLEs)

Cellular/Molecular Mechanism of Action: Function as a protected delivery format for fluorescent probes.
Route of Administration: Usually by intravascular injection.
Semi-Synthetic Methods: Spherical optical nanosensors, or PEBBLEs, can be produced in sizes ranging between 20 nm and 200 nm in diameter. The sensors are fabricated in a microemulsion and consist of fluorescent indicators entrapped in a polyacrylamide matrix. A generalized polymerization method has been developed that permits production of sensors containing any hydrophilic dye or combination of dyes in the matrix.
Interpretation and Analysis: The PEBBLE matrix protects the fluorescent dye from interference by proteins, allowing reliable *in vivo* dye calibration. Sensor response times are less than 1 ms. Cell viability assays indicate that PEBBLEs are biocompatible and exert negligible biological effects compared to control conditions. Several sensor (tracer) delivery methods have been studied, including liposomal delivery, gene gun bombardment, and pico-injection into single living cells.

Optical nanosensors for chemical analysis inside single living cells. 1. Fabrication, characterization, and methods for intracellular delivery of PEBBLE sensors. Clark HA, Hoyer M, Philbert MA, Kopelman R. Anal Chem 1999 Nov 1; 71 (21), pp. 4831–6.

Stabilization of Optical Tracer Agents
Indocyanine Green (ICG)

Cellular/Molecular Mechanism of Action: Indocyanine green absorbs and fluoresces in the near-infrared (NIR) range.
Background Information: One of the advantages of the application of NIR fluorescence light in imaging modalities for various *in vivo* biological processes is related to its ability to penetrate tissues more efficiently than light in the visible spectrum.
Application: Indocyanine green is a medically useful dye that can be applied as an optical marker (tracer) agent to evaluate liver function and assess cardiac output parameters.
Background Information: The poor stability of ICG in aqueous solution, especially at the high concentrations needed for bolus injection, has been a hindrance in clinical applications.
Implementation: Certain macromolecular additives can be formulated with ICG to improve the stability of this dye when formulated in aqueous solutions.
Interpretation and Analysis: Such non-covalent binding between dye and carrier molecules preserves substantially the dye in aqueous solutions for several weeks with no apparent changes in the measured *in vivo* biological properties.

Stabilization of the optical tracer agent indocyanine green using non-covalent interactions. Rajagopalan R, Uetrecht P, Bugaj JE, Achilefu SA, Dorshow RB. Photochem Photobiol 2000 Mar; 71 (3), pp. 347–50.

RENAL SYSTEM

Glomerulonephritis: Chronic/Prognostic Factors: Markovian Analysis
Phenolsulfonphthalein (PSP)

Cellular/Molecular Mechanism of Action: Phenolsulfonphthalein is secreted by the proximal renal tubular epithelium.

Application: Estimation of prognosis for conditions of chronic glomerulonephritis.
Implementation: Delineation of the incidence of hypertension in chronic glomerulonephritis and its influence on the clinical course of the disease applying statistic techniques (especially Markovian process).
Interpretation and Analysis: In cases of chronic glomerulonephritis, the incidence of hypertension is approximately 15.2% (e.g., n = 283/1,862), but if renal function is approximately one-half then the incidence increases to 50% (e.g., n = 930/1,862).

The average blood pressure in such disease states tends to become elevated with decreases in 15-minute values for PSP excretion tests and with increasing serum creatinine levels (Note: Variations are large). After a fall of renal function to approximately one-third of the normal, declines in the rate of the clinical course become markedly rapid. The prognosis for the progressive type of chronic glomerulonephritis is obviously shortened by the presence of hypertension. According to the results obtained by the Markovian approach, survival years of the patients with a wide range of renal function can be predicted with relative accuracy.

Prognosis of chronic glomerulonephritis with special reference to hypertension and renal function. Urakabe S, Shirai D, Orita Y, Ando A. Jpn Circ J 1975 Jul; 39 (7), pp. 815–21.

Tubular Absorption: Mechanism of Uptake
Phenolsulfonphthalein (PSP)/Paraaminohippuric Acid (PAH)/Cimetidine

Cellular/Molecular Mechanism of Action: Phenolsulfonphthalein is excreted into the urine by the proximal renal tubular epithelium. Alternatively, PAH is removed passively from the plasma and passes into the urinary filtrate at the level of the renal glomerulus.
Background Information: Phenolsulfonphthalein can be used to assess renal tubular function, but its excretion mechanism has not been elucidated.
Application: Evaluation of renal tubular function.
Implementation: Evaluation of the transporter-mediated excretion system for PSP using PAH (rat organic anion transporter1 [rOat1] substrate) and cimetidine (rOat3 substrate) as investigative molecular probes.
Interpretation and Analysis: Paraaminohippuric acid and cimetidine reduce the urinary excretion of PSP. The uptake of PSP by kidney slices involves individual tubular components. The IC50 values of rOat1 substrates are higher than those of rOat3 substrates. In the presence of cimetidine, Eadie-Hofstee plots produce a single straight line. The profile of the PSP uptake component in the presence of cimetidine is similar to that of the low-affinity component in the absence of cimetidine. Therefore, rOat1 and rOat3 appear to be involved in the renal uptake of PSP, and this agent is a high-affinity substrate for rOat3 but is a relatively low-affinity substrate for rOat1.

Major role of organic anion transporters in the uptake of phenolsulfonphthalein in the kidney. Itagaki S, Sugawara M, Kobayashi M, Nishimura S, Fujimoto M, Miyazaki K, Iseki K. Eur J Pharmacol 2003 Aug 15; 475 (1–3), pp. 85–92.

Tubular Excretion/Renal Glomerular Filtration
Phenolsulfonphthalein (PSP) Excretion/Inulin Clearance/Paraaminohippurate (PAH) Clearance

Cellular/Molecular Mechanism of Action: Phenolsulfonphthalein and PAH are secreted by the proximal renal tubule epithelium. Alternatively, inulin and PAH are removed passively from the plasma where at the level of the renal glomerulus it passes into the urinary filtrate.
Application: Detection of alterations in renal excretory capacity (phenolsulfonphthalein) and glomerular filtration (inulin and PAH).
Implementation: Evaluation of alterations in renal function induced by exposure to a toxic agent. Assessment of renal function can be determined by measuring (i) exogenous PSP excretion; (ii) inulin clearance; (iii) PAH clearance (monkeys only); (iv) endogenous serum creatinine; and (v) blood urea nitrogen (BUN) at multiple time points during the study.

Animal Model: Canine (beagle dog). Groups of male dogs are administered either a single oral dose of 5 mg/kg triclopyr or are fed a diet spiked with triclopyr at a dose of 5 mg/kg/day for 47 consecutive days.

Animal Model: Primate (male rhesus monkey). Toxic challenge is induced by exposure to triclopyr (3,5, 6-trichloro-2-pyridinyloxyacetic acid) in order to determine its influence on renal function following oral administration (e.g., beagle dog and rhesus monkey). Male rhesus monkeys are orally administered triclopyr by gavage at a dose of 5 mg/kg/day, 7 days/week for 28 days, after which the dosage is increased to 20 mg/kg/day for 102 consecutive days.
Interpretation and Analysis: Creatinine, BUN, and inulin clearance are within the normal range for both species following triclopyr administration, which indicates that repeated administration of triclopyr in the dog and monkey has no effect on glomerular filtration rate (GFR). In monkeys, the percentage of excreted PSP and PAH appears to increase following triclopyr administration (20 mg/kg/day), suggesting that these weak organic acids may be competing for the same plasma protein-binding site enhancing their clearance. More importantly, triclopyr does not appear to be competing with PSP or PAH for the active secretory site within the monkey kidney proximal tubules. Conversely, PSP clearance studies in dogs reveal that triclopyr administration (5 mg/kg) can significantly decrease the percentage PSP excretion even following a single-dose administration. The decrease in percentage PSP is reversible and inversely related to the plasma triclopyr concentration. Therefore, triclopyr appears to effectively compete with PSP for the active secretory site within the dog kidney proximal tubules. Conversely, the monkey is insensitive to the effects of triclopyr on the active secretory process even at doses 4-fold higher (20 mg/kg/day) than the effective dose in the dog (5 mg/kg/day). These findings suggest that the effect observed on PSP and PAH excretion in the dog represents a physiological competition for excretion and not toxicity.

Evaluation of renal function in rhesus monkeys and comparison to beagle dogs following oral administration of the organic acid triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid). Timchalk C, Finco DR, Quast JF. Fundam Appl Toxicol 1997 Mar; 36 (1), pp. 47–53.

Tubular Excretion/Renal Plasma Flow/Urine Flow/ Protein Binding: Non-Linear/Glomerular Filtration/ Tubular Secretion/Tubular Accumulation
Phenolsulfonphthalein (PSP)

Cellular/Molecular Mechanism of Action: Phenolsulfonphthalein is secreted by the proximal renal tubular epithelium.
Route of Administration: Phenolsulfonphthalein (intravenous).
Dosage: Phenolsulfonphthalein (1.0 g).
Implementation: Evaluation of the influence of probenecid or salicyluric acid (SUA) on the plasma kinetics and renal excretion of intravenous PSP. A physiologically based renal model has been developed that incorporates the functional characteristics of the kidney and is capable of determining the excretion of PSP (e.g., renal

plasma flow, urine flow, non-linear protein binding, glomerular filtration, tubular secretion, and tubular accumulation).

Animal Model: Canine (beagle dog).

Interpretation and Analysis: Pharmacokinetic analysis reveals that tubular secretion is the predominant route of excretion, and that secretion is inhibited by both SUA and probenecid. The model enables an accurate description and analysis of the measured plasma levels and renal excretion rates. The interaction with probenecid and SUA can be adequately described with the model by inhibition of the carrier-mediated uptake of PSP into the proximal tubular cells. However, both compounds clearly differ in their inhibitory action. Whereas probenecid shows simple competitive inhibition, for SUA a considerably more complex interaction (2-site competitive system) has to be taken into consideration. Especially in the interaction experiments, only satisfactory fits to the model are obtained when secretion is assumed to be dependent on unbound PSP concentrations. Model calculations show that in control experiments tubular secretion is accompanied by a pronounced accumulation of PSP within the proximal tubular cells, which is clearly diminished in the presence of SUA or probenecid. The predicted accumulation ratios are in good agreement with previous studies.

Physiologically based pharmacokinetic model for the renal clearance of phenolsulfonphthalein and the interaction with probenecid and salicyluric acid in the dog. Russel FG, Wouterse AC, van Ginneken CA. J Pharmacokinet Biopharm 1987 Aug; 15 (4), pp. 349–68.

Tubular Failure: Acute
Phenolsulfonphthalein (PSP)/Plasma Protein-Binding Changes with PSP in Acute Renal Failure (ARF)

Cellular/Molecular Mechanism of Action: Phenolsulfonphthalein is secreted by the proximal renal tubular epithelium.

Application: Phenolsulfonphthalein can be applied to detect alterations in renal tubular function such as those associated with acute and chronic kidney failure.

Route of Administration: Phenolsulfonphthalein (intravenous).

Implementation: Determine the influence of ARF on the disposition of PSP.

Animal Model: Rat (acute renal failure induced by subcutaneous injection of uranyl nitrate). In order to clarify the regulatory mechanisms of PSP excretion between liver and kidney in ARF, a simultaneous perfusion system of rat liver and kidney (rat) can be utilized that controls the flow rate and the constituent of the perfusate.

Interpretation and Analysis: Renal excretion of PSP decreases significantly in ARF compared to that in normal controls. In conditions of ARF, increases in biliary PSP excretion can be detected and reflect a process that compensates for reductions in renal excretion. Consequently no significant change can be appreciated in total body clearance of PSP between control and ARF. Binding investigations (*in vitro*) reveal that the binding fraction of PSP to plasma in conditions of ARF is significantly lower than observed in reference control plasma.

Applying the simultaneous perfusion system, neither biliary excretion nor the protein binding of PSP differs significantly between control and ARF, though its renal excretion decreases in ARF in a similar manner as observed *in vivo.* These results suggest that alterations in plasma protein binding as well as renal excretory function are determinants of PSP disposition in ARF.

Influence of acute renal failure on pharmacokinetics of phenolsulfonphthalein in rats: A comparative study *in vivo* and in the simultaneous perfusion system of liver and kidney. Yasuhara M, Katayama H, Fujiwara J, Okumura K, Hori R. J Pharmacobiodyn 1985 May; 8 (5), pp. 377–84.

Tubular Pharmaceutical Reabsorption/ Differentiation between Competitive vs Noncompetitive Tubular Absorption
Phenolsulfonphthalein (PSP)

Cellular/Molecular Mechanism of Action: Phenolsulfonphthalein is excreted by the proximal renal tubular epithelium.

Application: Evaluation of renal tubular function.

Implementation: Evaluation of the plasma kinetics and renal excretion of salicyluric acid (SUA, 0.8 g), administered intravenously with and without concomitant PSP administration.

Animal Model: Canine (beagle dog).

Interpretation and Analysis: Pharmacokinetic analysis reveals that tubular secretion is the predominant route of excretion, and that secretion is inhibited by PSP. A physiologically based kidney model can be utilized that possesses all the functional characteristics of the kidney and determine the excretion of SUA (renal plasma flow, urine flow, non-linear protein binding, glomerular filtration, tubular secretion, and tubular accumulation). The model facilitates an accurate description and analysis of measured plasma levels and renal excretion rates. The interaction with PSP can be adequately described with the model by non-competitive inhibition of a carrier-mediated uptake of SUA into the tubular cells. Furthermore, a small but significant reduction in non-renal SUA clearance can be observed. Model calculations show that, in controlled investigations, tubular secretion is accompanied by a pronounced accumulation of SUA within cells, which is clearly diminished in the presence of PSP. The predicted accumulation ratios are in good agreement with previously reported *in vitro* values.

Physiologically based pharmacokinetic model for the renal clearance of salicyluric acid and the interaction with phenolsulfonphthalein in the dog. Russel FG, Wouterse AC, van Ginneken CA. Drug Metab Dispos 1987 Sep–Oct; 15 (5), pp. 695–701.

Tubular Resorptive Capacity
Phenol Red/Bromophenol Blue/Bromothymol Blue

Cellular/Molecular Mechanism of Action: Phenol red, bromophenol blue, and bromothymol blue appear to be excreted by renal tubule acid transport mechanisms and are reabsorbed in a manner that is proportional to the glomerular load.

Application: Evaluation of renal tubule excretory function.

Route of Administration: Phenol red, bromophenol blue, and bromothymol blue (intravenous).

Implementation: Potential application includes the evaluation of renal tubule acid transport mechanisms.

Animal Model: Rabbit.

Interpretation and Analysis: Net tubular excretion of phenol red reaches a maximal value of 8 μmol/minute at a plasma concentration of ultrafilterable dye of about 0.1 mM and is decreased at higher plasma concentrations. Decreases in net tubular excretion at high plasma concentrations are also obtained for bromophenol blue and bromothymol blue, suggesting tubular reabsorption in addition to tubular secretion of the dye. Conclusive evidence for reabsorption is provided by administration of probenecid, which causes a fall in the excretion of the dyes below that filtered by the glomeruli. Tubular reabsorption of phenol red during probenecid administration appears to be proportional to the glomerular load and is increased under experimental conditions, leading to a decrease in urinary pH. Experiments involving efflux of phenol red from liposomes provides no evidence of a significant role of transmembrane passage by nonionic diffusion. The reabsorptive process is the result of preferential reabsorption of the acid as compared to the basic form of the indicator

dye across a hydrophilic pathway in the transporting membranes. Clearance ratio of phenol red to that of paraaminohippurate at low plasma concentrations is about 0.3. The low degree of extraction of phenol red from renal plasma is attributed both to tubular reabsorption and binding of the dye by plasma proteins.

Renal handling of phenol red. III. Bidirectional transport. Gerdes U, Kristensen J, Møller JV, Sheikh MI. J Physiol 1978 Apr; 277, pp. 115–29.

Urinary Bladder: Spontaneous Wall Activity
Polystyrene Fluorescent Spheres

Cellular/Molecular Mechanism of Action: Polystyrene fluorescent spheres can function as an *in vivo* detection/marker agent for evaluation of various physiological processes within body fluid compartments.
Background Information: Optical marker tracing methods have been applied successfully in recent years to quantify local (focal) mechanical deformation of heart tissue, skin, and striated muscles.
Application: Detection and characterization of physiological processes and pathological anomalies affecting body fluid compartments.
Route of Administration: Direct delivery.
Implementation: Evaluation of spontaneous bladder wall activity and responses following electrical stimulation of bladder innervating nerve fibers. One approach to acquiring such functional information can entail gluing polystyrene fluorescent spheres (d = 0.6 mm) to the ventral serosal bladder wall. Three-dimensional video registration of the polystyrene spheres can be used to calculate 2 directions of principal strain (epsilon-1, epsilon-2) on the bladder surface *in vivo.*
Animal Model: Rabbit (bladder serosal surface).
Interpretation and Analysis: During spontaneous activity, random contraction and relaxation occurs simultaneously and separately across the bladder wall for the 2 principal epsilon-1 and epsilon-2 strains. After extradural electrical stimulation of sacral nerve root S2, the principal strains epsilon-1 and epsilon-2 synchronize in time in such a way that epsilon-1 and epsilon-2 both represent contraction or both represent relaxation. One and the same bladder wall area passes through phases of contraction followed by relaxation and vice versa. After multiple stimulation periods, the coordination between the 2 principal strains during stimulation is reduced. The technique applied allows *in vivo* identification of local areas of contraction and relaxation in the intact bladder wall. Three-dimensional video detection of polystyrene fluorescent spheres to study bladder wall contraction and its relaxation proved to be a feasible technique, with which electrical stimulation effects and spontaneous activity could be measured.

Three-dimensional registration of mechanical bladder activity using polystyrene fluorescent spheres: A technical note. Kinder MV, Bos R, Willems PJ, Drost MR, Holtzer CA, Janknegt R, Marani E. Arch Physiol Biochem 1999 Jul; 107 (3), pp. 236–41.

REPRODUCTIVE SYSTEM

Amniotic Fluid Volume Estimation
Aminohippurate Sodium

Cellular/Molecular Mechanism of Action: Aminohippurate sodium can be applied as a tracer molecule to monitor physiological processes and estimate fluid compartment volumes.
Application: Utilization of aminohippurate sodium as a reagent for a dye-dilution technique suitable for accurately measuring amniotic fluid volume.
Route of Administration: Variable (amniotic fluid volume).
Implementation: Estimation of amniotic fluid volume assessed by the dye-dilution technique compared to direct measurements determined in singleton pregnancies with intact membranes undergoing a cesarean delivery.
Human Model: Women (n = 15; cases with amniocentesis at delivery n = 6; cesarean delivery n = 9 with samples collected 4–24 hours prior to surgery).
Interpretation and Analysis: Good concordance between the dye-determined and direct measurement of amniotic fluid volume can be appreciated in women undergoing amniocentesis (e.g., n = 6/6 cases; r = 0.99, p = < 0.001). Among women with varying times from amniocentesis to direct measurement, the correlation is not significant (e.g., n = 9 cases; r = 0.36, p = 0.08). The percent difference between dye-determined and directly measured amniotic fluid volume is significantly smaller in women undergoing amniocentesis just prior to delivery (7%) compared to women with varying times from amniocentesis to delivery (37%, p < 0.001). Dye-determined amniotic fluid volume accurately reflects actual amniotic fluid volume but the dye-determined concentrations, *in vivo,* may undergo rapid changes.

Dye-dilution techniques using aminohippurate sodium: Do they accurately reflect amniotic fluid volume? Magann EF, Whitworth NS, Files JC, Terrone DA, Chauhan SP, Morrison JC. J Matern Fetal Neonatal Med. 2002 Mar; 11 (3), pp. 167–70.

Mammary Gland Disease Differentiation/Optical Imaging
Indocyanine Green (ICG)/Gadolinium/Near-Infrared (NIR) Diffuse Optical Tomography

Cellular/Molecular Mechanism of Action: Indocyanine green is an albumin-bound absorbing dye.
Background Information: One of the advantages for applying NIR fluorescence light in imaging modalities for various *in vivo* biological processes is related to its ability to penetrate tissues more efficiently than light in the visible spectrum.
Application: Indocyanine green is an absorbing dye commonly used as a marker system within the plasma compartment. Under certain conditions, ICG possesses the potential to differentiate between diseased and normal tissues based on the quantified enhancement of suspicious lesions using magnetic resonance imaging (MRI) applied concurrently with NIR diffuse optical tomography (contrast enhancement properties).
Route of Administration: Intravascular.
Implementation: Near-infrared diffuse optical tomography (DOT) analysis of the mammary gland applied in combination/concurrently with MRI analysis. Results are then correlated with findings from histopathological evaluation of suspicious lesions.
Human Model: Cases scheduled for excisional biopsy or surgery.
Interpretation and Analysis: The ICG-enhanced optical images co-register accurately with gadolinium-enhanced magnetic resonance images, thereby validating the ability of DOT to image breast tissue. In contrast to simple transillumination, DOT appears to provide for localization and quantification of exogenous tissue chromophore concentrations. Additionally, ICG has demonstrated a potential to differentiate disease based on the quantified enhancement of suspicious lesions.

Concurrent MRI and diffuse optical tomography of breast after indocyanine green enhancement. Ntziachristos V, Yodh AG, Schnall M, Chance B. Proc Natl Acad Sci U S A. 2000 Mar 14; 97 (6), pp. 2767–72.

Sperm: Viability and Intracellular Calcium
Fluorescent Calcium Indicator [Indo-1]/ Chlortetracycline (CTC) Fluorescent Assay

Background Information: Cryopreserved bovine semen is less fertile than fresh semen for reasons that have not been fully elucidated. Cryopreservation is known to disrupt the sperm plasma membrane and it induces premature capacitation of sperm subpopulations, which may be a result of the increased internal calcium levels after thawing.
Application: Assessement of sperm viability.
Route of Administration: Topical *in vitro* bathing.
Implementation: Evaluation of the relationship between the fertility of cryopreserved/thawed sperm and (i) intracellular calcium levels assessed by the application of fluorescent calcium indicators (indo-1), and flow cytometry analysis; or (ii) their physiological status determined by the CTC fluorescent assay.

Animal Model: Bovine (sperm collected from fertile and nonfertile bulls).
Interpretation and Analysis: As detected by indo-1 fluorescence, intracellular calcium levels negatively correlate with bull fertility immediately after thawing ($p = 0.0362$; $n = 3$ ejaculates from each of 10 animals). Moreover, there is a significant difference between the 3 most and least fertile bulls in a herd over 4-hour incubation periods (e.g., $p < 0.05$; $n = 3$ ejaculates/bull). There is a positive correlation between sperm displaying the CTC acrosome reaction pattern and fertility ($p = 0.0014$; $n = 3$ ejaculates from each of 10 bulls).

Sperm calcium levels and chlortetracycline fluorescence patterns are related to the *in vivo* fertility of cryopreserved bovine semen. Collin S, Sirard MA, Dufour M, Bailey JL. J Androl 2000 Nov–Dec; 21 (6), pp. 938–43.

Urogenital: Cervical Cell Types: Biopsy Tissues/ Differentiation of Normal vs Abnormal/Cell Nuclei Visualization
Acetic Acid Enhancement

Cellular/Molecular Mechanism of Action: Acetic acid chemically modifies the physical properties of different cellular constituents.
Application: The use of high-resolution *in vivo* confocal imaging may offer a clinically useful adjunct to standard methods for the diagnosis and screening of epithelial precancers.
Implementation: Evaluate the feasibility of real-time confocal reflectance imaging of cervical tissue and the use of acetic acid as a contrast agent to increase visualization of cell nuclei.

***Ex Vivo* Model:** Confocal microscopy image evaluation of cervical cells and colposcopically normal and abnormal cervical biopsy specimens. Images are obtained before and after the application of 6% acetic acid.
Interpretation and Analysis: Confocal microscopy imaging can resolve subcellular detail throughout the entire epithelial thickness. Normal and abnormal tissues are clearly able to be differentiated. Addition of acetic acid enhances nuclear signals in all acquired images. High-contrast reflected light images of cervical tissue are attainable in near real time. Acetic acid significantly increases light scattering from cell nuclei, which may partially explain why acetowhitening occurs.

Laser scanning confocal microscopy of cervical tissue before and after application of acetic acid. Drezek RA, Collier T, Brookner CK, Malpica A, Lotan R, Richards-Kortum RR, Follen M. Am J Obstet Gynecol. 2000 May; 182 (5), pp. 1135–9.

RESPIRATORY SYSTEM

Surfactant Distribution
Fluorescent Bodipy-Labeled Surfactant Protein B

Cellular/Molecular Mechanism of Action: Surfactant lowers the viscous tension of pulmonary fluid in a manner that prevents collapse of terminal alveoli.
Application: Fluorescent Bodipy-labeled surfactant protein B can be applied to determine the distribution of endotracheally administered surfactant at the alveolar level in an animal model of acute respiratory distress syndrome.
Route of Administration: Intratracheal.
Semi-Synthetic Methods: Covalent conjugation/cross-linking of fluorescent Bodipy-labeled surfactant protein B.
Implementation: To measure surfactant distribution *in vitro,* a glass trough mimicking dichotomic lung anatomy can be used to determine the spreading properties of bovine lung surfactant extract supplemented with fluorescent Bodipy-labeled surfactant protein B. To measure surfactant distribution *in vivo,* anesthetized rats are exposed to aerosolized lipopolysaccharide (12 mg/kg body weight) to induce lung injury that resembles acute respiratory distress syndrome. In control rats, buffered saline is alternatively aerosolized. Twenty-four hours later rats are anesthetized, tracheotomized, and mechanically ventilated (peak airway pressure = 20 mbar; positive end-expiratory pressure = 6 mbar; inspiration time = expiration time = 0.6 seconds; Fio2 = 50%). Surfactant (bovine lung surfactant extract, supplemented with fluorescent Bodipy-labeled surfactant protein B; 50 mg/kg body weight) is given as a bolus; in control rats, a bolus of saline is administered. Rats are then ventilated for 5, 15, 30, or 60 minutes ($n = 8$ or 9 for each group) and their lungs excised and sliced. Lung slices, divided into aerated (open), underinflated (dystelectatic), or collapsed (atelectatic) alveolar areas are subsequently examined by both light and fluorescence microscopy.
Interpretation and Analysis: Surfactant spreads independent of glass trough geometry and lowers the surface tension to equilibrium values (25 mN/m) within a few seconds in *in vitro* preparations. Administration of surfactant *in vivo* distributes preferentially into underinflated and aerated alveolar areas. Surfactant distribution is not affected by length of mechanical ventilation. When conventional mechanical ventilation is used in lipopolysaccharide-induced lung injury, surfactant preferentially distributes into underinflated and aerated alveolar areas. Because surfactant rarely reaches collapsed alveolar areas, methods aiding in alveolar recruitment (e.g., open lung concept or body positioning) should precede surfactant administration.

In vitro and *in vivo* intrapulmonary distribution of fluorescently labeled surfactant. Diemel RV, Walch M, Haagsman HP, Putz G. Crit Care Med 2002 May; 30 (5), pp. 1083–90.

SEPSIS

Sepsis: *Escherichia Coli* (O157:H7)
Adenosine Triphosphate (ATP)/In Vivo Bioluminescence/Immunomagnetic Absorbance

Application: Development of methods for assessing the efficiency of immunomagnetic separation (IMS).

Implementation: Evaluation of the capturing efficiency of biosorbents for *E. coli* O157:H7, constructed using streptavidin-coated magnetic beads and biotinylated antibodies, using both *in vivo* and ATP bioluminescence.

Interpretation and Analysis: Both methods are suitable for the enumeration of bacteria captured by the biosorbents. The level of both ATP and *in vivo* bioluminescence depends on the media used but is unaffected by magnetic beads. The capture efficiency depends on time and sample volume but does not depend on the length of spacer arm of the biotinylation agent. For cell concentrations of $\leq$ 105 cfu/ml, in a 1-ml sample volume, nearly 80–85% recovery of the pathogen can be observed after 0.5 hours of incubation. For an 11-ml sample containing 104 cfu/ml, maximum recovery (50% of cells) is achieved only after 2 hours incubation. The detection limit of an ATP-based bioluminescent assay for *E. coli* O157:H7 can be reduced by 1 log cycle after optimization of IMS. The bioluminescent methods can be used for screening and testing the affinity of antibodies or other affinity elements of biosorbents toward live bacterial cells. Bioluminescent assays provide an easy way to optimize conditions for the capture of bacteria by biosorbents in real time.

Comparison of ATP and *in vivo* bioluminescence for assessing the efficiency of immunomagnetic sorbents for live *Escherichia coli* O157:H7 cells. Sun W, Khosravi F, Albrechtsen H, Brovko LY, Griffiths MW. J Appl Microbiol 2002; 92 (6), pp. 1021–7.

17 Magnetic Resonance Imaging (MRI)

MRI: GENERAL CONCEPTS AND PRINCIPLES

MRI: Concepts and Principles/Hepatic Disease

Magnetic resonance imaging relies on the physical properties of unpaired protons in tissues to generate images. Unpaired protons behave like tiny bar magnets and will align themselves in a magnetic field. Radiofrequency pulses will excite these aligned protons to higher energy states. As they return to their original state, they will release this energy as radio waves. The frequency of the radio waves depends on the local magnetic field, and by varying this over a subject, it is possible to arrange or build anatomical images. In general, MRI has not been sufficiently sensitive or specific in the assessment of diffuse liver disease for clinical use. However, because of the specific characteristics of fat and iron, it may be useful in the assessment of hepatic steatosis and iron overload. Magnetic resonance imaging is useful in the assessment of focal liver disease, particularly in conjunction with contrast agents. Hemangiomas have a characteristic bright appearance on T_2-weighted images because of the slow-flowing blood in dilated sinusoids. Focal nodular hyperplasia (FNH) has a homogenous appearance and enhances early in the arterial phase after gadolinium injection, while the central scar typically enhances late. Hepatic adenomas have a more heterogenous appearance and also enhance in the arterial phase, but less briskly than FNH. Hepatocellular carcinoma is similar to an adenoma but typically occurs in a cirrhotic liver and has earlier washout of contrast. The appearance of metastases depends on the underlying primary malignancy. Overall, MRI appears more sensitive and specific than computed tomography with contrast for the detection and evaluation of malignant lesions.

Magnetic resonance imaging and diseases of the liver and biliary tract. Part 1. Basic principles, MRI in the assessment of diffuse and focal hepatic disease. Macdonald GA, Peduto AJ. J Gastroenterol Hepatol 2000 Sep; 15 (9), pp. 980–91.

MRI: Concepts and Mechanisms/Hepatic Disease

Magnetic resonance cholangiography (MRC) relies on the strong T_2 signal from stationary liquids, in this case bile, to generate images. No contrast agents are required, and the failure rate and risk of serious complications is lower than with endoscopic retrograde cholangiopancreatography (ERCP). Data from MRC can be summated to produce an image much like the cholangiogram obtained by using ERCP. In addition, MRC and conventional MRI can provide information about the biliary and other anatomy above and below a biliary obstruction. This provides information for therapeutic intervention that is probably most useful for hilar and intrahepatic biliary obstruction. Magnetic resonance cholangiography (MRC) appears to be similar to ERCP with respect to sensitivity and specificity in detecting lesions causing biliary obstruction, and in the diagnosis of choledocholithiasis. It is also suited to the assessment of biliary anatomy (including the assessment of surgical bile-duct injuries) and intrahepatic biliary pathology. However, ERCP can be therapeutic as well as diagnostic, and MRC should be limited to situations where intervention is unlikely, where intrahepatic or hilar pathology is suspected, to delineate the biliary anatomy prior to other interventions, or after failed or inadequate ERCP. Magnetic resonance angiography (MRA) relies on the properties of flowing liquids to generate images. It is particularly suited to assessment of the hepatic vasculature and appears as good as conventional angiography. It has been shown to be useful in delineating vascular anatomy prior to liver transplantation or insertion of a transjugular intrahepatic portasystemic shunt. Magnetic resonance angiography may also be useful in predicting subsequent variceal hemorrhage in patients with esophageal varices.

Magnetic resonance imaging and diseases of the liver and biliary tract. Part 2. Magnetic resonance cholangiography and angiography and conclusions. Macdonald GA, Peduto AJ. J Gastroenterol Hepatol 2000 Sep; 15 (9), pp. 992–9.

MRI: Concepts for Masses (Soft Tissue)
Intravenous Contrast Agents/Oral Contrast Agents

Cross-sectional imaging of the abdomen in oncology patients presents unique challenges and opportunities. A close working relationship between the oncologist and radiologist is essential for the exchange of the clinical and imaging information necessary for optimizing patient diagnosis and management. Compared to helical computed tomography (CT), MRI of the abdomen and pelvis offers important advantages, including superior soft-tissue contrast. The multiplanar capabilities of MRI allow for direct coronal or sagittal imaging, providing a truer anatomic presentation of abdominal and pelvic masses. Recent advances in MRI, including the use of intravenous (i.v.) and oral contrast agents, the development of high-performance imagers, and improved surface coil designs, facilitate more rapid abdominal imaging with superior image quality. All of these features combine to produce a versatile imaging examination with exquisite sensitivity for depicting abdominal and pelvic tumors. In this context, they have clinical applicability for hepatic and extrahepatic abdominal MRI in the oncology patient. Techniques and protocols for MRI can be applied to most commercially available high-field MRI instrumentation.

Magnetic resonance imaging of the abdomen: Applications in the oncology patient. Low RN. Oncology (Huntingt) 2000 Jun; 14 (6 Suppl 3), pp. 5–14.

MRI: Concepts and Perspectives/Recent Trends in Contrast Agent Development for MRI/ Contrast-Enhancing Imaging Agents

Historically, small molecular weight gadolinium chelates have functioned as the predominant MRI contrast-enhancing agents. These 1st-generation MR contrast agents distribute into the intravascular and interstitial space, thus allowing the evaluation of physiological parameters, such as the status or existence of the blood-brain barrier or the renal function. Shortly after the 1st clinical use of paramagnetic metallochelates in 1983, several compounds were proposed as potential image-enhancing agents for the liver and cardiac infarcts. Meanwhile, liver-specific contrast agents based on gadolinium, manganese, or iron became a reality. Dedicated blood-pool agents will become available in the near future. These gadolinium or iron-based agents will be beneficial for longer-lasting MRA procedures, such as

cardiac imaging. Contrast-enhanced lymphography after interstitial or intravenous injection will facilitate achieving major steps forward in diagnostic imaging. Metastatic involvement will be seen either after the injection of ultrasmall superparamagnetic iron oxides or dedicated gadolinium chelates. The accumulation of both classes of compounds is triggered by intracellular uptake by macrophages. It is likely that similar agents will augment MRI of atheriosclerotic plaques, a systemic inflammatory disease of the arterial wall. Thrombus-specific agents based on small gadolinium-labeled peptides are currently in development. It is very obvious that the future of cardiovascular MRI will benefit from the development of new paramagnetic and superparamagnetic substances. The expectations for new tumor-, pathology-, or receptor-specific agents are high. However, it is not likely that such a compound will be available for daily routine MRI within the next decade.

Tissue-specific MR contrast agents. Weinmann HJ, Ebert W, Misselwitz B, Schmitt-Willich H. Eur J Radiol 2003 Apr; 46 (1), pp. 33–44.

Phantoms for Computed Tomograph (CT) and MRI
Barium Sulfate-Loaded Agarose Gel/Iodinated Contrast-Loaded Agarose Gel

Cellular/Molecular Mechanism of Action: Iodinated compounds and barium preparations possess radiopaque properties.
Application: Standardization of computed tomography and MRI analyses.
Considerations: Phantom studies are an important part of the evaluation of imaging techniques; however, presently available phantom construction materials are not adequate for studies involving both MRI and CT. It is possible to design a phantom constructed of material useful for multimodality imaging purposes.
Implementation: Iodinated contrast agent or $BaSO_4$ is added to preparations of agarose gels. Both CT and MRI are then performed, and T_1 and T_2 values and CT numbers (in Hounsfield units) are obtained for multiple combinations of contrast materials and agarose.
Interpretation and Analysis: The T_2 values of agarose gels span the range of those values found in biologic tissues. Phantoms containing iodinated contrast agent are not stable because the contrast agent diffuses across concentration gradients. Alternatively, $BaSO_4$-loaded agarose phantoms are stable, and varying barium concentrations produce phantoms that span the range of CT numbers found in biologic tissues. Addition of $BaSO_4$ does not substantially alter T_1 or T_2 values of agarose gels. Agarose concentration has only a small effect on the CT numbers of $BaSO_4$ suspensions.

Agarose gels loaded with $BaSO_4$ represent an effective material for constructing multimodality imaging phantoms. It provides adequate signal intensity for MRI and attenuation for CT, with independently variable contrast in both modalities.

$BaSO_4$-loaded agarose: A construction material for multimodality imaging phantoms. Litt HI, Brody AS. Acad Radiol 2001 May; 8 (5), pp. 377–83.

REACTIONS TO CONTRAST AGENTS

Adverse Reactions: Incidence and Frequency of Reactions to MRI Contrast Agents
Gadodiamide/Gadoteridol

Cellular/Molecular Mechanism of Action: Gadolinium-based compounds.
Application: Magnetic resonance contrast agent for angiography imaging procedures.
Implementation: Conduction of a survey by the American Society of Neuroradiology regarding the incidence of adverse reactions to gadolinium-based contrast agents, the contrast agents responsible, and the management of patients with allergy-like reactions to iodinated or gadolinium-based agents who required MR contrast agent administration.
Interpretation and Analysis: Review and interpetation of a survey from 105 centers (50.5% response rate) regarding a total of 687,255 gadopentetate dimeglumine injections. The approximate rate of adverse non-allergic reactions is 0.046% (e.g., n = 314/687,255) in addition to (i) 0.016% mild reactions (e.g., n = 107/687,255); (ii) 0.004% moderate (e.g., n = 28/687,255); and (iii) 0.001% severe (e.g., n = 5/687,255). Alternatively, of 74,275 recorded injections of gadodiamide, approximately (i) 0.015% (e.g., n = 11/74,275) non-allergic reactions; and (ii) 0.016% mild allergic-like reactions (e.g., n = 12/74,275) can be anticipated to occur. Of 64,005 gadoteridol administrations, reactions can be categorized as (i) 0.267% non-allergic reactions (e.g., n = 171/64,005); (ii) 0.077% mild (e.g., n = 49/64,005); (iii) 0.047% moderate (e.g., n = 29/64,005); and (iv) 0.017% severe (e.g., n = 11/74,275) allergy-like reactions. In one survey, twenty-six radiology departments take no precautions for cases with a previous history of allergy-like reactions to iodinated contrast material. Nineteen radiology departments do not premedicate cases that report previous reactions to gadolinium-based agents before repeat administration of MR contrast agents. Although MR contrast agents are safe, adverse reactions occur. Many centers have not adopted policies for cases with a history of adverse reactions.

Occurrence of adverse reactions to gadolinium-based contrast material and management of patients at increased risk: A survey of the American Society of Neuroradiology Fellowship Directors. Murphy KP, Szopinski KT, Cohan RH, Mermillod B, Ellis JH. Acad Radiol 1999 Nov; 6 (11), pp. 656–64.

Adverse Reactions: Cardiovascular and Hemodynamic Effects/Systemic and Pulmonary Circulation Pressure Flow Dynamics
Gadolinium ([Gd]-DTPA)/Diatrizoate/Iopamidol

Cellular/Molecular Mechanism of Action: Gadolinium and iodinated compound possess radiopaque properties.
Application: Conventionally, gadolinium in the form of [Gd]-DTPA, diatrizoate (ionic), and iopamidol (non-ionic) can be used as positive contrast image-enhancing agents for the detection of anatomical anomalies affecting the cardiovascular system (conventional radiography and MRI).
Route of Administration: [Gd]-DTPA, diatrizoate, and iopamidol can all be given as a bolus intravascular injection for the detection of anatomical anomalies affecting the cardiovascular system.
Dosage: Gadopentate dimeglumine ([Gd]-DTPA [Magnevist] at high dose = 0.6 mmol/kg or 1.2 ml/kg in swine). Diatrizoate (Urografin 76% at high dose = 0.6 mmol/kg or 1.2 ml/kg in swine). Iopamidol (Solutrast 370% at high dose = 0.6 mmol/kg or 1.2 ml/kg in swine).
Implementation: Evaluation of the hemodynamic reactions induced by high-dose gadopentetate dimeglumine compared with the side effects of high-dose ionic (diatrizoate: Urografin 76%) and non-ionic (iopamidol: Solutrast 370%) radiographic contrast media.
Study Design: Assessement of pressure and flow of the systemic and pulmonary circulation following intracardiac bolus injections delivered over a time period of 2 to 4 seconds (e.g., n = 18 swine).
Interpretation and Analysis: Gadolinium, diatrizoate, and iopamidol all transiently decrease aortic pressure ([Gd]-DTPA and

diatrizoate: −25%, iopamidol: −10%; $p < 0.01$). Pulmonary artery pressure, cardiac output, and stroke volume remain increased for several minutes while vascular resistance declines. Diatrizoate induces stronger and longer-lasting side effects ($p < 0.01$) than [Gd]-DTPA and iopamidol. Despite similar osmolalities, [Gd]-DTPA induces weaker side effects than equivolumetric applications of diatrizoate. Other than osmolality, other factors such as viscosity and chemotoxicity also influence the occurence of contrast media–associated side effects.

Gadopentetate dimeglumine and iodinated contrast media. Hemodynamic side effects after bolus injections in pigs. Kuhtz-Buschbeck JP, Ehrhardt K, Kohnlein S, Radtke W, Heintzen P. Invest Radiol 1997 Feb; 32 (2), pp. 111–9.

Adverse Reactions: Hemodialysis Cases
Gadolinium

Implementation: Meassurement of serum gadolinium concentrations in hemodialysis cases following administration of gadolinium MR contrast-image-enhancing agent (e.g., n = 70). Serum gadolinium concentrations are then measured before and after hemodialysis sessions. Cardiovascular, cutaneous, respiratory, psychoneurological, and digestive side effects are also evaluated in concert with any changes in liver and kidney functions, blood counts, and electrolytes.

Hemodialysis Schedule: Hemodialysis performed either on the same day (e.g., n = 16), day 1 (e.g., n = 34), day 2 (e.g., n = 14), or day 3 (e.g., n = 6), and then on a conventional schedule (3 times/week for 4 hours/session).

Interpretation and Analysis: Neither side effects nor blood changes can be appreciated in any of the hemodialysis cases. Average excretory rates are 78.2%, 95.6%, 98.7%, and 99.5% in the 1st to 4th hemodialysis sessions, respectively. Therefore, [Gd] contrast agents can be used in hemodialysis patients if hemodialysis is carried out promptly after performing diagnostic procedures.

Safety of gadolinium contrast agent in hemodialysis patients. Okada S, Katagiri K, Kumazaki T, Yokoyama H. Acta Radiol 2001 May; 42 (3), pp. 339–41.

Adverse Reactions: Renal Dysfunction
Gadolinium/Digital Subtraction Angiography (DSA)

Background Information: To prevent iodinated contrast medium–induced nephrotoxicity, gadolinium has been used increasingly for MR angiography (MRA) or conventional DSA to visualize arterial anatomy in patients undergoing vascular surgery who are considered at high risk because of chronic renal insufficiency.

Application: Postive contrast imaging agent in DSA and MRI procedures.

Dosage: Gadolinium-based contrast agents (≥ 0.25 mmol/kg).

Implementation: Contrast enhancement of images acquired by MR and DSA procedures.

Experimental Model: Single-center retrospective study evaluating the extent of nephrotoxicity induced during the course of MRA procedures (e.g., n = 218) and cases evaluated by DSA (e.g., n = 42) that applied gadolinium as the sole contrast agent (December 1999 to January 2001). Patient comorbid conditions, indications for vascular imaging, contrast dose, urine output, baseline and post-procedure serum creatinine concentration (SCr), and outcome are relevant variables that can provide insight into the relative toxicity of gadolinium contrast media and biological/physiological factors that may contribute to the development of such sequelae.

Interpretation and Analysis: In cases with preimage baseline chronic renal insufficiency, approximately 3.5% will develop acute renal failure following administration of gadolinium-based contrast preparations (e.g., n = 7/195; n = 3/153 MRA; n = 4/42 DSA). In cases classified as having baseline chronic renal insufficiency, serum creatinine concentrations can average approximately 38.2 ± 1.6 Ml/min/1.73 m^2, in contrast to values of 32.5 ± 7.8 Ml/min/1.73 m^2 for conditions of acute renal failure ($p = 0.33$). Doses for gadolinium in cases demonstrating renal failure postinjection can range from 0.31 to 0.41 mmol/kg for MRA and 0.27 to 0.42 mmol/kg for DSA procedures. Interestingly, acute renal failure rarely develops in cases with normal baseline serum creatinine levels (e.g., n = 0/65).

Despite reports of negligible nephrotoxicity, gadolinium-based contrast agents rarely cause acute renal failure in instances where underlying chronic renal insufficiency exist. Estimation of creatinine clearance alone does not enable accurate prediction of which cases are likely to develop acute renal failure. Cases at high risk should be identified, and prophylactic measures exercised to reduce the risk of induced nephrotoxicity.

Safety of gadolinium contrast angiography in patients with chronic renal insufficiency. Sam AD 2nd, Morasch MD, Collins J, Song G, Chen R, Pereles FS. J Vasc Surg 2003 Aug; 38 (2), pp. 313–8.

Adverse Reactions: Renal Dysfunction
Gadolinium Chelates

Background Information: Gadolinium chelates are mainly eliminated by the kidney.

Considerations: Limited information exists about the effects of gadolinium chelates eliminated by the kidney.

Implementation: Evaluation of the renal tolerance and renal function associated with these compounds in an *in vivo* rat model. A combination of renal ischemia and intrarenal iodinated contrast agent infusion (diatrizoate) can function as a reproducible and reversible model of acute renal failure (n = 5). Using this model, the renal tolerance of gadolinium DOTA ([Gd]-DOTA) (n = 10) and gadolinium DTPA ([Gd]-DTPA) (n = 10) can be evaluated, along with determination of the association between the administration of [Gd]-DOTA with diatrizoate (n = 5) and changes in renal function.

Interpretation and Analysis: [Gd]-DOTA induces no change in serum creatinine and creatinine clearance. Gadolinium DTPA induces a significant increase in serum creatinine (50–83 ± 5 and 70 ± 6 μmol/L) before and at 24 and 48 hours, respectively ($p < 0.05$), and a decrease in creatinine clearance from 1.6 ± 0.1 to 0.8 ± 0.1; 1.2 ± 0.1 Ml/Ml before and at 24 and 48 hours, respectively ($p < 0.05$). In this model, [Gd]-DOTA does not modify the renal tolerance of diatrizoate as assessed with serum creatinine and creatinine clearance. Gadolinium DOTA is not nephrotoxic and can be infused in association with iodinated contrast media. In this model, [Gd]-DTPA induces reversible renal failure.

Renal tolerance of gadolinium-DOTA and gadolinium-DTPA in rats. Brillet G, Dubois M, Beaufils H, Bourbouze R, Deray G. Invest Radiol 1994 Mar; 29 (3), pp. 352–4.

Adverse Reaction: Renal Dysfunction/Coronary Angiography: Catheterization Procedures
N-Acetylcysteine

Physiological Mechanism of Action: Traditionally, N-acetylcysteine is applied as a mucolytic agent and as an antidote in the treatment of acetaminophen poisoning, where it functions as a molecular strategy for replenishing hepatic glutathione stores. Glutathione is a reducing antioxidant reagent.

Application: N-acetylcysteine in cases undergoing computerized tomography with intravenous contrast to reduce the incidence of acute renal dysfunction.

Implementation: Administration of N-acetylcysteine (n = 3 doses) prior to cardiac catheterization studies.

Human Model: Cardiac catheterization cases (e.g., n = 5) and "historical" controls (e.g., n = 55) utilized for reference comparison (e.g., total n = 60 with baseline serum creatinine > 1.2 mg/dl and hydrated intravenously before and after catheterization procedures). Baseline creatinine values (N-acetylcysteine = 2.0 ± 0.7 vs no N-acetylcysteine 1.8 ± 0.4 mg/dl; p = 0.04). The amount or type of contrast agent used is consistent and uniform.

Interpretation and Analysis: Univariate analysis of clinical variables reveals no significant difference between cases receiving N-acetylcysteine and those that do not. The mean change in creatinine after 48 hours is −0.4 ± 0.3 (N- acetylcysteine) vs +0.1 ± 0.3 mg/dl for controls ($p < 0.001$). In cases with baseline creatinine > 2 mg/dl, the benefit is larger (−0.4 ± 0.4 vs +0.5 ± 0.3 mg/dl; $p < 0.001$). Multivariate analysis confirms speculation that pretreatment with N-acetylcysteine can produce information that can be applied as an independent predictor of renal protection ($p < 0.001$). Prophylactic use of acetylcysteine therefore prevents declines in renal function after coronary angiography. The benefit is greater in patients with baseline serum creatinine > 2 mg/dl.

Prevention of radiocontrast-induced nephropathy with N-acetylcysteine in patients undergoing coronary angiography. Tadros GM, Mouhayar EN, Akinwande AO, Campbell B, Wood C, Blankenship JA. J Invasive Cardiol 2003 Jun; 15 (6), pp. 311–4.

Adverse Reactions: Sequellae
Gadopentetate Dimeglumine

Route of Administration: Gadopentetate dimeglumine (intravascular).

Dosage: Gadopentetate dimeglumine (0.1 mmol/kg).

Implementation: Magnetic resonance imaging after intravenous administration of 0.1 mmol/kg gadopentetate dimeglumine (e.g., n = 15,496 cases).

Interpretation and Analysis: Adverse reactions are approximately 2.4% of all MRI procedures (e.g., n = 372/15,496). Symptoms abated the same or next day in 94.1% of cases (e.g., n = 350/372). Onset of signs occurs within 30 minutes after injection 49.7% of the time (e.g., n = 185/372), with the onset occurring more than 1 hour after injection in 44.9% of cases (e.g., n = 167/372). A very small percentage of cases may experience serious adverse reactions attributable to underlying disease conditions (e.g., n = 2/372). The rate of adverse reaction is 3.7% in patients with a history of asthma (e.g., n = 31/831 patients) or allergy (e.g., n = 144/3,860 patients). In scenarios where a history exists regarding previous reactions to MRI or iodinated contrast agents the adverse-reaction rate is approximately 21.3% (e.g., n = 16/75) and 6.3% (e.g., n = 54/857), respectively. The rate of adverse reaction is 2.2% with slow administration of gadopentetate dimeglumine compared to approximately 2.9% when injected rapidly.

Clinical safety of gadopentetate dimeglumine. Nelson KL, Gifford LM, Lauber-Huber C, Gross CA, Lasser TA. Radiology 1995 Aug; 196 (2), pp. 439–43.

Adverse Reactions/Sequellae and Relative Safety Compared to Computed Tomography (CT) Contrast Agents/Pancreatitis Animal Model in Rats
Gadolinium-DTPA ([Gd]-DTPA)/Fluorescein Isothiocyanate-Labeled Erythrocytes

Cellular/Molecular Mechanism of Action: The physical orientation of gadolinium is modified when subjected to magnetic energy fields that can be detected by MRI instrumentation.

Background Information: Contrast-enhanced computed tomography (CECT) has been proposed as a gold standard for early evaluation of acute necrotizing pancreatitis. However, iodinated contrast media used for CECT have been shown under certain circumstances to reduce pancreatic capillary flow and increase necrosis and mortality. Recent reports suggest that post-[GD] MRI provides images comparable to CECT in the assessment of severe acute pancreatitis.

Application: Potential MRI image-enhancing agent where it can be applied as a staging tool for assessing acute pancreatitis where it can provide improved levels of safety compared to CT contrast imaging agents.

Route of Administration: [Gd]-DTPA (bolus intravenous injection).

Dosage: [Gd]-DTPA (0.2 Ml/kg).

Implementation: Validation of the safety of [Gd]-DTPA through measurement of its effect on pancreatic capillary perfusion and acinar injury in acute pancreatitis. Evaluation of necrotizing pancreatitis utilizing [Gd]-DTPA for contrast-enhanced computed tomography imaging. Intravital microscopic quantitation of pancreatic capillary blood flow is performed using fluorescein isothiocyanate-labeled erythrocytes after induction of pancreatitis and 30 and 60 minutes after intravenous [Gd]-DTPA administration. Experimental and negative reference controls are compared with regard to mean arterial pressure, heart rate, arterial blood gases, hematocrit, amylase, lipase, and trypsinogen activation peptide production throughout the evaluation period.

Animal Model: Rat (e.g., n = 14 Wistar rats) utilized in a model for necrotizing pancreatitis induced by intraductal glycodeoxycholic acid (10 Mm/L) and intravenous caerulein (5 μg/kg/h) administered over a 6-hour infusion period.

Interpretation and Analysis: [Gd]-DTPA does not reduce capillary flow (1.93 ± 0.05 Nl/capillary/min) compared to the infusions with Ringer's solution (1.90 ± 0.06 Nl/capillary/min). Intravenous injection of [Gd]-DTPA does not further impair pancreatic microcirculation or increase acinar injury in acute necrotizing pancreatitis. Because of this advantage over CT contrast medium, further development of MRI as a staging tool in acute pancreatitis appears to be indicated.

The relative safety of MRI contrast agent in acute necrotizing pancreatitis. Werner J, Schmidt J, Warshaw AL, Gebhard MM, Herfarth C, Klar E. Ann Surg 1998 Jan; 227 (1), pp. 105–11.

Adverse Reactions/Subarachnoid Spaces/ Craniospinal and Cerebrospinal Fluid Leaks/ Subarachnoid Space Flow
Gadopentetate Dimeglumine/ Contrast Myelogram and Contrast Cisternogram

Dosage: Gadopentetate dimeglumine (0.5 ml, 0.7 ml, 0.8 ml or 1.0 ml: Magnevist; Schering, Berlin, Germany).

Implementation: Intrathecal contrast myelogram or cisternogram is performed via lumbar puncture (20–25 gauge needle). Approximately 3–5 mL/ml of cerebrospinal fluid is withdrawn and mixed with a single volume of 0.5 (n = 63), 0.7 (n = 13), 0.8 (n = 12), or 1.0 (n = 7) cc/mL of gadopentetate dimeglumine and then injected into the subarachnoid space. Immediate and delayed (up to 96 hours) T_1- and T_2-weighted MRI are acquired on superconductive, high-field (1.0–1.5 tesla) imaging units in 2 or 3 planes.

Human Model: Ninety-five cases presented with histories of cranial and spinal symptoms (age range: 1 month–78 years; sex: 50 males, 45 females). Following completion of diagnostic procedures, all cases are hospitalized over a 24-hour observation period in concert with a follow-up neurologic examination performed serially for a 6- to 12-month period.

Interpretation and Analysis: The incidence of gross behavioral changes, neurologic alterations, or seizure activity at any time following the procedure is very low (e.g., n = 0/95). Some cases will experience (i) postural postlumbar puncture headache (e.g., n = 19/95 or 20%); (ii) nausea (e.g., n = 6/95 or 6%); or (iii) episodes of vomiting (e.g., n = 2/95 or 2%). Each of these conditions invariably resolves with 24 hours of bed rest following lumbar puncture.

Low dose (0.5–1.0 mL/ml) intrathecal gadopentetate dimeglumine administration is safe, while still capable of providing diagnostic images. Potential clinical applications include the evaluation of obstructions and communications of the various subarachnoid spaces; spontaneous or traumatic/postsurgical craniospinal cerebrospinal fluid leaks; and subarachnoid space cerebrospinal fluid (CSF) flow and central nervous system parenchymal interstitial diffusion dynamics.

Intrathecal gadolinium (gadopentetate dimeglumine)-enhanced magnetic resonance myelography and cisternography: Results of a multicenter study. Tali ET, Ercan N, Krumina G, Rudwan M, Mironov A, Zeng QY, Jinkins JR. Invest Radiol 2002 Mar; 37 (3), pp. 152–9.

Adverse Reactions: Tissue Injury (Relative)
Gadopentetate Dimeglumine (Magnevist)/ Gadoteridol (ProHance)/Gadodiamide (Omniscan)/ Gadoversetamide (Optimark)

Cellular/Molecular Mechanism of Action: Gadolinium possesses radiopaque properties and its physical orientation is modified when exposed to magnetic energy fields.
Background Information: Increasing use of gadolinium-containing contrast agents in higher volumes and at faster injection rates, often with a power injector, elevates concerns regarding the potential for these diagnostic preparations to create extravascular tissue injury.
Application: Contrast image-enhancement agents to improve the diagnostic capabilities of MRI modalities.
Route of Administration (Research Toxicity Study): Gadopentetate dimeglumine (Magnevist), gadoteridol (ProHance), gadodiamide (Omniscan), and gadoversetamide (Optimark) each administered subcutaneously (e.g., mice).
Dosage: Gadopentetate dimeglumine (Magnevist), gadoteridol (ProHance), gadodiamide (Omniscan), and gadoversetamide (Optimark) each given at a dose rate of 0.3 ml per animal (e.g., mouse).
Implementation: Preparations of gadopentetate dimeglumine (Magnevist), gadoteridol (ProHance), gadodiamide (Omniscan), and gadoversetamide (Optimark) formulated as standard concentrations to facilitate comparisons of induced tissue injury in mice relative to reference controls (physiologic saline) and the conventional ionic radiographic contrast medium meglumine diatrizoate (Renografin 60).
Animal Model: Murine.
Each animal (mouse) receives a subcutaneous hindlimb injection of contrast media (e.g., n = 6 experimental groups; n = 15/group). After 48 hours, postmortem tissue samples are obtained for microscopic histopathology analysis.
Interpretation and Analysis: Of the 4 MR contrast agents, gadopentetate dimeglumine causes the greatest tissue damage, and gadoteridol and gadodiamide the lowest amount of tissue damage (e.g., 2 lowest osmolar agents). The difference is statistically significant in terms of both inflammation ($p = 0.0008$ for gadoteridol, and $p = 0.006$ for gadodiamide) and necrosis ($p = 0.0067$ for gadoteridol, and $p = 0.031$ for gadodiamide) when these agents are compared with gadopentetate dimeglumine. In regard to the control experiments, for all 3 variables (necrosis, edema, and inflammation), there is no statistically significant difference between the results with gadoteridol or gadodiamide and values observed for saline. In terms of both edema and inflammation, the effect of gadopentetate dimeglumine, although less, cannot be differentiated with any statistical significance from that of meglumine diatrizoate. Gadoversetamide, which has an osmolality between the ionic agent (gadopentetate dimeglumine) and the other 2 non-ionic agents, causes a reaction that cannot be differentiated from that seen with gadopentetate dimeglumine for both necrosis and edema. Only in the scoring of inflammation is the effect less using gadoversetamide compared to gadopentetate dimeglumine with any statistical significance ($p = 0.021$). The risk of tissue damage due to extravasation is not widely appreciated for gadolinium chelates. Care should be exercised during contrast injection to avoid inadvertent extravasation and its deleterious consequences, in particular with the 2 higher osmolar agents (gadopentetate dimeglumine and gadoversetamide).

Local tissue toxicity in response to extravascular extravasation of magnetic resonance contrast media. Runge VM, Dickey KM, Williams NM, Peng X. Invest Radiol 2002 Jul; 37 (7), pp. 393–8.

Adverse Reactions/Viscosity (High Viscosity of Iodinated Contrast Imaging Agents)/Hemodynamic Effects
Iodixanol

Application: Magnetic resonance image-enhancing contrast agent.
Implementation: High-viscosity, dimeric isoosmolar iodixanol iodinated contrast imaging agent can be injected intravenously to facilitate dynamic diffusion-weighted, echoplanar MRI obtained from b-value single-shot acquisitions and their isotropic apparent diffusion coefficient maps.
Animal Model: Rat (pentobarbital anesthetized).
Interpretation and Analysis: The mean baseline apparent diffusion coefficient value is $1.64 \pm 0.05 \times 10^{-3}$ mm^2/s for the cortex and $1.75 \pm 0.06 \times 10^{-3}$ mm^2/s for the medulla. Injections of iodixanol produce significant decreases in renal diffusion observed at 12 minutes and last for at least 24 minutes. Decreases in diffusion occur earlier for the cortex and last less than for the medulla. There is no significant modification in diffusion over time in the control group. Dynamic diffusion-weighted MRI can be used to non-invasively evaluate the *in vivo* renal hemodynamic response after injection of iodinated contrast (rats).

Hemodynamic effect of iodinated high-viscosity contrast medium in the rat kidney: A diffusion-weighted MRI feasibility study. Laissy JP, Menegazzo D, Dumont E, Piekarski JD, Karila-Cohen P, Chillon S, Schouman-Claeys E. Invest Radiol 2000 Nov; 35 (11), pp. 647–52.

CONTRAST AGENT PROFILES AND PROPERTIES

Contrast Agent Properties: Concentration Time Curve: Influential Variables
Dose vs Concentration

Application: Contrast image enhancement of brain imaging using MR instrumentation.
Dosage: Delineation of the quality of brain MR images as a function of increasing contrast media dosage.
Implementation: Delineation of the effect of varying both concentration and dosage of an intravenously administered MRI contrast agent on the concentration-time curve in brain tissue. Aside from injection time and injection rate, models have been designed that facilitate characterization of the distribution and transit time pertaining to the brain following injection but are independent from (not influenced by) pulse rate, heart volume, or other circulation parameters. The width of the transit-time distribution and the concentration-time

course in the brain can be computed according to the utilization of the indicator dilution theory.

Interpretation and Analysis: Increases in the dosage of administered contrast agent raise the maximum concentration of the agent in the brain, particularly if the injection time is short in duration. Increasing agent concentration (at fixed dosage and injection rate) also increases the maximum concentration within brain capillaries, particularly if the injection time is long. This increase, however, is less than that achieved by raising the dosage.

How do concentration and dosage of the contrast agent affect the signal change in perfusion-weighted magnetic resonance imaging? A computer simulation. Heiland S, Reith W, Forsting M, Sartor K. Magn Reson Imaging 2001 Jul; 19 (6), pp. 813–20.

Contrast Image-Enhancing Agent/MRI and Near-Infrared (NIR) Imaging/Biochemical Markers
Enzyme-Activated NIR Fluorescent Magnetic Nanoparticles/[Indocyanine Dye Cy5.5]

Background Information: A number of quantitative 3-dimensional tomographic NIR fluorescence imaging techniques have recently been developed and combined with MRI to yield highly detailed anatomic and molecular information in living organisms.

Application: Can be utilized as magnetic nanoparticle-based MR contrast agents that have NIR fluorescence (NIRF) capabilities that are activated by certain biological enzymes.

Route of Administration: Subcutaneous (example), but other prototypes could potentially be given intravascularly.

Semi-Synthetic Methods: Probes are prepared by the conjugation of arginyl peptides to cross-linked iron oxide amine (amino-CLIO), either by a disulfide linkage or a thioether linker, followed by the attachment of the indocyanine dye Cy5.5. The NIRF of disulfide-linked conjugate is activated by 1,4-dithio-l-threitol (DTT), while the NIRF of thioether-linked conjugate is activated by trypsin. Fluorescent quenching of the attached fluorochrome occurs in part due to interaction with iron oxide, as evident by the activation of fluorescence with DTT in the presence of nanoparticles that have less than 1 dye molecule attached per particle.

Implementation: Evaluate the potential for enzyme-activated near-IR fluorescent magenetic nanoparticles to detect specific biochemical entities expressed by specific cell populations.

Interpretation and Analysis: Following subcutaneous injection of the probe, axillary and brachial lymph nodes become darkened on MR images and are easily delineated by NIRF imaging. The probes may provide the basis for a new class of so-called smart nanoparticles, capable of pinpointing their position through their magnetic properties, while providing information on their environment by optical imaging techniques.

Near-infrared fluorescent nanoparticles as combined MR/optical imaging probes. Josephson L, Kircher MF, Mahmood U, Tang Y, Weissleder R. Bioconjug Chem 2002 May–Jun; 13 (3), pp. 554–60.

Contrast Image-Enhancing Agent/Properties
[Gd]-TREN-Me-3,2-HOPO/2-Hydroxyisophthalamide/2,3-Dihydroxyterephthalamide/Bis(acetate)/Salicylamide

Cellular/Molecular Mechanism of Action: Tris[(3-hydroxy-1-methyl-2-oxo-1,2-didehydropyridine-4-carboxamido)ethyl]amine (TREN-Me-3,2-HOPO) can function as a tripodal ligand that can form a stable complex with $[Gd^{3+}]$. Salicylamide, 2-hydroxyisophthalamide, 2,3-dihydroxyterephthalamide, and *bis*(acetate) can function as derivatizable chelator groups.

Background Information: TREN-Me-3,2-HOPO has a low level of water solubility that prevents detailed magnetic characterization and practical applicability.

Application: Magnetic resonance imaging contrast agent.

Semi-Synthetic Methods: Mixed ligand systems based on the TREN-Me-3,2-HOPO platform that possess (i) 2 hydroxypyridinone (HOPO) chelators; and (ii) 1 chelator that can be easily functionalized. Ligands can use salicylamide, 2-hydroxyisophthalamide, 2,3-dihydroxyterephthalamide, and *bis*(acetate) as the derivatizable chelators.

Implementation: Evaluation of ligands possessing (i) 2 HOPO chelators groups; and (ii) a chelator that can easily be functionalized.

Interpretation and Analysis: Salicylamide-, 2-hydroxyisophthalamide-, and 2,3-dihydroxyterephthalamide-based ligands possess sufficient thermodynamic stability for *in vivo* applications. The relaxivities of the 3 corresponding $[Gd^{3+}]$ complexes range from 7.2 to 8.8 mM/s at 20 MHz, 25°C, and pH 8.5, significantly higher than the values for clinically employed polyaminocarboxylate complexes (3.5–4.8 mM/s). The high relaxivities of these complexes are consistent with their faster rates of water exchange (< 100 ns), higher molecular weights (> 700), and greater numbers of inner-sphere coordinated water molecules ($q = 2$) relative to those of polyaminocarboxylate complexes. A mechanism for the rapid rates of water exchange has been proposed to involve a low energy barrier between the 8- and 9-coordinate geometries for lanthanide complexes of HOPO-based ligands. The pathway is supported by the crystal structure of La[TREN-Me-3,2-HOPO] [triclinic P1: $Z = 4$, $a = 15.6963(2)$ A, $b = 16.9978(1)$ A, $c = 17.1578(2)$ A, $\alpha = 61.981(1)°$, $\beta = 75.680(1)°$, $\gamma = 71.600(1)°$], which shows both 8- and 9-coordinate metal centers in the asymmetric unit, demonstrating that these structures are very close in energy. These properties make heteropodate $[Gd^{3+}]$ complexes promising candidates for use in macromolecular contrast media, particularly at higher magnetic field strengths.

Syntheses and relaxation properties of mixed gadolinium hydroxypyridinonate MRI contrast agents. Cohen SM, Xu J, Radkov E, Raymond KN, Botta M, Barge A, Aime S. Inorg Chem 2000 Dec 11; 39 (25), pp. 5747–56.

Contrast Image-Enhancing Agent/Properties and Improvement
[Gd]-PCP2A(H_2O_2)

Cellular/Molecular Mechanism of Action: The PCP2A moiety of [Gd]-PCP2A(H_2O_2) serves to chemically fix [Gd] so that the paramagnetic ion does not exist free within biological systems. The orientation of paramagnetic ion gadolinium [Gd] becomes physically altered when subjected to magnetic energy fields.

Application: The agent [Gd]-PCP2A(H_2O_2) can be applied as an (improved) paramagnetic image-enhancing agent for application in MRI.

Route of Administration: [Gd]-PCP2A (intravenous).

Semi-Synthetic Methods: Techniques have been decribed for the synthesis of pyridine-containing macrocycle bearing 2 acetic and 1 methylenephosphonic arms (PCP2A). An efficient synthesis of PCP2A is based on the macrocyclization reaction between 2,6-bis(chloromethyl)pyridine and a 1,4, 7-triazaheptane derivative bearing a methylenephosphonate group on N-4.

Interpretation and Analysis: The Gd(III) complex of PCP2A possesses properties that make it a very promising contrast agent for improved applications in MRI based on the observation that it displays: (i) a very high stability constant ($\log K_{GdPCP2A} = 23.4$) that should guarantee against *in vivo* release of toxic-free Gd(III) ions and free ligand molecules; in addition to (ii) a relaxivity that is about

2 times higher than the values reported for contrast agents frequently used in clinical practice. Its high relaxivity is the result of the presence of 2 water molecules in the inner coordination sphere and a significant contribution from water molecule(s) hydrogen bonded to the phosphonate group. Moreover, the inner sphere water molecules are involved in an exchange with bulk water that is relatively fast. This property is important for the attainment of an even higher relaxivity once the molecular reorientation rate of the [Gd]-PCP2A(H_2O_2) (−) moiety is lengthened by means of conjugation to a macromolecular substrate.

[GdPCP2A(H_2O_2)](−): A paramagnetic contrast agent designed for improved applications in magnetic resonance imaging. Aime S, Botta M, Frullano L, Geninatti Crich S, Giovenzana G, Pagliarin R, Palmisano G, Sirtori FR, Sisti M. J Med Chem 2000 Oct 19; 43 (21), pp. 4017–24.

Contrast Image-Enhancing Agent/ Properties: Physiological, Pharmacology, Toxicology *Gadolinium*

Cellular/Molecular Mechanism of Action: Gadolinium is a lanthanide element that has its physical orientation modified when subjected to magnetic energy fields.
Application: Gadolinium chloride has been (i) applied as a positive contrast agent (gadolinium chelates); (ii) widely used as an inhibitor of stretch-activated ion channels and physiological responses of tissues to mechanical stimulation; and (iii) employed *in vivo* as a selective macrophage inhibitor.
Considerations: Gadolinium can both provide pharmacological benefits and exert toxicological properties. Although adverse reactions to these agents are uncommon, it is known that gadolinium salts can bring about a wide variety of physiological alterations.

Basic experimental studies and clinical aspects of gadolinium salts and chelates. Adding LC, Bannenberg GL, Gustafsson LE. Cardiovasc Drug Rev 2001 Spring; 19 (1), pp. 41–56.

Contrast Image-Enhancing Agent/ MRI Agent: Oxygen-Carrying Compounds *Perfluorochemicals (PFCs)*

Cellular/Molecular Mechanism of Action: Perfluorochemicals possess oxygen-carrying properties. The characteristics that make them unique diagnostic agents pertain to their lack of hydrogen atoms, immiscibility with water, low surface tension, compressibility, and long intravascular persistence when emulsified and given intravenously.
Application: Perfluorochemicals can function as versatile contrast agents with important applications related to radiology (x-ray), ultrasound, or MR. When made radiopaque, they are visible with x-ray computed tomography (CT) and standard radiography.
Interpretation and Analysis: Because the neat liquid is inert, it can be ingested, instilled in the lung, or introduced into any hollow organ to image the lumen without untoward effects. The long intravascular persistence allows the imaging of blood vessels and vascularized tissues. Small or deep vessels become visible on color Doppler imaging and angiographic images of any vascular tree including the coronaries can be rendered from the serial CT images. As PFCs accumulate within reticuloendothelial cells, specific liver and spleen enhancement is achieved, allowing the detection of small tumors within these organs. When injected interstitially, the particles find their way to the draining lymph nodes, providing detail of the internal architecture to detect the presence or absence of tumor involvement on both CT and sonography. Using 19F MR, tissue perfusion and tissue pO_2 measurements can be achieved. As can be seen, the applications of PFC in diagnosis are vast, unique, and important. These new capabilities will carry radiological tools to new horizons.

The potential role of perfluorochemicals (PFCs) in diagnostic imaging. Mattrey RF. Artif Cells Blood Substit Immobil Biotechnol 1994; 22 (2), pp. 295–313.

CARDIOVASCULAR SYSTEM

Angiography: Pulmonary Vasculature/2D Phase-Contrast/Time-of-Flight Sequences/Phased-Array Torso Coils/Contrast-Enhanced Helical Computed Tomography (CT)

In the past few years MR angiography (MRA) of the pulmonary vasculature has advanced from a research tool to a clinically relevant imaging modality. Early 2D phase-contrast and time-of-flight (TOF) sequences without the use of contrast agents were time-consuming and limited by considerable imaging and motion artifacts. Since the introduction of MR scanners with stronger gradients (> 20 mT/m) and contrast-enhanced techniques, imaging of the pulmonary vasculature with adequate spatial resolution within a single breath-hold is now possible. In the detection of pulmonary embolism in the lobar or segmental arteries, contrast-enhanced MRA is now on the verge of being considered an established modality, possibly competing with conventional pulmonary angiography and contrast-enhanced helical CT. In the future, utilization of phased-array torso coils, the application of navigator pulse sequences, and 3D time-resolved ultrafast MRA will overcome the final limitations of current techniques. Blood-pool MR contrast agents may provide a "one-stop-shopping" approach to the investigation of lower extremity veins and pulmonary arteries in venous thromboembolism.

Pulmonary magnetic resonance angiography. Hoffmann U, Schima W, Herold C. Eur Radiol 1999; 9 (9), pp. 1745–54.

Cardiac Imaging/Coronary Artery Stenoses/ Coronary Artery Bypass Grafts/Anomalous Coronary Arteries/Vessel Wall Imaging *Intravascular MRI Contrast Agents*

Multiple cardiovascular MR (CMR) techniques have been developed that make it possible to overcome the substantial difficulties associated with the imaging of coronary arteries. Tortuous small coronary arteries are imaged in 3D-volume data sets. Cardiac motion is reduced by diastolic gating with ultrafast sequences. Respiration is suppressed by breath-holding or respiratory gating. Signal-to-noise can be increased with contrast agents. In clinical trials CMR has been successfully used to assess coronary artery stenoses, coronary artery bypass grafts, and anomalous coronary arteries. Recent developments in steady state imaging, volume selective imaging with tracking, parallel imaging techniques, vessel wall imaging, and intravascular contrast agents may soon enable CMR of the coronary arteries to become an effective and widespread clinical tool.

MR coronary angiography: 2001 update. Bunce NH, Lorenz CH, Pennell DJ. Rays 2001 Jan–Mar; 26 (1), pp. 61–9.

Cardiac Imaging/Myocardial Infarct Characterization *[^{87}Rb]/Evans Blue Dye/Triphenyl Tetrazolium Chloride/Glibenclamind/Pinacidil/Bumetanide*

Cellular/Molecular Mechanism of Action: (i) Rubidium ion (Rb^+) is a K^+ tracer element; (ii) glibenclamide is a blocker of

ATP-sensitive K^+ channels; (iii) pinacidil is a K^+(ATP) opener; (iv) bumetanide is a $K^+/Na^+/2Cl^-$ co-transporter inhibitor.

Application: [^{87}Rb] can be applied in MRI procedures to identify and characterize myocardial infarcts. Evans blue and triphenyl tetrazolium chloride staining can be applied to delineate areas at risk (AAR) and areas of necrosis, respectively.

Dosage: Glibenclamide (3 μM); pinacidil (10 μM); bumetanide (10 μM).

Implementation: Delineation of the effect of drugs that can modulate passive K^+ permeability into cardiomyocytes in normal and reperfusion-damaged cardiac muscle. Rubidium ion is used as a K^+ tracer and [^{87}Rb]-MRI can serve as a method of detection and characterization. Preparations are incubated in buffer contained either in (i) glibenclamide; (ii) pinacidil; (iii) bumetanide; or (iv) no drug (control). Upon reperfusion 3-dimensional [^{87}Rb] MR images are acquired to obtain kinetic parameters for Rb^+ uptake and its distribution. Areas at risk and areas of necrosis are determined by Evans blue and triphenyl tetrazolium chloride staining, respectively.

Animal Model: Swine (left anterior descending artery [LAD] of isolated pig hearts perfused with Krebs-Henseleit buffer [KHB] occluded for 2 hours and subsequently reperfused for 2 hours with KHB containing 4.7 mM RbCl instead of KCl).

Interpretation and Analysis: The kinetics remain monoexponential in the affected anterior wall and the uptake rates are approximately 32, 36, 37, and 21% of that in the posterior wall in the control, Glib, Pin, and Bum groups, respectively. Infarct sizes determined histologically as a percentage of total ventricular (left + right) mass (14–22%) correspond to sizes of areas with 20 to 40% of maximal Rb image intensity [I(Rb)(max), 15–22%], except for values observed with pinacidil (12.5 vs 21%). The sizes of areas with 20 to 50% of I(Rb)(max) (30–36%) closely correlates with those of AAR determined histologically (31–33%). Lactate dehydrogenase release does not differ in all preparations. Therefore, (i) reperfusion damage quickly inhibits Rb^+ uptake; (ii) Rb^+ uptake in normal and reperfused tissue does not significantly depend on K^+(ATP) or the $K^+/Na^+/2Cl^-$ co-transporter; and (iii) areas with 20% to 40% of maximal image intensity correspond to infarct areas.

The effects of drugs modulating K^+ transport on Rb^+ uptake and distribution in pig hearts following regional ischemia: ^{87}Rb MRI study. Kupriyanov VV, Xiang B, Sun J, Jilkina O. NMR Biomed 2002 Aug; 15 (5), pp. 348–55.

Cardiac Imaging/Myocardial Infarct Detection and Characterization
Gadophrin-2/Gadolinium-DTPA ([Gd]-DTPA)

Application: Gadophrin-2 functions as a necrosis-avid contrast agent (NACA); [Gd]-DTPA is a non-specific agent that can be applied to determine myocardial viability (MV) in acute myocardial infarction (AMI) applying MRI analysis.

Route of Administration: [Gd]-DTPA (intracoronary artery); gadophrin-2 (intracoronary artery).

Dosage: [Gd]-DTPA (0.1 mmol/kg); gadophrin-2 (0.005 mmol/kg).

Implementation: [Gd]-DTPA or gadophrin-2 can be delivered into the coronary artery by either fast (n = 3 dogs) or slow bolus (n = 3 dogs). Serial electrocardiogram (ECG)-triggered cardiac MRI of T_1-weighted segmented turbo fast low-angle shot (FLASH) sequence is performed and compared with results observed with triphenyltetrazolium chloride (TTC) histochemical staining. The contrast ratio and infarct size are quantified and analyzed statistically.

Animal Model: Canine (n = 12 dogs subjected to induction of reperfused acute myocardial infarction through the use of a transcatheter balloon occlusion of the coronary artery).

Interpretation and Analysis: Cardiovascular side effects can rarely be appreciated with the local delivery of either [Gd]-DTPA or gadophrin-2 contrast agents. After administration, gadophrin-2 induces a strong (CR ≥ 1.78) and persistent (≥ 10 hours) contrast enhancement of infarcted region. The infarct size defined with gadophrin-2 is almost identical to that observed with TTC staining throughout the postcontrast period. With a dose 20 times higher, [Gd]-DTPA also strongly enhances infarct-to-normal contrast; however, the enhancement diminishes over time (e.g., from early strong to later faint enhancement and eventual loss of contrast). Size of the delineated infarct is also unstable (e.g., from early overestimation to later underestimation and eventual disappearance of the enhanced infarct). In combination with a percutaneous transluminal coronary angioplasty (PTCA) procedure, intracoronary artery–administered MRI contrast agents may prove useful for postprocedure verification of diagnosis. The NACA-enhanced MRI may serve as an *in vivo* surrogate of postmortem histochemical staining for the determination of MV. Although applicable in a clinical setting, cardiac MRI with nonspecific [Gd]-DTPA is less reliable and should be performed within 1 hour after contrast agent injection.

Intracoronary delivery of [Gd]-DTPA and gadophrin-2 for determination of myocardial viability with MR imaging. Ni Y, Pislaru C, Bosmans H, Pislaru S, Miao Y, Bogaert J, Dymarkowski S, Yu J, Semmler W, Van de Werf F, Baert AL, Marchal G. Eur Radiol 2001; 11 (5), pp. 876–83.

Cardiac Imaging/Myocardial Infarction: Acute/Reperfusion Injury
Gadolinium/Gadolinium-DTPA ([Gd]-DTPA)

Background Information: Pixel-by-pixel calculation of T_1 values (T_1 mapping) has been used in different tissues to focus on T_1 changes in a quantitative fashion.

Application: Establish T_1 mapping of human myocardium (1.5 Tesla system) and diagnostic detection and characterization of acute myocardial infarction (AMI).

Implementation: Evaluation of the diagnostic effectiveness of multi-breath-holding provocative test in conditions of reperfused AMI (3 days ± 1 day) established with MRI (1.5 Tesla system) and [Gd]-DTPA applied as a contrast image-enhancing agent (e.g., n = 8 cases). Sets of 5 images with varying T_1 weighting are acquired prior to and after the administration of contrast agent to generate images from calculated T_1 values (T_1 mapping).

Interpretation and Analysis: Prior to contrast agent administration, all cases display T_1 prolongation in the area of infarction, which can be identified in separate measurements using the delayed enhancement approach. Compared to non-infarcted areas, T_1 values in the infarcted areas are increased by 18 ± 7% (standard error, $p < 0.05$). The spatial extent of the area of T_1 prolongation is larger than that of the hyperenhanced areas in conventional contrast-enhanced images. T_1 maps obtained after the application of [Gd]-DTPA reveal a T_1 reduction of approximately 27 ± 4% in infarcted tissue compared to non-infarcted areas ($p < 0.05$). The areas showing T_1 reduction are in agreement with hyperenhanced regions in conventional T_1-weighted images. T_1 mapping can visualize changes in the longitudinal relaxation time induced by AMI. T_1 mapping can detect myocardial necrosis without the use of contrast media. Information that can be extracted from a combination of pre- and postcontrast T_1 maps exceeds that from conventional contrast studies.

T_1 mapping in patients with acute myocardial infarction. Messroghli DR, Niendorf T, Schulz-Menger J, Dietz R, Friedrich MG. J Cardiovasc Magn Reson 2003; 5 (2), pp. 353–9.

Cardiac Imaging/Myocardial Infarction/Acute vs Chronic Myocardial Infarction Differentiation
Gadopentetate Dimeglumine Magnevist/Gadophrin-3

Cellular/Molecular Mechanism of Action: Gadolinium is a component of gadophrin-3 and its spatial orientation becomes altered when subjected to magnetic energy fields.
Background Information: Contrast-enhanced MRI can accurately quantify infarct size; however, some controversies persist about which contrast medium is best suited for this purpose.
Application: Detection and differentiation between acute and chronic myocardial infarction lesions using gadophrin-3 and Magnevist either individually or in combination.
Dosage: Gadophrin-3 (50 μmol/kg 24 hours before image acquisition); Magnevist (100 μmol/kg).
Implementation: Determination of the enhancement profile of a necrosis-specific contrast agent (gadophrin-3) compared to a standard extracellular agent on T_1-weighted MR images in acute and chronic myocardial infarctions (MIs).

Image analysis can be performed at 48 hours and at 6 weeks following induction of infarction lesions created by permanent occlusion of a branch of the left coronary artery. Image analysis by MRI can be performed with the aid of gadophrin-3 as an MRI contrast-enhancement agent. Continuous short-axis views are collected using an inversion recovery turbo fast low angle shot sequence. Imaging is repeated 5–10 minutes following an additional Magnevist injection. The area of hyperenhancement demarcated following gadophrin-3 injection is then compared with the region of hyperenhancement seen on gadophrin-3 plus Magnevist enhanced image using triphenyltetrazolium chloride (TTC) staining as the standard of reference.

Animal Model: Rabbit (n = 13 thoracotomy and permanent occlusion of a branch of the left coronary artery. Note: N = 11 animals survived to 48 hours, and n = 5 survived for 6 weeks).
Interpretation and Analysis: In an acute MI model, the mean difference in size of hyperenhancement seen on 2 different *in vivo* MR scans is approximately $-1.8 \pm 6.0\ mm^2$ ($p > 0.05$). Both measurements show excellent agreement with TTC staining. Chronic myocardial infarctions show no enhanced detection with gadophrin-3, whereas application of Magnevist results in hyperenhancement. Standard extracellular contrast agents do not overestimate the size of acute MI. The combination of gadophrin-3 and Magnevist can distinguish acute and chronic myocardial injury because chronic MIs do not enhance with gadophrin-3.

Imaging of myocardial infarction: Comparison of Magnevist and gadophrin-3 in rabbits. Barkhausen J, Ebert W, Debatin JF, Weinmann HJ. J Am Coll Cardiol 2002 Apr 17; 39 (8), pp. 1392–8.

Cardiac Imaging/Myocardial Infarct Size Delineation [Gadolinium-Based Media]/ Viable Myocyte Labeling: [Magnesium-Based Media]/Myocardial Integrity
Gadoversetamide/Gadopentetate Dimeglumine/ Albumin-Bound [Gd]-Based Media/ Magnesium-Based Contrast Media

Cellular/Molecular Mechanism of Action: The orientation of magnesium and gadolinium atoms becomes altered when subjected to a magnetic energy field.
Application: Magnesium and gadolinium atoms can serve as positive contrast-enhancing agents for MRI techniques. In this context, they can be used in formulations for the detection and characterization of lesions associated with myocardial infarction.
Route of Administration: Magnesium and gadolinium-based positive contrast-enhancing imaging agents are delivered by intravascular injection.
Implementation: Serial image analysis of ligated coronary arteries with segmented inversion-recovery gradient-echo MRI (repetition time msec/echo time msec/inversion time msec [fixed], 5.2/2.5/430; flip angle, 15°) for a period of 1 hour after administration of contrast media facilitated by the use of a 1.5 Tesla MR unit. Serial measurements of the longitudinal relaxation are performed by using the Look-Locker approach (repetition time msec/echo time msec, 1,000/3.5; flip angle, 10°). Detection and size of infarction can be evaluated at each time point and compared with end point histologic findings.

Animal Model: Coronary ligation performed in rats (e.g., n = 30).
Interpretation and Analysis: All manganese-based contrast media clear from the blood pool rapidly. Manganese-based contrast media allow precise labeling of viable cardiomyocytes within 30 minutes, and the labeling persists for at least 1 hour. Accumulation of gadoversetamide in the infarct area is apparent after 5 minutes, and the peak contrast-to-noise ratio (CNR) between infarct and myocardium is comparable to the peak CNR of manganese-based contrast agents. Extracellular gadopentetate dimeglumine provides excellent infarct detection but precise sizing of infarcts can only best be achieved during a small imaging window if a fixed inversion time of 430 msec is used. Albumin-binding gadolinium-based contrast media provides a longer imaging window, but infarct size is overestimated because of the non-specific distribution of unbound gadolinium agent. When extracellular gadolinium-based agents are used for infarct size measurement, imaging parameters and timing are important because the kinetics of both normal and irreversibly injured myocardium must be considered. Manganese-based agents are highly specific and less sensitive to timing for infarct size determination, but further studies are required to determine if they are feasible for human use.

Characterization of viable and non-viable myocardium at MRI: Comparison of gadolinium-based extracellular and blood pool contrast materials vs manganese-based contrast materials in a rat myocardial infarction model. Flacke S, Allen JS, Chia JM, Wible JH, Periasamy MP, Adams MD, Adzamli IK, Lorenz CH. Radiology 2003 Mar; 226 (3), pp. 731–8. Epub 2003 Jan 24.

Cardiac Imaging/Myocardial Perfusion/Tracer Kinetics
CMD-A2-Gd-DOTA

Cellular/Molecular Mechanism of Action: The physical orientation of molecular gadolinium is altered when placed within a magnetic energy field that can be detected by MRI instrumentation.
Background Information: Recent developments in MRI using specific contrast media can facilitate the assessment of myocardial perfusion.
Application: Gadolinium is used as a tracer element that can be detected in biological systems by MRI modalities. CMD-A2-Gd-DOTA is an imaging agent that can be utilized to characterize myocardial perfusion.
Route of Administration: Intravascular.
Implementation: CMD-A2-Gd-DOTA can be applied as an intravascular MRI tracer agent for evaluating myocardial perfusion. To validate the intravascular properties of CMD-A2-Gd-DOTA, the agent can be simultaneously injected with [^{99m}Tc]-labeled red blood cells. Options for subsequent analyses include (i) tracer kinetics of both compounds assessed by coronary sinus effluent sampling, radioactivity counting, and concentration determination in samples

for 1st-pass time curves measurements; and (ii) 2-slice turboFLASH sequence on a 1.5 Tesla whole-body MRI in order to evaluate 1st-pass CMD-A2-Gd-DOTA signal intensity (SI) vs time curves.

Animal Model: Swine (isolated heart preparation perfused at various controlled flow rates). Group 1 (5 hearts: simultaneous injection of CMD-A2-Gd-DOTA and [^{99m}Tc]-labeled red blood cells). Group 2 (5 hearts: 2-slice turboFLASH sequence on a 1.5 Tesla whole-body MRI for evaluation of 1st-pass CMD-A2-Gd-DOTA SI vs time curves).

Interpretation and Analysis: First-pass concentration curves for CMD-A2-Gd-DOTA display flow rates ranging from 0.8 to 3.1 ml/min/g and are typical for an intravascular contrast agent. Parameters for MRI studies reveal an upslope and mean transit time of SI time curves that correlate strongly with myocardial perfusion processes. Within the physiologic range of flows, CMD-A2-Gd-DOTA is able to demonstrate tracer kinetics for *in vivo* assessment of myocardial perfusion using MRI.

Kinetic characterization of CMD-A2-Gd-DOTA as an intravascular contrast agent for myocardial perfusion measurement with MRI. Canet EP, Casali C, Desenfant A, An MY, Corot C, Obadia JF, Revel D, Janier MF. Magn Reson Med 2000 Mar; 43 (3), pp. 403–9.

Cardiac Imaging/Myocardial Perfusion Angiography/Contrast Agent Options/ Microvascular Permeability/Timing of Contrast Agent Injection

Small Molecular Weight Agents/Blood-Pool Agents/ Gadolinium-Chelate/Ultrasmall Superparamagnetic Particles of Iron Oxide (USPIOs)

Cellular/Molecular Mechanism of Action: Gadolinium is classified as a paramagnetic metallic ion and its spatial orientation becomes physically altered when subjected to magnetic energy fields.

Background Information: Currently available MR contrast agents are not confined to the intravascular space because of their small molecular size. These agents produce peak vascular enhancement for only a short period. Conversely, blood-pool agents have longer intravascular residence time and higher relaxivity. Therefore, these agents provide a greater degree of flexibility, versatility, and accuracy in performing MR angiography (MRA) procedures. In the utilization of blood-pool agents, the timing of contrast injection becomes less significant because the optimal imaging window is in tens of minutes rather than seconds. In addition, larger anatomic regions can be imaged optimally.

Application: Gadolinium-chelate that binds *in vivo* to albumin and USPIOs has been applied as blood-pool agents for MRI enhancement. Such preparations can be utilized in MRI methodologies for analysis of anatomical abnormalities affecting vascular structures, including the coronary artery, pulmonary artery, and peripheral arterial networks. Additionally, their use can facilitate the evalution of parameters such as relative myocardial perfusion.

Route of Administration: Gadolinium-chelate (intravascular) and USPIOs (intravascular) are selectively delivered by intravascular injection facilitated by direct catheterization of arterial or venous structures. The timing of contrast injection for blood-pool agents becomes less significant because the optimal imaging window is in tens of minutes rather than seconds. In addition, larger anatomic regions can be imaged optimally.

Interpretation and Analysis: Preliminary evidence appears to support the notion that blood-pool agents may play a diagnostic role in coronary, peripheral, and pulmonary angiography. Blood-pool agents are currently being evaluated in human trials with relevant examples, including gadolinium-chelate that binds *in vivo* to albumin to form blood-pool agents and USPIOs. In this context, contrast-enhanced 3-D MRA permits comprehensive assessment of the supra-aortic arteries as well as the arterial system in the chest, abdomen, and lower extremities. Three-dimensional MRA combines intravenous injection of a non-nephrotoxic, paramagnetic, extracellular contrast agent that increases the signal intensity of blood by shortening its T_1 value with the acquisition of a fast 3D data set. High contrast between the vascular lumen and surrounding tissues, inherent 3-dimensionality, and the ability to collect image data in the chest and abdomen under apnea conditions all contribute to excellent image quality. Besides their ability to increase vascular contrast, blood-pool agents provide physiologic information, including rate of entry, rate of accumulation, and rate of elimination. These qualities of MR blood-pool agents have proven to be of significant value in the assessments of myocardial perfusion and microvascular permeability.

Blood-pool MR contrast agents for cardiovascular imaging. Saeed M, Wendland MF, Higgins CB. J Magn Reson Imaging 2000; 12, pp. 890–8.

MR-angiography: The role of contrast agents. Goyen M, Ruehm SG, Debatin JF. Eur J Radiol 2000 Jun; 34 (3), pp. 247–56.

Cardiac Imaging/Myocardial Perfusion/ Coronary Angiography/Pulmonary Angiography/ Peripheral Angiography/Myocardial Perfusion/ Occlusive vs Reperfused Infarction/ Reversible vs Irreversible Myocardial Injury/ Microvascular Integrity Assessment

Blood-Pool MRI Contrast Agents

Magnetic resonance imaging has emerged as a new diagnostic technique for evaluation of the cardiovascular system. The diagnostic benefits of MR contrast agents have been demonstrated in the laboratory and in clinical research that has taken place during the past 12 years. Contrast agents enhance the sensitivity of MR angiography and improve early detection and characterization of myocardial and microvascular injuries. Clinically approved MR contrast agents are not confined to the intravascular space due to their small molecular size (standard extracellular MR contrast agents) and, therefore, they produce peak vascular enhancement for only a short period of time (, 30 minutes). In recent years remarkable progress has been made in the development of blood-pool agents for MRI. Impetus for creation of these agents has come in part from a growing appreciation of MR angiography. Vascular mapping has become an important application of medical imaging. Ample experimental evidence has shown that blood-pool agents may play a significant role in coronary, peripheral, and pulmonary angiography. In addition to their ability to increase vascular contrast, blood-pool agents provide physiological information such as rate of entry, rate of accumulation, and rate of elimination. In the heart, blood-pool contrast agents are useful for estimating relative myocardial perfusion, discriminating between occlusive and reperfused infarction, differentiating between reversible and irreversible myocardial injury, and detecting microvascular integrity. Blood-pool agents are currently being investigated in humans. Initial clinical trials have demonstrated their safety and feasibility and, hopefully, future trials will demonstrate their efficacy.

Value of blood-pool MR contrast agents in imaging of the heart and blood vessels. Saeed M. Drugs Today (Barc) 1999 Nov; 35 (11), pp. 879–92.

Cardiac Imaging/Myocardial Sarcoidosis/ Myocardial Inflammation and Interstitial Edema/ Therapeutic Efficacy Monitoring (Prednisolone) *Gadolinium-DTPA ([Gd]-DTPA)*

Background Information: Cardiac involvement is an important prognostic factor in patients with sarcoidosis.
Application: [Gd]-DTPA-enhanced MRI ([Gd]-MRI) can be used for the diagnosis of cardiac sarcoidosis and monitoring responses to prednisolone therapy.
Implementation: Analysis of conditions of sarcoidosis with cardiac involvement applying Gd-MRI technology with a 1.5 Tesla superconducting magnet system T_1-weighted spin-echo sequence (e.g., n = 16 cases).

Human Model: Cases suffering from cardiac sarcoidosis diagnosed based on histological examination of tissue samples or fulfillment of specific patient profile criteria.
Interpretation and Analysis: Gadolinium can enhance the intensity of localized signals detected by MRI that correspond to lesions associated with (i) interstitial edema within the left ventricle (e.g., n = 8/16); and (ii) thinning of the left ventricular septal wall (e.g., n = 2/16). Monitoring of cases following a 1-month period of prednisolone therapy (60 mg every other day or 30–40 mg s.i.d.) reveals markedly diminished localized high-intensity signals. Images of the heart obtained by gadolinium MRI may reflect active inflammation with interstitial edema in patients with sarcoidosis. Gadolinium MRI may be a useful non-invasive method for early detection of cardiac sarcoidosis and for evaluating the effects of steroid therapy.

Diagnosis of cardiac sarcoidosis and evaluation of the effects of steroid therapy by gadolinium-DTPA-enhanced magnetic resonance imaging. Shimada T, Shimada K, Sakane T, Ochiai K, Tsukihashi H, Fukui M, Inoue S, Katoh H, Murakami Y, Ishibashi Y, Maruyama R. Am J Med 2001 May; 110 (7), pp. 520–7.

Cardiac Imaging/Myocardium Infarction/Detailed Segmental Left Ventricle (LV) Function *Iron Oxide–Loaded Myogenic Precursor Cells*

Cellular/Molecular Mechanism of Action: Myogenic precursor cells detect, migrate to, and accumulate within lesions of myocardial generation.
Application: Evaluation of myocardial implants of myogenic precursor cells (MPC) loaded with iron oxide to determine how reliably they can be detected by *in vivo* cardiac MRI.
Route of Administration: Direct (selective) catheterization.
Implementation: Evaluation of the ability for iron oxide–loaded myogenic precursor cells to detect myocardial lesions. Such an objective can be approached by electromechanical mapping of the LV and performing guided microinjections into normal and infarcted myocardium utilizing autologous MPC delivered through a percutaneous catheter. Cardiac MRI can be used to detect implanted iron oxide nanoparticle-loaded MPC preparations. Magnetic resonance imaging data are then compared to LV electromechanical mapping findings and cross-registered pathology.

Animal Model: Swine (n = farm pigs; n = 4/7 with anterior myocardial infarction).
Interpretation and Analysis: The vast majority of injections into normal (e.g., n = 9/9) and locally damaged (e.g., n = 12/12) myocardium can be detected on T_2-weighted spin echo and inversion-recovery true-fisp MRI (low signal areas) with good anatomical concordance with sites of implantation on electromechanical maps. All sites of injection can be confirmed by correlation with pathology findings, which in all infarct animals reveal iron-loaded MPC at the center and periphery of the infarct as expected from MRI analysis. In summary, targeted catheter-based implantation of iron-loaded MPC into locally infarcted LV myocardium is accurate and can be reliably demonstrated by *in vivo* cardiac MRI. The ability to non-invasively identify intramyocardial cell implantation may be a determinant for future experimental studies designed to analyze subsequent effects of such therapy on detailed segmental LV function.

Magnetic resonance imaging of targeted catheter-based implantation of myogenic precursor cells into infarcted left ventricular myocardium. Garot J, Unterseeh T, Teiger E, Champagne S, Chazaud B, Gherardi R, Hittinger L, Guéret P, Rahmouni A. J Am Coll Cardiol 2003 May 21; 41 (10), pp. 1841–6.

Cardiovascular: Contrast-Enhanced Imaging of Cardiovascular Anatomical Structures/ Intervention MRI/Angioplasty/Gene Delivery/ Growth Factor Delivery

Magnetic resonance imaging can provide a non-invasive combined diagnostic examination of different modalities, including visualization and measurement of cardiac and vascular anatomy, in addition to assessment of blood flow, left ventricular mass, function, perfusion, and viability. Contrast agents for cardiovascular imaging are used to enhance the capability of MRI in assessing perfusion, tissue viability, infarction, and vascular stenosis. Furthermore, contrast agents may be used for guiding procedures and therapies in the evolving field of interventional MRI, such as angioplasty and delivery of genes and growth factors. Targeted MR contrast agents are needed to further improve the specificity of MRI and to monitor pathophysiological processes including necrosis, inflammation, or angiogenesis. The research and development of MR contrast agents will likely continue at the cellular and molecular levels over the next decade.

Contrast agents for cardiovascular magnetic resonance imaging. Current status and future directions. Schalla S, Higgins CB, Saeed M. Drugs R D 2002; 3 (5), pp. 285–302.

Cardiovascular: Myocardial Infarction/ Discrimination: Normal vs Infarcted Myocardium *Superparamagnetic Iron Oxide (SPIO)*

Application: Discrimination between infarcted and normal myocardial tissues using SPIO and high field MRI (7 Tesla).
Dosage: Superparamagnetic iron oxide nanoparticles (T_1 weighted = 1 mg Fe/kg; T_2 weighted = 5 or 10 mg Fe/kg).
Implementation: Evaluation of the capacity for SPIO nanoparticles in combination with T_1-weighted gradient echo sequence to visualize myocardial infarctions 48 hours after ligature. In addition, MRI can be performed using a T_2-weighted RARE sequence and nanoparticles injected 5, 24, or 48 hours after infarction.

Animal Model: Rat (n = 6 induced permanent myocardial infarction for T_1 weighted; and n = 36 rats for T_2 weighted).
Interpretation and Analysis: No changes in contrast between normal and infarcted myocardium can be observed after nanoparticle injection on T_1-weighted images. However, nanoparticles can induce a significant contrast increase between normal and infarcted myocardium on T_2-weighted images whatever the delay is between induced infarction and imaging (2.99 ± 1.66 preinjection vs 7.82 ± 1.96 after SPIO injection at a dose of 5 mg Fe/kg 5 hours postinfarction, p = 0.0001). In summary, it is possible through the application

of injected nanoparticles to discriminate normal from infarcted myocardium on T_2-weighted images. However, the high magnetic field prevents the visualization of the T_1 effect of SPIO nanoparticles.

High field magnetic resonance imaging evaluation of superparamagnetic iron oxide nanoparticles in a permanent rat myocardial infarction. Chapon C, Franconi F, Lemaire L, Marescaux L, Legras P, Saint-André JP, Denizot B, Le Jeune JJ. Invest Radiol 2003 Mar; 38 (3), pp. 141–6.

Vascular Imaging/Adhesion Molecule Expression/ Endothelial Intercellular Adhesion Molecule (ICAM)/ Microvascular (Endothelial) Leukocyte Receptor Complex Expression/Autoimmune Encephalitis *[Anti-ICAM]-Paramagnetic Liposomes (ACPL)*

Application: The *in vivo* targeting of molecules expressed on vascular endothelium, while providing sufficient signal enhancement at these sites for detection by MRI.
Implementation: [Anti-ICAM]-paramagnetic liposomes targeted to ICAM-1 administered *in vivo* for delineating endothelial leukocyte receptor upregulation in the cerebral microvasculature during conditions of autoimmune encephalitis.

Animal Model: Mouse (autoimmune encephalitis).
Interpretation and Analysis: Fluorescence microscopy analysis reveals that fluorescently tagged ACPLs are localized within the central nervous system (CNS) microvasculature in a pattern consistent with ICAM-1 upregulation. Such findings closely correlate with immunohistochemical analyses. High resolution *ex vivo* MRI of mouse brain demonstrates that ACPL binding patterns confer significant enhancement of signal intensity (SI) compared to control images. Preparations of ACPLs therefore appear to be a useful MRI contrast agent for visualization of specific molecules expressed on vascular endothelium during various disease states.
Instrumentation: Magnetic resonance imaging instrumentation.

ICAM-1 expression in autoimmune encephalitis visualized using magnetic resonance imaging. Sipkins DA, Gijbels K, Tropper FD, Bednarski M, Li KC, Steinman L. J Neuroimmunol 2000 Apr 3; 104 (1), pp. 1–9.

Vascular Imaging/Angiogenesis of Endothelium/ Atherosclerosis *Alpha$_v$Beta$_3$ ($\alpha_V\beta_3$)-Integrin-Targeted Paramagnetic Nanoparticles*

Background Information: Angiogenesis is a critical feature of plaque development in atherosclerosis and might play a key role in both the initiation and subsequent rupture of plaques that lead to myocardial infarction and stroke. The precursory molecular or cellular events that initiate plaque growth and that ultimately contribute to plaque instability, however, cannot be detected directly with any current diagnostic modality.
Application: Detection of alterations in vascular angiogenesis.
Route of Administration: Intravascular.
Implementation: Evaluation of angiogenesis phenomenon using $\alpha_V\beta_3$-integrin-targeted paramagnetic nanoparticles injected intravenously.

Animal Model: New Zealand White rabbits (atherosclerosis model dietarily induced by feeding 1% cholesterol for approximately 80 days).
Interpretation and Analysis: Alpha$_V$beta$_3$-integrin-targeted paramagnetic nanoparticles can specifically detect neovasculature structures within 2 hours by routine MRI at a clinically relevant field strength (1.5 Tesla). Increased angiogenesis can be detected as a 47 $\pm$ 5% enhancement in MRI signal averaged throughout the abdominal aortic wall following $\alpha_V\beta_3$-targeted paramagnetic nanoparticle administration. Pretreatment of atherosclerotic rabbits with $\alpha_V\beta_3$-targeted non-paramagnetic nanoparticles competitively blocks specific contrast enhancement of the $\alpha_V\beta_3$-targeted paramagnetic agent. Magnetic resonance imaging analysis can detect patterns of increased $\alpha_V\beta_3$-integrin distribution within the atherosclerotic wall that is spatially heterogeneous along both transverse and longitudinal planes of the abdominal aorta. Histology and immunohistochemistry can detect marked proliferation of angiogenic vessels within the aortic adventitia, coincident with prominent, neointimal proliferation among cholesterol-fed, atherosclerotic *in vivo* models in comparison with a sparse incidence of neovasculature in reference controls. Such approaches for molecular imaging may provide a method for defining the burden and evolution of atherosclerosis in susceptible individuals as well as responsiveness of individual conditions to antiatherosclerotic therapies.

Molecular imaging of angiogenesis in early-stage atherosclerosis with $\alpha_v\beta_3$-integrin-targeted nanoparticles. Winter PM, Morawski AM, Caruthers SD, Fuhrhop RW, Zhang H, Williams TA, Allen JS, Lacy EK, Robertson JD, Lanza GM, Wickline SA. Circulation 2003 Nov 4; 108 (18), pp. 2270–4.

Vascular Imaging: Angiogenesis (Example: Neoplastic Disease)/Tumor Dimethylarginine Dimethylaminohydrolase (DDAH) Overexpression *NC100150/Hoechst 33342*

Cellular/Molecular Mechanism of Action: The orientation of NC100150 becomes altered within a magnetic field. Alternatively, Hoechst 33342 emits fluorescent light energy.
Background Information: Intracellular factors that regulate nitric oxide (NO) synthesis represent important targets in tumor progression. Overexpression of DDAH, which metabolizes the endogenous inhibitors of NO synthesis asymmetric dimethylarginine and N-monomethyl-L-arginine, results in C6 gliomas with enhanced growth rates compared to wild cell types.
Application: NC100150 can be utilized as an intravascular blood-pool contrast agent that is detectable by MRI methodologies. Hoechst 33342 can be used as a fluorescent perfusion-marker dye that can be measured by flourescent detection systems.
Route of Administration: Utilization of NC100150 and Hoechst 33342 for neoplastic lesion angiogenesis requires that these be delivered intravascularly.
Implementation: Determine the (i) *in vitro* effects of DDAH on tumor vascular morphogenesis by measurement of the transverse relaxation rates R_2* and R_2 in clone D27 gliomas overexpressing DDAH and C6 wild-type gliomas using intrinsic susceptibility MRI sensitive to changes in endogenous deoxyhemoglobin; (ii) determine susceptibility contrast-enhanced MRI using NC100150 as an intravascular blood-pool contrast agent; and (iii) compare results with fluorescence microscopy of the tumor uptake of the perfusion marker Hoechst 33342.
Interpretation and Analysis: The baseline R_2* is significantly faster in D27 tumors, consistent with a greater vascular development ($p < 0.02$, ANOVA). There is no significant difference between the response of the 2 tumor types to hypercapnia (5% CO_2/95% air), used as a probe for vascular maturation, or hyperoxia (5% CO_2/95% O_2), used as a probe for vascular function. NC100150 increases the R_2* and R_2 rates of both tumor types and detects a significantly larger blood volume in the D27 tumors ($p < 0.02$, ANOVA). This correlates with a significantly greater uptake of Hoechst 33342 in the D27 tumors compared to C6 wild-type tumors ($p < 0.02$, ANOVA). Despite increased tumor blood volume, the Delta R_2*/Delta R_2 ratio,

an index of microvessel size, shows that the capillaries in the 2 tumor types are of a similar caliber. Such observations demonstrate the potential of susceptibility MRI-derived quantitative end points to non-invasively assess tumor angiogenesis. Application of intravascular blood-pool contrast agents such as NC100150 for such purposes appears very promising. Overexpression of DDAH results in increased *in vivo* neovascularization of C6 gliomas. The lack of significant difference in hypercapnic/hyperoxic response between C6 and D27 tumors and the similar vessel caliber are also consistent with a role for DDAH in the initial stages of vasculogenesis.

Instrumentation: Magnetic resonance imaging and fluorescence microscopy instrumentation.

Effects of overexpression of dimethylarginine dimethylaminohydrolase on tumor angiogenesis assessed by susceptibility magnetic resonance imaging. Kostourou V, Robinson SP, Whitley GS, Griffiths JR. Cancer Res 2003 Aug 15; 63 (16), pp. 4960–6.

Vascular Imaging: Angiogenic Markers Expressed by Endothelium/Alpha$_V$Beta$_3$ ($\alpha_v\beta_3$) Integrin
Anti-$\alpha_V\beta_3$-Integrin-Linked [Gd]-Perfluorocarbon Nanoparticles

Cellular/Molecular Mechanism of Action: Anti-$\alpha_V\beta_3$ integrin monoclonal antibody (anti-$\alpha_v\beta_3$ MoAb) recognizes and physically binds to $\alpha_V\beta_3$ integrin complexes. Gadolinium is an element that undergoes changes in physical orientation when subjected to magnetic energy fields. Nanoparticles can serve as a selective delivery modality for a compound and/or diminish elimination or metabolism within the plasma compartment.

Background Information: Alpha$_v$beta$_3$ integrin is a molecular marker that can be utilized to detect and characterize angiogenic endothelium (e.g., neoplastic lesions).

Application: Site-directed contrast image enhancement of (micro)angiogenic vessels. Gadolinium can be detected by MRI capabilities.

Route of Administration: Intravenous.

Animal Model: Rabbit (corneal micropocket with neovascularization induced using basic fibroblast growth factor).

Interpretation and Analysis: Selective *in vivo* targeting of contrast agent results in a 25% increase in the average MR signal intensity after 90 minutes. Control groups in which nanoparticles are either used alone, linked to an isotype-matched antibody, or linked to DM101 and administered following receptor blocking do not display MR contrast enhancement at similar dose levels. Therefore, antibody-mediated targeting of agents enhances MR signal intensity in the capillary bed when utilized in a corneal micropocket model for angiogenesis, and is selectively retained within the angiogenic region via specific interaction with $\alpha_V\beta_3$ epitopes.

Magnetic resonance contrast enhancement of neovasculature with $\alpha_v\beta_3$-targeted nanoparticles. Anderson SA, Rader RK, Westlin WF, Null C, Jackson D, Lanza GM, Wickline SA, Kotyk JJ. Magn Reson Med 2000 Sep; 44 (3), pp. 433–9.

Vascular Imaging/Angiography: Vascular Perfusion/Imaging Artifacts
Dynamic Susceptibility Contrast MRI/Contrast Agent Concentration

Background Information: Quantification of cerebral perfusion with dynamic susceptibility contrast MRI (DSC-MRI) requires the measurement of arterial input function (AIF) parameters. Conventionally, it is possible to derive the contrast concentration from the DSC sequence by monitoring changes in either the amplitude or the phase signal on the assumption that the signal arises completely from blood. In practice, partial volume artifacts are inevitable because a compromise has to be reached between the temporal and spatial resolution of the DSC acquisition. As the concentration of the contrast agent increases, the vector of the complex blood signal follows a spiral-like trajectory. In the case of a partial-volume voxel, the spiral is located around the static contribution of the surrounding tissue. If the static contribution of the background tissue is disregarded, estimations of the contrast concentration will be incorrect. By optimizing the correspondence between phase information and amplitude information 1 can estimate the origin of the spiral, and thereupon correct for partial volume artifacts. This correction is shown to be accurate at low spatial resolutions for phantom data and to improve the AIF determination in a clinical example.

Correcting partial volume artifacts of the arterial input function in quantitative cerebral perfusion MRI. van Osch MJ, Vonken EJ, Bakker CJ, Viergever MA. Magn Reson Med 2001 Mar; 45 (3), pp. 477–85.

Vascular Imaging/Angiography: Pulmonary Embolism
Blood-Pool Contrast Agents

Magnetic resonance angiography (MRA) has been established as a powerful non-invasive imaging modality. Its applications to the study of the pulmonary vasculature have been hampered by a multitude of factors, such as respiratory and cardiac motion artifacts, saturation problems, long acquisition times, and limited spatial resolution. The recent introduction of contrast-enhanced MRA (CE-MRA) has greatly improved the potential for possible investigation of the pulmonary arteries under clinical conditions. Three-dimensional sequences with minimum TR and TE, a flip angle between 20° and 60°, and minimum slice thickness can be considered an optimal approach for breath-hold imaging combined with the automatic injection of contrast medium. Early studies have demonstrated the superiority of CE-MRA over non-enhanced techniques. The major indication for CE-MRA of the pulmonary vasculature is pulmonary embolism. Here a sensitivity of 85% and specificity of 95% can be obtained. It can be complemented by perfusion imaging, ventilation imaging, functional measurements of the right ventricle, and MR venography of the pelvic and femoral veins. Blood-pool contrast agents will open new perspectives in the future. Reference articles have reviewed the technical aspects of CE-MRA of the pulmonary vasculature, pathologic findings, and their interpretation, as well as present and future clinical applications.

Contrast-enhanced magnetic resonance angiography of the pulmonary vasculature: A review. Kauczor HU. Invest Radiol 1998 Sep; 33 (9), pp. 606–17.

Vascular Imaging: Angiography of Abdominal Vasculature/Relative Effective Diagnostic Window/Relative Contrast-to-Noise Ratio/In-Plane Vascular Imaging
[FeO]-BPA Ultrasmall Superparamagnetic Iron Oxide Agent/WIN 22181 Gadolinium Polymer/Blood-Pool Agent Image Half-Lives

Route of Administration: Ultrasmall superparamagnetic iron oxide agent (FeO-BPA: intravenous); gadolinium polymer (WIN 22191: intravascular).

Implementation: Evaluation of the anatomical integrity of abdominal vasculature applying blood-pool contrast image-enhancing agents utilized in concert with MRI techniques. Coronal T_1-weighted

3-dimensional fast field-echo images can be obtained with a conventional 1.5 Tesla MRI system before and after injection of FeO-BPA. Comparison studies involve conduction of MR angiograms acquired using the gadolinium polymer (WIN 22181). Enhanced images are obtained 5–210 minutes after injection of FeO-BPA and 1–120 minutes after injection of WIN 22181.

Animal Model: Swine (e.g., n = 7 for MRI and n = 7 for MRI angiograms).

Interpretation and Analysis: The ultrasmall superparamagnetic iron oxide and gadolinium polymer blood-pool agents can be used effectively to obtain detailed angiogram images of the abdominal vascular tree when 2-minute image acquisition times are employed. In-plane running vessels can be imaged without saturation effects and with equivalent maximum signal-to-noise ratios. Half the maximum signal-to-noise ratio is reached 150 minutes after FeO-BPA injection, whereas this interval is approximately 70 minutes for gadolinium polymer. Because of these different imaging half-life values, the effective diagnostic window provided by FeO-BPA is 6 to 8 times longer than that of WIN 22181. In the liver, the vascular T_1 shortening and the parenchymal T_2* effect of FeO-BPA complement each other, resulting in an optimal contrast-to-noise ratio that is significantly higher than that achieved with gadolinium polymer (WIN 22181). Such a "double-contrast effect" in the liver is especially helpful when obtaining detailed MR portograms. The blood-pool agents FeO-BPA and gadolinium polymer (WIN 22181) can be used to produce high-quality abdominal MR angiograms on standard MRI equipment. The contrast-to-noise ratio of hepatic vessels is best on iron oxide-enhanced images because of a T_1-T_2* synergistic effect in the liver. The longer diagnostic window provided by FeO-BPA coupled with the option of in-plane imaging suggests the usefulness of FeO-BPA in future MRI-guided vascular interventions.

Abdominal MR angiography performed using blood pool contrast agents: Comparison of a new superparamagnetic iron oxide nanoparticle and a linear gadolinium polymer. Nolte-Ernsting C, Adam G, Bucker A, Berges S, Bjornerud A, Gunther RW. AJR Am J Roentgenol 1998 Jul; 171 (1), pp. 107–13.

Vascular Imaging: Angiography and Neurophysiology/Enhanced Contrast-to-Noise Ratio/Improved Resolution
Magnetite Dextrane Nanoparticles: Blood-Pool Agent/ Functional MRI (fMRI)

Cellular/Molecular Mechanism of Action: Magnetite dextran nanoparticles following direct injection intravascularly remain within this body compartment for a prolonged period of time. The metallic component of the complexes undergoes spacial reorientation when subjected to magnetic energy fields that can be detected by MRI modalities.

Background Information: Functional MRI in awake-behaving primates is an emerging tool for bridging the gap between human fMRI and neurophysiology information from non-human primates.

Application: Magnetite dextran nanoparticles (Feridex) can be applied as a blood-pool agent to enhance fMRI contrast-to-noise ratios (CNRs) and achieve improved levels of resolution.

Dosage: Magnetite dextran nanoparticles.

Implementation: Intravascular injection of magnetite dextran nanoparticles (Feridex) as a blood-pool agent to delineate its capacity to enhance fMRI CNRs.

Animal Model: Primate (macaque monkey).

Interpretation and Analysis: Compared to lanthanide chelates, the intravascular half-life of magnetite dextran is relatively long ($T_{1/2}$ = 198 minutes) with a shortened T_2 relaxation observed in blood and cerebral cortex. Greater than 3-fold enhancement in the percentage MR signal change is observed using nanoparticles (13%) compared with conventional blood oxygen level–dependent (BOLD) fMRI (4%). The calculated regional cerebral blood volume in the primary visual cortex increases 32% with photic stimulation. The increased CNR allows greater flexibility in the design of awake-behaving primate fMRI studies with the potential for improvements in resolution and significantly shortened imaging times.

Enhancing fMRI contrast in awake-behaving primates using intravascular magnetite dextran nanoparticles. Dubowitz DJ, Bernheim KA, Chen DY, Bradley Jr WG, Andersen RA. Neuroreport 2001 Aug 8; 12 (11), pp. 2335–40.

Vascular Imaging/Arteriography of Peripheral and Renal Arteries/Alternative Non-Iodinated Contrast Image-Enhancing Agent/Digital Subtraction Angiography
Gadolinium

Cellular/Molecular Mechanism of Action: Gadolinium formulations possess radiopaque properties and their spatial orientation becomes altered when subjected to magnetic energy fields.

Application: Image enhancement of vascular anatomical abnormalities affecting peripheral and renal arterial vessels utilizing digital subtraction angiography (DSA) as an imaging modality.

Route of Administration: Gadodiamide (intra-arterial).

Dosage: Gadodiamide (mean volume = 136 ± 46 mL [range 60–200]).

Implementation: Peripheral and renal intra-arterial DSA facilitated by the administration of gadodiamide as a positive contrast image-enhancing agent in cases where iodinated contrast media is contraindicated (e.g., n = 17; n = 10 males; mean age 74 years, range 68–83 years). Detection of vascular anatomical abnormalities can subsequently serve as a guide for performing percutaneous intervention procedures (e.g., balloon angioplasty, stent placement).

Interpretation and Analysis: Serious side effects are rarely observed (e.g., n = 0/17 with no detectable changes in renal or thyroid function, and lack of exanthema or other allergic types of reactions). In conditions not requiring renal artery intervention, serum creatinine at discharge remains unchanged (2.57 ± 1.43 mg/dL to 2.40 ± 1.28 mg/dL, p = nonsignificant). In cases undergoing angioplasty/stenting of renal artery stenoses, serum creatinine decreases significantly (e.g., 3.53 ± 1.75 mg/dL to 2.36 ± 1.15 mg/dL [$p < 0.01$]). The diagnostic quality of the peripheral digital subtraction angiographs is often (usually) rated as being "good," but corresponding renal images are often graded as only being of "sufficient" diagnostic quality. In situations where the diagnostic administration of iodinated contrast image-enhancing reagents are contraindicated, gadodiamide may be a suitable alternative for renal or peripheral DSA followed by angioplasty.

Gadodiamide as an alternative contrast agent during angioplasty in patients with contraindications to iodinated media. Zeller T, Muller C, Frank U, Burgelin K, Sinn L, Horn B, Flugel PC, Roskamm H. J Endovasc Ther 2002 Oct; 9 (5), pp. 625–32.

Vascular Imaging/Atherosclerotic Plaque Lesions
Ultrasmall Superparamagnetic Particles of Iron Oxide (USPIOs)/(Sinerem, Guerbet)

Cellular/Molecular Mechanism of Action: Iron oxide particles change their spatial orientation when placed within an intense magnetic energy field. Macrophages perceive such particles as a for-

eign substance and internalize them through phagocytosis mechanisms.

Background Information: Agents classified as USPIOs are phagocytosed by cells of the mononuclear phagocytic system.

Application: Detection and characterization of atherosclerotic plaques.

Dosage: Ultrasmall superparamagnetic particles of iron oxide (Sinerem, Guerbet at 1 mmol Fe/kg).

Implementation: Evaluation of USPIO agents as potential detection agents that recognize biomarkers for atherosclerosis-associated inflammatory changes in the vessel wall before the development of vascular luminal narrowing. One approach to achieving this objective can entail characterization of the thoracic aorta using 3-dimensional MR angiography (MRA) with the enhancement of images using a paramagnetic contrast agent. One week later, all rabbits except 1 of the hyperlipidemic animals are then injected with a USPIO contrast agent (Sinerem, Guerbet) at a dose of 1 mmol Fe/kg.

***Ex Vivo* Model:** Rabbit aorta preparations.

Animal Model: Rabbit (n = 6 heritable hyperlipemic New Zealand White; and n = 3 New Zealand White control rabbits).

Interpretation and Analysis: Three-dimensional MRA data sets collected over 5 days show increasing signal in the aortic lumen. No vascular lesions can be detected at the time of initial imaging. The aortic wall of the control rabbits remains smooth and bright. Marked susceptibility effects become evident on day 4 within the aortic walls of hyperlipidemic rabbits. Results observed *in vivo* can be confirmed *ex vivo* by imaging aortic specimens. Histopathology can detect marked Fe uptake in macrophages embedded in atherosclerotic plaques in scenarios of hyperlipidemia, and electron microscopy analysis reveals multiple cytoplasmic Fe particles within macrophages. No such changes can be appreciated in controls or in conditions of hyperlipidemia not receiving Sinerem. Ultrasmall superparamagnetic particles of iron oxide are phagocytosed by macrophages in atherosclerotic plaques of the aortic wall of hyperlipidemic rabbits in a quantity sufficient to recognize susceptibility effects that can be detected by MRI.

Magnetic resonance imaging of atherosclerotic plaque with ultrasmall superparamagnetic particles of iron oxide in hyperlipidemic rabbits. Ruehm SG, Corot C, Vogt P, Kolb S, Debatin JF. Circulation 2001 Jan 23; 103 (3), pp. 415–22.

Vascular Imaging/Blood-Pool Agent/Neoplastic Lesion Vasculature Networks
Iohexol Liposomes

Background Information: Although soluble non-ionic iodine compounds with low systemic toxic effects have been developed for use in computed tomography (CT), they have short residence times of a few minutes or mere seconds, which is an insufficient period of time for blood-pool imaging, even with high-speed multidetector row spiral CT. Moreover, potential renal toxic effects preclude repeated administration of these contrast agents during imaging, as well as their use in patients with compromised renal function.

Implementation: Develop and evaluate a CT contrast agent for blood-pool imaging that remains in the blood for more than 3 hours and that is relatively non-toxic to the kidneys. A relevant agent in this regard includes liposomal iohexol formulations.

Semi-Synthetic Methods: Determination of the encapsulation efficiency in terms of milligrams of iodine per milliliter of lipid formulation and delineation of preparation *in vitro* stability in phosphate buffer solutions and human plasma. Complementary investigations can assess formulation *in vivo* stability, residence time, and enhancement of contrast on images of various organ systems.

Animal Model: Rabbit.

Interpretation and Analysis: Formulations containing 34.8 mg of iodine per milliliter of liposomal iohexol solution retain stability in blood plasma both *in vitro* and *in vivo* postinjection. An intravenous dose of 475 mg of iodine per kilogram of body weight produces contrast enhancement in the rabbit model of approximately 130 HU in the aorta and liver cortex and approximately 100 HU in the kidney cortex. Contrast enhancement can be maintained for 3 hours postinjection, and minimal clearance of the contrast agent via the kidneys is observed. The liposomal iohexol formulations have a sufficient residence time for blood-pool imaging (rabbit models). Future experiments with long-residence-time iohexol formulations may lead eventually to applications in cardiac imaging and in early tumor detection.

Long-residence-time nano-scale liposomal iohexol for X-ray-based blood-pool imaging. Kao CY, Hoffman EA, Beck KC, Bellamkonda RV, Annapragada AV. Acad Radiol 2003 May; 10 (5), pp. 475–83.

Vascular Imaging/Brain Reperfusion Injury with Ischemia/Oxidative Stress/Brain Tumors/ Neurodegenerative Disorders
MC-PROXYL/Carbamoyl-PROXYL

Background Information: Several investigations have reported that central nervous system (brain) free radicals generation is involved in various brain dysfunctions, including ischemia-reperfusion injury, brain tumors, and neurodegenerative diseases. It has been reported that the spin probe MC-PROXYL can penetrate the blood-brain barrier (BBB) and can be useful for evaluating oxidative stress in the brain.

Application: The agent MC-PROXYL functions as a spin probe for imaging changes in ischemia-reperfusion injury. The agent carbamoyl-PROXYL functions as a BBB impermeable imaging probe.

Route of Administration: Intravenous.

Implementation: Evaluation of reperfusion injury and models of stroke-prone spontaneous hypertensive conditions.

Animal Model: Murine (*in vivo*) and rat (isolated brain: stroke-prone spontaneously hypertensive model).

Interpretation and Analysis: The agent MC-PROXYL, but not carbamoyl-PROXYL, is widely distributed throughout the brain. Rapid decay of 2-dimensional electron spin resonance (ESR) images of MC-PROXYL in isolated stroke-prone spontaneous hypertensive rat (SHRSP) brain can be observed, in contrast to Wistar-Kyoto rats (WKYs), using the ESR imaging system. Furthermore, by using L-band ESR non-invasively, the decay rate of MC-PROXYL in the head region can be found to be faster in live SHRSPs than in live Wistar-Kyoto rats. Taken together, the high oxidative stress sustained by oxygen radical generation in SHRSPs may cause alterations in MC-PROXYL metabolism within the brain. Such results suggest that *in vivo* ESR could be applied for the assessment of antioxidant effects on oxidative stress events in the brain (e.g., animal disease models for SHRSP).

Meassurement of oxidative stress in stroke-prone spontaneously hypertensive rat brain using *in vivo* electron spin resonance spectroscopy. Miyazaki H, Shoji H, Lee MC. Redox Rep 2002; 7 (5), pp. 260–5.

Vascular Imaging/Thromboses (Arterial)
RGD-Ultrasmall Superparamagnetic Particles of Iron Oxide (USPIOs)

Cellular/Molecular Mechanism of Action: The peptide RGD recognizes and physically binds to the exterior surface of activated

platelet populations. The physical orientation of iron ions becomes altered when subjected to magnetic energy fields.

Application: Detection of platelet thromboses, especially those formed within the arterial vasculature, which have a relatively higher platelet composition than do venous thromboses.

Semi-Synthetic Methods: Preparation of USPIOs coupled to an RGD peptide (RGD-USPIO) that selectively recognizes activated platelet populations.

Implementation: Evaluation of thrombus lesions applying USPIOs coupled to an RGD peptide (RGD-USPIO) targeted for activated platelets, applied in combination with MRI modalities. Thrombus visualization *ex vivo* is then compared using RGD-USPIO and a non-targeted USPIO. Relevant variables that can be assessed include determining the influence of the duration of thrombus exposure to RGD-USPIO (*ex vivo*) on thrombus visualization and spatial resolution of MR images (*ex vivo* and *in vivo*).

***Ex Vivo* Model:** Thrombus (*ex vivo* and *in vivo* preparations).

Interpretation and Analysis: RGD-USPIOs provide superior thrombus visualization compared to non-targeted USPIOs *ex vivo*, and maximum enhancement can be achieved after exposure of vascular thrombses to RGD-USPIO preparations for periods of approximately 1 hour. The ability to visualize clots is highly dependent on the spatial resolution of the image. *In vivo*, an in-plane level of resolution of less than 0.2×0.2 mm^2 is required for good clot visualization after contrast enhancement. Achievable resolution and sensitivity is a potential limitation for the usefulness of active vascular MRI targeting.

A targeted contrast agent for magnetic resonance imaging of thrombus: Implications of spatial resolution. Johansson LO, Bjornerud A, Ahlstrom HK, Ladd DL, Fujii DK. J Magn Reson Imaging 2001 Apr; 13 (4), pp. 615–8.

Vascular Thromboses
Gadolinium-DTPA ([Gd]-DTPA) Lipid-Encapsulated Perfluorocarbon Nanoparticle

Cellular/Molecular Mechanism of Action: Fibrin monomer naturally undergoes multimeric (non-covalent) polymerization with other strands of fibrin monomer following the cleavage of a small peptide fragment from fibrinogen by the biochemical activity of thrombin.

Application: Detection and characterization of vascular thrombi.

Route of Administration: Intended for intravascular delivery.

Implementation: Evaluation of the sensitivity of lipid-encapsulated perfluorocarbon nanoparticle with numerous [Gd]-DTPA complexes incorporated into the outer surface in order to function as a contrast imaging agent for detecting intravascular fibrin deposits. One approach for evaluating such properties includes utilizing the agent to determine its capacity to detect fibrin strands in a defined *in vitro* human thrombosis model.

Interpretation and Analysis: After binding to fibrin clots, scanning electron microscopy of treated clots reveals patterns of accumulations of dense nanoparticles on the clot surface. Fibrin clots with sizes ranging from 0.5 to 7.0 mm are imaged at 4.7 Tesla with or without the targeted contrast agent. Regardless of size, untreated clots are not easily detected by T_1-weighted MRI, while targeted contrast agent dramatically improves the detection of all clots. Decreases in T_1 and T_2 relaxation times (20–40%) are measured relative to the surrounding media and reference control clots.

High-resolution MRI characterization of human thrombus using a novel fibrin-targeted paramagnetic nanoparticle contrast agent. Yu X, Song SK, Chen J, Scott MJ, Fuhrhop RJ, Hall CS, Gaffney PJ, Wickline SA, Lanza GM. Magn Reson Med 2000 Dec; 44 (6), pp. 867–72.

CENTRAL NERVOUS SYSTEM

Central Nervous System (CNS)
Ferrimagnetic Iron Oxide Nanoparticles

Application: Potential application for brain imaging.

Route of Administration: Intravascular (proposed).

Semi-Synthetic Methods: Nanoparticles can be prepared by co-precipitation of ferric and ferrous ions in the presence of either dextran or polyvinyl alcohol. The method of preparation results in loosely packed clusters (dextran) or branched chains (polyvinyl alcohol) of particles containing on the order of 600 and 400 particles, respectively.

Implementation: Meassurement of proton transverse relaxation rates (R2) in agar gels with varying concentrations of ferrimagnetic iron oxide nanoparticles in a field strength of 1.5 Tesla.

Interpretation and Analysis: For both methods of particle preparation, concentrations of ferrimagnetic iron in agar gel less than 0.01 mg/ml have no measurable effect on the value of R2 for the gel. The results indicate that MRI-based R2 measurements using 1.5 Tesla clinical scanners are not quite sensitive enough to detect the very low concentrations of nanoparticulate biogenic magnetite reported in human brain tissue.

Instrumentation: Clinical MRI system.

Detection limits for ferrimagnetic particle concentrations using magnetic resonance imaging based proton transverse relaxation rate measurements. Pardoe H, Chua-anusorn W, St Pierre TG, Dobson J. Phys Med Biol 2003 Mar 21; 48 (6), pp. N89–95.

Central Nervous System (CNS)/Amyloid Beta (Abeta) Plaques (Brain): Alzheimer's Disease (AD)
Gadolinium (Gd) Abeta$_{1\text{-}40}$ Nanoparticles/ Monocrystalline Iron Oxide Abeta$_{1\text{-}40}$ Nanoparticles

Cellular/Molecular Mechanism of Action: Abeta$_{1\text{-}40}$ has a high (non-covalent) binding avidity for abeta amyloid.

Background Information: The presence of abeta plaques in the brain is a hallmark pathological feature of AD. Transgenic mice that overexpress mutant amyloid precursor protein (APP), or both mutant APP and presenilin-1 (APP/PS1), develop abeta plaques similar to those in AD patients and have been proposed as animal models in which to test experimental therapeutic approaches for the clearance of abeta. However, there are few *in vivo* whole-brain imaging methods available for detecting abeta plaques in mice or men.

Application: Detection and characterization of abeta plaque deposits *in vivo* within the brain.

Route of Administration: Intra-arterial injection with mannitol to facilitate transient opening of the blood-brain barrier (BBB).

Implementation: Evaluation of the capacity for abeta$_{1\text{-}40}$ peptide magnetically labeled with either gadolinium (Gd) or monocrystalline iron oxide nanoparticles (MION) to detect *in vivo* amyloid plaque deposits.

Animal Model: Murine (transgenic).

Interpretation and Analysis: Magnetically labeled abeta$_{1\text{-}40}$ with mannitol enables the detection of many abeta plaques. Furthermore, the numerical density of abeta plaques detected by MR microimaging (μMRI) and by immunohistochemistry shows excellent correlation. This approach represents an *in vivo* method for detecting abeta in AD transgenic mice and suggests that diagnostic MRI methods to detect abeta in AD patients may ultimately be feasible.

Instrumentation: Magnetic resonance microimaging.

Detection of Alzheimer's amyloid in transgenic mice using magnetic resonance microimaging. Wadghiri YZ, Sigurdsson EM, Sadowski M, Elliott JI, Li Y, Scholtzova H, Tang CY, Aguinaldo G, Pappolla M, Duff K, Wisniewski T, Turnbull DH. Magn Reson Med 2003 Aug; 50 (2), pp. 293–302.

Central Nervous System (CNS)/Blood-Brain Barrier (BBB) Diffusion
Superparamagnetic Solid Lipid Nanoparticles (SLN)

Cellular/Molecular Mechanism of Action: Solid lipid nanoparticles, due to their lipophilic properties, difuse into the lymph following duodenal administration and diffusion across the BBB into the CNS.
Application: Solid lipid nanoparticles can be used to selectively "target" pharmaceutical agents for the lymph.
Route of Administration: Solid lipid nanoparticles (duodenal).
Semi-Synthetic Methods: Preparation of superparamagnetic SLN that are colloidal in size and *in vitro* analysis reveal they possess relaxometric properties similar to Endorem.
Implementation: Magnetic resonance imaging of the CNS with superparamagnetic SLN.
Interpretation and Analysis: Magnetic resonance imaging of the CNS with superparamagnetic SLN reveals they have a slower blood clearance than Endorem. Magnetic resonance imaging data are consistent with CNS uptake of SLN lasting up to a duration of 135 minutes. Therefore, SLN are capable of passively diffusing across an intact BBB, which is a feature that could be utilized for the molecular design of CNS MRI contrast agents.

In vitro and *in vivo* study of solid lipid nanoparticles loaded with superparamagnetic iron oxide. Peira E, Marzola P, Podio V, Aime S, Sbarbati A, Gasco MR. J Drug Target 2003 Jan; 11 (1), pp. 19–24.

Central Nervous System (CNS)/Blood-Brain Barrier Penetration/Transferrin Receptor Complexes/ Demyelinated Axons
Oligodendrocyte Progenitor Cells/[Transferrin Magnetic Nanoparticles]

Cellular/Molecular Mechanism of Action: Oligodendrocyte progenitor cells naturally migrate to specific foci within the CNS, including demyelinated axons.
Background Information: Demyelination is a common pathological finding in human neurological diseases and frequently persists as a result of failure of endogenous repair. Transplanted oligodendrocytes and their precursor cells can (re)myelinate axons, raising the possibility of therapeutic intervention. The migratory capacity of transplanted cells is of key importance in determining the extent of (re)myelination and can, at present, be evaluated only by the use of invasive and irreversible procedures.
Application: Potential delivery modality that recognizes demyelinated axons.
Route of Administration: Presumably intravascular.
Implementation: Delineation of the capacity for transferrin receptors to function as an effective "target" that can facilitate the intracellular delivery of magnetic nanoparticles and guide the migration of transplanted tagged oligodendrocyte progenitor cells into the spinal cord with myelin-deficient neurons.
Animal Model: Rat (myelin-deficient neurons).
Interpretation and Analysis: Cell migration can be easily detected by using 3-dimensional MR microscopy, which demonstrates a close correlation between the areas of contrast enhancement and degree of "new" myelination in an *in vivo* myelin-deficient model. In this context, investigations have demonstrated the feasibility of successfully using MR to track transplanted oligodendrocyte progenitor cells. The technique has the potential of being easily extended into other neurotransplantation research investigations involving different precursor cell types.

Neurotransplantation of magnetically labeled oligodendrocyte progenitors: Magnetic resonance tracking of cell migration and myelination. Bulte JW, Zhang S, van Gelderen P, Herynek V, Jordan EK, Duncan ID, Frank JA. Proc Natl Acad Sci U S A 1999 Dec 21; 96 (26), pp. 15256–61.

Central Nervous System (CNS)/Blood-Brain Barrier (BBB) Permeability/Multiple Sclerosis/Brain Tumor Growth
Intravascular Contrast Agent

Background Information: A non-invasive technique for measuring the permeability of the BBB to water could help to evaluate changes in the functional integrity of the BBB that occur in different pathological conditions, such as multiple sclerosis or growth of brain tumor. Some clinical investigators have proposed an MR method to measure BBB permeability based on the T_1 reductions induced by injecting various doses of paramagnetic contrast agent. However, this method may be difficult to implement in a clinical environment.
Implementation: Utilization of a 2-point technique that applies a spatially selective inversion to measure T_1 prior to and after injection of an intravascular contrast agent. Measurements acquired are compared to numerical simulations generated with a physiological model that accounts for blood flow and includes 2 different blood volumes (e.g., non-exchanging and exchanging).
Animal Model: Rat brain.
Interpretation and Analysis: The permeability of the BBB can be evaluated from the change in T_1 caused by vascular contrast agent. This technique might provide an approach for monitoring changes in BBB permeability to water in clinical studies.

A model of blood-brain barrier permeability to water: Accounting for blood inflow and longitudinal relaxation effects. Barbier EL, St Lawrence KS, Grillon E, Koretsky AP, Decorps M. Magn Reson Med 2002 Jun; 47 (6), pp. 1100–9.

Central Nervous System (CNS)/Cerebral Perfusion/ Induced Vasoconstriction/Blood Oxygen Level–Dependent (BOLD) Baseline Resting Signal
Caffeine / Functional MRI (fMRI)

Physiological Mechanism of Action: Caffeine functions as a cerebral vasoconstrictor.
Background Information: Previous research has demonstrated that caffeine has the ability to reset the level of coupling between blood flow and neuronal activity.
Application: Investigation of the effect of disease processes and pharmaceutical agents on cerebral blood flow.
Implementation: Evaluation of the influence that caffeine has improving BOLD signal response in fMRI-based techniques.
Interpretation and Analysis: Caffeine causes a decrease in cerebral perfusion by as much as 13.2% without a change in performance. Caffeine is a cerebral vasoconstrictor that causes an increase in the concentration of deoxyhemoglobin and thus a decrease in the BOLD baseline resting signal by 4.4%. During activation, the vasculature responds from below-normal baseline levels with a normal increase in blood flow and volume, resulting in an overall increase in BOLD contrast. Such increases can be as large as 22–37% during the performance of a visually cued motor task. The benefit of such a large increase in the BOLD contrast could be used

to improve image resolution, the acquisition scheme, or the task design of fMRI experiments. Caffeine has the potential to be used as a contrast booster for fMRI experiments.

On the use of caffeine as a contrast booster for BOLD fMRI studies. Mulderink TA, Gitelman DR, Mesulam MM, Parrish TB. Neuroimage 2002 Jan; 15 (1), pp. 37–44.

Central Nervous System (CNS)/Dopamine D_2 Receptor Complexes/Bilateral Frontal Cortex Blood Oxygen Level–Dependent (BOLD) Signal Increases/ Psychoactive Pharmacological Agent Efficacy Evaluation
Functional MRI (fMRI) / Sulpiride

Cellular/Molecular Mechanism of Action: Sulpiride functions as a selective D_2-like receptor antagonist.
Physiological Mechanism of Action: Sulpiride promotes increases in frontal dopaminergic function by antagonism of presynaptically located dopamine D_2 receptors within this region of the brain and is consistent with its known therapeutic mechanism of action.
Application: Sulpiride.
Route of Administration: Sulpiride (intravenous).
Dosage: Sulpiride (3 mg/kg vs 10 mg/kg vs 30 mg/kg).
Implementation: Blood oxygen level–dependent contrast fMRI was used to study the effects of the D_2-like receptor selective antagonist, sulpiride, at 2 Tesla in the brain of the alpha-chloralose anesthetized rat.
Interpretation and Analysis: Region of interest (ROI) analysis indicates significant ($p < 0.05$) bilateral increases in BOLD signal intensity in the frontal cortex following a single administration of sulpiride (10 mg/kg). Blood oxygen level–dependent signal changes are slow in onset and increase gradually, reaching $8.0 \pm 0.5\%$ (mean $\pm$ standard error of measurement) above preinjection control values 165 minutes after drug administration. Signal increases remain high up to 3 hours post–sulpiride administration. Sulpiride (30 mg/kg) has a similar effect in the frontal cortex, reflected by signal increases $5.2 \pm 1.8\%$ above control values by 174 minutes. However, the effects of sulpiride at this dosage are more variable (between rats), but this observation is not statistically significant. Sulpiride (3 mg/kg) has no significant effect upon BOLD signal intensity within any region of the brain. No dose of sulpiride within the ranges evaluated induces any significant BOLD signal changes within the striatum or cerebellum. Such observations support the concept that sulpiride causes an increase in frontal dopaminergic function by antagonism of presynaptically located dopamine D_2 receptors within this brain region, consistent with its therapeutic action. Furthermore, the utility of BOLD contrast fMRI as a means of detecting changes in neuronal activity contingent upon the administration of a psychoactive pharmacological agent has been demonstrated.

Detection of pharmacologically mediated changes in cerebral activity by functional magnetic resonance imaging: The effects of sulpiride in the brain of the anesthetised rat. Preece M, Mukherjee B, Huang CL, Hall LD, Leslie RA, James MF. Brain Res 2001 Oct 19; 916 (1–2), pp. 107–14.

Central Nervous System (CNS)/Endothelial ICAM Expression in the CNS/Microvascular (Endothelial) Leukocyte Receptor Complex Expression
[Anti-ICAM]-Paramagnetic Liposomes/Fluorescent-Tagged [Anti-ICAM]-Paramagnetic Liposomes (ACPLs)

Application: The *in vivo* targeting of membrane-associated ICAM complexes expressed by vascular endothelium to create sufficient signal enhancement at these sites for detection by MRI instrumentation.
Implementation: [Anti-ICAM]-paramagnetic liposomes targeted to intercellular adhesion molecule-1 (ICAM-1) administered *in vivo* for delineating the upregulation of endothelial leukocyte receptor complexes of the cerebral microvasculature during conditions of autoimmune encephalitis.
Animal Model: Mouse (autoimmune encephalitis).
Interpretation and Analysis: Fluorescence microscopy analysis reveals that fluorescently tagged ACPLs are localized within the CNS microvasculature in a pattern consistent with ICAM-1 upregulation. Such findings closely correlate with immunohistochemical analyses. High-resolution MRI of mouse brains *ex vivo* demonstrates that ACPL binding patterns confer significant enhancement of signal intensity (SI) as compared to control images. Preparations of ACPLs therefore appear to be a useful MRI contrast agent for visualization of specific molecules expressed on vascular endothelium during various disease states.
Instrumentation: Magnetic resonance imaging instrumentation.

ICAM-1 expression in autoimmune encephalitis visualized using magnetic resonance imaging. Sipkins DA, Gijbels K, Tropper FD, Bednarski M, Li KC, Steinman L. J Neuroimmunol 2000 Apr 3; 104 (1), pp. 1–9.

Central Nervous System (CNS)/Gene Mapping Model: *In Vivo* β-Galactosidase Expression
Galactopyranose Functional Group/Chelated Paramagnetic Ion

Cellular/Molecular Mechanism of Action: Galactopyranose can function as a substrate for the indicator (marker) gene β-galactosidase.
Background Information: High-resolution *in vivo* imaging of gene expression is not possible applying existing techniques.
Application: High-resolution *in vivo* MRI imaging of "reporter" gene expression.
Semi-Synthetic Methods: Preparation of an MRI contrast agent in which the access of water to the 1st coordination sphere of a chelated paramagnetic ion is blocked with a substrate that can be removed by enzymatic cleavage. Following cleavage, the paramagnetic ion can interact directly with water protons to increase the MR signal.
Implementation: Detection and characterization of *in vivo* gene expression.
Interpretation and Analysis: Galactopyranose can function as an effective blocking group that can render an MRI contrast agent sensitive to detecting the expression of β-galactosidase (marker gene). Pertaining to cellular resolution, regions of higher intensity in MR images correlate with regions expressing the marker enzyme. These results offer the promise of *in vivo* mapping of gene expression in transgenic animals and validate a general approach for constructing a family of MRI contrast agents that respond to biological activity.

In vivo visualization of gene expression using magnetic resonance imaging. Louie AY, Huber MM, Ahrens ET, Rothbacher U, Moats R, Jacobs RE, Fraser SE, Meade TJ. Nat Biotechnol 2000 Mar; 18 (3), pp. 321–5.

Central Nervous System (CNS)/Gliomatosis Cerebri
Gadopentetate Dimeglumine

Application: Magnetic resonance imaging characteristics of gliomatosis cerebri reiterate the diffuse nature of this tumor, but such features are non-specific and thus may pose a diagnostic challenge.

Because perfusion MRI can provide a physiologic map of the microcirculation, it is possible to compare the measured relative cerebral blood volume (rCBV) at the time of perfusion imaging with histopathologic findings in gliomatosis cerebri.

Considerations: First-pass bolus infusion utilizing gadopentetate dimeglumine in conjunction with MRI (dynamic contrast-enhanced T_2*-weighted gradient-echo echo-planar images).

Implementation: Retrospective analysis of conventional and perfusion MR images of proven cases of gliomatosis cerebri. Conventional MR images are evaluated for the presence or absence of contrast enhancement, necrosis, and extent of T_2-weighted signal intensity abnormality. Dynamic contrast-enhanced T_2*-weighted gradient-echo echo-planar images are acquired during the 1st pass of a bolus injection of gadopentetate dimeglumine. The rCBV is calculated by using non-diffusible tracer kinetics and expressed relative to normal-appearing white matter. Pathologic findings are reviewed in all patients and compared with the MR perfusion data. Multivoxel 2-dimensional chemical shift imaging proton MR spectroscopic data can also be acquired (e.g., n = 3 cases), as can single-voxel data (e.g., n = 1 case).

Human Model: Cases of gliomatosis cerebri (n = 7).

Interpretation and Analysis: Conventional MR images reveal the presence of diffuse abnormalities in all cases and absence of contrast enhancement in all but 1 case. Average rCBV range is 0.75–1.26 (mean, 1.02 ± 0.42 standard deviation). Magnetic resonance spectroscopic data reveal spectra consistent with the presence of tumoral disease. Histopathologic evaluations reveal an absence of vascular hyperplasia in all specimens. Conclusion: The low MR rCBV measurements of gliomatosis cerebri are in concordance with the lack of vascular hyperplasia found at histopathologic examination; thus, perfusion MRI provides useful adjunctive information that is not available from conventional MRI techniques.

Instrumentation: Magnetic resonance imaging systems.

Dynamic contrast-enhanced T_2*-weighted MR imaging of gliomatosis cerebri. Yang S, Wetzel S, Law M, Zagzag D, Cha S. AJNR Am J Neuroradiol 2002 Mar; 23 (3), pp. 350–5.

Gadopentetate-dimeglumine-enhanced MR imaging of gliomatosis cerebri: Appearance mimicking leptomeningeal tumor dissemination. Rippe DJ, Boyko OB, Fuller GN, Friedman HS, Oakes WJ, Schold SC. AJNR Am J Neuroradiol 1990 Jul–Aug; 11 (4), pp. 800–1.

Central Nervous System (CNS)/Ischemia (Cerebral): Infarct Lesions
$H_2{}^{17}O$/Gadolinium-DTPA ([Gd]-DTPA)

Cellular/Molecular Mechanism of Action: The agent $H_2{}^{17}O$ functions as an analog of water in biological systems.

Background Information: Oxygen-17 enriched water acts as a T_2 shortening contrast agent whose effect can be suppressed by decoupling at the [^{17}O] frequency during TE interval in a spin-echo MR image.

Application: Quantifying low concentrations of $H_2{}^{17}O$ *in vivo* and exploring its utility for assessing cerebral ischemia.

Route of Administration: $H_2{}^{17}O$ (intravenous).

Dosage: $H_2{}^{17}O$ (bolus of 1 ml of 10 atom% $H_2{}^{17}O$).

Implementation: Serial T_2-weighted echo planar images are acquired in phantoms and rat brain with decoupler power alternated every 8 images. The resulting periodic signal change (proportional to $H_2{}^{17}O$ concentration) is subsequently detected by cross-correlating the square-wave decoupler power time course with the signal intensity in each voxel. Natural abundance (0.037 atom%) images of $H_2{}^{17}O$ are then generated (rat brain).

Animal Model: Rat (brain focal ischemic model).

Interpretation and Analysis: The transverse relaxivity of $H_2{}^{17}O$ in brain has been estimated to be $R_2 = 2.4 \pm 0.5\ s^{-1}$ atom%$^{-1}$. Estimated brain $H_2{}^{17}O$ concentrations are 0.06 ± 0.01 atom%. In the rat focal ischemia model, ^{17}O cross-correlation maps compare well with diffusion and [Gd]-DTPA perfusion images for the detection of infarct locations.

MRI of focal cerebral ischemia using ^{17}O-labeled water. de Crespigny AJ, D'Arceuil HE, Engelhorn T, Moseley ME. Magn Reson Med 2000 Jun; 43 (6), pp. 876–83.

Central Nervous System (CNS)/Ischemic Insult to the Brain/Neurologic Ischemia Model/Relative Uptake and Neurotoxicity
Iopromide/Gadobenate Dimeglumine/Gadodiamide

Background Information: Because contrast agent (CA) formulations are injected intravenously to patients who may have a disrupted blood-brain barrier, their neurotolerability ideally should be evaluated.

Route of Administration: Iopromide (intravascular); gadobenate dimeglumine (intravascular); and gadodiamide (intravascular).

Dosage: Iopromide (7 g iodine/kg: 10 times higher than the maximum clinical dose in rats); gadobenate dimeglumine (2.0 mmol/kg; 20 times clinical dose in rats); and gadodiamide (2.0 mmol/kg; 20 times the clinical dose in rats).

Considerations: Iopromide is a poorly neurotolerated iodinated contrast agent.

Implementation: Rats were prepared for electroencephalogram (EEG) recording about 15 days before induction of ischemia in the right hemisphere by 2-hour middle cerebral artery (MCA) occlusion and reperfusion for 3 days. Rats with evidence of CNS ischemia on day 3 after transient MCA occlusion are subsequently selected for neurotolerability studies. Model validation in terms of EEG on day 3 after MCA occlusion is performed in concert with iopromide iodinated contrast agent. Recordings for EEG tracings are analyzed for pathological tracings and for changes in spectral profiles in terms of the frequency index (FI) at 1, 2, and 3 hours after test compound injection. Comparison evaluations between gadobenate dimeglumine and gadodiamide are performed at 2.0 mmol/kg with D-mannitol applied as a reference control. The presence of contrast agent in the brain (rat) is verified by measuring the total gadolinium content by using inductively coupled plasma-atomic emission spectrometry analysis. Given the absence of metabolism for both contrast agents, the values of gadolinium content can be interpreted as representing unmetabolized contrast agent.

Animal Model: Rat (brain ischemia model utilizing EEG as an adjunct detection modality following validation for appropriateness).

Interpretation and Analysis: On days 1, 2, and 3 following transient MCA occlusion, the lesioned hemispheres (rat) display decreases in frequency-index values relative to basal parameters. Intact (unlesioned) hemispheres, after a slight, non-significant decrease in fractional index values on the 1st 2 days, present with normal frequency index values on day 3. Iopromide injected intravenously into ischemic rats at a dose 10 times higher than the maximum clinical dose causes bilateral spikes on EEG tracings and increases in frequency index values for the unlesioned hemisphere without affecting the lesioned hemisphere. Gadobenate dimeglumine, like gadodiamide, when injected into ischemic rats does not cause spikes or further changes in the frequency index values for the lesioned hemisphere and does not modify the normal frequency index value of unlesioned hemispheres. No significant differences between gadobenate dimeglumine, gadodiamide, and D-mannitol

can be appreciated when postinjection frequency index values are compared. Finally, higher levels of gadolinium are found in the lesioned hemisphere with respect to the unlesioned hemisphere after both gadobenate dimeglumine and gadodiamide administration. In summary, (i) EEG tracings are a valuable modality for assessing ischemia induced by transient MCA occlusion in a model designed to evaluate contrast agent neurotolerability because ischemic and contrast agent effects can be clearly differentiated; (ii) the higher level of contrast agent in the lesioned hemisphere compared with the unlesioned 1 (2 to 3 times), even 3 hours after injection, demonstrates that contrast agents effectively penetrated the brain and if they were neurotoxic, any negative effects would be detected; and (iii) gadobenate dimeglumine, like gadodiamide, injected intravenously at a dose 20 times higher than the intended clinical dose for brain MRI is well tolerated and, also like gadodiamide, is suitable for use in neurological diseases for which contrast-enhanced MRI is indicated.

Instrumentation: Inductively coupled plasma-atomic emission spectrometry analysis.

Neurotolerability of contrast agents in rats with brain ischemia induced by transient middle cerebral artery occlusion: EEG evaluation. Frigeni V, Miragoli L, Grotti A, Lorusso V. Invest Radiol 2001 Jan; 36 (1), pp. 1–8.

Central Nervous System (CNS)/Ischemia: Regional *Iopromide/Gadobenate Dimeglumine/Gadobutrol*

Application: Gadobenate dimeglumine possesses a safety profile that demonstrates its suitability for characterizing neurological disease states when contrast-enhanced MRI is indicated.

Route of Administration: Iopromide (intravenously); gadobenate dimeglumine (intravenous); and gadobutrol (intravenous).

Dosage: Iopromide (7 g I/kg [rat]); gadobenate dimeglumine (2 mmol/kg [rat]); and gadobutrol (2 mmol/kg [rat]).

Implementation: Injection of iopromide 3 days following occlusion of the middle cerebral artery (MCA) (rats) to determine its capacity to detect regional areas of ischemia within the CNS.

Rats are prepared for somatosensory-evoked potential recording at least 5 days before ischemia induction. Ischemia is induced by occlusion of the right MCA for a period of 30 minutes following reperfusion for 3 days. The somatosensory-evoked potential responses generated contralaterally relative to the stimulated upper limb are recorded from the primary somatosensory cortical area. Iopromide (poorly neurotolerated iodinated contrast agent) is then injected intravenously for the detection of regions of CNS ischemia on day 3 postocclusion. Comparative imaging analysis is then performed utilizing gadobenate dimeglumine and gadobutrol. Somatosensory-evoked potential responses are then measured as peak latencies (P2 and N2) and peak-to-peak amplitude (P2N2). Brain concentration of iopromide are measured by high-performance liquid chromatography, whereas amounts of the gadolinium contrast agent are quantified by inductively coupled plasma-atomic emission spectrometry analysis; given the absence of metabolism for both contrast agents, the gadolinium content values can be interpreted as representing unmetabolized contrast agent.

Interpretation and Analysis: In the ischemic rats, somatosensory-evoked potential responses of the lesioned hemisphere show significant increases in P2, N2, and interpeak N2-P2 latencies and a significant reduction in P2N2 amplitude. Iopromide dramatically increases the P2N2 amplitude of the somatosensory-evoked potential responses recorded from both hemispheres of ischemic rats without affecting the P2, N2, and interpeak N2-P2 latencies. The iopromide effect coincides with high concentrations of the contrast agent in the brain. Iopromide has no effect on healthy rats. Gadobenate dimeglumine does not induce any alteration in somatosentory-evoked potential components of either the lesioned or unlesioned hemisphere of ischemic rats. No significant difference can be appreciated between responses obtained before and after gadobenate dimeglumine injection. Gadobutrol, administered at the same dose, on the whole shows the same behavior as gadobenate dimeglumine, although a slight but significant decrease in the P2 latency, a sign of excitatory activity, is observed 2 hours after injection. Analytic data indicates the presence of higher levels of contrast agent in the lesioned hemisphere relative to unlesioned hemispheres 2 hours after injection. In summary, (i) evaluation of somatosensory-evoked potentials in ischemic rats is a useful tool for assessing the potential neurological effects of a new contrast agent because ischemic and contrast agent effects can be clearly differentiated; (ii) investigative strategies applied allow the contrast agents to penetrate into the brain, where their activity can be manifested and evaluated; and (iii) complete absence of neurological activity of gadobenate dimeglumine shows its safety profile and confirms its suitability for use in neurological diseases for which contrast-enhanced MRI is indicated.

Comparative study between gadobenate dimeglumine and gadobutrol in rats with brain ischemia: Evaluation of somatosensory-evoked potentials. Frigeni V, Miragoli L, Grotti And A, Lorusso V. Invest Radiol 2001 Oct; 36 (10), pp. 561–72.

Central Nervous System (CNS)/Lesion Detection: Subtle Intracranial Alterations *Pilocarpine*

Application: Pilocarpine can be used to induced status epilepticus. In this context, pilocarpine can be used as an agent for evaluating the ability of imaging modalities to detect subtle pathology within brain parenchyma, including the identification of (i) edematous brain alterations that follow sustained seizures; (ii) regional hyperperfusion; and (iii) neurodegeneration.

Implementation: Functional MRI (fMRI) analysis of the brain at 12 hours following arrest of pilocarpine-induced status epilepticus maintained over a period of 4 hours duration. Regional cerebral blood volume (rCBV) maps are generated using T_2*-weighted gradient-echo images and an ultrasmall superparamagnetic iron oxide contrast agent. Histopathological data, obtained immediately following MRI analysis, is then correlated with the images through careful evaluation of tissues.

Animal Model: Rat (induction of brain status epilepticus applying pilocarpine).

Interpretation and Analysis: Diffusion-weighted and T_2-weighted imaging shows changes throughout the cerebral cortex, hippocampus, amygdala, and medial thalamus. However, only T_2-weighted imaging, based on rapid acquisition relaxation-enhanced sequences, reveals in the cortex inhomogeneous hyperintensity that is highest in a band corresponding to layer V. In the cortex, rCBV peaks in superficial and deep bands exhibiting a distribution complementary to the highest T_2-weighted intensity. Selective rCBV increases are also detectable within the hippocampus and subcortical structures. In tissue sections, alterations indicative of marked edema are found with Nissl staining in areas corresponding to the highest T_2-weighted intensity. Degenerating neurons, detected by Fluoro-JadeB histochemistry, are concentrated in tissue exhibiting hyperperfusion in rCBV maps, such as hippocampal subfields and dentate gyrus, cortical layers II/III and VI, and medial thalamus. Therefore, (i) T_2-weighted imaging provides a sensitive tool to investigate edematous brain alterations that follow sustained seizures; (ii) rCBV maps reveal regional hyperperfusion; and (iii) rCBV peaks in tissue exhibiting marked neurodegeneration may not be selectively revealed by structural MRI. The findings provide an interpretation of

the brain response to sustained seizures revealed *in vivo* by different strategies of MRI analysis.

Magnetic resonance imaging of changes elicited by status epilepticus in the rat brain: Diffusion-weighted and T_2-weighted images, regional blood volume maps, and direct correlation with tissue and cell damage. Fabene PF, Marzola P, Sbarbati A, Bentivoglio M. Neuroimage 2003 Feb; 18 (2), pp. 375–89.

Central Nervous System (CNS)/Lesions: Intracranial/ Relative Image Quality/Adverse Effects and Safety
Gadoteridol/Gadopentetate Dimeglumine

Application: Detection and characterization of intracranial lesions applying gadoteridol and gadopentetate dimeglumine as MR contrast-enhancing imaging agents.
Dosage: Gadoteridol (0.1 mmol/kg); gadopentetate dimeglumine (0.1 mmol/kg).
Implementation: Utilization of gadoteridol or gadopentetate dimeglumine as positive contrast-enhancing imaging agents for the detection of intracranial lesions applying MR capabilities. The presence of pathology, degree of enhancement, and location and number of lesions, as well as additional information gained, are compared for each subject's unenhanced and enhanced scans for both the gadoteridol and gadopentetate dimeglumine examination. Safety is evaluated in all treated subjects by means of pre- and postdose vital signs along with laboratory tests and monitoring for adverse events.

Human Model: Evaluation by MRI of 80 subjects suspected of suffering from intracranial lesions. Contrast agents are assigned in random order, separated by a 3- to 7-day period between administrations.
Interpretation and Analysis: There is no significant difference in the number of lesions visualized pre- and postcontrast for either gadoteridol or gadopentetate dimeglumine. A high degree of correlation exists between different clinicians evaluating images. When postcontrast image sets are compared between contrast agents, there is no significant difference in superiority of 1 agent over the other ($p > 0.05$). No significant difference for any safety parameter can be appreciated between the 2 agents. In summary, gadoteridol and gadopentetate dimeglumine appear to be effective and well tolerated for use in contrast-enhanced MRI of the CNS at a dose of 0.1 mmol/kg.

Phase III, randomized, double-blind, cross-over comparison of gadoteridol and gadopentetate dimeglumine in magnetic resonance imaging of patients with intracranial lesions. Greco A, Parker JR, Ratcliffe CG, Kirchin MA, McNamara MT. Australas Radiol 2001 Nov; 45 (4), pp. 457–63.

Central Nervous System (CNS)/Meningitis: Idiopathic Hypertrophic Cranial Pachymeningitis/ Corticosteroid Response (± Pulsed Administration)
Gadolinium

Cellular/Molecular Mechanism of Action: Gadolinium is an element that possesses paramagnetic properties.
Background Information: Idiopathic hypertrophic cranial pachymeningitis is a rare disease, of undetermined pathogenesis, that is characterized by inflammation and fibrosis of the dura mater.
Application: Detection and characterization of idiopathic hypertrophic cranial pachymeningitis conditions and their response to therapeutic intervention.
Implementation: Evaluation of idiopathic hypertrophic cranial pachymeningitis conditions to establish clinical presentation, radiological findings, and therapeutic intervention.

Human Model: Cases of idiopathic hypertrophic cranial pachymeningitis ($n = 6$).

Interpretation and Analysis: In cases of idiopathic hypertrophic cranial pachymeningitis, 1 of the main manifestations is cranial nerve palsies and headache (e.g., $n = 6/6$). Three associated conditions are often present, namely optic neuropathy, Tolosa-Hunt syndrome, and diabetes insipidus. Gadolinium-enhanced MRI is of diagnostic benefit and reveals intense dural enhancement in a linear or nodular pattern. Response to corticosteroid therapy is better for cases exhibiting linear, rather than nodular, dural enhancement. In some cases, surgical decompression of the superior orbital fissure provides lasting relief. The course of the disease frequently progresses aaccording to 1 of 3 patterns: (i) sustained remission; (ii) relapse with corticosteroid independence; or (iii) relapse with corticosteroid dependence. Pulse corticosteroid therapy provides significant relief, while reducing the daily corticosteroid requirement and avoiding side effects, for a corticosteroid-dependent relapsing patient. Idiopathic hypertrophic cranial pachymeningitis can progress with a varied clinical course. It is important to prevent irreversible cranial neuropathy during the active phase of the disease, using daily administration of corticosteroids, pulse corticosteroid therapy, or surgical decompression.

Idiopathic hypertrophic cranial pachymeningitis: Clinicoradiological spectrum and therapeutic options. Hatano N, Behari S, Nagatani T, Kimura M, Ooka K, Saito K, Yoshida J. Neurosurgery 1999 Dec; 45 (6), pp. 1336–42; discussion 1342–4.

Central Nervous System (CNS)/Multiple Sclerosis (MS)
Gadolinium (Gd)/[Gd]-DOTA/ Dynamic Susceptibility Contrast (DSC) MR

Background Information: Since the pathogenesis of MS lesions is not yet fully understood, the development of DSC MR perfusion imaging has potential for better characterizing lesion pathology.
Implementation: Utilization of single doses of [Gd]-DOTA contrast agent for the detection and characterization of MS plaque lesions. Magnetic resonance instrumentation (1.5 Tesla scanner) is applied to acquire echoplanar images every 0.5 seconds over a time period of 1 minute. From the signal intensity vs time curves, the relative cerebral blood volume (rCBV) can be evaluated for regions in plaques and in gray and white matter. The rCBV calculated for acute, [Gd]-enhancing plaques is corrected for the effects of blood-brain barrier leakage, using a correction algorithm.

Human Model: Multiple sclerosis patients (e.g., $n = 25$).
Interpretation and Analysis: Acute plaques have significantly higher blood volumes than normal-appearing white matter ($p < = 0.01$). Chronic plaques that appear hypointense on T_1-weighted images have lower rCBV than T_1-isointense plaques ($p \leq 0.03$). Acute phases in MS are accompanied by vasodilation. In the later stages of gliosis, vascular perfusion decreases with increasing axonal injury. Although the DSC technique is less sensitive than conventional MRI, the information provided is essentially different from that obtained with any other MR methods.

Dynamic susceptibility contrast MR imaging of plaque development in multiple sclerosis: Application of an extended blood-brain barrier leakage correction. Haselhorst R, Kappos L, Bilecen D, Scheffler K, Möri D, Radü EW, Seelig J. J Magn Reson Imaging 2000 May; 11 (5), pp. 495–505.

Central Nervous System (CNS)/Multiple Sclerosis (MS)/Active vs Inactive Stage of Disease
Gadodiamide/Single vs Double vs Triple Injection Schemes

Background Information: A triple dose (TD) of gadolinium chelate is a highly sensitive approach for detecting lesion activity in

MS. However, individual TD injections do not provide data on the severity of the pathologic process in a population of lesions, and their clinical use is limited by cost-benefit considerations. One aim of the studies is to determine whether the use of 3 subsequent single doses (SDs) of a gadolinium chelate in brain MRI is useful in detecting MS lesions with different patterns of enhancement.

Application: Detection and characterization of CNS plaque lesions in conditions of MS in order to determine if cases are in the active or inactive stages of disease.

Route of Administration: Gadodiamide (intravascular).

Dosage: Gadodiamide (0.1 mmol/kg).

Implementation: Acquisition of T_1-weighted spin-echo images before and after 3 intravenous injections of gadodiamide in cases of MS.

Human Model: Cases of MS (n = 10).

Interpretation and Analysis: In the vast majority of cases with MS, SD images can detect multiple enhanced lesions. In 1 clinical study, SD gadodiamide was able to facilitate the detection of 6 enhancing plaque lesions in each of 10 MS cases. Double-dose (DD) images detected 13 plaque lesions, and TD images detected 22 plaque lesions. Differences between SD and TD and between DD and TD are usually significant ($p < 0.018$). Approximately (i) 27% of MS plaque lesions are enhanced with all 3 doses (e.g., n = 6); (ii) 32% of MS plaque lesions are enhanced with both DD and TD (n = 7); and (iii) 9 MS plaque lesions enhance with only TD (n = 9). The proportions of cases with at least 1 enhancing lesion are (i) SD n = 4/10; (ii) DD n = 7/10; and (iii) TD n = 9/10. In defining active disease case populations, all that is needed is 19 SD injections, compared to 30 SD injections if a TD administration protocol is implemented. Utilizing 3 subsequent SD injections, the number of enhancing lesions progressively increases. This approach allows the distinction of 3 levels of enhancement, and it reduces the amount of contrast agent needed to distinguish patients with active MS from those with non-active MS.

Three subsequent single doses of gadolinium chelate for brain MR imaging in multiple sclerosis. Sardanelli F, Iozzelli A, Losacco C, Murialdo A, Filippi M. AJNR Am J Neuroradiol 2003 Apr; 24 (4), pp. 658–62.

Central Nervous System (CNS)/Multiple Sclerosis (MS)/Detection of Enhancing Lesions: Magnetic Transfer (±)
Gadoteridol (Gd-HP-DO3A)

Application: Imaging of MS lesions residing within the brain and spinal cord. In this context, Gd-HP-DO3A can function as a 2-compartment paramagnetic contrast agent for MRI.

Dosage: Gadoteridol (Gd-HP-DO3A: 0.1 mmol/kg).

Implementation: Evaluate the possibility of using image subtraction to enhance the detection of MS-associated lesions in brain MR scans with and without magnetic transfer (MT). One approach to achieving this objective involves performing MR brain imaging (1.5 Tesla) with spin-echo T_1-weighted sequences with and without MT, followed by performing a 2nd imaging procedure after the administration of Gd-HP-DO3A. Precontrast and postcontrast images are then digitally recorded. Enhancing lesions are counted on the postcontrast images only (post-Gd), comparing precontrast and postcontrast images by direct visual control (pre-/post-Gd), and on the subtracted images (SI) only.

Human Model: Multiple sclerosis cases (n = 10).

Interpretation and Analysis: Comparisons reveal that the number of enhancing lesions detected post-Gd and pre-/post-Gd is greater with the benefit of MT (e.g., 36 vs 69 and 36 vs 52, respectively). However, the number of enhancing lesions detected with SI is higher in the absence of MT (e.g., 59 vs 50). Significant differences can be appreciated for (i) pre-/post-Gd without MT compared to SI without MT ($p = 0.028$); (ii) pre-/post-Gd without MT compared to pre-/post-Gd with MT ($p = 0.012$); as well as for (iii) pre-/post-Gd with MT compared to post-Gd with MT ($p = 0.028$). Magnetic transfer in combination with pre-/post-Gd allows for the detection of 1.6 enhancing lesions per case more than can be achieved without MT. Subtraction imaging without MT allows the detection of an increased number of enhancing lesions. Conversely, SI with MT does not. Final assessment reveals calculated sensitivity and positive predictive values for (i) no MT, 63 and 94% (post-Gd); (ii) 67 and 100% (pre-/post-Gd); (iii) 96 and 88% (SI); (iv) with MT, 93 and 73% (post-Gd); (v) 96 and 100% (pre-/post-Gd); and (vi) 91 and 98% (SI), respectively. Therefore, SI seems to increase the sensitivity without MT; moreover, it can be used to correct the pseudoenhancement that impairs post-Gd images with MT.

Evaluation of Gd-enhancement in brain MR of multiple sclerosis: Image subtraction with and without magnetization transfer. Sardanelli F, Losacco C, Iozzelli A, Renzetti P, Rosso E, Parodi RC, Bonetti M, Murialdo A. Eur Radiol 2002 Aug; 12 (8), pp. 2077–82. Epub 2002 Feb 05.

Central Nervous System (CNS)/Multiple Sclerosis (MS)/Plaque Formation Monitoring within the CNS
Gadolinium (Example)

Background Information: The time courses for total creatine (Cr), N-acetylaspartate (NAA), choline (Cho), and myo-inositol have not previously been characterized while performing follow-up contrast-enhanced evaluations of MS plaques.

Implementation: Evaluation over a period of 2 years of comparisons for the absolute concentrations of the metabolites Cr, NAA, Cho, and myo-inositol between patients treated with a placebo or 15 ± deoxyspergualin (DSG) and clinical groups with relapsing-remitting or secondary-progressive MS. Diagnostic characterization can involve the application of stimulated-echo acquisition mode sequence (TR/TE = 3,000/20).

Human Model: Deoxyspergualin treatment group (n = 16) compared to normal reference controls (n = 11). The selected cases initially display a contrast-enhancing plaque that is monitored for a 2-year period.

Interpretation and Analysis: In contrast-enhancing plaques, Cho is significantly elevated and shows a significant reduction after both 3 and 12 months. The initially normal Cr significantly increases between 3 and 12 months and is negatively correlated with plaque volume on T_1-weighted MR images. NAA initially shows normal values, a significant decrease at 1 month, and a slow recovery over 2 years. Myo-inositol does not show a clear tendency. Placebo groups do not differ from treatment groups, nor does the relapsing-remitting group differ from the secondary-progressive group. The contradictory time courses of Cr and NAA show that an absolute quantification in proton MR spectroscopy in MS is necessary to avoid a false interpretation of reduced NAA/Cr ratios. The increase in Cr is probably due to remyelination. The initial dip and later recovery of NAA seem to be related to diminishing edema and remyelination.

Serial proton MR spectroscopy of contrast-enhancing multiple sclerosis plaques: Absolute metabolic values over 2 years during a clinical pharmacological study. Mader I, Roser W, Kappos L, Hagberg G, Seelig J, Radue EW, Steinbrich W. AJNR Am J Neuroradiol 2000 Aug; 21 (7), pp. 1220–7.

Central Nervous System (CNS)/Myelogram (Lumbar): T_1 and T_2 Shortening
Iodinated Contrast Media Side-Chain Structure/ Imaging Schedule: < 24 Hours vs 24 Hours vs 48 Hours

Cellular/Molecular Mechanism of Action: Iodinated contrast medias are radiodense elements.
Application: Detection and characterization of anatomical variations within the cerebrospinal fluid (CSF) space surrounding the spinal cord.
Route of Administration: Iodinated contrast media formulations are administered by injection directly into the CSF space surrounding the spinal cord.
Implementation: Lumbar myelography studies applying MRI imaging modalities at completion of contrast agent injection and then at 8 hours and 24 hours postadministration (e.g., n = 20 cases). Measurements are then recorded for the parameters of signal intensities, calculated T_1- and T_2-relaxation times of different iodinated contrast agents, a dilution row of iopamidol, and mixtures of CSF and iotrolan compared with physiological saline solution using different T_1- and T_2-weighted sequences. [^{1}H]-spectroscopy is then performed with several solutions containing iodine or other reagent formulations.
Interpretation and Analysis: A fluid-fluid level of the CSF exists in the lumbar dural sac in the vast majority of cases immediately after lumbar myelography with a non-ionic iodinated contrast agent. Increased signal intensity on T_1-weighted and decreased signal intensity on T_2-weighted sequences can be appreciated for all contrast agents, as well as for the dilution row, compared to physiological saline solution. The structure of the side chains of the contrast agents is responsible for the T_1- and T_2-shortening effect. It is important to be aware of the effect of iodinated contrast agents in MRI. To avoid misinterpretation of atypical findings, MRI of the spine should not be performed earlier than 24 hours after myelography.

Effects of iodinated contrast agents in MR imaging. Hergan K, Doringer W, Langle M, Oser W. Eur J Radiol 1995 Nov; 21 (1), pp. 11–7.

Central Nervous Sytem/Neurophysiology/ Neuroanatomy
Monocrystalline Iron Oxide Nanoparticles

Application: Characterization of primate neurophysiology and neuroanatomy to improve understanding of human neuroimaging modalities.
Implementation: Mapping of functional MRI (fMRI) signals produced by moving stationary stimuli (random → dots or lines).
Animal Model: Primate (fixating monkeys).
Interpretation and Analysis: Functional sensitivity is increased by a factor of approximately 5-fold relative to the blood oxygen level–dependent (BOLD) technique by the injection of monocrystalline iron oxide nanoparticle (MION) as a contrast agent. Areas identified as motion sensitive include V2, V3, MT/V5, vMST, FST, VIP, and FEF (with moving dots), as well as V4, TE, LIP, and PIP (with random lines). These regions sensitive for moving dots are largely in agreement with monkey single unit data and (except for V3A) with human fMRI results. Moving lines activate some regions that have not been previously implicated in motion processing. Overall, there is a defined relationship between a motion pathway and the dorsal stream in primates.

Visual motion processing investigated using contrast agent-enhanced fMRI in awake-behaving monkeys. Vanduffel W, Fize D, Mandeville JB, Nelissen K, Van Hecke P, Rosen BR, Tootell RB, Orban GA. Neuron 2001 Nov 20; 32 (4), pp. 565–77.

Central Nervous System (CNS)/Neurotoxicity: Cytarabine/Acute Leukemia: Childhood Onset/ Cerebral Perfusion/Relative Efficacy of Single Photon Emission Computed Tomography (SPECT) vs MRI
[^{99m}Tc]-Hexamethyl-Propylene-Amine Oxime

Background Information: Cytarabine (ara-C) is 1 of the most effective chemotherapeutic agents in patients with acute leukemia (AL), with a clear dose effect. Use of high-dose ara-C is hampered, however, by a noticeable toxicity, particularly to the CNS.
Application: Assess the usefulness of CNS perfusion imaging with technetium-99m [^{99m}Tc]-hexamethyl-propylene-amine oxime (HMPAO) SPECT concurrent to MRI to specifically assess the effects of standard- and high-dose ara-C in children with AL.
Implementation: Evaluation of cerebral perfusion by [^{99m}Tc]-HMPAO SPECT in cases of childhood AL after induction therapy consisting of a (i) standard-dose ara-C; (ii) immediately after consolidation with high-dose ara-C; and (iii) later during follow-up (range, 6–44 months). The chemotherapy-related adverse events are monitored and correlated to SPECT and MRI.
Human Model: Cases of childhood AL (n = 12; age range = 4–15 years; n = 26 perfusion studies).
Interpretation and Analysis: After the induction phase, all children are neurologically normal on MRI. On SPECT imaging, a number of children (e.g., n = 4/12) display a slightly heterogeneous perfusion pattern. After high-dose ara-C (4–36 g/m^2), slightly more than a third of affected children have regressive neurologic signs of potential toxic origin (n = 5/12), and of these cases, a small percentage will have an abnormal MRI scan (n = 1/5), whereas all cases display evidence of diffuse cerebral and/or cerebellar heterogeneous perfusion on SPECT. Some cases without any neurologic symptoms (e.g., n = 7) and normal MRI scans also display normal SPECT profiles (n = 3/7 normal; n = 4/7 abnormal). On follow-up analysis of cases initially presented with clinical neurological toxicity, (i) a relatively large number display SPECT images that improve (n = 3/4); (ii) a small percentage remain unchanged (e.g., n = 1/4 or 25%); and (iii) some display delayed abnormalities (T_2 white matter hypersignal and cerebellar atrophy) that appear on MRI scans (e.g., n = 2/4). Diffuse heterogeneous brain hypoperfusion is often the sole early objective imaging feature identified by SPECT of high-dose ara-C neurotoxicity, where MRI still demonstrates normal pictures.

Contribution of single-photon emission computed tomography in the diagnosis and follow-up of CNS toxicity of a cytarabine-containing regimen in pediatric leukemia. Véra P, Rohrlich P, Stiévenart JL, Elmaleh M, Duval M, Bonnin F, Bok B, Vilmer E. J Clin Oncol 1999 Sep; 17 (9), pp. 2804–10.

Central Nervous System (CNS)/Neurotoxicity: Kainate-Induced
Paramagnetic Gadolinium (III) Ion: [Gd]-DTPA/ Gadomer-17: Blood-Pool Agent/Polylysine-[Gd]-DTPA: Blood-Pool Agent

Application: Detection and characterization of toxin-induced CNS lesions applying MRI instrumentation.
Implementation: Utilization of MRI in combination with paramagnetic gadolinium^{+3} ion ([Gd]-DTPA) and prototype agents for blood-pool enhancement (Gadomer-17 and polylysine-[Gd]-DTPA) to detect lesions in the CNS. Magnetic resonance is performed with

T_1-weighted spin-echo sequence before and after the administration of kainite and contrast media.

Animal Model: Rat (kainite infused intravenously to induce CNS lesions).

Interpretation and Analysis: Enhancement of lesion rims and other brain tissue abnormalities due to kainite with [Gd]-DTPA, Gadomer-17, and polylysine-[Gd]-DTPA can be observed mainly in the region of hippocampus and in the areas not protected by the blood-brain barrier.

Assessment of kainite toxicity using contrast-enhanced magnetic resonance imaging. Beravs K, Frangez R, Demsar F. Pflugers Arch 2000; 440 (5 Suppl), pp. R153–4.

Central Nervous System (CNS)/Neurotransmitter Dynamics/Relative Cerebral Blood Flow/Drug Abuse/Schizophrenia/Epilepsy/Neurodegeneration
Superparamagnetic Iron Oxide/Amphetamine/Cocaine Analog 2β-Carbomethoxy-3b-(4-Fluorophenyl)Tropane (CFT)

Background Information: The use of functional MRI (fMRI) techniques for evaluating pharmacological stimuli has great potential for understanding neurotransmitter dynamics pertaining to a number of brain disorders, such as drug abuse, schizophrenia, epilepsy, and neurodegeneration. Unfortunately, blood oxygenation level–dependent (BOLD) imaging at common fields strengths, such as 1.5 or 3 Tesla, has very low sensitivity and low contrast-to-noise ratios (CNRs).

Application: Detection and characterization of alterations in neurotransmitter dynamics and relative cerebral blood flow for various conditions affecting the CNS, including drug abuse, schizophrenia, epilepsy, and neurodegeneration.

Route of Administration: Intravascular.

Implementation: Evaluation of the ability for a superparamagnetic iron oxide contrast agent with a long plasma half-life to detect hemodynamic changes related to dopaminergic stimuli using amphetamine or the cocaine analog CFT as provocative diagnostic agents. The technique is sometimes referred to as increased relaxation with iron oxide nanoparticles (IRON).

Interpretation and Analysis: Results indicate that even at field strengths as high as 4.7 Tesla, it is possible to obtain increases in CNR by factors of 2 to 3 over BOLD imaging, which can provide a greater than 1 order of magnitude increase in statistical power with greatly increased sensitivity to hemodynamic changes in brain regions difficult to observe using BOLD imaging. Furthermore, use of the intravascular contrast agent allows for a meaningful physiologic parameter to be measured (relative cerebral blood volume [rCBV]), in contrast to conventional BOLD imaging.

Improved mapping of pharmacologically induced neuronal activation using the IRON technique with superparamagnetic blood pool agents. Chen YC, Mandeville JB, Nguyen TV, Talele A, Cavagna F, Jenkins BG. J Magn Reson Imaging 2001 Nov; 14 (5), pp. 517–24.

Central Nervous System (CNS)/Sarcoidosis/ Optimization of High-Dose Corticosteroid Therapy/ Differentiation of Reversible and Irreversible CNS Lesions/Prevention of Irreversible CNS Damage
Gadolinium

Cellular/Molecular Mechanism of Action: Gadolinium possesses paramagnetic properties.

Background Information: Corticosteroids are applied in the management of CNS-associated sarcoidosis lesions.

Application: Detection and characterization of sarcoidosis affecting the CNS in a manner that can be used to optimize decision making related to responses to therapeutic intervention.

Implementation: Evaluate and document changes observed utilizing sequential MRI for sarcoidosis lesions located within the CNS and determine their response to corticosteroid therapy (mean follow-up period = 36 months). Results are compared before and after therapeutic periods (n = 75) divided into attack (high-dose), upkeep (decreased-dose), and minimal (low-dose) periods. Parenchymal lesions are classified as Type 1 (enhanced with gadolinium), Type 2 (demyelinating), or Type 3 (lacunar), and are assessed (classified) as regressing, stable, or progressing.

Human Model: Cases of sarcoidosis affecting the CNS (n = 24).

Interpretation and Analysis: Slightly greater than 33% of cases have several types of lesions (e.g., n = 7/24). An absence of neurologic defect is only recognized with isolated Type 3 lesions (e.g., n = 6 cases). In approximately half of affected cases with Type I the lesions regress in essentially all attack periods (e.g., n = 13/24 cases; n = 22/22 attack periods), but progress in approximately one-third of upkeep and minimal periods (n = 9/27). Type 2 (n = 7 cases) and Type 3 (n = 13 cases) lesions remain stable in essentially all therapeutic periods (n = 68/68). Type 1 lesions appear in a small number of cases with Type 2 and Type 3 lesions during 2 upkeep and 3 minimal periods (e.g., n = 3 patients). Findings observed on follow-up MRI analysis can function as a basis for (i) reintroducing high-dose corticosteroid therapy (e.g., n = 8 cases); (ii) differentiation between reversible and irreversible lesions in CNS sarcoidosis; and (iii) a reference parameter for adjusting treatment to prevent irreversible CNS damage.

Central nervous system sarcoidosis: Follow-up at MR imaging during steroid therapy. Dumas JL, Valeyre D, Chapelon-Abric C, Belin C, Piette JC, Tandjaoui-Lambiotte H, Brauner M, Goldlust D. Radiology 2000 Feb; 214 (2), pp. 411–20.

Central Nervous System (CNS)/Somatosensory Stimulation Mapping
Manganese Chloride ($MnCL_2$)/Activity-Induced Manganese (Mn^{+2})-Dependent Contrast MRI/Dynamic Activity-Induced Mn^{+2}-Dependent Contrast MRI

Cellular/Molecular Mechanism of Action: In the brain, $MnCl_2$ accumulates in the active region(s) by entering active cells through voltage-gated Ca^{+2} channels.

Background Information: Activity-induced manganese-dependent contrast (AIM) MRI is a hemodynamic-independent functional MRI method that uses manganese ion as an MR-detectable contrast agent.

Application: Activity-induced manganese-dependent contrast MRI of the CNS.

Route of Administration: The agent $MnCl_2$ is infused intra-arterially after modification of the functionality of the blood-brain barrier (BBB) with a hyperosmolar agent.

Implementation: Development of a dynamic activity-induced manganese-dependent (DAIM) paradigm that utilizes sequential MR scans during $MnCl_2$ infusion, prior to and following functional brain stimulation. Time-course analysis can be used to produce stimulation-specific functional maps within the brain. The methodology can be applied to test reactions to glutamate administration and electric stimulation (e.g., rat forepaw).

Interpretation and Analysis: Functional stimulation of the brain facilitates Mn^{+2} accumulation in active region(s) by entering active cells through voltage-gated Ca^{+2} channels, causing local signal increases in T_1-weighted images. The contrast of AIM MRI depends strongly on the depth of anesthesia, and the low levels used in somatosensory stimulation studies can lead to significant non-specific accumulation of manganese ion throughout the brain. Dynamic activity-induced manganese-dependent contrast MRI maps are better confined to the specific region of brain activated by somatosensory stimulation compared to AIM MRI.

Dynamic activity-induced manganese-dependent contrast magnetic resonance imaging (DAIM MRI). Aoki I, Tanaka C, Takegami T, Ebisu T, Umeda M, Fukunaga M, Fukuda K, Silva AC, Koretsky AP, Naruse S. Magn Reson Med 2002 Dec; 48 (6), pp. 927–33.

Central Nervous System (CNS)/Stroke: Acute Vascular/Bolus Tracking MRI

Bolus tracking MRI is a powerful technique for measuring perfusion and is playing an increasing role in the investigation of acute stroke. However, limitations have been reported when assessing patients with steno-occlusive disease. The presence of a steno-occlusive disease in the artery may cause bolus dispersion, which has been shown to introduce significant errors in cerebral blood flow (CBF) quantification. Bolus dispersion is commonly described by a vascular transport function, but the function that properly characterizes the dispersion is unknown. Relevant methods have been described to quantify bolus dispersion errors on perfusion measurements. A realistic patient-specific model can be constructed from anatomical and physiologic MR data, and the arterial blood flow pattern and the transport of the bolus of contrast agent are computed using finite element analysis. Methodologies can also be used to evaluate the accuracy of 3 simple vascular models. A methodology has already been tested on MR data from 2 normal subjects and 2 subjects with mild carotid artery stenosis. The estimated CBF errors are on the order of 15 to 20%. However, the presence of stenosis does not necessarily introduce larger dispersion (not only the geometrical model but also the particular physiologic conditions influence the degree of bolus dispersion). The method described will contribute to a better understanding of errors introduced by dispersion effects, to the assessment and validation of vascular models, and to the development of new methods for the correction of dispersion errors in CBF quantification.

Estimation of bolus dispersion effects in perfusion MRI using image-based computational fluid dynamics. Calamante F, Yim PJ, Cebral JR. Neuroimage 2003 Jun; 19 (2 Pt 1), pp. 341–53.

Central Nervous System (CNS)/Stroke: Vascular/Postcontrast Diffusion-Weighted Images
Gadopentetate Dimeglumine

Background Information: Diffusion-weighted imaging (DWI) is usually done before administration of intravenous contrast agents. Repetition of DWI is occasionally necessary after administration, but the effects of contrast material on DWI and apparent diffusion coefficient (ADC) values have not yet been fully examined.
Application: Detection and characterization of vascular anomalies within the CNS.
Route of Administration: Gadopentetate dimeglumine (intravenous).
Dosage: Gadopentetate dimeglumine (0.1 mmol per 1 kg body weight).
Implementation: Determine if the administration of gadolinium-based contrast material significantly affects DWI and ADC values. In this context, diffusion-weighted images are acquired for conditions of CNS vascular stroke. Scan images are acquired at the acute or subacute stages of infarct from 3 hours to 5 days after onset of symptoms, applying gadopentetate dimeglumine as an injectable contrast image-enhancing agent. Diffusion-weighted images are obtained using single-shot echo-planar imaging with a "b" value of 1,000 s/mm^2. The signal-to-noise ratios are then examined for normal regions of the brain and infarct lesions, in addition to delineation of the contrast-to-noise ratio for each lesion. Other parameters measured include the comparison of the ADC values calculated from DWI before and after administration of contrast. The statistical significance of differences between precontrast and postcontrast administration are determined by use of a paired t test.
Human Model: Cases of vascular stroke affecting the CNS (e.g., n = 39; mean age = 67.9 years; range = 34–87 years).
Interpretation and Analysis: The signal-to-noise and contrast-to-noise ratio of the diffusion-weighted images is not significantly different before and after administration of contrast agent. The ADC values are slightly lower after administration of contrast agent for both normal brain ($p = 0.0011$) and infarcts ($p = 0.038$). The estimated differences in the ADC values are approximately 1.3% and 3.5% for normal brain and infarcts, respectively. The lack of a significant difference between the signal-to-noise and contrast-to-noise ratios of diffusion-weighted images before and after administration of contrast agent indicates the feasibility of postcontrast DWI.

Effect of intravenous gadolinium-DTPA on diffusion-weighted images: Evaluation of normal brain and infarcts. Yamada K, Kubota H, Kizu O, Nakamura H, Ito H, Yuen S, Tanaka O, Kubota T, Makino M, Van Cauteren M, Nishimura T. Stroke 2002 Jul; 33 (7), pp. 1799–802.

Central Nervous System (CNS)/Vocal Centers (High) of the CNS/Nucleus Robustus Archistriatalis/Area X
Manganese (Mn^{+2})/Calcium Analogue

Cellular/Molecular Mechanism of Action: Manganese and calcium analogs have their spatial orientation altered when subjected to magnetic energy fields.
Application: Manganese can be applied as a tracing agent for CNS paramagnetic tract applicable for MRI methodologies.
Route of Administration: Manganese and calcium analogs are injected into the high vocal center of the CNS.
Implementation: Central nervous imaging performed by the injection of Mn^{+2} and a calcium analog into the high vocal center of starlings.
Animal Model: Avian/starling (high vocal center injection).
Interpretation and Analysis: Injection of Mn^{+2} and a calcium analog into the high vocal center of starlings within a few hours labels the nucleus robustus archistriatalis and area X as observed by *in vivo* MRI. Structures highlighted by Mn^{+2} accumulation assume the expected tridimensional shape of the nucleus robustus archistriatalis and area X as identified by classical histological or neurochemical methods. The volume of these CNS nuclei can be accurately calculated by segmentation of the areas highlighted by Mn^{+2}. Besides confirming previously established volumetric sex differences, Mn^{+2} uptake into these nuclei reveals new functional sex differences affecting Mn^{+2} transport. A faster transport is present in males than in females and different relative amounts of Mn^{+2} are transported to the nucleus robustus archistriatalis and area X in males compared to females. Such an *in vivo* approach allows for the acquisition of repeated measures, opens alternative opportunities for

studying seasonal plasticity in size and activity of song-control nuclei, and correlates neuronal activity with behavior. It also provides greater insight into the *in vivo* axonal transport and neuronal activity in song-control nuclei of oscines.

In vivo manganese-enhanced magnetic resonance imaging reveals connections and functional properties of the songbird vocal control system. Van der Linden A, Verhoye M, Van Meir V, Tindemans I, Eens M, Absil P, Balthazart J. Neuroscience 2002; 112 (2), pp. 467–74.

Central Nervous System (CNS) Behavior: Awake-Behaving Function/Regional Cerebral Blood Volume/Primary Visual Cortex
Magnetite Dextran Nanoparticles

Cellular/Molecular Mechanism of Action: Magnetite dextran nanoparticles are passively transported throughout the intravascular compartment and their location within the body can be detectable by MRI instrumentation.
Background Information: Functional MRI (fMRI) in awake-behaving primates is an emerging tool for bridging the gap between human fMRI and neurophysiology information from non-human primates.
Application: Blood-pool agent to enhance fMRI contrast-to-noise (CNR) in mammalian fMRI analyses.
Route of Administration: Intravascular.
Implementation: Evaluation of behavioral changes as a function of various physiological parameters associated with the CNS, including regional cerebral blood flow and volume.
Animal Model: Primate (e.g., macaque).
Interpretation and Analysis: The intravascular half-life of magnetite dextran is long compared to lanthanide chelates ($T_{1/2}$ = 198 minutes) with shortened T_2 relaxation observed in blood and the cerebral cortex. Greater than 3-fold enhancement in the percent change in MR signal can be observed using nanoparticles (13%) compared with conventional BOLD fMRI (4%). The calculated regional cerebral blood volume in primary visual cortex increases 32% with photic stimulation. The increased CNR allows greater flexibility in the design of awake-behaving primate fMRI studies with the potential for improvements in resolution and significantly shortened imaging times.

Enhancing fMRI contrast in awake-behaving primates using intravascular magnetite dextran nanoparticles. Dubowitz DJ, Bernheim KA, Chen DY, Bradley WG Jr, Andersen RA. Neuroreport 2001 Aug 8; 12 (11), pp. 2335–40.

Neoplasia (Central Nervous System [CNS]): Glioma/Microglial Cell Detection and Characterization
Ultrasmall Superparamagnetic Particles of Iron Oxide (USPIOs)

Cellular/Molecular Mechanism of Action: Ultrasmall superparamagnetic particles of iron oxide are perceived as foreign material and internalized (phagocytized) by microglial cell populations residing within the CNS.
Background Information: Microglia are intrinsic immunoeffector cells of the CNS that play a very sensitive, crucial role in the response to almost any brain pathology. Upon stimulation, they are transformed into a state of elevated phagocytic activity.
Application: Reveal the extent of microglial tumors and assess the status of immunologic defense processes.
Implementation: Evaluate the presence and distribution of activated microglial cells residing within the CNS utilizing USPIO preparations in combination with MRI instrumentation.
Animal Model: Glioma cell cultures and rat glioma *in vivo* model.
Interpretation and Analysis: Using USPIOs in cell culture experiments and in a rat glioma model, microglia can be labeled magnetically and detected by confocal microscopy within and around tumors in a typical border-like pattern. Quantitative *in vitro* studies reveal that microglia cells internalize amounts of USPIOs that are significantly higher than those incorporated by tumor cells and astrocytes. Labeled microglia can be detected and quantified with MRI in cell phantoms, and the extent of the tumor can be seen *in vivo* in glioma-bearing rats. Magnetic labeling of microglia provides a potential tool for MRI of gliomas that more precisely reflects tumor morphology. Furthermore, the results suggest that MRI may yield functional data on the immunologic reaction within the CNS.

Magnetic labeling of activated microglia in experimental gliomas. Fleige G, Nolte C, Synowitz M, Seeberger F, Kettenmann H, Zimmer C. Neoplasia 2001 Nov–Dec; 3 (6), pp. 489–99.

Neoplasia (Central Nervous System [CNS]): Gliomas
Contrast-Enhanced Dynamic Susceptibility MRI

Background Information: Abnormalities in the recirculation phase of the passage of a contrast agent bolus have been identified in tumors and have been proposed to represent vascular tortuosity and hypoperfusion in areas of angiogenic neovascularization.
Application: Detection and characterization (grading) of the microcirculation profiles of gliomas within the CNS as a function of vascular tortuosity and regions of hypoperfusion.
Implementation: Characterization of glioma lesions by contrast-enhanced dynamic susceptibility MRI. Residual relaxivity effects are minimized by the injection of contrast agent before the acquisition of dynamic images. Maps of relative cerebral blood volume (rCBV) and relative recirculation (rR) are calculated, and values from enhancing tumor tissue compared with tumor grade.
Human Model: Human glioma cases (e.g., n = 27).
Interpretation and Analysis: Histologic grade categories include grade II-astrocytoma (n = 3); grade III-anaplastic astrocytoma (n = 10); and grade IV-glioblastoma multiforme (n = 14). Relative cerebral blood volume values vary among tumor grades, with higher mean values associated with higher grade tumors ($p < 0.001$). Mean rR values in grade II tumors are not significantly different from those in normal gray and white matter. Mean rR values in grades III and IV tumors are similar and significantly higher than those in grade II tumors ($p < 0.01$). The distribution of the pixel values of rR reveals significant differences between grades III and IV tumors ($p < 0.001$), with low values of skewness in keeping with a normal distribution in grade III tumors and higher values in grade IV tumors. Variation in the recirculation characteristics of a contrast agent bolus is related to tumor grade in gliomas. Abnormalities in contrast agent recirculation appear to provide independent information concerning the microcirculation in imaging studies of angiogenesis and may be of value as surrogate markers in trials of antiangiogenic therapy.

Abnormalities in the recirculation phase of contrast agent bolus passage in cerebral gliomas: Comparison with relative blood volume and tumor grade. Jackson A, Kassner A, Annesley-Williams D, Reid H, Zhu XP, Li KL. AJNR Am J Neuroradiol 2002 Jan; 23 (1), pp. 7–14.

Neoplasia (Central Nervous System [CNS]): Glioma of Murine Origin
Blood-Pool Contrast Agents: MP-2269 and MS-325/ Extracellular Contrast Agent: OptiMARK

Cellular/Molecular Mechanism of Action: MP-2269 and MS-325 are 2 small molecular weight serum albumin-binding blood-pool contrast agents. OptiMARK functions as a compound that remains in extracellular fluid compartments with essentially no intracellular penetration.
Application: Intravenous MRI contrast agents are commonly used to improve the detection of intracranial tumors and other CNS lesions for diagnosis and treatment planning.
Route of Administration: MP-2269 and MS-325 (intravenous), and OptiMARK (intravenous).
Dosage: MP-2269 and MS-325 (30 μmol/kg: small molecular weight, albumin-binding blood-pool contrast agents). OptiMARK (100 μmol/kg: extracellular imaging agent).
Implementation: Detection of CNS neoplastic lesions (glioma) with the small molecular weight blood-pool contrast agents MP-2269 and MS-325 compared to the extracellular contrast agent OptiMARK, utilizing 1.5 Tesla electromagnetic resonance imaging analysis.
Animal Model: Mouse glioma.
Interpretation and Analysis: Tumor image contrast is significantly enhanced and long-lived following administration of MP-2269 and MS-325 blood-pool agents. More specifically, contrast enhancement peaks slowly at 25 to 30 minutes following administration, remains constant for $>$ 3 hours, and returns to baseline within 20 hours. Comparable but "transient" enhancement can be achieved using an extracellular contrast agent (OptiMARK), which demonstrates rapid peaks in contrast enhancement at 2 to 5 minutes following administration and then declines over a 40-minute period. The blood-pool contrast agents demonstrate an approximate 3-fold increase in dose effectiveness and a lengthened window of tumor contrast enhancement compared to commonly available extracellular contrast agents. This demonstrates the potential of alternative contrast-enhanced MRI examination protocols for tumor detection.

Albumin-binding MR blood-pool agents as MRI contrast agents in an intracranial mouse glioma model. Adzamli K, Yablonskiy DA, Chicoine MR, Won EK, Galen KP, Zahner MC, Woolsey TA, Ackerman JJ. Magn Reson Med 2003 Mar; 49 (3), pp. 586–90.

Neoplasia (Central Nervous System [CNS]): Gliosarcoma
Rhodaminated Long-Circulating Dextran-Coated Iron Oxide (LCDIO)/Hoechst 33258 Dye/Green Fluorescent Protein

Cellular/Molecular Mechanism of Action: Hoechst 33258 dye can be applied as an intravital endothelial stain. Rhodaminated LCDIO particles can preferentially localize intracellularly within certain malignant neoplastic cell types (e.g., gliosarcoma).
Application: Detection and characterization of CNS neoplastic lesions (e.g., gliosarcoma).
Route of Administration: Intravascular.
Implementation: Determination of the distribution of an LCDIO preparation in tumors. Accumulation of LCDIO in tissue sections is evaluated using multichannel fluorescence microscopy with rhodaminated LCDIO, green fluorescent protein as a tumor marker, and Hoechst 33258 dye as an intravital endothelial stain. Uptake into tumor cells is correlated (validated) with results from immunohistochemical and cell culture uptake analyses. The effect of intratumoral LCDIO uptake on MRI signal intensity is evaluated with a 1.5 Tesla superconducting magnet.
Animal Model: Rodent gliosarcoma.
Interpretation and Analysis: Tumoral accumulation of LCDIO is 0.11% $\pm$ 0.06 of the injected dose per gram of tissue in brain tumors and sufficient for detection using MRI. In tumor sections, LCDIO preferentially localizes in tumor cells (49.0% $\pm$ 4.6) but is also taken up by macrophages in tumors (21.0% $\pm$ 3.1) and by endothelial cells in the areas of active angiogenesis (6.5% $\pm$ 1.4). In cell culture, LCDIO uptake strongly correlates with growth rate of tumor cell lines. Tumoral LCDIO accumulation is not negligible and helps explain MRI signal intensity changes observed in clinical trials. Microscopically, LCDIO accumulates predominantly in tumor cells and tumor-associated macrophages. Uptake into tumor cells appears to be directly proportional to cellular proliferation rate.
Instrumentation: Magnetic resonance imaging signal intensity evaluated with a 1.5 Tesla superconducting magnet.

Tumoral distribution of long-circulating dextran-coated iron oxide nanoparticles in a rodent model. Moore A, Marecos E, Bogdanov A Jr, Weissleder R. Radiology 2000 Feb; 214 (2), pp. 568–74.

Neoplasia (Central Nervous System [CNS]): Medulloblastoma
Gadolinium-Based Agent

Background Information: Experimental mouse models are emerging as useful systems for the study of human brain tumors. Nuclear MRI methods can non-invasively provide images of complex heterogeneous tissues such as experimental brain tumors.
Route of Administration: Gadolinium-based agents (intravenous).
Implementation: Demonstration of the feasibility of longitudinal high-resolution MRI in detecting and characterizing brain tumors.
Animal Model: Two mouse brain tumor models: patched heterozygous (ptc $\pm$) mice with spontaneously arising posterior fossa tumors that resemble human medulloblastoma, and homozygous nude mice implanted with intracerebral xenografts of human medulloblastoma cell lines.
Interpretation and Analysis: Methods have been optimized to achieve favorable volumetric comparison with histologic methods and submillimeter resolution, improved by contrast enhancement with intravenous administration of a gadolinium-based agent.

Magnetic resonance imaging of patched heterozygous and xenografted mouse brain tumors. Nelson AL, Algon SA, Munasinghe J, Graves O, Goumnerova L, Burstein D, Pomeroy SL, Kim JY. J Neurooncol 2003 May; 62 (3), pp. 259–67.

Neoplastic Disease (Central Nervous System [CNS])/Angiogenesis of Gliomas/Dimethylarginine Dimethylaminohydrolase (DDAH) Overexpression
NC100150/Hoechst 33342

Cellular/Molecular Mechanism of Action: The orientation of NC100150 becomes altered within a magnetic field. Alternatively, Hoechst 33342 emits fluorescent light energy.
Background Information: Intracellular factors that regulate nitric oxide (NO) synthesis represent important targets in tumor progression. Overexpression of DDAH, which metabolizes the endogenous inhibitors of NO synthesis asymmetric dimethylarginine and

N-monomethyl-L-arginine, results in C6 gliomas with enhanced growth rates compared to wild cell types.

Application: NC100150 can be utilized as an intravascular blood-pool contrast agent that is detectable by MRI methodologies. Hoechst 33342 can be used as a fluorescent perfusion-marker dye that can be measured by flourescent detection systems.

Route of Administration: Utilization of NC100150 and Hoechst 33342 for neoplastic lesion angiogenesis requires that these be delivered intravascularly.

Implementation: Determine the (i) *in vitro* effects of DDAH on tumor vascular morphogenesis by measurement of the transverse relaxation rates R_2* and R_2 in clone D27 gliomas overexpressing DDAH and C6 wild-type gliomas using intrinsic susceptibility MRI sensitive to changes in endogenous deoxyhemoglobin; (ii) determine susceptibility contrast-enhanced MRI using NC100150 as an intravascular blood-pool contrast agent; and (iii) compare results with fluorescence microscopy of the tumor uptake for the perfusion marker Hoechst 33342.

Interpretation and Analysis: The baseline R_2* is significantly faster in D27 tumors, consistent with a greater vascular development ($p < 0.02$, ANOVA). There is no significant difference between the response of the 2 tumor types to hypercapnia (5% CO_2/95% air), used as a model for vascular maturation, or hyperoxia (5% CO_2/95% O_2), used as a model for vascular function. NC100150 increases the R_2* and R_2 rates of both tumor types and detects a significantly larger blood volume in the D27 tumors ($p < 0.02$, ANOVA). This correlates with a significantly greater uptake of Hoechst 33342 in the D27 tumors compared to C6 wild-type tumors ($p < 0.02$, ANOVA). Despite increased tumor blood volume, the Delta R_2*/Delta R_2 ratio, an index of microvessel size, shows that the capillaries in the 2 tumor types are of a similar diameter. Such observations demonstrate the potential of susceptibility MRI-derived quantitative end points to non-invasively assess tumor angiogenesis. Application of intravascular blood-pool contrast agents like NC100150 for such purposes appears very promising. Overexpression of DDAH results in increased *in vivo* neovascularization of C6 gliomas. The lack of significant difference in hypercapnic/hyperoxic responses between C6 and D27 tumors and the similar vessel caliber are also consistent with a role for DDAH in the initial stages of vasculogenesis.

Instrumentation: Magnetic resonance imaging and fluorescence microscopy instrumentation.

Effects of overexpression of dimethylarginine dimethylaminohydrolase on tumor angiogenesis assessed by susceptibility magnetic resonance imaging. Kostourou V, Robinson SP, Whitley GS, Griffiths JR. Cancer Res 2003 Aug 15; 63 (16), pp. 4960–6.

Neoplastic Disease/Brain Tumor Diagnostic and Therapeutic Implications
Gadobenate Dimeglumine ([Gd]-BOPTA)

Background Information: Gadobenate dimeglumine has a significantly greater [Gd] uptake by brain tumor tissue than gadopentate dimeglumine ([Gd]-DTPA).

Application: The agent [Gd]-BOPTA is assimilated at relatively high concentrations within certain neoplastic lesions that affect the central nervous system.

Route of Administration: Gadobenate dimeglumine and [Gd]-DTPA (intralesionally and subcutaneous in rat research investigations).

Dosage: Gadobenate dimeglumine and [Gd]-DTPA (0.05 mmol/g tumor weight).

Implementation: Evaluate whether [Gd]-BOPTA is an efficient agent for neutron capture therapy (NCT). Delineation of the imaging properties of [Gd]-BOPTA and [Gd]-DTPA injected intralesionally into tumors. At the peak of [Gd] uptake, thermal neutron irradiation is applied.

Animal Model: Rat (Fisher 344 strain). Four groups of Fisher 344 rats (control, neutron (n), n+ [Gd]-DTPA, n+ [Gd]-BOPTA) injected subcutaneously with 9L gliosarcoma cells in both hind legs.

Interpretation and Analysis: A pronounced delay in tumor growth can be appreciated with [Gd+] formulations compared to control and neutron analyses ($p = 0.0053$, 0.0064, respectively). Furthermore, BOPTA is associated with a significantly prolonged delay of tumor growth compared to DTPA ($p = 0.033$). In addition, [Gd]-NCT demonstrates that [Gd]-BOPTA can function as an effective compound for NCT. Better cytocidal effects of [Gd]-BOPTA warrant further investigation of subcellular [Gd] distribution.

In vivo gadolinium neutron capture therapy using a potentially effective compound ([Gd]-BOPTA). Matsumura A, Zhang T, Yamamoto T, Yoshida F, Sakurai Y, Shimojo N, Nose T. Anticancer Res 2003 May–Jun; 23 (3B), pp. 2451–6.

CENTRAL NERVOUS SYSTEM: SPECIAL SENSES

Special Senses/Cochlear Noise-Related Injury/Damage
GdDTPA-BMA

Cellular/Molecular Mechanism of Action: The element gadolinium (Gd) is a paramagnetic ion that has its physical (special) orientation altered when subjected to magnetic energy fields.

Application: Detection and characterization of normal and noise-damaged cochlea using GdDTPA-BMA in a manner that can facilitate its application as a T_1 contrast agent for MRI modalities.

Route of Administration: GdDTPA-BMA (intravenous).

Implementation: The time course and distribution of the *in vivo* uptake of the gadodiamide chelate bound paramagnetic [Gd] ion (GdDTPA-BMA) throughout the membranous labyrinth of normal and impulse noise-damaged guinea pig cochleas are measured by MRI (4.7 Tesla).

Interpretation and Analysis: Simultaneous signal enhancement of the basal, medial, and apical scala tympani (ST) and scala vestibuli (SV) can be observed within 10 minutes following injection, with maximal levels reached around 100 minutes. Evaluation by ANOVA and post hoc paired t tests reveals statistically significant differences in the levels and rates of [Gd] uptake-enhancement between the scalae. The basal, medial, and apical ST reveal the most rapid and extensive enhancement occurs throughout the period of active [Gd] uptake, while the SV show comparatively slower and less enhancement, and the intact scala media (SM) indicate insignificant enhancement. The *in vivo* penetration of [Gd] and enhancement of the membranous SM increases significantly in the noise-damaged cochlea, suggesting lesioning of the cochlear membranes.

Magnetic resonance imaging of the membranous labyrinth during *in vivo* gadolinium (GdDTPA-BMA) uptake in the normal and lesioned cochlea. Counter SA, Bjelke B, Borg E, Klason T, Chen Z, Duan ML. Neuroreport 2000 Dec 18; 11 (18), pp. 3979–83.

GASTROINTESTINAL SYSTEM

Abdominal Imaging/Oral Contrast Agents
Intravenous Contrast Agent

Cross-sectional imaging of the abdomen in oncology patients presents unique challenges and opportunities. A close working relationship between the oncologist and radiologist is essential for the

exchange of the clinical and imaging information necessary for optimizing patient diagnosis and management. Compared to helical computed tomography (CT), MRI of the abdomen and pelvis offers important advantages, including superior soft-tissue contrast. The multiplanar capabilities of MRI allow for direct coronal or sagittal imaging, providing a truer anatomic presentation of abdominal and pelvic masses. Recent advances in MRI, including the use of intravenous and oral contrast agents, the development of high-performance imagers, and improved surface coil designs, facilitate more rapid abdominal imaging with superior image quality. All of these features combine to produce a versatile imaging examination with exquisite sensitivity for depicting abdominal and pelvic tumors. In this context, they have clinical applicability for hepatic and extrahepatic abdominal MRI in the oncology patient. Techniques and protocols for MRI can be applied to most commercially available high-field MRI instrumentation.

Magnetic resonance imaging of the abdomen: Applications in the oncology patient. Low RN. Oncology (Huntingt) 2000 Jun; 14 (6 Suppl 3), pp. 5–14.

Intestinal/Rectal Carcinoma/Tumor Staging/ Comparison of Sonography with MRI
Gadolinium/Superparamagnetic Iron Oxide

Application: Superparamagnetic iron oxide and gadolinium can be applied in MRI of conditions of rectal cancer, especially in conditions involving proximal and stenotic lesions.
Implementation: Evaluate conditions of rectal cancer that have undergone transrectal sonography performed with a 10-MHz endoanal probe and MRI (1.0 Tesla or 1.5 Tesla) using a whole-body coil. After rectal administration of a superparamagnetic iron oxide MR contrast agent, T_1- and T_2-weighted images and gadolinium-enhanced double-contrast images are obtained. The results of examinations are then compared with the histology of resected specimens.

Human Model: Rectal carcinoma (e.g., n = 39; n = 19/39 females, n = 20 males).
Interpretation and Analysis: Histopathology analyses can detect T_1, T_2, T_3, and T_4 stage tumors (e.g., n = 4 stage-T_1, n = 11 stage-T_2, n = 18 stage-T_3, n = 6 stage-T_4 tumors) using the tumor-node-metastasis (TNM) staging system. Nodal metastases can be seen in a large percentage of cases (e.g., n = 16/39). Transrectal sonography cannot be performed in all conditions due to various factors, including "high" (proximal) tumor location (e.g., n = 11/36). In cases where transrectal sonography is feasible (e.g., n = 28/39), the accuracy of image detection for T-stage conditions is approximately 64% overall (patients not receiving radiation, 69%; patients receiving radiation, 60%) and 70% for N-stage tumors. Conversely, double-contrast MRI can correctly identify T-stage tumors with an accuracy of 64% overall (e.g., n = 39; patients not receiving radiation, 75%; patients receiving radiation, 53%) and N-stage with a 62% level of accuracy. The assessment of rectal wall penetration (Dukes' classification A vs B) predicts sensitivity, specificity, and accuracy levels of 93%, 71%, and 82%, respectively, for transrectal sonography and 100%, 60%, and 85% for MRI. In scenarios where it is technically feasible, transrectal sonography is an accurate method for staging rectal cancer. In proximal or stenotic tumors, double-contrast MRI is the method of choice. Diagnostic accuracy of transrectal sonography and MRI is high for predicting bowel wall penetration.

Comparison of transrectal sonography and double-contrast MR imaging when staging rectal cancer. Fuchsjager MH, Maier AG, Schima W, Zebedin E, Herbst F, Mittlbock M, Wrba F, Lechner GL. AJR Am J Roentgenol 2003 Aug; 181 (2), pp. 421–7.

GENETIC

Gene Mapping Model: *In Vivo* β-Galactosidase Expression
Galactopyranose Functional Group/Chelated Paramagnetic Ion

Cellular/Molecular Mechanism of Action: Galactopyranose can function as a substrate for the indicator (marker) gene β-galactosidase.
Background Information: High-resolution *in vivo* imaging of gene expression is not possible applying existing techniques.
Application: High-resolution *in vivo* MRI imaging of "reporter" gene expression.
Semi-Synthetic Methods: Preparation of an MRI contrast agent in which the access of water to the 1st coordination sphere of a chelated paramagnetic ion is blocked with a substrate that can be removed by enzymatic cleavage. Following cleavage, the paramagnetic ion can interact directly with water protons to increase the MR signal.
Implementation: Detection and characterization of *in vivo* gene expression.
Interpretation and Analysis: Galactopyranose can function as an effective blocking group that can render an MRI contrast agent sensitive to detecting the expression of β-galactosidase (marker gene). Pertaining to cellular resolution, regions of higher intensity in MR images correlate with regions expressing the marker enzyme. These results offer the promise of *in vivo* mapping of gene expression in transgenic animals and validate a general approach for constructing a family of MRI contrast agents that respond to biological activity.

In vivo visualization of gene expression using magnetic resonance imaging. Louie AY, Huber MM, Ahrens ET, Rothbacher U, Moats R, Jacobs RE, Fraser SE, Meade TJ. Nat Biotechnol 2000 Mar; 18 (3), pp. 321–5.

HEMATOLOGY

Hematopoietic Progenitor Cell Migration
Hematopoietic Progenitor Cells/Magnetic Polysaccharide Nanoparticles-Transferrin/ Gadopentetate Dimeglumine Liposomes/P7228 Liposomes/Ferumoxides/Ferumoxtran

Application: Labeling of human hematopoietic progenitor cells with various MRI contrast-enhancing agents to facilitate acquisition of images at 1.5 Tesla.
Route of Administration: Intravascular.
Semi-Synthetic Methods: Labeling of hematopoietic progenitor cells with ferumoxides, ferumoxtran, magnetic polysaccharide nanoparticles-transferrin, P7228 liposomes, and gadopentetate dimeglumine liposomes.
Implementation: Evaluation of the organ/tissue biodistribution of labeled hematopoietic progenitor cells using MRI. Data can be analyzed by measuring MR signal intensities and R1 and R2* relaxation rates of labeled cells and non-labeled reference control cells. Mean quantitative data for the various contrast agents are assessed to detect significant differences compared to reference control cells by means of the Scheffe statistical analysis model. As a reference standard, MRI data can be compared with electron microscopic and spectrometric data.

Interpretation and Analysis: For all contrast agents, the intracellular assimilation into the cytoplasm can be demonstrated with electron microscopy and quantified by spectrometry. Compared with non-labeled control cells, progenitor cells labeled with iron oxides show significantly ($p < 0.05$) increased R2*. Cells labeled with gadopentetate dimeglumine liposomes show significantly increased R1. Detection thresholds are approximately 5×10^5 cells for gadopentetate dimeglumine liposomes and ferumoxtran, 2.5×10^5 cells for ferumoxides and P7228 liposomes, and 1×10^5 cells for magnetic polysaccharide nanoparticles-transferrin. Therefore, hematopoietic progenitor cells can be labeled with MR contrast agents and their detection is possible with a standard 1.5 Tesla MR imager.

Instrumentation: Magnetic resonance imaging with T_1- and T_2-weighted spin-echo and fast field-echo sequences.

Targeting of hematopoietic progenitor cells with MR contrast agents. Daldrup-Link HE, Rudelius M, Oostendorp RA, Settles M, Piontek G, Metz S, Rosenbrock H, Keller U, Heinzmann U, Rummeny EJ, Schlegel J, Link TM. Radiology 2003 Sep; 228 (3), pp. 760–7.

HEPATIC SYSTEM

Hepatic/Backdiffusion Phenomenon of Hepatic Contrast Agent
Diethyl Iminodiacetic Analogues (Diethyl IDA)/ Mebrofenin

Background Information: The kinetics of organic anions are well described and backdiffusion from hepatocyte to plasma is an accepted phenomenon. Although IDAs, as organic anions, should also show bidirectional transport between hepatocyte and plasma, this has not been directly demonstrated.

Application: Hepatic contrast-image-enhancing agent for MRI modalities.

Route of Administration: Diethyl IDAs (intravascular); mebrofenin (intravascular).

Implementation: Evaluate backdiffusion characteristics of hepatic contrast agents into the intravascular compartment and quantify the corresponding fractional rate constant. Kinetics of diethyl IDA can be characterized through the analysis of femoral artery and hepatic venous samples collected following the injection of tracer preparations into a (i) peripheral vein, or (ii) hepatic artery or portal vein. Arterial time-concentration curves can also be compared between peripheral venous and either hepatic arterial or portal venous injections (canine). Complementary findings can be obtained by generating time-activity curves recorded from regions of interest (ROIs) over the cardiac blood-pool and peripheral hepatic parenchyma during routine IDA hepatobiliary imaging with diethyl IDA or mebrofenin and fractional rate constants for clearance of IDA from the hepatocyte compared to compartmental and deconvolution analyses (e.g., $n = 30$ cases).

Animal Model: Anesthetized dogs (e.g., $n = 3$).

Human Model: Hepatobiliary imaging with diethyl IDA ($n = 30$ cases).

Interpretation and Analysis: Following peripheral injection, there is an early arteriovenous concentration gradient that develops across the liver indicating a hepatocyte extraction fraction of 0.9 (dog #1), 0.8 (dog #2), and 0.6 (dog #3). The net extraction fraction decreases exponentially over a 40-minute period. Time-concentration curves for the hepatic vein and femoral artery are virtually superimposed following intrahepatic injections. Peripheral arterial curves, however, have different shapes according to whether injections are delivered intrahepatically or peripherally, and are consistent with the occurrence of significant backdiffusion. In human clinical cases, the blood disappearance curves can be fitted as the sum of 2 exponentials and the liver curves as the difference of 2 exponentials (rate constants = alpha1h and alpha2h). Based on compartmental analysis of the blood curves, the sum of the fractional rate constants of tracer movement from hepatocyte to bile canaliculus (k_{32}) and to plasma (k_{12}) is similar to and correlates with the rate constant, alpha, for the hepatocyte impulse response function ($r = 0.62$, $n = 30$, $p < 0.001$). In contrast, alpha1h and alpha2h are respectively clearly greater and smaller than alpha. Moreover, neither of these hepatic rate constants correlates with alpha. Diffusion of IDA from hepatocyte into the blood is significant and even in the presence of normal liver function accounts for about 50% of IDA transport out of the hepatocyte. It should be taken into account in pharmacokinetic studies based on either compartmental or deconvolution analysis.

Bidirectional transport of iminodiacetic organic anion analogues between plasma and hepatocyte. Peters AM, Myers MJ, Mohammadtaghi S, Mubashar M, Mathie RT. Eur J Nucl Med 1998 Jul; 25 (7), pp. 766–73.

Hepatic/Cholestasis/Tumor Contrast Enhancement Pattern/Invasiveness and Degree of Malignancy: Peritumor Rim Sign
Mangafodipir Trisodium/Manganese Dipyridoxal Diphosphate/Gadoxetic Acid/Gadolinium Ethoxybenzyl Diethylenetriaminopentaacetic Acid/Gadolinium Butylbenzyl Diethylenetriaminepentaacetic Acid

Background Information: Because of the demand to extend the range of diagnostic uses for currently available extracellular MRI contrast agents, a new generation of tissue-specific (i.e., hepatobiliary) contrast agents has been successfully developed to enhance the performance of hepatic MRI. These agents include mangafodipir trisodium (formerly manganese dipyridoxal diphosphate [Mn-DPDP]); gadoxetic acid (formerly gadolinium ethoxybenzyl diethylenetriaminopentaacetic acid—[Gd]-EOB-DTPA); and gadolinium butylbenzyl diethylenetriaminepentaacetic acid (MS-264).

Implementation: Delineation of the imaging behaviors of (i) mangafodipir trisodium (formerly Mn-DPDP); (ii) gadoxetic acid (formerly [Gd]-EOB-DTPA); and (iii) MS-264, for different types of cholestasis and focal liver lesions when applied in combination with MRI modalities. In addition, comparisons are made between agents to identify their similarities or dissimilarities in their possible mechanisms of action and the clinical implications of such observations.

Interpretation and Analysis: The intravenous administration of (i) mangafodipir trisodium (formerly Mn-DPDP); (ii) gadoxetic acid (formerly [Gd]-EOB-DTPA); and (iii) MS-264 can facilitate the identification of both global and local obstructive cholestases in postcontrast images. Liver tumors of different orgins and grades of cellular differentiation can be detected and classified according to their contrast enhancement patterns appreciated in certain postcontrast phases. The invasiveness or degree of malignancy of a given hepatic tumor type can also be presumed as a function of the presence or absence of a peritumoral rim pattern. Such diagnostic information, otherwise only invasively achievable, may prove to be invaluable in clinical decision making.

Enhanced magnetic resonance imaging for tissue characterization of liver abnormalities with hepatobiliary contrast agents: An overview of preclinical animal experiments. Ni Y, Marchal G. Top Magn Reson Imaging 1998 Jun; 9 (3), pp. 183–95.

Hepatic/Hepatocyte Mechanisms of Gadolinium Uptake
Gadolinium [Gd] Complexes/[Gd]-DTPA/ [Gd]-EOB-DTPA/[Gd]-BOPTA/[Gd]-B 20790

Application: Gadolinium complexes can be used as contrast image-enhancing agents for MRI of hepatic structures.
Implementation: Evaluation of the hepatic transport of different gadolinium complexes through delineation of the uptake/assimilation of [Gd]-DTPA, [Gd]-BOPTA, [Gd]-EOB-DTPA, and [Gd]-B 20790 by basolateral rat hepatocyte plasma membrane vesicles. Complementary investigations entail assessment of the rate and extent of gadolinium agent uptake by *Xenopus laevis* oocytes in response to the injection of 5 ng of human OATP cRNA.
Interpretation and Analysis: In basolateral rat hepatocyte plasma membrane vesicles, [Gd]-DTPA uptake is indistinguishable from non-specific binding to vesicles; [Gd]-BOPTA and [Gd]-EOB-DTPA enter plasma membrane vesicles according to a linear, concentration-dependent mechanism up to 1.5 mM of substrate. By contrast, [Gd]-B 20790 uptake follows a saturative kinetic with an apparent Km of 92 $\pm$ 15 μM and a Vmax of 143 $\pm$ 42 pmol/mg prot/15 seconds, and can occur in an osmotic-sensitive space. Sulfobromophthalein and taurocholate, but not unconjugated bilirubin, inhibit the uptake rate of [Gd]-B 20790 but not that of [Gd]-DTPA, [Gd]-BOPTA, or [Gd]-EOB-DTPA.

Injection into *Xenopus laevis* oocytes of 5 ng of human OATP cRNA after 3 days results in a $\geq$ 2-fold stimulation ($p < 0.001$) of transport of [Gd]-B 20790 but not of [Gd]-BOPTA or [Gd]-EOB-DTPA. Hepatic uptake of the MRI contrast agent [Gd]-B 20790 is a carrier-mediated mechanism operated by OATP, while MRI compounds with other chemical structures enter the hepatocyte by other mechanisms.

Molecular mechanisms for the hepatic uptake of magnetic resonance imaging contrast agents. Pascolo L, Cupelli F, Anelli PL, Lorusso V, Visigalli M, Uggeri F, Tiribelli C. Biochem Biophys Res Commun 1999 Apr 21; 257 (3), pp. 746–52.

Hepatic/Hepatocyte Mechanisms of Manganese (Mn^{+2}) Uptake
Manganese Dipyridoxyl Diphosphate ([Mn]-DPDP)/ Manganese Chloride ($MnCl_2$)

Cellular/Molecular Mechanism of Action: A fraction of free paramagnetic Mn^{+2} ion penetrates the cell membrane of hepatocytes through Ca^{+2} channels and then strongly interacts with molecular ATP.
Implementation: Spectroscopic comparisons of the mechanisms of the hepatic uptake for $MnCl_2$ and [Mn]-DPDP. Alterations in the phosphorus-31 [^{31}P]-NMR spectrum for intracellular adenosine 5′-triphosphate (ATP) can be used to monitor internalization of manganese by hepatic tissues.
***Ex Vivo* Model:** Isolated and perfused rat liver.
Interpretation and Analysis: Delivery of Mn^{+2} as $MnCl_2$ in the perfusate rapidly enters hepatocytes, where it strongly interacts with ATP and induces a broadening of the [^{31}P] lines. Nifedipine inhibition of the process confirms the concept that manganese ions cross the cellular membrane at least partly through Ca^{+2} channels. The agent [Mn]-DPDP induces weaker but still significant changes in the ATP spectrum. The inability of pyridoxine to compete for the uptake of manganese confirms that the vitamin B_6 carrier is not involved in the internalization process of the paramagnetic complex. Lastly, preincubation of [Mn]-DPDP with blood does not increase the extent of dissociation. The alterations of the [^{31}P] spectrum in the liver subsequent to the administration of [Mn]-DPDP are attributable to a fraction of free Mn^{+2} released by the chelate and delivered to the hepatocytes.

Dynamic evaluation of the hepatic uptake and clearance of manganese-based MRI contrast agents: A ^{31}P NMR study on the isolated and perfused rat liver. Colet JM, Vander Elst L, Muller RN. J Magn Reson Imaging 1998 May–Jun; 8(3), pp. 663–9.

Hepatic/Portal and Hepatic Vein Selective Imaging
DAB-Am64-(1B4M-Gd)(64)/PAMAM-G4D-(1B4M-Gd)(64)

Cellular/Molecular Mechanism of Action: Hydrophobic molecules reportedly accumulate in the liver more extensively than do hydrophilic compounds.
Background Information: A generation-4 polypropylenimine diaminobutane dendrimer (e.g., DAB-Am64) should theoretically be more hydrophobic than a generation-4 polyamidoamine dendrimer (e.g., PAMAM-G4D). Systemically administered hydrophobic molecules reportedly accumulate extensively within the liver and thus are potentially useful as liver MRI contrast agents.
Semi-Synthetic Methods: A generation-4 polypropylenimine diaminobutane dendrimer (DAB-Am64) is used to synthesize a conjugate with 2-(*p*-isothiocyanatobenzyl)-6-methyl-diethylenetriaminepentaacetic acid (1B4M) [DAB-Am64-(1B4M-Gd)(64)] for the purpose of complexing with Gd(III) ions.
Animal Model: Murine hepatic intravascular image preparations.
Implementation: Evaluation of the relative ability of the DAB conjugate, DAB-Am64-(1B4M-Gd)(64), to accumulate within the liver in addition to other characteristics. Parameters are then compared to those for the PAMAM conjugate PAMAM-G4D-(1B4M-Gd)(64), which is known to be a useful vascular MRI contrast agent, in regard to its availability as a liver MRI contrast agent.
Interpretation and Analysis: This DAB conjugate, DAB-Am64-(1B4M-Gd)(64), quickly accumulates within the liver. DAB-Am64-(1B4M-Gd)(64) accumulates to a significantly greater extent in the liver and less in the intravascular blood pool than does PAMAM-G4D-(1B4M-Gd)(64) ($p < 0.001$). Contrast-enhanced MRI with DAB-Am64-(1B4M-Gd)(64) is able to homogeneously enhance liver parenchyma and visualize both portal and hepatic veins of 0.5 mm diameter in mice. In conclusion, DAB-Am64-(1B4M-Gd)(64) is a good candidate as a liver MRI contrast agent.

Novel liver macromolecular MR contrast agent with a polypropylenimine diaminobutyl dendrimer core: Comparison to the vascular MR contrast agent with the polyamidoamine dendrimer core. Kobayashi H, Kawamoto S, Saga T, Sato N, Hiraga A, Ishimori T, Akita Y, Mamede MH, Konishi J, Togashi K, Brechbiel MW. Magn Reson Med 2001 Oct; 46 (4), pp. 795–802.

Hepatic Adenocarcinoma (VX-2 Metastatic Model)/ Gallbladder Imaging/Hepatocellular Clearance
[Gd]-HP-DO3A/[Gd]-2,5-BPA-DO3A

Application: Detection and characterization of gallbladder abnormalities and conditions of adenocarcinoma.
Route of Administration: The contrast image enhancement agents [Gd]-2,5-BPA-DO3A (15d) and [Gd]-HP-DO3A are both delivered by intravascular injection.
Implementation: Evaluate the effect of substituent lipophilicity, substituent position, and overall charge on the hepatobiliary clearance

and tolerance of a series of aromatic ring-containing macrocyclic [Gd] chelates for the purpose of selecting a candidate compound for evaluation as a hepatobiliary imaging agent. One approach to achieving this objective can involve delineation of tissue distribution patterns (mice) and T_1-weighted pulse sequences or T_1-weighted breath-hold pulse sequences (cats, rabbits, rhesus monkeys) using [Gd]-2,5-BPA-DO3A (15d) and [Gd]-HP-DO3A as a reference control agent.

Animal Model: Cats, rabbits, rat, and rhesus monkey (rabbit: VX-2 adenocarcinoma tranplant model for metastatic liver disease).

Interpretation and Analysis: Each compound is excreted bimodally. [Gd]-2,5-BPA-DO3A (15d) possesses an optimal combination of hepatobiliary clearance (47% in rats, 29% in mice) and tolerance (minimum lethal dose 5.0 mmol/kg). Initial imaging studies demonstrate the feasibility of [Gd]-2,5-BPA-DO3A for hepatic imaging (cat). In VX-2 adenocarcinoma implants, [Gd]-2,5-BPA-DO3A provides sustained hepatic signal intensity (SI) enhancement and lesion conspicuity over a 120-minute imaging time course (rabbit metastatic liver disease model). In normal liver function, sustained hepatic SI enhancement and a time-dependent increase in gallbladder SI over the entire 90-minute imaging time course can be afforded by [Gd]-2,5-BPA-DO3A (rhesus monkey). Dramatic and sustained SI enhancement of hepatic tissue is observed with [Gd]-2,5-BPA-DO3A that is superior in all respects to the extracellular space MRI agent, [Gd]-HP-DO3A, employed as a control (cats, rabbits, rhesus monkey).

Synthesis, characterization, and imaging performance of a new class of macrocyclic hepatobiliary MR contrast agents. Marinelli ER, Neubeck R, Song B, Wagler T, Ranganathan RS, Sukumaran K, Wedeking PW, Nunn A, Runge VM, Tweedle MF. Invest Radiol 2000 Jan; 35 (1), pp. 8–24.

Hepatic Free Radical Production/Hepatic Vein and Inferior Vena Cava Nitric Oxide (NO) Synthase and NO/Endotoxin Shock Model
N-Methyl-D-Glucamine Dithiocarbamate $(MGD)_2$-Fe^{+2}-NO Complex

Cellular/Molecular Mechanism of Action: $(MGD)_2$-Fe^{+2}-NO complexes bind to and function as a competitive inhibitor of nitric oxide synthetase.

Application: Detection of free radical species such as those produced at elevated levels within the liver during conditions of septic inflammation (e.g., endotoxemia/endotoxic shock).

Semi-Synthetic Methods: Nitric oxide is complexed with the Fe^{+2}-chelate spin trap, MGD, and the resulting product $(MGD)_2$-Fe^{+2}-NO is verified by EPR.

Implementation: Evaluation of the distribution of molecular radical formation visualized by MRI applying the agent $(MGD)_2$-Fe^{+2}-NO. Nitric oxide synthase can be verified as the source of NO in a septic shock protocol by the preadministration of the competitive inhibitor N-monomethyl-L-arginine.

Animal Model: Rat endotoxic shock protocol.

Interpretation and Analysis: Complexes of $(MGD)_2$-Fe^{+2}-NO accumulate in the liver (rats) and provide significant contrast-enhanced profiles for vascular structures including the hepatic vein and inferior vena cava. Preadministration with N-monomethyl-L-arginine (NO competitive inhibitor of NO synthase) results in reduced hepatic $(MGD)_2$-Fe^{+2}-NO complex enhancement. The NO complex is more stable *in vivo* and a more effective MRI contrast agent than other stable nitrogen-containing radicals (e.g., nitroxides). The MRI spin-trapping method should be a powerful tool for visualizing spatial distributions of free radicals in pathologic organs and tissues when combined with the appropriate radical complexing agent, such as $(MGD)_2$-Fe^{+2} preparations.

In vivo imaging of spin-trapped nitric oxide in rats with septic shock: MRI spin trapping. Fujii H, Wan X, Zhong J, Berliner LJ, Yoshikawa K. Magn Reson Med 1999 Aug; 42 (2), pp. 235–9.

Hepatic and Pancreatic Lesions
Mangafodipir Trisodium

Background Information: The detection and characterization of pancreatic tumors as well as the reliable staging of pancreatic cancer are important clinical radiographic objectives. Contrast-enhanced helical computed tomography (CT) has been the standard modality for pancreatic imaging at many institutions, but MRI has gained a considerable role in the evaluation of patients with equivocal CT findings.

Application: Detection and characterization of disease states affecting the liver and pancreas, including neoplastic conditions employing MRI analysis.

Interpretation and Analysis: Contrast-enhanced MRI has proven to be very helpful in detecting small tumors or the identification of tumor-like lesions in patients with equivocal CT findings. Mangafodopir may improve the staging of pancreatic cancer by increasing the sensitivity of MRI in the detection of liver metastases. Reviews have been published that summarize the potential of contrast-enhanced MRI and its limitations compared with contrast-enhanced helical CT.

Evaluation of focal pancreatic masses: Comparison of mangafodipir-enhanced MR imaging and contrast-enhanced helical CT. Schima W, Fugger R. Eur Radiol 2002 Dec; 12 (12), pp. 2998–3008. Epub 2002 Jul 12.

Hepatic Imaging/Renal Accumulation/Blood Retention/Liver Accumulation/Intratumor Fine Vessel Resolution/Influence of Molecular Size
Macromolecular Polyamidoamine Dendrimer Cores/ G6D-(1B4M-Gd)(256)/G7D-(1B4M-Gd)(512)/ G8D-(1B4M-Gd)(1024)/G9D-(1B4M-Gd)(2048)

Background Information: Pharmacokinetic characteristics of intravascular macromolecular MRI contrast agents with polyamidoamine dendrimer cores smaller than generation-7 have previously been described in the literature.

Application: Detection and characteriation of hepatic pathology.

Route of Administration: Intravascular.

Semi-Synthetic Methods: The MRI contrast agents GxD-(1B4M-Gd)(2(x+2)) synthesized with generation-7, -8, and -9 polyamidoamine dendrimers and 2-(*p*-isothiocyanatobenzyl)-6-methyl-diethylenetriaminepentaacetic acid (1B4M).

Implementation: Evaluate the effects of greater hepatic uptake on the pharmacokinetics of the larger generation dendrimers [e.g., GxD-(1B4M-Gd)(2(x+2)) synthesized with generation-7, -8, and -9 polyamidoamine dendrimers and 2-(*p*-isothiocyanatobenzyl)-6-methyl-diethylenetriaminepentaacetic acid (1B4M)].

Animal Model: Murine (pharmacokinetic).

Interpretation and Analysis: Delineation of the pharmacokinetic characteristics of G6D-(1B4M-Gd)(256) reveals that in biodistribution and dynamic micro-MRI studies, significantly less renal accumulation of G7D-(1B4M-Gd)(512), G8D-(1B4M-Gd)(1024), and G9D-(1B4M-Gd)(2048) can be appreciated compared to G6D-(1B4M-Gd)(256) ($p < 0.01$). There is a significantly greater accumulation of G8D-(1B4M-Gd)(1024) and G9D-(1B4M-Gd)(2048) in

the liver compared to G6D-(1B4M-Gd)(256) and G7D-(1B4M-Gd)(512) ($p < 0.01$). The highest blood retention of all dendrimer-based MRI contrast agents is exhibited by G7D-(1B4M-Gd)(512) ($p < 0.01$). The normal and intratumoral fine vessels of approximately 100-μm diameter can be visualized in normal or tumor-bearing mice by high resolution 3-dimensional-micro-MR angiographs with G7D-(1B4M-Gd)(512) and G8D-(1B4M-Gd)(1024) with good vessel-to-soft tissue contrast. Therefore, increased accumulation in the liver with concomitant decreased uptake in the kidney is caused by increased molecular sizes of the dendrimer-based MRI contrast agents.

Micro-MR angiography of normal and intratumoral vessels in mice using dedicated intravascular MR contrast agents with high generation of polyamidoamine dendrimer core: Reference to pharmacokinetic properties of dendrimer-based MR contrast agents. Kobayashi H, Kawamoto S, Saga T, Sato N, Hiraga A, Konishi J, Togashi K, Brechbiel MW. J Magn Reson Imaging 2001 Dec; 14 (6), pp. 705–13.

Hepatic Imaging/Selective Visualization
$K_9GdW_{10}O_{36}/K_{11}[Gd(PW_{11}O_{39})_2]$

Cellular/Molecular Mechanism of Action: The basis for the liver-specific properties of $K_9GdW_{10}O_{36}$ and $K_{11}[Gd(PW_{11}O_{39})_2]$ have not been definitively established.

Application: Liver-specific MRI contrast-enhancing agent. But further refinement of $K_9GdW_{10}O_{36}$ and $K_{11}[Gd(PW_{11}O_{39})_2]$ is required in order to diminish their degree of toxicity.

Route of Administration: $K_9GdW_{10}O_{36}$ and $K_{11}[Gd(PW_{11}O_{39})_2]$ are given intravenously.

Implementation: Evaluation of the 2 gadolinium polyoxometalates, $K_9GdW_{10}O_{36}$ and $K_{11}[Gd(PW_{11}O_{39})_2]$, in both *in vitro* and *in vivo* environments to determine their potential as candidates for tissue-specific MRI contrast agents.

Animal Model: Rat (Sprague-Dawley).

Interpretation and Analysis: T_1-relaxivities of 6.89 mM/s for $K_9GdW_{10}O_{36}$ and 5.27 mM/s for $K_{11}[Gd(PW_{11}O_{39})_2]$ are slightly higher than that of the commercial MRI contrast agent ([Gd]-DTPA). Both compounds bind to bovine serum albumin and human serum transferrin. In Sprague-Dawley rats, the agents display favorable liver-specific contrast enhancement when evaluated by MRI methodologies. Imaging studies demonstrate that both agents have a long residence time, showing MR signal enhancement in the liver for more than 40 minutes, which is longer than many commercially available contrast agents. Results from *in vivo* and *in vitro* assays reveal that GdW_{10} and $Gd(PW_{11})_2$ are promising liver-specific MRI contrast agents and GdW_{10} may be used in the diagnosis of pathological states. However, with the higher acute toxicity, the 2 gadolinium polyoxometalates need to be modified and studied further before clinical use.

An evaluation of gadolinium polyoxometalates as possible MRI contrast agent. Feng J, Li X, Pei F, Sun G, Zhang X, Liu M. Magn Reson Imaging 2002 Jun; 20 (5), pp. 407–12.

Hepatic Macrophages/Mononuclear Phagocytic System Activity
Superparamagnetic Iron Oxide (AMI-25)/[^{99m}Tc]-Phytate

Cellular/Molecular Mechanism of Action: Superparamagnetic iron oxide (AMI-25) is non-selectively assimilated intracellularly by the hepatic mononuclear phagocytic system.

Application: Assessment of hepatic phagocytic activity with potential applications for also evaluating the pulmonary phagocytic system.

Route of Administration: Intravascular.

Implementation: Evaluation of the potential for measuring hepatic phagocytic activity by dynamic superparamagnetic iron oxide-enhanced MRI. One approach that can be utilized for achieving this objective includes the application of dynamic MRI analysis in concert with performing a radioisotope study with [^{99m}Tc]-phytate. In a dynamic MR study of this nature, the ratio (Rv) of the distribution volumes of iron oxide (Vm/Ve) can be obtained by applying a 3-compartment model to data obtained simultaneously from the kidney and liver. Changes in Rv caused by silica injection and by dosing superparamagnetic iron oxide, AMI-25, can subsequently be evaluated. In the dynamic MR study using an *in vivo* model the input function can be calculated from data obtained from the hepatic artery and portal vein.

Animal Model: Rabbits (New Zealand White) randomly allocated to normal and silica-treated groups.

Animal Model: Beagle dog (hepatic artery and portal vein).

Interpretation and Analysis: The ratio of the distribution volumes for iron oxide (Vm/Ve) reaches a maximum value at around 80 minutes after AMI-25 injection. The Rv of the normal controls groups is approximately 5.06 ± 1.53, whereas the Rv of the silica-treated group is approximately 2.13 ± 1.20. The results are similar to tissue count data obtained in radioisotope study. The Rv value is not dependent on the injected dose of AMI-25. The rate of transport constants (k1, k2, k3) cannot be estimated with a 3-compartment model regardless of obtaining the input function. It has therefore been concluded that the ratio of the distribution volumes for iron oxide (Vm/Ve) may be a quantitative index reflecting decreases in phagocytic activity within the liver as determined by dynamic superparamagnetic iron oxide–enhanced MRI.

Pharmacokinetic modeling of phagocytic activity of the liver using superparamagnetic iron oxide nanoparticles in dynamic MR imaging. Na JB, Suh JS, Huh YM, Kim SJ, Kim SH, Cha SH, Lee SH. Yonsei Med J 2003 Jun 30; 44 (3), pp. 429–37.

Hepatic Necrosis/Reperfusion Infarct
Gadophrin-2/MP-2229

Cellular/Molecular Mechanism of Action: The mechanisms of action leading to specific localization of necrosis-avid contrast agents (NACAs) such as gadophrin-2 are not well defined. It has been suggested recently that agents with a high degree of serum albumin binding may also serve as NACAs by virtue of non-specific hydrophobic interactions. Both MP-2269 and gadophrin-2 possess albumin-binding avidities and MP-2269 can function as a blood-pool agent.

Application: Gadophrin can be applied to detect and characterize regions of infarction, including artificially-induced reperfused hepatic infarcts that can be recognized by MRI analysis. The agent MP-2269 can function as an albumin-binding blood-pool agent.

Route of Administration: MP-2269 (intravenous); gadophrin-2 (intravenous).

Dosage: MP-2269 (0.05 mmol/kg); gadophrin-2 (0.05 mmol/kg).

Implementation: Evaluation of hepatic reperfusion infarcts with serial T_1-weighted MRI performed before and after administration of MP-2269. The imaging procedure is repeated again 24 hours after intravenous injection of gadophrin-2 at the same dosage (0.05 mmol/kg). Individual MR images are matched with corresponding histomorphological findings. The signal intensity and contrast ratio

of infarcted and normal hepatic lobes are quantified and compared between the 2 agents during the postcontrast phase.

Animal Model: Surgical induction of reperfused infarcts in the right liver lobe of rats (e.g., n = 6).

Interpretation and Analysis: Before contrast, the infarcted lobe is indiscernible from normal liver on T_1-weighted MRI. Shortly after injection of both MP-2269 and gadophrin-2, a negative contrast pattern develops between infarcted and normal liver because of a strong liver signal intensity enhancement and an inferior uptake within necrotic liver tissues. During delayed phase analysis (> 60 minutes), a necrosis-specific contrast enhancement (contrast ratio 1.6) develops with gadophrin-2 but not with MP-2269. The MR images closely correspond with histomorphological findings. Although both MP-2269 and gadophrin-2 possess binding avidity for serum albumin fractions, only gadophrin-2 displays a persistent necrosis-specific contrast enhancement in the rat model of reperfused liver infarction. The role of albumin binding in the mechanisms of NACAs should be reevaluated.

MRI contrast enhancement of necrosis by MP-2269 and gadophrin-2 in a rat model of liver infarction. Ni Y, Adzamli K, Miao Y, Cresens E, Yu J, Periasamy MP, Adams MD, Marchal G. Invest Radiol 2001 Feb; 36 (2), pp. 97–103.

Hepatic Neoplasia

Contrast-Enhancing Agents: [Mn]-DPDP and Ferumoxides/Reticuloendothelial System–Specific Agents: Iron Oxide Particulates/Combined Perfusion/Hepatocyte-Selective: Gadolinium ([Gd])-BOPTA and [Gd]-EOB-DTPA/Computed Tomography during Arterial Portography (CTAP)

Background Information: Optimal detection of focal hepatic lesions in patients with metastases can alter patient management and result in significant cost savings by reducing the number of unnecessary laparotomies for unresectable disease. Comparisons of reticuloendothelial agents and hepatobiliary agents for imaging liver metastases are lacking in the literature. Further studies comparing MRI enhanced with liver-specific contrast agents to CTAP are needed to determine if hepatic MRI can replace CTAP for the preoperative evaluation of hepatic metastases.

Considerations: Most clinical experience is with non-specific extracellular gadolinium chelates. The relatively low cost, safety, good patient tolerance, and ability to help detect and characterize a wide range of liver diseases have greatly contributed to the common use of gadolinium chelates as image-enhancing agents.

Application: A wide variety of MRI agents can be applied for the detection and characterization of hepatic lesions in conditions of hepatic metastatic disease. Examples include contrast agents (ferumoxides & [Mn]-DPDP); non-specific extracellular fluid image contrast agents (gadolinium chelates), reticuloendothelial agents, CTAP, and hepatocellular agents ([Gd]-BOPTA, [Gd]-EOB-DTPA, [Gd]-DTPA).

Route of Administration: Essentially all of the diagnostic image-enhancing agents applied in the detection and characterization of hepatic metastatic disease are delivered by intravascular injection.

Interpretation and Analysis: Liver-specific MRI contrast agents (reticuloendothelial and hepatobiliary agents) offer greater lesion-to-liver contrast on T_2-weighted MR images than the conventional extracellular fluid space MRI contrast agents (gadolinium chelates), which have a non-specific distribution. In effect, this increases the conspicuity of focal hepatic lesions with negligible reticuloendothelial cells (e.g., metastases). Hepatocyte-selective agents increase the signal intensity of background liver on T_1-weighted images, which increases the conspicuity of focal lesions that do not contain hepatocytes (e.g., metastases). For the detection of hepatic metastases, although the work of Seneterre et al suggests that the accuracy of ferumoxide-enhanced MRI is equivalent to that of CTAP, other studies have found CTAP to be superior. For the characterization of focal liver lesions, [Mn]-DPDP and ferumoxides have been added to the small list of FDA-approved contrast agents, and both can help to increase diagnostic specificity. Two of the hepatobiliary agents that are not yet approved, [Gd]-BOPTA and [Gd]-EOB-DTPA, have the potential of characterizing liver lesions during dynamic contrast enhancement (similar to [Gd]-DTPA) and during the hepatocyte phase (similar to [Mn]-DPDP), and may increase the detection of focal liver lesions.

Contrast agents for MR imaging of the liver. Semelka RC, Helmberger TK. Radiology 2001 Jan; 218 (1), pp. 27–38.

MR imaging in the evaluation of hepatic metastases. Imam K, Bluemke DA. Magn Reson Imaging Clin N Am 2000 Nov; 8 (4), pp. 741–56.

Hepatic Neoplasia/Differentiation Neoplasia vs Parenchyma/Hepatic Lesions and Central Nervous System (CNS) Neoplasia

Gadobenate Dimeglumine ([Gd]-BOPTA)/[Gd]-DTPA

Cellular/Molecular Mechanism of Action: The dimeglumine component of the paramagnetic contrast agent [Gd]-BOPTA is partially eliminated by biliary excretion.

Background Information: After the advent of extracellular contrast media, hepatobiliary-specific gadolinium chelates were developed to improve the diagnostic value of MRI of the liver.

Application: Gadobenate dimeglumine can be used as a contrast image-enhancing agent for improved visualization of the liver and CNS (e.g., brain). In this latter capacity, [Gd]-BOPTA can aid in the detection and characterization of neoplastic lesions affecting the CNS.

Route of Administration: Gadobenate dimeglumine is administered by intravascular injection.

Implementation: Gadobenate dimeglumine through partial biliary excretion can produce prolonged visual enhancement of liver parenchyma on T_1-weighted images.

Interpretation and Analysis: [Gd]-BOPTA shows better MRI enhancement in brain tumors than can be provided by [Gd]-DTPA at every time course up until 2 hours after administration, and no enhancement in peritumoral tissue and normal brain. Conversely, inductively coupled plasma atomic emission spectroscopy (ICP-AES) demonstrates significantly greater uptake of [Gd]-BOPTA in tumor samples than that in peritumoral tissue and normal brain 5 minutes following administration. Gadolinium is retained for a longer period of time within brain tumors when [Gd]-BOPTA rather than [Gd]-DTPA is administered. [Gd]-BOPTA is a useful contrast agent for MRI in brain tumors and possibly an effective absorption agent for neutron capture therapy.

Comparison of gadobenate dimeglumine and gadopentetate dimeglumine: A study of MR imaging and inductively coupled plasma atomic emission spectroscopy in rat brain tumors. Zhang T, Matsumura A, Yamamoto T, Yoshida F, Nose T, Shimojo N. AJNR Am J Neuroradiol 2002 Jan; 23 (1), pp. 15–8.

Hepatic Neoplasia/Hepatocellular Carcinoma/Hepatocellular Adenoma/Focal Nodular Hyperplasia/Hepatic Metastases/Mass Differentiation

Superparamagnetic Iron Oxide Particles (SPIOs)/Ultrasmall Superparamagnetic Iron Oxide Particles (USPIOs)

Application: Limited detection and characterization of hepatic metastases, hepatocellular carcinoma, hepatocellular adenoma, and focal nodular hyperplasia.

Route of Administration: Superparamagnetic iron oxide particles and USPIOs given intravenously.

Implementation: Evaluation of cases with focal hepatic disease utilizing (i) SPIO (e.g., n = 53); and (ii) USPIO (e.g., n = 27) superparamagnetic iron oxide particles in combination with spin-echo T_1- and T_2-weighted imaging (T_1WI, T_2WI) obtained at 1.0 Tesla, before and after intravenous contrast agent administration. The percentage of signal-to-noise ratio (SNR) change and lesion-to-liver contrast (LLC) are then measured and statistically compared.

Human Model: Cases of focal liver disease (e.g., metastases, hepatocellular carcinoma [HCC]; hepatocellular adenoma [HCA]; focal nodular hyperplasia [FNH]).

Interpretation and Analysis: Changes in liver signal intensity (SI) on T_1WI are approximately −28% and +16% following SPIO and USPIO infusion, respectively. On T_2WI, liver decreases in SI on postcontrast images with both agents (−78% SPIO, −73% USPIO), and the difference is not statistically significant ($p \leq 0.07$). Both SPIO and USPIO provide > 500% improvement in LLC on T_2WI. On T_1WI, LLC increases with metastases (120%) and HCC (325%) with SPIO. Post-USPIO, LLC is increased on T_1WI only in metastases (> 500%). Both SPIO and USPIO show excellent hepatic uptake that is presumed to be secondary to reticuloendothelial activity, based on the degree of percent change in SI appreciated in the liver after administration of contrast agent on T_2WI. However, USPIO preparations exhibit blood-pool activity that may aid in further characterization of focal liver lesions, as is evidenced by their greater T_1 effect in the liver and in some focal liver lesions.

MRI in focal liver disease: A comparison of small and ultrasmall superparamagnetic iron oxide as hepatic contrast agents. Mergo PJ, Engelken JD, Helmberger T, Ros PR. J Magn Reson Imaging 1998 Sep–Oct;8 (5), pp. 1073–8.

Hepatic Neoplasia/Metastatic
Carboxydextran Iron-Oxide Nanoparticles (SHU-555A/ Resovist)/Eovist (Gadolinium [Gd]-EOB-DTPA)

Cellular/Molecular Mechanism of Action: Intracellularly internalized by mechanisms of phagocytosis by macrophages (e.g., Kupffer cells) that comprise the hepatic reticuloendothelial system (RES or mononuclear phagocytic system).

Background Information: Injections of Eovist ([Gd]-EOB-DTPA) are selectively taken up by hepatocytes, which increase the signal intensity of normal liver parenchyma on T_1-weighted images. Relative lesion-to-liver contrast is thereby improved because malignant tumors either do not contain hepatocytes or their function has become significantly modified.

Application: Detection and characterization of hepatic neoplastic metastases.

Route of Administration: Intended for intravascular injection.

Dosage: Clinical trials have evaluated the safety and efficacy of Eovist injected at doses up to 100 mmol/kg body weight. Resovist Injection (SHU-555A) contains iron-oxide nanoparticles coated with carboxydextran, which is administered as an intravenous bolus injected as a fixed-volume dose that is dependent on body weight.

Considerations: Following intravenous bolus injection, Eovist is eliminated by both the renal and biliary routes of excretion.

Interpretation and Analysis: The uptake of injected Resovist by RES cells results in a decrease of signal intensity in normal liver parenchyma on both T_2- and T_1-weighted images. Due to the altered phagocytic distribution and activity, the signal intensity in most metastatic tumors is not affected, resulting in improved lesion-to-liver contrast. Both Resovist and Eovist exhibit acceptable safety profiles in clinical trials and have the potential to provide additional information regarding lesion detection, classification, and characterization.

Eovist Injection and Resovist Injection: Two new liver-specific contrast agents for MRI. Mintorovitch J, Shamsi K. Oncology (Huntingt) 2000 Jun; 14 (6 Suppl 3), pp. 37–40.

Hepatic Radiation-Induced Injury/Diminished Hepatic Imaging Secondary to Radiation Therapy
Ferumoxide

Cellular/Molecular Mechanism of Action: Ferumoxide particulate matter within the intravascular compartment is perceived as "foreign" by the reticuloendothelial (mononuclear phagocytic) system and is subsequently phagocytized by cell populations lining the hepatic sinousoids.

Application: Particulate reticuloendothelial MR contrast agents can be utilized to image the liver.

Route of Administration: Ferumoxide particulate reticuloendothelial MR contrast agent (intravenous).

Implementation: Hepatic imaging with a particulate ferumoxide preparation.

Human Model: Radiation therapy–induced hepatic injury (e.g., management of metastatic colon carcinoma).

Interpretation and Analysis: Radiation-induced liver injury can be detected utilizing a particulate reticuloendothelial MR contrast agent. In a relevant example, cases with systemic forms of neoplasia that have been managed with radiation therapy (e.g., metastatic colon carcer) are subject to secondary radiation damage to hepatic parenchyma. The image pattern visualized with ferumoxide preparations applied in combination with MRI fails to produce a darkened image following contrast agent administration. Such findings suggest that detection of metastatic disease after ferumoxide contrast agents may be impaired in patients who have previously received upper abdominal radiation therapy.

Radiation-induced liver injury detected by particulate reticuloendothelial contrast agent. Padhani AR, Husband JE, Gueret Wardle D. Br J Radiol 1998 Oct; 71 (850), pp. 1089–92.

Hepatic Radiation-Induced Injury/Relative Sensitivity
Superparamagnetic Reticuloendothelial System (RES)-Directed Agents/Polycrystalline Iron Oxide Nanocompounds (PION)/Carboxydextran-Coated Maghemite (DDM128N/389 or DDM128)

Route of Administration: Particle-sized superparamagnetic RES-directed agents (i) PION (31 nm) and (ii) DDM128N/389 or DDM128 (59 nm) are delivered by intravenous injection.

Implementation: Compare liver contrast-enhancing characteristics of the 2 particle-sized superparamagnetic RES-directed agents (i) PION and (ii) DDM128N/389 or DDM128 in conditions of hepatic dysfunction. T_2-weighted spin-echo MRI is performed 3 days after x-irradiation at 30 minutes postcontrast.

Animal Model: Experimental protocol for focal radiation-induced hepatitis involves the delivery of a single x-irradiation exposure varying from 10 to 60 Gy on 1 side of the liver.

Interpretation and Analysis: Using the RES-directed PION, the normal, non-irradiated portions of the liver have decreases in signal intensity with a maximum negative enhancement of −66%, while the irradiated portions of liver display decreases in signal intensity of approximately −24% (60 Gy). Signal intensity declines in irradiated liver tissue using PION are dose dependent but can be appreciated at all radiation dose levels (10–60 Gy). The difference in

signal intensity between irradiated (−63%) and non-irradiated (−82%) portions is also statistically different using DDM128 at 60 Gy. However, lower irradiation doses (10 and 30 Gy) fail to produce a statistically significant difference in the enhancement of irradiated and non-irradiated portions of the liver. Sensitivity of liver enhancement with RES-directed agents is size dependent. The smaller particle (PION) is more sensitive for detecting radiation-induced hepatitis compared to larger particle contrast agents (DDM128). The relative insensitivity of DDM128 enhancement for diffuse liver injury is more clinically advantageous for detecting focal lesions in the presence of diffuse hepatic injury.

Contrast-enhanced MR imaging of 2 superparamagnetic RES-contrast agents: Functional assessment of experimental radiation-induced liver injury. Stiskal M, Demsar F, Muhler A, Schwickert HC, Roberts TP, Szolar D, Fischer H, Brasch RC. J Magn Reson Imaging 1999 Jul; 10 (1), pp. 52–6.

MRI of Hepatic Structures
Gadolinium

Following the introduction of rapid, high-quality scan techniques and the development of new, tissue-specific contrast agents, the applications of MRI for liver imaging continue to grow. Magnetic resonance imaging using a T_1-weighted sequence, T_2-weighted sequence, and serial gadolinium-enhanced gradient echo sequences is very effective at both detecting and characterizing various hepatic lesions. The use of a growing number of contrast agents has the potential to increase the sensitivity and specificity of liver MRI in numerous pathological conditions by improving morphological and functional information. Review of the current status of liver MRI, highlighting technical and methodological approaches, contrast agents, and main clinical applications, reveals that MRI appears to be an ideal diagnostic tool for the detection and characterization of liver disease.

MRI evaluation of the liver. Beavers KL, Semelka RC. Semin Liver Dis 2001 May; 21 (2), pp. 161–77.

ISCHEMIA AND INFARCTION

Necrosis of Tissue/Reperfusion Infarct
Gadophrin-2/MP-2269

Cellular/Molecular Mechanism of Action: The mechanisms of action leading to specific localization of necrosis-avid contrast agents (NACAs) such as gadophrin-2 are not well defined. It has been suggested recently that agents with a high degree of serum albumin binding may also serve as NACAs by virtue of non-specific hydrophobic interactions. Both MP-2269 and gadophrin-2 possess albumin-binding avidities and MP-2269 can function as a blood-pool agent.
Application: Gadophrin can be applied to detect and characterize regions of infarction, including artificially-induced reperfused hepatic infarcts that can be recognized by MRI analysis. The agent MP-2269 can function as an albumin-binding blood-pool agent.
Route of Administration: MP-2269 (intravenous); gadophrin-2 (intravenous).
Dosage: MP-2269 (0.05 mmol/kg); gadophrin-2 (0.05 mmol/kg).
Implementation: Evaluation of hepatic reperfusion infarcts with serial T_1-weighted MRI performed before and after administration of MP-2269. The imaging procedure is repeated again 24 hours after intravenous injection of gadophrin-2 at the same dosage (0.05 mmol/kg). Individual MR images are compared with corresponding histomorphological findings. The signal intensity and contrast ratio of infarcted and normal hepatic lobes are quantified and compared between the 2 agents during the postcontrast phase.

Animal Model: Surgical induction of reperfused infarcts in the right liver lobe of rats (e.g., n = 6).
Interpretation and Analysis: Before contrast, the infarcted lobe is indiscernible from normal liver on T_1-weighted MRI. Shortly after injection of both MP-2269 and gadophrin-2, a negative contrast pattern develops between infarcted and normal liver because of a strong liver signal intensity enhancement and an inferior uptake within necrotic liver tissues. During delayed phase analysis (> 60 minutes), a necrosis-specific contrast enhancement (contrast ratio 1.6) develops with gadophrin-2 but not with MP-2269. The MR images closely correspond with histomorphological findings. Although both MP-2269 and gadophrin-2 possess binding avidity for serum albumin fractions, only gadophrin-2 displays a persistent necrosis-specific contrast enhancement in the rat model of reperfused liver infarction. Therefore, the role of albumin binding in the mechanisms of NACAs should be reevaluated.

MRI contrast enhancement of necrosis by MP-2269 and gadophrin-2 in a rat model of liver infarction. Ni Y, Adzamli K, Miao Y, Cresens E, Yu J, Periasamy MP, Adams MD, Marchal G. Invest Radiol 2001 Feb; 36 (2), pp. 97–103.

Oxygen Tension Indicator
Hemoglobin (Hb)

Cellular/Molecular Mechanism of Action: Iron ions contained within Hb fractions bind molecular oxygen.
Application: Exogenous molecular Hb is an effective indicator of relative local oxygen tension in MR microscopy studies performed *in vivo*.
Implementation: Injection of Hb to delineate its ability to permeate through relatively dense neural tissues.

Experimental Model: Insects (flies).
Interpretation and Analysis: Exogenous molecular Hb is more sensitive than other MRI oximetry methods, and therefore can be used at higher resolutions and in specimens with no blood oxygen level–dependent (BOLD) effects.

Hemoglobin is able to permeate through relatively dense neural tissue but does not detectably disrupt normal neurophysiology. Large changes in signal intensity (40–50%) occur when external O_2 levels can be manipulated artificially from 0% to 21% with Hb injections. Oxygen-dependent contrast changes produced by exogenous Hb can be detected by T_2-weighted imaging analysis and can be roughly calibrated if necessary. Therefore, it may be feasible to apply Hb as a contrast agent that may be useful in functional MRI (fMRI) studies for detecting alterations in metabolism at minute resolution levels (e.g., tens of microns).

In vivo oxygen detection using exogenous hemoglobin as a contrast agent in magnetic resonance microscopy. Sun PZ, Schoening ZB, Jasanoff A. Magn Reson Med 2003 Apr; 49 (4), pp. 609–14.

MUSCULOSKELETAL SYSTEM

Arthrography: MR/Contrast Media Compatibility/ Intra-Arterial Placement Confirmation
Gadopentetate Dimeglumine/Methyl Thymol Blue Indicator

Application: Confirmation of the intra-arterial placement of contrast material before performing MR arthrography.

Implementation: Analysis of mixtures of non-ionic and ionic iodinated contrast agent, gadopentetate dimeglumine, lidocaine, and epinephrine using spectrophotometric titration with a gadolinium ion titrant and methyl thymol blue indicator.

Interpretation and Analysis: No significant dissociation of gadolinium ion occurs when gadopentetate dimeglumine is mixed with iodinated contrast agents, lidocaine, or epinephrine over a range of dilutions. Therefore, gadopentetate dimeglumine and iodinated contrast material can be mixed before MRI without any release of free gadolinium and are safe for confirming the intra-articular placement of contrast material before MR arthrography.

Is a mixture of gadolinium and iodinated contrast material safe during MR arthrography? Brown RR, Clarke DW, Daffner RH. AJR Am J Roentgenol 2000 Oct; 175 (4), pp. 1087–90.

Cartilage Integrity/Injury Detection
Gadolinium ([Gd]-DTPA)

Cellular/Molecular Mechanism of Action: Gadolinium possesses radiopaque properties.

Application: Detection of alteration in the molecular integrity of the matix structure/composition of cartilaginous tissue through the identification of changes in magnetized transfer utilizing MRI modalities.

Implementation: Measurement of magnetic transfer (MT) and T_1 and T_2 relaxation of normal, trypsinized, and interleukin-1beta (IL-1β)-treated cartilage in the absence and presence of [Gd]-DTPA.

Interpretation and Analysis: Without the addition of [Gd]-DTPA, neither T_1 nor T_2 detect any significant change with cartilage damage. However, in the presence of [Gd]-DTPA, trypsinized cartilage exhibits substantially shorter T_1 than normal cartilage, as expected due to the glycosaminoglycan (GAG) loss in these samples and associated increases in [Gd]-DTPA concentration. The T_2 results are similar but less dramatic. The MT pseudo 1st-order exchange rate, RM(0B), does not depend on the contrast agent concentration, as expected, and is significantly faster for trypsinized and slower for IL-1β-treated cartilage. In both cases, the MT fraction of the macromolecular pool M(0B) decreases, while only trypsinized cartilage shows an increase in MT exchange rate [R]. The MT ratio (MTR) decreases with increasing [Gd]-DTPA concentration. However, interpretation of the MTR results in the presence of [Gd]-DTPA is complicated due to competing effects of increased longitudinal relaxivity and MT exchange. Therefore, in a cartilage sample with an unknown degree of GAG depletion and some collagen damage, a full MT analysis might be used to probe the molecular state or character of cartilage, but it is not possible to use a simple MTR measurement after the administration of [Gd]-DTPA to differentially determine the amount of cartilage degradation in the sample.

Can MTR be used to assess cartilage in the presence of [Gd]-DTPA 2? Henkelman RM, Stanisz GJ, Menezes N, Burstein D. Magn Reson Med 2002 Dec; 48 (6), pp. 1081–4.

Cartilage Integrity/Glycosaminoglycans (GAGs)/ Cartilage Degradation: Trypsin- and Interleukin-1-Induced Injury
Gadolinium-DTPA^{-2} ([Gd]-DTPA^{-2})

Background Information: Glycosaminoglycans are the main source of tissue fixed charge density (FCD) in cartilage and are lost early in arthritic diseases.

Application: Detect and characterize cartilage degradation.

Implementation: Evaluate the hypothesis that, like Na^+, the charged contrast agent [Gd]-DTPA^{-2} (and hence proton T_1) can be used to measure tissue FCD and hence GAG concentration.

Interpretation and Analysis: Nuclear MR spectroscopy studies of cartilage explants demonstrate that there is a strong correlation ($r > 0.96$) between proton T_1 in the presence of [Gd]-DTPA^{-2} and tissue sodium and GAG concentrations. An ideal 1-compartment electrochemical (Donnan) equilibrium model can be utilized as a means of quantifying FCD from [Gd]-DTPA^{-2} concentration. This approach yields a value 50% less but linearly correlated with the validated method of quantifying FCD from Na^+ ion and can be used as the basis of an empirical model with which to quantify FCD from [Gd]-DTPA^{-2} concentration, or a more sophisticated physical model could be developed. Spatial distributions of FCD can be easily observed in T_1-weighted MRI studies of trypsin- and interleukin-1-induced cartilage degradation, with good histological correlation. Therefore, equilibration of the tissue with [Gd]-DTPA^{-2} gives the opportunity to directly image (through T_1 weighting) the concentration of GAG, a major and critically important macromolecule in cartilage. Pilot clinical studies demonstrate penetration of [Gd]-DTPA^{-2} into cartilage, suggesting that this technique is clinically feasible.

[Gd]-DTPA^{-2} as a measure of cartilage degradation. Bashir A, Gray ML, Burstein D. Magn Reson Med 1996 Nov; 36 (5), pp. 665–73.

Cartilage Integrity/Proteoglycan (PG) Depletion: Detection and Characterization/Papain-Induced Cartilage Injury
Gadolinium-DTPA^{-2} ([Gd]-DTPA^{-2})

Background Information: Loss of PGs from the extracellular matrix of cartilage is an early event that occurs in the initial stages of osteoarthritis.

Application: Detection and characterization of cartilage degradation relevant to degenerative joint disease (DJD).

Implementation: Evaluate the capability of [Gd]-DTPA^{-2}-enhanced MRI to quantitatively assess PG content in a model of cartilage degeneration. One approach to assessment of cartilage PG content can entail the application of [Gd]-DTPA^{-2} as an MRI contrast image-enhancing agent. A series of knee flexions are performed post-[Gd]-DTPA^{-2} injection to facilitate penetration of the contrast agent into cartilage structures.

Animal Model: Goat knee (stifle joint) preparations. Partial to total PG depletion induced by an intra-articular injection of papain 1 day prior to MRI sessions (cartilage degeneration model).

Interpretation and Analysis: A close correlation can be found between the extent of PG loss and [Gd]-DTPA^{-2}-induced T_1 decreases. Papain-induced PG depletion can be confirmed by postmortem histological and biochemical assessments. A 2-hour delay following [Gd]-DTPA^{-2} injection results in optimal accuracy in quantitating cartilage defects. However, DeltaT_1s observed in cartilage of exercised goat knees are not affected by papain or interleukin-1beta pretreatment. Therefore, as long as a preinjection T_1 map is obtained, the [Gd]-DTPA^{-2}-enhanced MRI technique provides good sensitivity in detecting partial loss of PGs from within articular cartilage. Such a pattern appears to be true only when the animal is maintained in a resting state during diffusion of the [Gd]-DTPA^{-2}. This approach is of particular interest for long-term evaluations of cartilage degeneration and regeneration.

In vivo assessment of macromolecular content in articular cartilage of the goat knee. Laurent D, Wasvary J, Rudin M, O'Byrne E, Pellas T. Magn Reson Med 2003 Jun; 49 (6), pp. 1037–46.

Discography: Intervertebral Disc Pathology/MRI: Alternative to Computed Tomography (CT) Discography
Gadolinium

Background Information: Magnetic resonance arthrography with gadolinium has been used to evaluate shoulder, elbow, wrist, hip, knee, and other joints. Gadolinium has not been extensively used as an intradiscal contrast agent. Preliminary results using gadolinium as an intradiscal contrast agent for MR discography have been encouraging.

Application: Gadolinium can be used to perform MR discography to detect and characterize intervertebral disc pathology. Furthermore, MR discography with gadolinium can be recommended for patients allergic to iodinated contrast agents and for individuals who wish to limit the level of radiation exposure normally experienced with CT discography procedures.

Implementation: Evaluation of intervertebral disc disease utilizing water-soluble iodinated contrast and gadopentetate dimeglumine injected at each intravertebral disc level. Following discography, anteroposterior and lateral radiographs, CT scans, and T_1-weighted MRI are performed. Interscan and interobserver interpretation variability is determined for MR discography and CT discography using the Pearson correlation-coefficient r test.

Human Model: Cases with suspected intervertebral disc disease (n = 13; n = 42 disc spaces).

Interpretation and Analysis: Interscan variability is highly correlated between CT discography and MR discography when used to determine disc normality (r = 0.87), general degeneration (r = 0.87), anular fissure (r = 0.89), disc herniation (r = 0.92), and contrast leakage (r = 0.77). Interscan variability can be assessed by the Pearson test, which usually reveals that all "r" values for all examiners noted to be statistically significant at p values = 0.01 or less. Interobserver variability significantly correlates with professional specialization and level of diagnostic experience. Interobserver variability also significantly correlates with CT and MRI discography for disc normality (CT r = 0.60; MRI r = 0.56), general degeneration (CT r = 0.76; MRI r = 0.71), anular fissure (CT r = 0.79; MRI r = 0.84), and disc herniation (CT r = 0.63; MRI r = 0.64). The readings for contrast leakage do not reach statistical significance for CT or MR discography. The high interscan and interobserver correlation rates obtained for MR discography using gadolinium, as compared with the standard CT discography technique, indicate that MR discography may be an acceptable substitute for the imaging of disc pathology. Magnetic resonance discography with gadolinium may be recommended for patients allergic to iodinated contrast agents and for patients who wish to limit their radiation exposure.

Gadopentetate dimeglumine as an intradiscal contrast agent. Huang TS, Zucherman JF, Hsu KY, Shapiro M, Lentz D, Gartland J. Spine 2002 Apr 15; 27 (8), pp. 839–43.

Osteoarthritis/Detection of Mechanical and Enzyme-Induced Lesions
Manganese Chloride Liposomes/ Gadolinium-DTPA ([Gd]-DTPA) Liposomes

Cellular/Molecular Mechanism of Action: Gadolinium and manganese are elements that possess paramagnetic properties.

Application: After intra-articular application of [Gd]-DTPA the visualization cartilage surface roughness is limited because of diffusion into the cartilage. Liposome-entrapped contrast agents can be employed to improve the sensitivity of MR arthrography for the diagnostic detection of cartilage surface abnormalities.

Route of Administration: [Gd]-DTPA, [Gd]-DTPA-liposomes, manganese chloride, and manganese chloride liposomes are all administered intra-articularly.

Implementation: Evaluation of joint structures applying the paramagnetic contrast agents [Gd]-DTPA and manganese chloride in either a free form or entrapped within liposomes utilizing high resolution MRI (7.1 Tesla).

Animal Model: Swine temporomandibular joint and rabbit knee joint.

Interpretation and Analysis: After intra-articular injection of liposome-entrapped contrast agents an excellent contrast between cartilage surface and joint space can be achieved. Diffusion of the contrast agent into the cartilage layer is prevented, and visualization of cartilage surfaces is markedly improved. Small mechanically and enzymatically induced cartilage lesions can be assessed reliably. Intra-articular injection of liposome-entrapped contrast agents can improve the potential of MR arthrography for the detection of early osteoarthritic cartilage changes.

Instrumentation: Magnetic resonance imaging instrumentation (7.1 tesla).

Improved nuclear magnetic resonance microscopic visualization of joint cartilage using liposome entrapped contrast agents. Gründer W, Biesold M, Wagner M, Werner A. Invest Radiol 1998 Apr; 33 (4), pp. 193–202.

Rheumatoid Arthritis (RA)/Bone Erosion Detection/ Synovial Hypertrophy/Synovitis
Contrast Image-Enhancing Agent

Application: Detection and characterization of RA conditions.

Route of Administration: Magnetic resonance contrast image enhancement agent (intravenous).

Implementation: Evaluate affected joints analyzed by radiography (1D) and MRI with the later modality performed before and after the administration of a contrast image-enhancing agent. Image examinations are performed on the 2nd–5th metacarpophalangeal (MCP) joints and wrist. The number of erosions on radiographic examination and MRI are then calculated, and synovitis in the MCP joints and wrists graded semiquantitatively.

Human Model: Cases of RA joint pathology of $\leq$ 1 year duration (e.g., n = 25 and untreated) involving the MCP joints and wrist.

Interpretation and Analysis: Extremity MRI (E-MRI) can detect substantially more bone erosion lesions than conventional radiographic examination (e.g., n = 57 vs 6, respectively; 9.5 to 1 ratio). Synovial hypertrophy grades are significantly higher in RA joints with clinical signs of joint inflammation, including swelling and/or tenderness (median 3, 5th–95th centile 1–4) than without these clinical signs (median 2, 5th–95th centile 1–3), $p < 0.001$. Fifty-one percent of the joints without clinical signs of synovitis show synovial hypertrophy on E-MRI. There is a positive correlation between MRI scores of synovitis and the number of erosions detected by MRI in MCP joints (Spearman $r_s = 0.31$, $p < 0.01$). Healthy controls have no evidence of erosions or synovitis based on MRI interpretations. Joint destruction starts very early in RA and E-MRI allows detailed evaluation of inflammatory and destructive changes in wrists and MCP joints in patients with incipient RA.

Low field dedicated magnetic resonance imaging in untreated rheumatoid arthritis of recent onset. Lindegaard H, Vallø J, Hørslev-Petersen K, Junker P, Östergaard M. Ann Rheum Dis 2001 Aug; 60 (8), pp. 770–6.

Rheumatoid Arthritis (RA)/Synovitis/Joint Effusion/ Fibrovascular Connective Tissue Proliferation/ Pannus Formation

Magnetic resonance imaging is the modality of choice in early diagnosis and management of RA. The pathologic processes in RA involve synovitis, joint effusion, proliferation of fibrovascular connective tissue, and the formation of pannus. Other imaging techniques available for imaging of RA include ultrasound, scintigraphy, computed tomography, and plain radiography (PR). Magnetic resonance imaging provides a high level of sensitivity in detecting inflammatory changes in joint structures. Several studies have reported high intra- and interobserver reliability and low variation for MRI. Magnetic resonance imaging allows detection and, in some cases, quantification of synovial changes. Dynamic MRI is a new technique that utilizes rate of synovial enhancement in evaluation of inflammatory changes. Magnetic resonance imaging allows visualization of erosions in 3 orthogonal planes. It has been shown in many studies to have much greater sensitivity than PR in detecting erosions. Use of a contrast agent further increases the sensitivity in detecting erosions and differentiates and outlines synovial proliferation from fluid collection. Other manifestations of RA such as intraosseous cysts, tenosynovitis, bone marrow edema, and carpal tunnel syndrome can also be visualized on MRI. Advances in MRI include contrast-enhancement, dynamic, and quantitative techniques. Magnetic resonance imaging assists in the early detection of RA, which allows earlier initiation of treatment with disease-modifying therapies.

Magnetic resonance imaging in early detection of rheumatoid arthritis. Tehranzadeh J, Ashikyan O, Dascalos J. Semin Musculoskelet Radiol 2003 Jun; 7 (2), pp. 79–94.

NEOPLASIA

Mammary Carcinoma
Ultrasmall Superparamagnetic Particles of Iron Oxide (USPIO) Enhancement

Application: Ultrasmall superparamagnetic iron oxide–enhanced MRI has the potential to become an adjunct to conventional MRI of the breast for preoperative assessment of axillary lymph nodes in patients with breast cancer.
Implementation: Magnetic resonance imaging of mammary carcinoma lesions within 24 to 36 hours after the start of intravenous slow-drip infusion of USPIO followed by gadolinium-enhanced MRI (1.5 Tesla system). Lymph nodes are staged prospectively by using established criteria, and results are correlated with histologic findings (standard reference control) for comparison with MRI findings.
Human Model: Mammary carcinoma cases (e.g., n = 18).
Interpretation and Analysis: Axillary staging with USPIO-enhanced MRI produces true-positive results in about 50% of cases (e.g., n = 9/18). Approximately 39%, 0%, and 11% of cases are categorized as true-negatives; false-positives; or false-negatives, respectively (sensitivity, 82%; specificity, 100%; positive predictive value, 100%; 2nd reader, = 1.0). Magnetic resonance imaging analysis is capable of detecting a large number of lymph nodes (e.g., n = 405). Lymph node–based sensitivity can vary between 83% and 73% and specificity between 96% and 97% depending on the evaluator (κ = 0.68). Ultrasmall superparamagnetic particles of iron oxide as an intravascular contrast agent cannot replace gadolinium for assessment of the primary tumor; however, no clinically relevant interaction can be appreciated. Thus, an integrated imaging approach is feasible in all patients.

Preoperative breast cancer staging: MR imaging of the axilla with ultrasmall superparamagnetic iron oxide enhancement. Michel SC, Keller TM, Frohlich JM, Fink D, Caduff R, Seifert B, Marincek B, Kubik-Huch RA. Radiology 2002 Nov; 225 (2), pp. 527–36.

Mammary Gland Disease Differentiation
Gadolinium/Indocyanine Green (ICG)/Near-Infrared (NIR) Diffuse Optical Tomography (DOT)

Cellular/Molecular Mechanism of Action: Indocyanine green is an albumin-bound absorbing dye within the intravascular compartment.
Application: Indocyanine green is an absorbing dye commonly used as a marker system within the plasma compartment. Under certain conditions, ICG possesses the potential to differentiate disease based on the quantified enhancement of suspicious lesions using MRI concurrently with NIR DOT (contrast enhancement properties).
Route of Administration: Intravascular.
Implementation: Near-infrared DOT analysis of the mammary gland applied in combination or concurrently with MRI. Results are then correlated with histopathological evaluation of suspicious lesions.
Human Model: Cases scheduled for excisional biopsy or surgery.
Interpretation and Analysis: The ICG-enhanced optical images co-register accurately with gadolinium-enhanced MR images, thereby validating the ability of DOT to image breast tissue. In contrast to simple transillumination, DOT appears to provide for localization and quantification of exogenous tissue chromophore concentrations. Additionally, ICG has demonstrated a potential to differentiate disease based on the quantified enhancement of suspicious lesions.

Concurrent MRI and diffuse optical tomography of breast after indocyanine green enhancement. Ntziachristos V, Yodh AG, Schnall M, Chance B. Proc Natl Acad Sci U S A 2000 Mar 14; 97 (6), pp. 2767–72.

Necrotic Tissues: Neoplastic Lesions
Carborane [Gd]-DTPA 2

Cellular/Molecular Mechanism of Action: [Gd]-carborane complex 2 can function as a carrier complex for boron.
Application: Magnetic resonance *in vivo* imaging of neoplastic lesions that are undergoing active necrosis.
Implementation: Determine *in vivo* the ability for carborane [Gd]-DTPA 2 to detect neoplasic lesions. Validation of *in vivo* findings can be established applying *in vitro* alpha-autoradiography.
Animal Model: Rat (Donryu strain: tumor-bearing).
Interpretation and Analysis: Carborane [Gd]-DTPA 2 is metabolized slower in the body than [Gd]-DTPA 1. The results from inductively coupled plasma atomic emission spectroscopy (ICP-AES) methods indicate that compound 2 is incorporated into normal tissues and metabolized quickly, whereas it does not accumulate in tumor or brain tissues. Alpha-autoradiography reveals that high levels of boron accumulate within internal organs and in necrotic tumor tissue.

In vivo evaluation of carborane gadolinium-DTPA complex as an MR imaging boron carrier. Nakamura H, Fukuda H, Girald F, Kobayashi T, Hiratsuka J, Akaizawa T, Nemoto H, Cai J, Yoshida K, Yamamoto Y. Chem Pharm Bull (Tokyo) 2000 Jul; 48 (7), pp. 1034–8.

Neoplasia/Vascular and Lymphatic Endothelium (Associated)
Nanocrystals/Semiconductor Quantum Dots (qdots)/ Tumor Vasculature-Targeting Peptides/Peptide-Coated ZnS-Capped CdSe qdots/[Nanoparticles]

Background Information: Inorganic nanostructures that interface with biological systems have recently attracted widespread interest in biology and medicine. Nanoparticles are thought to have potential as novel intravascular delivery probes for both diagnostic (e.g., imaging) and therapeutic (e.g., chemotherapy) purposes. Critical issues for successful nanoparticle delivery include the ability to target specific tissues and cell types and the discovery of approaches for escaping the biological particulate filter known as the reticuloendothelial system.
Application: Detection and characterization of vascular and lymphatic structures associated with neoplastic lesions.
Route of Administration: Intravenous.
Implementation: Explore the feasibility of *in vivo* semiconductor qdot targeting. Qdots are small (< 10 nm) inorganic nanocrystals that possess unique luminescent properties because their fluorescence emission is stable and optimized by varying the particle size or composition.
Animal Model: Murine.
Interpretation and Analysis: ZnS-capped CdSe qdots coated with a lung-targeting peptide accumulate in the lungs of mice after intravenous injection, whereas 2 other peptides specifically direct qdots to blood vessels or lymphatic vessels in tumors. Addition of polyethylene glycol to the qdot coating prevents non-selective accumulation of qdots in reticuloendothelial tissues. These results encourage the construction of more complex nanostructures with capabilities such as disease sensing and drug delivery.

Nanocrystal targeting *in vivo*. Akerman ME, Chan WC, Laakkonen P, Bhatia SN, Ruoslahti E. Proc Natl Acad Sci U S A 2002 Oct 1; 99 (20), pp. 12617–21.

Neoplasia/Vascularity of Tumors/Mammary Carcinoma Transplants
Gadolinium: [Gd]-DTPA for MRI/Galactose-Based Ultrasound (US) Contrast Agent

Cellular/Molecular Mechanism of Action: Gadolinium is classified as a paramagnetic metallic ion and its spatial orientation becomes altered when placed within a magnetic energy field.
Background Information: Angiogenesis is essential for spread and growth of malignant tumors. Because non-invasive methods for observing tumor vascularization are limited, most of the current knowledge regarding this field of study is founded on previous results from histology-based investigations.
Application: Dynamic parameters obtained using intermittent contrast-enhanced Doppler sonography and dynamic MRI can be compared and correlated with microvessel density in neoplastic lesions.
Route of Administration: [Gd]-DTPA (intravenous for MRI); galactose-based contrast image-enhancing agent (intravenous for Doppler US).
Implementation: Comparison of dynamic parameters obtained using intermittent contrast-enhanced Doppler sonography and dynamic MRI to determine the degree to which findings correlate with patterns of microvessel density.

Transplanted tumors can be examined with dynamic T_1-weighted sequences using [Gd]-DTPA and images obtained by MR in concert with intermittent power Doppler sonography after a single bolus of galactose-based contrast agent. Following imaging, tumors are harvested for examination aided by immunofluorescence microscopy (e.g., $n = 6$ CD31-type lesions). Using a 2-compartment model, the MR parameters amplitude (reflecting plasma volume) and k_{ep} (influenced by the vessel permeability) are calculated and compared with maximal enhancement (max) and perfusion (p) measured by ultrasound.

Animal Model: Tumor-bearing nude mice (e.g., $n = 11$).
Interpretation and Analysis: The MR amplitude significantly correlates with the max ultrasound parameter ($r = 0.61$; $p = 0.01$): max ($r = 0.67$; $p = 0.01$), amplitude ($r = 0.72$; $p = 0.01$); while perfusion ($r = 0.62$; $p = 0.05$) correlates with microvessel density. Values for k_{ep} moderately correlate with max but not with perfusion and microvessel density. Dynamic MRI and contrast-enhanced ultrasound are complementary methods for examining perfusion and vascularity of experimental tumors.
Instrumentation: Magnetic resonance scanner (1.5 tesla).

Comparing dynamic parameters of tumor vascularization in nude mice revealed by magnetic resonance imaging and contrast-enhanced intermittent power Doppler sonography. Kiessling F, Krix M, Heilmann M, Vosseler S, Lichy M, Fink C, Farhan N, Kleinschmidt K, Schad L, Fusenig NE, Delorme S. Invest Radiol 2003 Aug; 38 (8), pp. 516–24.

Neoplasia: Carcinoma (Prostatic)/Gene Expression Imaging/c-myc Messenger RNA (mRNA) Transcription
Gadolinium ([Gd])-Anti-mRNA Oligonucleotide Strand/ Trans-Membrane Carrier Peptide

Cellular/Molecular Mechanism of Action: The physical orientation of gadolinium changes when placed within a magnetic energy field. Trans-membrane carrier proteins are transported across cell membrane structures by specific membrane-associated complexes. Oligonucleotides can be designed as the complementary sequence to specific mRNA strands and the resulting cDNA/mRNA double strand effectively prevents translation of the corresponding mRNA strand. Diagnostically, complementary strands of cDNA theoretically accumulate within the cytosol of cells that are actively transcribing the corresponding mRNA sequences that cDNA strands have been designed to recognize (hybridize/bend).
Background Information: The term "molecular imaging" can be broadly defined as the *in vivo* characterization and measurement of biological processes at the cellular and molecular level.
Application: Concept and methodology (strategy) for diagnostically detecting *in vivo* gene expression by MRI. Example: Detection of specific oncogenes expressed within neoplastic cell types.
Route of Administration: Theoretically would be administered most commonly by intravenous infusion.
Semi-Synthetic Methods: Development of a novel intravital and intracellular MRI contrast agent composed of a gadolinium complex, an oligonucleotide sequence (peptide nucleic acid [PNA]), and a trans-membrane carrier peptide designed to correspond to a peptide sequence similar to that of the homeodomain of the Antennapedia protein.
Implementation: Determine if a [Gd]-oligonucleotide complex (i) can accumulate in tumor cells *in vitro* and *in vivo*; and (ii) whether the specificity of a PNA for the upregulated c-myc mRNA in the cell's cytoplasm has an effect on contrast agent retention within tumor cells.
Tissue Culture Models: HeLa cell lines.

Animal Model: Rat (Dunning R3327 AT1 rat prostate adenocarcinoma).
Interpretation and Analysis: Using the c-myc-specific and a c-myc-non-specific control PNA, an increase in signal intensity in the tumor cells can be observed after 10 minutes *in vitro* and *in vivo* (maximum reached *in vitro* in HeLa within 60 minutes compared to Dunning R3327 AT1 rat prostate adenocarcinoma cells *in vivo* within 30 minutes). Such increases of signal intensity can be maintained *in vitro* in HeLa cells for only 4 hours and in Dunning R3327 AT1 rat prostate adenocarcinoma cells *in vivo* at least for 5 hours by using the c-myc mRNA-specific PNA as a "retention" agent.

Intracellular visualization of prostate cancer using magnetic resonance imaging. Heckl S, Pipkorn R, Waldeck W, Spring H, Jenne J, von der Lieth CW, Corban-Wilhelm H, Debus J, Braun K. Cancer Res 2003 Aug 15; 63 (16), pp. 4766–72.

Neoplasia: Carcinoma (Prostatic)/Lymph Node Metastases
Lymphotropic Superparamagnetic Nanoparticles

Cellular/Molecular Mechanism of Action: Superparamagnetic nanoparticles have been designed that have high lymphotropic properties.
Background Information: Accurate detection of lymph-node metastases in prostate cancer is an essential component of the approach to treatment.
Application: Detection of small nodal metastases using highly lymphotropic superparamagnetic nanoparticles.
Route of Administration: Intravenous.
Dosage: Lymphotropic superparamagnetic nanoparticles (2.6 mg of iron per kilogram of body weight).
Implementation: Detection of small nodal metastases utilizing highly lymphotropic superparamagnetic nanoparticles that gain access to lymph nodes by means of interstitial-lymphatic fluid transport. All case candidates are examined by MRI before and 24 hours after the intravenous administration of lymphotropic superparamagnetic nanoparticles. Imaging results are then correlated with histopathological findings.

Human Model: Cases with presurgical clinical stage T_1, T_2, or T_3 prostate cancer that have had surgical lymph-node resection or biopsy (n = 80).
Interpretation and Analysis: Analysis of lymph nodes either resected or biopsied (e.g., n = 334 lymph nodes) reveals that approximately 18.9% (e.g., n = 63/334) from 41% of cases (e.g., n = 33/80) have histopathological detectable evidence of metastases. Of these nodes (e.g., n = 63), approximately 71.4% (e.g., n = 45/63) do not fulfill the usual imaging criteria for malignancy. Magnetic resonance imaging with lymphotropic superparamagnetic nanoparticles can correctly identify the vast majority of cases with nodal metastasis (e.g., n = 33/33 or 100%). Node-by-node analysis has a significantly higher level of sensitivity than does conventional MRI (90.5% vs 35.4%, $p < 0.001$) or nomograms. Therefore, high-resolution MRI with magnetic nanoparticles allows the detection of smaller and otherwise undetectable lymph-node metastases in conditions of prostate cancer.
Instrumentation: High-resolution MRI.

Non-invasive detection of clinically occult lymph-node metastases in prostate cancer. Harisinghani MG, Barentsz J, Hahn PF, Deserno WM, Tabatabaei S, van de Kaa CH, de la Rosette J, Weissleder R. N Engl J Med 2003 Jun 19; 348 (25), pp. 2491–9.

Neoplasia: Carcinoma (Squamous Cell Type)/Esophogeal Squamous Cell Carcinoma (SCC) Epidermal Growth Factor Receptor (EGFR) Expression
Anti-EGFR Coated Supermagnetic Particles

Cellular/Molecular Mechanism of Action: Anti-EGFR monoclonal antibody recognizes and physically binds to membrane-associated EGFR.
Background Information: Epidermal growth factor receptors are overexpressed in esophageal SCC.
Application: Enhanced detection of esophageal SCC employing an endoluminal surface coil.
Route of Administration: Anti-EGFR-coated supermagnetic particles (intravascular).
Semi-Synthetic Methods: Coating superparamagnetite particles with monoclonal antibodies (MAbs) directed against EGFRs.
Animal Model: Rat (athymic bearing TE8 or H69 tumors).
Human Model: Cases of esophageal cancer (n = 30).
Interpretation and Analysis: In terms of depth of cancer invasion, the accuracy rate of MRI using the endoluminal surface coil is approximately 83%. MRI with an endoluminal surface coil will be a useful examination approach for detecting esophageal cancer. Anti-EGFR-coated supermagnetic particles possess EGFR-specific MRI contrast capacity *in vivo* in tumor-bearing rats. Immunospecific MRI using magnetite particles coated with MAbs against EGFR seems to be useful for diagnosing SCC of the esophagus.
Instrumentation: Endoluminal 1.5 tesla serconductive MR system.

What's new in imaging? New magnetic resonance imaging of esophageal cancer using an endoluminal surface coil and antibody-coated magnetite particles. Ozawa S, Imai Y, Suwa T, Kitajima M. Recent Results Cancer Res 2000; 155, pp. 73–87.

Neoplasia (Central Nervous System [CNS])/Brain Tumor Diagnostic and Therapeutic Implications
Gadobenate Dimeglumine ([Gd]-BOPTA)

Background Information: [Gd]-BOPTA has a significantly greater [Gd] uptake by brain tumor tissue than gadopentate dimeglumine ([Gd]-DTPA).
Application: The agent [Gd]-BOPTA is assimilated at relatively high concentrations within certain neoplastic lesions that affect the CNS.
Route of Administration: [Gd]-BOPTA and [Gd]-DTPA (intralesionally and subcutaneously in rat research investigations).
Dosage: [Gd]-BOPTA and [Gd]-DTPA (0.05 mmol/g tumor weight).
Implementation: Evaluate whether [Gd]-BOPTA is an efficient agent for neutron capture therapy (NCT).

Delineate the imaging properties of [Gd]-BOPTA and [Gd]-DTPA injected intralesionally into tumors. At the peak of [Gd] uptake, thermal neutron irradiation is applied.

Animal Model: Rat (Fisher 344 strain). Four groups of Fisher 344 rats (control, neutron (n), n+ [Gd]-DTPA, n+ [Gd]-BOPTA) injected subcutaneously with 9L gliosarcoma cells in both hind legs.
Interpretation and Analysis: A pronounced delay in tumor growth can be appreciated with [Gd+] formulations compared to control and neutron analyses (p = 0.0053, 0.0064, respectively). Furthermore, BOPTA is associated with a significantly prolonged

delay of tumor growth compared to DTPA ($p = 0.033$). In addition, [Gd]-NCT demonstrates that [Gd]-BOPTA can function as an effective compound for NCT. Better cytocidal effects of [Gd]-BOPTA warrant further investigation of subcellular [Gd] distribution.

In vivo gadolinium neutron capture therapy using a potentially effective compound ([Gd]-BOPTA). Matsumura A, Zhang T, Yamamoto T, Yoshida F, Sakurai Y, Shimojo N, Nose T. Anticancer Res 2003 May–Jun; 23 (3B), pp. 2451–6.

Neoplasia (Central Nervous System [CNS]): Glioma/ Microglial Cell Detection and Characterization
Ultrasmall Superparamagnetic Particles of Iron Oxide (USPIOs)

Cellular/Molecular Mechanism of Action: Ultrasmall superparamagnetic particles of iron oxide are perceived as foreign material and internalized (phagocytized) by microglial cell populations residing within the CNS.

Background Information: Microglia are intrinsic immunoeffector cells of the CNS that play a very sensitive, crucial role in the response to almost any brain pathology. Upon stimulation, they are transformed into a state of elevated phagocytic activity.

Application: Reveal the extent of microglial tumors and assess the status of immunologic defense processes.

Implementation: Evaluate the presence and distribution of activated microglial cells residing within the CNS utilizing USPIO preparations in combination with MRI instrumentation.

Animal Model: Glioma cell cultures and rat glioma *in vivo* model.

Interpretation and Analysis: Using USPIOs in cell culture experiments and in a rat glioma model, microglia can be labeled magnetically and detected by confocal microscopy within and around tumors in a typical border-like pattern. Quantitative *in vitro* studies reveal that microglia cells internalize amounts of USPIOs that are significantly higher than those incorporated by tumor cells and astrocytes. Labeled microglia can be detected and quantified with MRI in cell phantoms, and the extent of the tumor can be seen *in vivo* in glioma-bearing rats. Magnetic labeling of microglia provides a potential tool for MRI of gliomas that more precisely reflects tumor morphology. Furthermore, the results suggest that MRI may yield functional data on the immunologic reaction within the CNS.

Magnetic labeling of activated microglia in experimental gliomas. Fleige G, Nolte C, Synowitz M, Seeberger F, Kettenmann H, Zimmer C. Neoplasia 2001 Nov–Dec; 3 (6), pp. 489–99.

Neoplasia (Central Nervous System [CNS]): Gliomas/ Angiogenic Neovascularization
Contrast-Enhanced Dynamic Susceptibility MRI

Background Information: Abnormalities in the recirculation phase of the passage of a contrast agent bolus have been identified in tumors and have been proposed to represent vascular tortuosity and hypoperfusion in areas of angiogenic neovascularization.

Application: Detection and characterization (grading) of the microcirculation profiles of gliomas within the CNS as a function of vascular tortuosity and regions of hypoperfusion.

Implementation: Characterization of glioma lesions by contrast-enhanced dynamic susceptibility MRI. Residual relaxivity effects are minimized by the injection of contrast agent before the acquisition of dynamic images. Maps of relative cerebral blood volume (rCBV) and relative recirculation (rR) are calculated, and values from enhancing tumor tissue compared with tumor grade.

Human Model: Human glioma cases (e.g., $n = 27$).

Interpretation and Analysis: Histologic grade categories include grade II-astrocytoma ($n = 3$); grade III-anaplastic astrocytoma ($n = 10$); and grade IV-glioblastoma multiforme ($n = 14$). Relative cerebral blood volume values vary among tumor grades, with higher mean values associated with higher grade tumors ($p < 0.001$). Mean rR values in grade II tumors are not significantly different from those in normal gray and white matter. Mean rR values in grades III and IV tumors are similar and significantly higher than those in grade II tumors ($p < 0.01$). The distribution of the pixel values of rR reveals significant differences between grades III and IV tumors ($p < 0.001$), with low values of skewness in keeping with a normal distribution in grade III tumors and higher values in grade IV tumors. Variation in the recirculation characteristics of a contrast agent bolus is related to tumor grade in gliomas. Abnormalities in contrast agent recirculation appear to provide independent information concerning the microcirculation in imaging studies of angiogenesis and may be of value as surrogate markers in trials of antiangiogenic therapy.

Abnormalities in the recirculation phase of contrast agent bolus passage in cerebral gliomas: Comparison with relative blood volume and tumor grade. Jackson A, Kassner A, Annesley-Williams D, Reid H, Zhu XP, Li KL. AJNR Am J Neuroradiol 2002 Jan; 23 (1), pp. 7–14.

Neoplasia (Central Nervous System [CNS]): Glioma of Murine Origin
Blood-Pool Contrast Agents: MP-2269 and MS-325/ Extracellular Contrast Agent: OptiMARK

Cellular/Molecular Mechanism of Action: MP-2269 and MS-325 are 2 small molecular weight serum albumin-binding blood-pool contrast agents. OptiMARK functions as a compound that remains in extracellular fluid compartments with essentially no intracellular penetration.

Background Information: Intravenous MRI contrast agents are commonly used to improve the detection of intracranial tumors and other CNS lesions for diagnosis and treatment planning.

Application: Intravenous MRI contrast agents are commonly used to improve the detection of intracranial tumors and other CNS lesions for diagnosis and treatment planning.

Route of Administration: MP-2269 and MS-325 (intravenous), and OptiMARK (intravenous).

Dosage: MP-2269 and MS-325 (30 μmol/kg: small molecular weight, albumin-binding blood-pool contrast agents). OptiMARK (100 μmol/kg: extracellular imaging agent).

Implementation: Detection of CNS neoplastic lesions (glioma) with the small molecular weight blood-pool contrast agent, MP-2269 and MS-325, compared to the extracellular contrast agent, OptiMARK, utilizing 1.5 Tesla electromagnetic resonance imaging analysis.

Animal Model: Mouse glioma.

Interpretation and Analysis: Tumor image contrast is significantly enhanced and long-lived following administration of MP-2269 and MS-325 blood-pool agents. More specifically, contrast enhancement peaks slowly at 25 to 30 minutes following administration, remains constant for > 3 hours, and returns to baseline within 20 hours. Comparable but "transient" enhancement can be achieved using an extracellular contrast agent (OptiMARK), which demonstrates rapid peaks in contrast enhancement at 2 to 5 minutes following administration and then declines over a 40-minute period. The blood-pool contrast agents demonstrate an approximate 3-fold increase in dose-effectiveness and a lengthened window of tumor contrast enhancement compared to commonly available extracellular

contrast agents. This demonstrates the potential of alternative contrast-enhanced MRI examination protocols for tumor detection.

Albumin-binding MR blood pool agents as MRI contrast agents in an intracranial mouse glioma model. Adzamli K, Yablonskiy DA, Chicoine MR, Won EK, Galen KP, Zahner MC, Woolsey TA, Ackerman JJ. Magn Reson Med 2003 Mar; 49 (3), pp. 586–90.

Neoplasia (Central Nervous System [CNS]): Gliosarcoma
Rhodaminated LCDIO/Hoechst 33258 Dye/Green Fluorescent Protein

Cellular/Molecular Mechanism of Action: Hoechst 33258 dye can be applied as an intravital endothelial stain. Rhodaminated long-circulating dextran-coated iron oxide (LCDIO) particles can preferentially localize intracellular within certain malignant neoplastic cell types (e.g., gliosarcoma).
Application: Detection and characterization of CNS neoplastic lesions (e.g., gliosarcoma).
Route of Administration: Intravascular.
Implementation: Determination of the distribution of a LCDIO preparation in tumors. Accumulation of LCDIO in tissue sections is evaluated using multichannel fluorescence microscopy with rhodaminated LCDIO, green fluorescent protein as a tumor marker, and Hoechst 33258 dye as an intravital endothelial stain. Uptake into tumor cells is correlated (validated) with results from immunohistochemical and cell culture uptake analyses. The effect of intratumoral LCDIO uptake on MRI signal intensity is evaluated with a 1.5 tesla superconducting magnet.
Animal Model: Rodent gliosarcoma.
Interpretation and Analysis: Tumoral accumulation of LCDIO is 0.11% ± 0.06 of the injected dose per gram of tissue in brain tumors and sufficient for detection using MRI. In tumor sections, LCDIO preferentially localizes in tumor cells (49.0% ± 4.6) but is also taken up by macrophages in tumors (21.0% ± 3.1) and by endothelial cells in the areas of active angiogenesis (6.5% ± 1.4). In cell culture, LCDIO uptake strongly correlates with growth rate of tumor cell lines. Tumoral LCDIO accumulation is not negligible and helps explain MRI signal intensity changes observed in clinical trials. Microscopically, LCDIO accumulates predominantly in tumor cells and tumor-associated macrophages. Uptake into tumor cells appears to be directly proportional to cellular proliferation rate.
Instrumentation: Magnetic resonance imaging signal intensity evaluated with a 1.5 Tesla superconducting magnet.

Tumoral distribution of long-circulating dextran-coated iron oxide nanoparticles in a rodent model. Moore A, Marecos E, Bogdanov A Jr, Weissleder R. Radiology 2000 Feb; 214 (2), pp. 568–74.

Neoplasia (Central Nervous System [CNS]): Medulloblastoma
Gadolinium-Based Agent

Background Information: Experimental mouse models are emerging as useful systems for the study of human brain tumors. Nuclear MRI methods can non-invasively provide images of complex heterogeneous tissues such as experimental brain tumors.
Route of Administration: Gadolinium-based agents (intravenous).
Implementation: Demonstration of the feasibility of longitudinal high-resolution MRI in detecting and characterizing brain tumors.
Animal Model: Two mouse brain tumor models—patched heterozygous (ptc ±) mice with spontaneously arising posterior fossa tumors that resemble human medulloblastoma, and homozygous nude mice implanted with intracerebral xenografts of human medulloblastoma cell lines.
Interpretation and Analysis: Methods have been optimized to achieve favorable volumetric comparison with histologic methods and submillimeter resolution, improved by contrast enhancement with intravenous administration of a gadolinium-based agent.

Magnetic resonance imaging of patched heterozygous and xenografted mouse brain tumors. Nelson AL, Algon SA, Munasinghe J, Graves O, Goumnerova L, Burstein D, Pomeroy SL, Kim JY. J Neurooncol 2003 May; 62 (3), pp. 259–67.

Neoplastic Disease/Diagnostic and Therapeutic Implications
Gadobenate Dimeglumine ([Gd]-BOPTA)

Background Information: Gadobenate dimeglumine has a significantly greater [Gd] uptake by brain tumor tissue than gadopentate dimeglumine ([Gd]-DTPA).
Application: The agent [Gd]-BOPTA is assimilated at relatively high concentrations within certain neoplastic lesions that affect the central nervous system (CNS).
Route of Administration: [Gd]-BOPTA and [Gd]-DTPA (intralesionally and subcutaneously in rat research investigations).
Dosage: [Gd]-BOPTA and [Gd]-DTPA (0.05 mmol/g tumor weight).
Implementation: Evaluate whether [Gd]-BOPTA is an efficient agent for neutron capture therapy (NCT). Complementary investigations can involve delineation of the imaging properties of [Gd]-BOPTA and [Gd]-DTPA injected intralesionally into tumors. At the peak of [Gd] uptake, thermal neutron irradiation is applied.
Animal Model: Rat (Fisher 344 strain). Four groups of Fisher 344 rats (control, neutron (n), n+ [Gd]-DTPA, n+ [Gd]-BOPTA) injected subcutaneously with 9L gliosarcoma cells in both hind legs.
Interpretation and Analysis: A pronounced delay in tumor growth can be appreciated with [Gd+] formulations compared to control and neutron analyses ($p = 0.0053$, 0.0064, respectively). Furthermore, BOPTA is associated with a significantly prolonged delay of tumor growth compared to DTPA ($p = 0.033$). In addition, [Gd]-NCT demonstrates that [Gd]-BOPTA can function as an effective compound for NCT. Better cytocidal effects of [Gd]-BOPTA warrant further investigation of subcellular [Gd] distribution.

In vivo gadolinium neutron capture therapy using a potentially effective compound ([Gd]-BOPTA). Matsumura A, Zhang T, Yamamoto T, Yoshida F, Sakurai Y, Shimojo N, Nose T. Anticancer Res 2003 May–Jun; 23 (3B), pp. 2451–6.

Neoplastic Disease (Central Nervous System [CNS])/ Angiogenesis of Gliomas/Dimethylarginine Dimethylaminohydrolase (DDAH) Overexpression
NC100150/Hoechst 33342

Cellular/Molecular Mechanism of Action: The orientation of NC100150 becomes altered within a magnetic field. Alternatively, Hoechst 33342 emits fluorescent light energy.
Background Information: Intracellular factors that regulate nitric oxide (NO) synthesis represent important targets in tumor progression. Overexpression of DDAH, which metabolizes the endogenous inhibitors of NO synthesis asymmetric dimethylarginine and N-monomethyl-L-arginine, results in C6 gliomas with enhanced growth rates compared to wild cell types.

Application: NC100150 can be utilized as an intravascular blood-pool contrast agent that is detectable by MRI methodologies. Hoechst 33342 can be used as a fluorescent perfusion-marker dye that can be measured by flourescent detection systems.
Route of Administration: Utilization of NC100150 and Hoechst 33342 for neoplastic lesion angiogenesis requires that these be delivered intravascularly.
Implementation: Determine the (i) *in vitro* effects of DDAH on tumor vascular morphogenesis by measurement of the transverse relaxation rates R_2* and R_2 in clone D27 gliomas overexpressing DDAH and C6 wild-type gliomas using intrinsic susceptibility MRI sensitive to changes in endogenous deoxyhemoglobin; (ii) determine susceptibility contrast-enhanced MRI using NC100150 as an intravascular blood-pool contrast agent; and (iii) compare results with fluorescence microscopy of the tumor uptake of the perfusion marker Hoechst 33342.
Interpretation and Analysis: The baseline R_2* is significantly faster in D27 tumors, consistent with a greater vascular development ($p < 0.02$, ANOVA). There is no significant difference between the response of the 2 tumor types to hypercapnia (5% CO_2/95% air), used to evaluate vascular maturation, or hyperoxia (5% CO_2/95% O_2), used to characterize vascular function. NC100150 increases the R_2* and R_2 rates of both tumor types and detects a significantly larger blood volume in the D27 tumors ($p < 0.02$, ANOVA). This correlates with a significantly greater uptake of Hoechst 33342 in the D27 tumors compared to C6 wild-type tumors ($p < 0.02$, ANOVA). Despite increased tumor blood volume, the Delta R_2*/Delta R_2 ratio, an index of microvessel size, shows that the capillaries in the 2 tumor types are of a similar caliber. Such observations demonstrate the potential of susceptibility MRI-derived quantitative end points to non-invasively assess tumor angiogenesis. Application of intravascular blood-pool contrast agents such as NC100150 for such purposes appears very promising. Overexpression of DDAH results in increased *in vivo* neovascularization of C6 gliomas. The lack of a significant difference in hypercapnic/hyperoxic response between C6 and D27 tumors and the similar vessel caliber are also consistent with a role for DDAH in the initial stages of vasculogenesis.
Instrumentation: Magnetic resonance imaging and fluorescence microscopy instrumentation.

Effects of overexpression of dimethylarginine dimethylaminohydrolase on tumor angiogenesis assessed by susceptibility magnetic resonance imaging. Kostourou V, Robinson SP, Whitley GS, Griffiths JR. Cancer Res 2003 Aug 15; 63 (16), pp. 4960–6.

Neoplastic Disease Angiogenesis/Tumor Dimethylarginine Dimethylaminohydrolase (DDAH) Overexpression
NC100150/Hoechst 33342

Cellular/Molecular Mechanism of Action: The orientation of NC100150 becomes altered within a magnetic field. Alternatively, Hoechst 33342 emits fluorescent light energy.
Background Information: Intracellular factors that regulate nitric oxide (NO) synthesis represent important targets in tumor progression. Overexpression of DDAH, which metabolizes the endogenous inhibitors of NO synthesis asymmetric dimethylarginine and N-monomethyl-L-arginine, results in C6 gliomas with enhanced growth rates compared to wild cell types.
Application: NC100150 can be utilized as an intravascular blood-pool contrast agent that is detectable by MRI methodologies. Hoechst 33342 can be used as a fluorescent perfusion-marker dye that can be measured by flourescent detection systems.
Route of Administration: Utilization of NC100150 and Hoechst 33342 for neoplastic lesion angiogenesis requires that these be delivered intravascularly.
Implementation: Determine the (i) *in vitro* effects of DDAH on tumor vascular morphogenesis by measurement of the transverse relaxation rates R_2* and R_2 in clone D27 gliomas overexpressing DDAH and C6 wild-type gliomas using intrinsic susceptibility MRI sensitive to changes in endogenous deoxyhemoglobin; (ii) determine susceptibility contrast-enhanced MRI using NC100150 as an intravascular blood-pool contrast agent; and (iii) compare results with fluorescence microscopy of the tumor uptake of the perfusion marker Hoechst 33342.
Interpretation and Analysis: The baseline R_2* is significantly faster in D27 tumors, consistent with a greater vascular development ($p < 0.02$, ANOVA). There is no significant difference between the response of the 2 tumor types to hypercapnia (5% CO_2/95% air), used as a probe for vascular maturation, or hyperoxia (5% CO_2/95% O_2), used as a probe for vascular function. NC100150 increases the R_2* and R_2 rates of both tumor types and detects a significantly larger blood volume in the D27 tumors ($p < 0.02$, ANOVA). This correlates with a significantly greater uptake of Hoechst 33342 in the D27 tumors compared to C6 wild-type tumors ($p < 0.02$, ANOVA). Despite increased tumor blood volume, the Delta R_2*/Delta R_2 ratio, an index of microvessel size, shows that the capillaries in the 2 tumor types are of a similar caliber. Such observations demonstrate the potential of susceptibility MRI-derived quantitative end points to non-invasively assess tumor angiogenesis. Application of intravascular blood-pool contrast agents such as NC100150 for such purposes appears very promising. Overexpression of DDAH results in increased *in vivo* neovascularization of C6 gliomas. The lack of significant difference in hypercapnic/hyperoxic response between C6 and D27 tumors and the similar vessel caliber are also consistent with a role for DDAH in the initial stages of vasculogenesis.
Instrumentation: Magnetic resonance imaging and fluorescence microscopy instrumentation.

Effects of overexpression of dimethylarginine dimethylaminohydrolase on tumor angiogenesis assessed by susceptibility magnetic resonance imaging. Kostourou V, Robinson SP, Whitley GS, Griffiths JR. Cancer Res 2003 Aug 15; 63 (16), pp. 4960–6.

PANCREAS

Lesions: Pancreatic and Hepatic
Mangafodipir Trisodium

Background Information: The detection and characterization of pancreatic tumors as well as the reliable staging of pancreatic cancer are important objectives for radiologic evaluation. Contrast-enhanced helical computed tomography (CT) has been the standard modality for pancreatic imaging at many institutions, but MRI has gained a considerable role in the evaluation of patients with equivocal CT findings.
Application: Detection and characterization of disease states affecting the liver and pancreas, including neoplastic conditions utilizing MRI analysis.
Interpretation and Analysis: Contrast-enhanced MRI has proven to be very helpful in the detection of small tumors or the identification of tumor-simulating lesions in patients with equivocal CT findings. Mangafodopir may improve the staging of pancreatic cancer by increasing the sensitivity of MRI in the detection of liver metastases.

Evaluation of focal pancreatic masses: Comparison of mangafodipir-enhanced MR imaging and contrast-enhanced helical CT. Schima W, Fugger R. Eur Radiol 2002 Dec; 12 (12), pp. 2998–3008.

Pancreatic Duct Function: Pancreatography
Secretin

Cellular/Molecular Mechanism of Action: Secretin promotes increases in exocrine pancreatic function.
Background Information: Magnetic resonance pancreatography is a new modality for visualizing the pancreatic duct. Prolonged dilation of the pancreatic duct following secretin administration may suggest obstruction at the level of the pancreatic duct orifice.
Application: Enhance visualization of pancreatic duct exocrine flow.
Implementation: Pancreatography of idiopathic acute recurrent pancreatitis aided by secretin-mediated stimulation of exocrine function in concert with subsequent endoscopic retrograde pancreatogram with or without manometry (e.g., n = 10 cases).
Interpretation and Analysis: Visualization of the main pancreatic duct is possible in most cases without evidence of chronic disease (e.g., n = 10/10). Similarly, it is possible to detect cases of pancreas divisum (e.g., n = 2), and prolonged dilation of the pancreatic duct on secretin-stimulated MR pancreatography accompanied by evidence of pancreatic duct outflow obstruction (e.g., n = 3). Some cases of pancreatic duct outflow obstruction have normal secretin-stimulated MR pancreatography (e.g., n = 4). Therefore, secretin-stimulated MR pancreatography provides high-quality pancreatic duct images and has high specificity but low sensitivity for diagnosing pancreatic duct outflow obstruction using manometric/clinical criteria.

Secretin-stimulated magnetic resonance pancreaticogram to assess pancreatic duct outflow obstruction in evaluation of idiopathic acute recurrent pancreatitis: A pilot study. Khalid A, Peterson M, Slivka A. Dig Dis Sci 2003 Aug; 48 (8), pp. 1475–81.

Pancreatitis: Acute Biliary/ Cholangiopancreatography: Pancreatic Morphology Alterations
Secretin

Cellular/Molecular Mechanism of Action: Secretin stimulates exocrine pancreatic function.
Application: Assessment of the potential for acute biliary pancreatitis progressing to pancreatic morphological alterations applying MR cholangiopancreatography. Secretin can be applied to evaluate exocrine pancreatic function and improve visualization of the exocrine pancreatic tree.
Implementation: Characterization of biliary pancreatitis applying MR cholangiopancreatography. Secretin is administered in order to determine if the pancreatic duct is incomplete or absent, and ductal morphology before and after secretin stimulation compared (e.g., n = 16 cases).
Human Model: Cases having undergone cholecystectomy but devoid of alterations of pancreatic function 5 years after the last episode of pancreatitis (e.g., n = 25/40 female, n = 15/40 male; n = 27/40 mild, n = 13/40 severe).
Interpretation and Analysis: Significant differences can be observed when the diameter and length of the main pancreatic duct are compared in cases with reference controls, and can be completely visualized in 60% of cases, in addition to being seen in all cases following secretin stimulation. The comparative statistical analysis of the length and diameter of the pancreatic duct before and after the secretin stimulation shows significant differences. Acute biliary pancreatitis leads to morphological alterations, such as scar lesions that do not become chronic in nature. Secretin stimulation improves the visualization of the main pancreatic duct.

Main pancreatic duct: Morphology after acute biliary pancreatitis with magnetic resonance cholangiopancreatography after secretin stimulation. Pareja E, Artigues E, Mir J, Fabra R, Martinez V, Vazquez A, Trullenque R. Rev Esp Enferm Dig 2003 Jun; 95 (6), pp. 395–400, 389–94 (different versions of same article).

Pancreatitis (Acute): Prognostic Features

Application: Detection, characterization, and prediction of prognosis for survival in cases suffering from acute pancreatitis.
Implementation: Characterization of elevations in peripancreatic signal on fat-suppressed T_1-weighted images. Abdominal MRI from cases of acute pancreatitis are assessed to determine the presence of elevated signal intensity within the peripancreatic fat on fat-suppressed T_1-weighted spooled gradient echo images. The degrees of signal change are then graded as mild, moderate, or severe (e.g., n = 31 cases).
Interpretation and Analysis: Abnormally high signal intensity within the peripancreatic fat on fat-suppressed T_1-weighted images can be detected in approximately 30% of cases (e.g., n = 10/30). A modest number of cases display signal changes graded as mild (e.g., n = 3/10), moderate (e.g., n = 1/10), and severe (e.g., n = 6/10). The mortality rate within a 68-day period for cases graded with severe elevations in peripancreatic soft tissue signal is very high (e.g., n = 6/6). Alternatively, the survival rate for cases without evidence of elevated peripancreatic fat signal is very high (e.g., n = 21/21). The correlation between the presence and severity of elevated peripancreatic MR signal and patient outcome is significant ($p < 0.05$). Therefore, increases in peripancreatic image signals appear to be closely associated with a progressively poorer prognosis for survival in cases suffering from acute pancreatitis. Such techniques may represent a simplified method for prognostic cross-sectional imaging.

High signal peripancreatic fat on fat-suppressed spoiled gradient echo imaging in acute pancreatitis: Preliminary evaluation of the prognostic significance. Martin DR, Karabulut N, Yang M, McFadden DW. J Magn Reson Imaging 2003 Jul; 18 (1), pp. 49–58.

Pancreatitis: Early or Mild Chronic Form
Gadolinium Chelate

Application: Gadolinium chelate image-enhancing agents can be used to diagnose early or mild chronic pancreatitis by dynamic MRI, especially before apparent pancreatic morphologic or signal intensity changes become apparent.
Route of Administration: Gadolinium chelate (intravascular).
Implementation: Evaluation of suspected cases of early or mild chronic pancreatitis classified according to the imaging criteria of (i) equivocal chronic pancreatitis (ultrasound, computed tomography, or endoscopic retrograde cholangiopancreatography [ERCP]) grading; (ii) having dynamic MRI that includes a lack of enhancement; (iii) arterial dominant; and (iv) early venous and late venous phases of contrast enhancement. The signal intensity is measured at the pancreatic head, body, and tail on all phases, and for each, the signal intensity ratio (SIR, the signal intensity in postcontrast divided by that in precontrast) is individually calculated. Images are reviewed for pancreatic morphologic abnormalities without knowing the results of signal intensity measurements.

Human Model: Cases of suspected early or mild chronic pancreatitis (e.g., n = 24). Reference control subjects consist of cases without pancreatic diseases (e.g., n = 20).

Interpretation and Analysis: On unenhanced images, there is no significant difference of signal intensity between control and pancreatitis cases ($p < 0.05$). In pancreatitis conditions, but not controls, the unenhanced signal intensity of the pancreatic head and body are significantly higher than that of the tail ($p < 0.05$). In control cases, the greatest enhancement (highest SIR) after injection is in the arterial phase (1.89 ± 0.31), which is significantly higher than in the early venous phase (1.68 ± 0.17, $p < 0.01$) and in the late venous phase (1.61 ± 0.15, $p < 0.001$). Pancreatitis conditions however, have an arterial phase SIR (1.65 ± 0.23) that is significantly lower than its early venous phase SIR (1.75 ± 0.22, $p < 0.05$) and lower than the arterial phase SIR of the control group ($p < 0.01$). The presence of an SIR < 1.73 in the arterial phase and/or a delayed peak enhancement after contrast agent administration has a sensitivity and specificity of diagnosing early or mild chronic pancreatitis of 92% and 75%, respectively. This sensitivity is significantly higher than the sensitivity of 50% for diagnosis based on morphologic abnormalities ($p < 0.05$). Measuring pancreatic signal intensity on gadolinium chelate dynamic MRI is helpful for diagnosing early or mild chronic pancreatitis, especially before apparent pancreatic morphologic or signal intensity changes are present.

Suspected early or mild chronic pancreatitis: Enhancement patterns on gadolinium chelate dynamic MRI. Zhang XM, Shi H, Parker L, Dohke M, Holland GA, Mitchell DG. J Magn Reson Imaging 2003 Jan; 17 (1), pp. 86–94.

RENAL SYSTEM

Renal Abscesses and Asymptomatic Masses
Gadolinium-DTPA ([Gd]-DTPA)/LumenHance

Implementation: Magnetic resonance imaging of asymptomatic renal masses, with enhancement sufficient to effectively exclude simple and hemorrhagic cysts.

Considerations: Because the MRI appearances of renal cell neoplasm and renal abscess overlap, both must be considered in the differential diagnosis.

Interpretation and Analysis: Focal and multifocal renal abscesses can be positively detected by MRI utilizing [Gd]-DTPA functioning as an imaging contrast-enhancing agent .

Instrumentation: Computer-integrated MRI capabilities.

Asymptomatic renal abscess: Evaluation with gadolinium DTPA-enhanced MRI. Cyran KM, Kenney PJ. Abdom Imaging 1994 May–Jun; 19 (3), pp. 267–9.

Renal Structures/Renal Cancer Staging
Renal Eliminated Paramagnetic Contrast

Magnetic resonance imaging differs fundamentally from other imaging techniques, because for the 1st time 1 method permits simultaneous assessment of macroscopic changes in the kidneys and evaluation of renal functional derangements. To provide morphological information that may compete with the results of computed tomography (CT) and ultrasonography (US), special techniques like artifact compensation, fast imaging, and fat suppression have to be employed. The additional use of a renal-eliminated paramagnetic contrast agent permits assessment of parenchymal perfusion and visualization of the passage of the contrast medium, providing information about renal excretory function. Results and clinical utility of state-of-the-art MRI can be summarized as follows: (i) MR is not a screening method; sensitivity is, however, similar to CT when fat suppression and contrast agents are used. (ii) Classification of renal masses is almost restricted to the differentiation of cystic, fat-containing, and solid lesions. (iii) Contrast-enhanced MRI helps to assess large retroperitoneal masses and to classify them as intra- or extrarenal. (iv) Accurate staging of renal cancers is possible; thus, MRI is a good alternative when CT yields uncertain findings and for patients with known contraindications to the administration of iodinated contrast agents. (v) Contrast-enhanced dynamic MRI studies provide semiquantitative evaluation of global and segmental renal function. (vi) MR angiography offers the possibility to evaluate renal arteries and veins.

Magnetic resonance imaging of the kidneys: Current status. Krestin GP. Magn Reson Q 1994 Mar; 10 (1), pp. 2–21.

Renal Transplant Rejection
Ultrasmall Superparamagnetic Particles of Iron Oxide (USPIOs)

Cellular/Molecular Mechanism of Action: Preparations of USPIOs are perceived as foreign material and internalized by macrophage populations. Prussian blue detects intracellular iron deposits.

Application: Indirect monitoring of the integrity of renal transplants through the detection of macrophage accumulation in situations of kidney rejection.

Implementation: Evaluation of renal transplants non-invasively applying MRI in combination with image-enhancing infusion of USPIOs preparations. In this manner it is possible to determine whether accumulation of immune cells like macrophages in renal transplants can be detected *in vivo* while a kidney transplant is in the process of being rejected. Logistically, this is accomplished by infusion of USPIOs with MRI performed before, immediately after, and 1 day following administration.

Animal Model: Rat (major histocompatibility disparate DA to BN male rat renal transplantation recipients).

Interpretation and Analysis: Infusion of USPIO on the 4th day posttransplantation reveals some rejecting allografts detected as a decrease of MR signal intensity 1 day later. Isografts and allografts with triple immunosuppressant treatment have no MR signal reduction. Immunohistologic staining for ED1+ macrophages and CD4+ and CD8+ T cells in allogeneic transplanted kidneys indicates accumulation of these immune cells as a feature of acute rejection progress. Morphological studies by electron microscopy confirm the existence of iron inside macrophage lysosomes residing within rejected kidneys, while Prussian blue staining detects the presence of iron plaques in macrophages. Isografts and allografts with a triple immunosuppressant treatment exhibit smaller MR signal reductions with minimal histologic changes. The concurrence of MR signal reduction following USPIO infusion with pathological manifestation in a rat renal allograft model suggests the possibility that renal transplantation status may be assessed by MRI using USPIO particles as markers for the accumulation of immune cells, such as macrophages.

Magnetic resonance imaging detection of rat renal transplant rejection by monitoring macrophage infiltration. Zhang Y, Dodd SJ, Hendrich KS, Williams M, Ho C. Kidney Int 2000 Sep; 58 (3), pp. 1300–10.$eref4

Renal/Urinary Bladder and Kidney
Piperidinyl Nitroxide Stable Free Radical Derivative (TES)

Application: Contrast-enhancing pharmaceutical for nuclear magnetic resonance (NMR) procedures.

Implementation: Evaluation of a piperidinyl nitroxide stable free radical derivative, TES, to determine its diagnostic efficacy as an NMR contrast enhancer of renal structures in conditions of normal health, experimentally-induced unilateral renal ischemia, renal vascular congestion, and hydronephrosis.
Interpretation and Analysis: Physiologic measurements indicate that TES is rapidly excreted in the urine with a clearance rate equal to the glomerular filtration rate. Because the compound is strongly paramagnetic, it increases the observable NMR intensity within the kidneys and urine at relatively low doses (0.04–0.9 g/kg). TES-enhanced spin-echo renal images can clearly demonstrate the presence of disease and functional abnormalities in diseased kidneys. These abnormalities are either not evident or only indirectly suggested on non-enhanced NMR images.

Work in progress: Nuclear magnetic resonance study of a paramagnetic nitroxide contrast agent for enhancement of renal structures in experimental animals. Brasch RC, London DA, Wesbey GE, Tozer TN, Nitecki DE, Williams RD, Doemeny J, Tuck LD, Lallemand DP. Radiology 1983 Jun; 147 (3), pp. 773–9.

Renal/Urinary Bladder and Kidney
Tempo Carboxylic Acid (TCA)

Cellular/Molecular Mechanism of Action: Functions as a nitroxide diagnostic agent.
Application: Contrast-enhancing pharmaceutical for MRI procedures.
Considerations: The meglumine salt of TCA (piperidinyl nitroxide stable free radical derivative) functions as an nuclear magnetic resonance (NMR) contrast enhancer.
Interpretation and Analysis: Enhances the signal intensity of the kidney and bladder.

Ideal imaging in MR: Contrast-enhancing pharmaceuticals. Evaluation of tempo carboxylic acid as nitroxide diagnostic agent. Lamarque JL, Almès C, Rouanet JP, Prat X, Bruel JM, Pujol J, Rodière MJ, Martin JM, Lopez P, Rossi M. Eur J Radiol 1986 Feb; 6 (1), pp. 48–52.

Renal Vascular Perfusion: Cortex and Medulla
Iodixanol

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Iodixanol is a high-viscosity, dimeric isoosmolar iodinated contrast medium.
Route of Administration: Iodixanol (intravenous).
Implementation: Assess the abilities of dynamic diffusion-weighted MRI to demonstrate the *in vivo* effects of the high-viscosity iodinated contrast agent (iodixanol) on medullary and cortical blood flow in the kidney. Dynamic diffusion-weighted, echoplanar MR images are acquired from 5 b-value single-shot images and their apparent isotropic diffusion coefficient maps established before and after intravenous injection of iodixanol, and compared with those obtained from negative reference controls.

Animal Model: Rat (pentobarbital-sedated; n = 9 administered iodixanol; n = 4 negative saline controls).
Interpretation and Analysis: The mean baseline apparent diffusion coefficient values are $1.64 \pm 0.05 \times 10^{-3}$ mm^2/s for the cortex and $1.75 \pm 0.06 \times 10^{-3}$ mm^2/s for the medulla. In the iodixanol group, a significant decrease in renal diffusion can be observed at 12 minutes and lasts at least 24 minutes. The decrease in diffusion occurs earlier for the cortex and lasts less than for the medulla. There is no significant modification in diffusion over time in the control group. Preliminary experience in rats reveals that dynamic diffusion-weighted MRI can be used to non-invasively evaluate *in vivo* renal hemodynamic response after injection of iodinated contrast agent.

Hemodynamic effect of iodinated high-viscosity contrast medium in the rat kidney: A diffusion-weighted MRI feasibility study. Laissy JP, Menegazzo D, Dumont E, Piekarski JD, Karila-Cohen P, Chillon S, Schouman-Claeys E. Invest Radiol 2000 Nov; 35 (11), pp. 647–52.

RESPIRATORY SYSTEM

Pulmonary Embolism
Monocrystalline Iron Oxide

Cellular/Molecular Mechanism of Action: The spatial orientation of monocrystalline iron oxide becomes altered within magnetic energy fields, which can be detected by MRI instrumentation.
Background Information: Contrast-enhanced MR angiography could be used as a non-invasive imaging technique to determine the therapeutic effect and end point in thrombolysis of acute pulmonary embolism.
Application: Contrast-enhanced MR angiography. Relevant examples include utilization as a non-invasive imaging technique to determine the therapeutic effect and end point in thrombolysis of acute pulmonary embolism in an animal model.
Route of Administration: Monocrystalline iron oxide (intravenous).
Dosage: Monocrystalline iron oxide (2 mg).
Implementation: Three-dimensional time of flight (TOF) MR angiograms are obtained after intravenous administration of Fe (2 mg) of a long-circulating monocrystalline iron oxide. Animals then receive 5,000 IU heparin and recombinant tissue plasminogen activator (1.3 mg/kg) intravenously. Magnetic resonance angiography imaging is repeated 30 minutes and 60 minutes after initiation of thrombolytic therapy.

Animal Model: New Zealand White rabbits anesthetized and mechanically ventilated (e.g., n = 18). Single (n = 12 emboli) or dual (n = 12 emboli in n = 6 animals) pulmonary emboli are created by the injection of autologous thrombi through a right internal jugular vein.
Interpretation and Analysis: Monocrystalline iron oxide nanoparticle (MION)-enhanced MR angiography accurately detects pulmonary emboli (e.g., rabbits). Thrombolysis during the observation period is successful less than 50% of the time (e.g. n = 8/18). In single emboli, the revascularization rate is approximately 50% (e.g., n = 6/12 emboli). The rate is 33% (e.g., n = 4/12 emboli) with multiple emboli. Magnetic resonance angiography allows determination of thrombus resolution or thrombus persistence. Therefore, it is entirely feasible to diagnose pulmonary embolism accurately and to monitor thrombolysis of pulmonary emboli by MION-enhanced MR angiography.

Efficacy of thrombolytic therapy in pulmonary embolism determined by MION-enhanced MRA: An experimental study in rabbits. Frank H, Loewe R, Loewe C, Oberhuber G, Schwaighofer B, Huber K, Weissleder R. Invest Radiol 1998 Dec; 33 (12), pp. 853–7.

Pulmonary/Thoracic Imaging

The application of MRI for thoracic investigations has become increasingly appealing. This resurgence has been triggered by the enormous improvements in image quality with the development of scanners with faster data-acquisition capabilities and the routine use of contrast agents. In particular, MR pulmonary angiography, an examination that once had been hampered by blurring from respiratory and cardiac motion and magnetic-susceptibility-related signal

loss, has become a robust technique in this scenario. Initial efforts and current trends have been described that focus on fast imaging protocols employing contrast agents that provide exquisite images of the pulmonary vasculature. In addition, the recent introduction of MR lung perfusion and ventilation scans has made available potentially new methods for characterizing diseases that, combined with MR pulmonary angiography, may provide a practical approach for the diagnosis of difficult conditions such as pulmonary embolism, which has long been awaited.

Magnetic resonance pulmonary angiography. Wielopolski PA. Coron Artery Dis 1999 May; 10 (3), pp. 157–75.

Pulmonary Imaging
Intravenous Contrast Agents/Hyperpolarized Noble Gases or Oxygen

Background Information: The lung has long been neglected by MRI. This is due to unique intrinsic difficulties: (i) signal loss due to cardiac pulsation and respiration; (ii) susceptibility artifacts caused by multiple air-tissue interfaces; and (iii) low proton density.
Application: Imaging of the lung to detect and characterize various disease states using MRI methodologies. Such diagnostic approaches can be used on a routine basis for (i) characterization of pulmonary nodules; (ii) staging of bronchogenic carcinoma, in particular assessment of chest wall invasion; (iii) evaluation of inflammatory activity in interstitial lung disease; (iv) acute pulmonary embolism; (v) chronic thromboembolic pulmonary hypertension; (vi) vascular involvement in malignant disease; and (vii) vascular abnormalities.
Implementation: Evaluation of stratigies that can be employed to overcome the problems of MRI of the lung, including (i) breath-hold imaging; (ii) respiratory and cardiac gating procedures; (iii) use of short repetition and echo times; (iv) increase the relaxivity of existing spins by administration of intravenous contrast agents; and (v) enrich spin density by hyperpolarized noble gases or oxygen.
Interpretation and Analysis: Improvements in scanner performance and frequent use of contrast media have increased the interest in MRI and MR angiography of the lung. Future applications include perfusion imaging using extracellular or intravascular (blood-pool) contrast agents and ventilation imaging using inhaled hyperpolarized noble gases, of paramagnetic oxygen or of aerosolized contrast agents. These techniques represent new approaches for functional lung imaging. The combination of visualization of morphology and functional assessment of ventilation and perfusion is unequaled by any other technique.

Contrast-enhanced MRI of the lung. Kauczor HU, Kreitner KF. Eur J Radiol 2000 Jun; 34 (3), pp. 196–207.

Pulmonary Imaging: Aerozolized Contrast Image Enhancement Agents/Signal Increase and Signal-to-Noise Ratios (SNRs)/Relative Aerosolized Effectiveness
Gadoteridol/Gadobutrol/Gadopentetate Dimeglumine (Magnevist)/Gadobenate Dimeglumine/ Gadopentetate Dimeglumine

Cellular/Molecular Mechanism of Action: Gadolinium atom orientation becomes altered when exposed to magnetic energy fields
Application: Detection and characterization of pulmonary disease and alterations in normal respiratory physiology.
Route of Administration: Ventilation of aerosolized contrast agent.
Semi-Synthetic Methods: Utilization of a jet nebulizer to generate aerosolized formulations.
Implementation: Aerozoliation of gadopentetate dimeglumine (Magnevist; Berlex Imaging, Montville, NJ, USA), gadobutrol (Gadovist; Schering, Jarfalla, Sweden), gadoteridol (ProHance; Bracco-Byk Gulden, Konstanz, Germany), gadobenate dimeglumine (MultiHance; Bracco-Byk Gulden, Konstanz, Germany), and gadopentetate dimeglumine (formulated with mannitol and a surface active detergent) can function as potential pulmonary ventilation contrast media for MRI procedures. In this context, aerosolized contrast agents including gadopentetate dimeglumine, gadobutrol, gadoteridol, gadobenate dimeglumine, and gadopentetate dimeglumine (formulated with mannitol and a surface active detergent) are delivered by mechanical ventilation followed by MRI (1.5 Tesla fields). T_1-weighted turbo-spin-echo (TSE) images using respiratory gating are obtained before and after a 10-minute ventilation period. Pulmonary signal intensity (SI) and SNR changes are measured for both lungs.

Animal Model: Domestic swine intubated and administered aerosolized contrast agents delivered by mechanical ventilation (n = 12 animals).
Interpretation and Analysis: Mean increases in SI range between 13.5% and 45.8% (right lung) and 14% and 39.8% (left lung). The SNR changes range from +14.7% to +46.8% and from +13.1% to +40.5% for the right and left lung, respectively. The highest SI and SNR increases are observed with the administration of gadoteridol.

The use of gadolinium for MR ventilation imaging is primarily hindered by its high viscosity and, thus, its capability of aerosolization. The medium with the lowest viscosity at room temperature (gadoteridol) shows the most promising enhancement results. Gadolinium-based contrast agents can potentially be applied as a pulmonary imaging alternative. With a reduction of ventilation duration down to 10 minutes, the method appears tolerable in a clinical setting.

Gadolinium-containing contrast agents for pulmonary ventilation magnetic resonance imaging: Preliminary results. Haage P, Karaagac S, Adam G, Spuntrup E, Pfeffer J, Gunther RW. Invest Radiol 2002 Mar; 37 (3), pp. 120–5.

Pulmonary Imaging
Hyperpolarized Noble Gases/Helium (^{3}He)/ Xenon (^{129}Xe)

Semi-Synthetic Methods: Non-radioactive noble gases with a nuclear spin 1/2 (^{3}He, ^{129}Xe) can be hyperpolarized by optical pumping. Polarization is transferred from circularly polarized laser light to the noble-gas atoms via alkali-metal vapors (spin exchange) or metastable atoms (metastability exchange). Hyperpolarization results in a non-equilibrium polarization 5 orders of magnitude higher than the Boltzmann equilibrium compensating for the several 1,000 times lower density of noble gases as compared with liquid state hydrogen concentrations in tissue, and allows for short imaging times. Hyperpolarization can be stored for a sufficiently long period of time (3 hours to 6 days) to allow for transport and application.
Implementation: Hyperpolarized gases are administered as inhalative "contrast agents," thereby facilitating the imaging of airways and airspaces. Besides the known anesthetic effect of xenon, no adverse effects are associated with its application. Pulse sequences are optimized to effectively use the non-renewable hyperpolarization before it decays or is destroyed, using fast low-flip-angles strategies to allow for dynamic/breath-hold imaging of highly diffusible (He) or soluble (Xe) gases with *in vivo* T_1-times well below 1 minute.

Since helium is not absorbed in considerable amounts, its application is restricted to the lung. Xenon has also been evaluated for imaging of diseases affecting the white matter of the central nervous system and functional studies of cerebral vascular perfusion.

Interpretation and Analysis: Magnetic resonance imaging using hyperpolarized gases is emerging as a technical challenge and opportunity for the MR community. Preliminary experience suggests potential for functional imaging of pulmonary ventilation and cerebral perfusion.

Instrumentation: Magnetic resonance systems equipped with broadband radio-frequency system and dedicated coils.

MRI using hyperpolarized noble gases. Kauczor H, Surkau R, Roberts T. Eur Radiol 1998; 8 (5), pp. 820–7.

Pulmonary Signal Intensity/Aerosol Distribution Homogeneity

Aerosolized Gadopentetate Dimeglumine/Aerosolized Gadoteridol

Route of Administration: Inhalation as aerosolized gadoteridol and gadopentetate dimeglumine.

Implementation: Compare aerosolized gadoteridol and gadopentetate dimeglumine with regard to homogeneity of aerosol distribution and relative pulmonary signal intensity (SI) changes after a 10-minute mechanical ventilation period. Logistically, 1 approach to achieving this objective can entail mechanical aerosolization with nebulized contrast medium (e.g., n = 20 procedures; n = 10 swine). Images are obtained using a respiratory-gated T_1-weighted turbo-spin-echo (TSE) sequence.

Animal Model: Intubated domestic swine (n = 10) mechanically aerosolized with nebulized contrast media.

Interpretation and Analysis: Pulmonary SI increases for the right and left lung are lower for gadopentetate dimeglumine (e.g., 26% ± 25% and 25% ± 12%, respectively), compared to gadoteridol (e.g., 45% ± 12% and 43% ± 10%, respectively). Aerosol distribution is almost invariably homogeneous in character. Therefore, both contrast agents in aerosolized form are capable of depicting pulmonary ventilation in healthy animals with lung capacities comparable to those of humans. Signal intensity differences between gadoteridol and gadopentetate dimeglumine can be explained by their considerable difference in viscosity.

Comparison of aerosolized gadoteridol and gadopentetate dimeglumine for MR ventilation imaging of the lung. Haage P, Karaagac S, Adam G, Glowinski A, Gunther RW. Magn Reson Med 2001 Oct; 46 (4), pp. 803–6.

Pulmonary Vasculature: Spin-Echo MR

Early attempts to image the pulmonary vasculature with spin-echo MRI were hampered by severe image degradation related to respiratory and cardiac pulsation artifact, susceptibility at interfaces between lung parenchyma and vessel wall, and poor contrast between flowing blood and intravascular filling defects of emboli. With the development of gradient-echo MR angiographic techniques, some of these limitations have been overcome; however, the need for multiple breath-holds and the frequent occurrence of flow-related artifacts that could simulate pulmonary emboli have diminished their clinical utility. With the development of contrast-enhanced MR angiography, many of the limitations of earlier techniques have been addressed. Images of both lungs with high signal-to-noise ratios and high contrast between flowing blood and pulmonary emboli can be acquired in a single breath-hold, during "1st-pass" imaging with extracellular contrast agents in the coronal plane. However, subsegmental vessels cannot be assessed with this approach. The technique has been refined further by imaging each lung separately in the sagittal plane; this offers higher resolution and total lung coverage and requires a shorter breath-hold. Finally, several investigators have reported preliminary data on imaging of the pulmonary vasculature with blood-pool agents, exploiting respiratory triggering or navigator echoes to eliminate the need for breath-holding for the detection of pulmonary emboli.

Pulmonary magnetic resonance angiography. Meaney JF, Johansson LO, Ahlstrom H, Prince MR. J Magn Reson Imaging 1999 Sep; 10 (3), pp. 326–38.

Respiratory: Anatomical Structures and Function/ Chronic Obstructive Pulmonary Disease (COPD)/ Ventilation Parameters

Aerosolized MRI Contrast Agents/Sulfur Hexafluoride/ Perfluorcarbons/ Hyperpolarized Noble Gases (^{3}He and ^{129}Xe)

Magnetic resonance imaging instrumentation and techniques have been developed for functional ventilation imaging. The current status and future perspectives in research and clinical applications are summarized. Morphological lung imaging is based on chest radiography and computed tomography, whereas scintigraphy is used for ventilation imaging. During recent years, MRI has emerged as a new means for functional imaging of ventilation. Aerosolized contrast agents and oxygen are used in proton imaging, whereas non-proton imaging relies on fluorine compounds, such as sulfur hexafluoride and perfluorcarbons, or on hyperpolarized noble gases, such as helium-3 [^{3}He] or xenon-129 [^{129}Xe]. All the gases are administered as inhaled "contrast agents" for imaging of the airways and airspaces. In general, straightforward images demonstrate the homogeneity of ventilation in a breath-hold and allow for determination of ventilated lung. The different properties of the different compounds enable the measurement of additional functional parameters. They comprise airspace size, regional oxygen partial pressure, and analysis of ventilation distribution, ventilation/perfusion ratios, and gas exchange, including oxygen uptake. Novel MRI techniques provide the potential for functional imaging of ventilation. The next steps include definition of the value and the potential of the different contrast mechanisms as well as determination of the significance of the functional information with regard to physiological research and patient management in COPD and others.

Assessment of lung ventilation by MR imaging: Current status and future perspectives. Kauczor HU, Hanke A, Van Beek EJ. Eur Radiol 2002 Aug; 12 (8), pp. 1962–70. Epub 2002 May 24.

EXPERIMENTAL IMAGING CONTRAST-ENHANCING AGENTS

MRI Agent (Experimental)

Polygalacturonic Acid

Cellular/Molecular Mechanism of Action: Linear carbohydrate polymer of monomeric galacturonic acid.

Considerations: Pectin and polygalacturonic acid are used extensively as gelling agents and stabilizers by the food industry. In addition, they have applications as therapeutic and diagnostic pharmaceutical agents with examples including the MRI agent LumenHance. Commercially available preparations are composed of apple and citrus pectins that consist of a mixture of partially methoxylated and/or amidated polygalacturonic acids with molecular weights ranging from 25,000 to > 100,000 kDa.

Implementation: Pectin can be chemically or enzymatically hydrolyzed to yield polygalacturonic acid fractions of diverse average molecular weight ranges and polydispersities for a variety of

uses. A simple high-performance size exclusion chromatography (HPSEC) method employing commonly available non-specialized high-performance liquid chromatography (HPLC) instrumentation can be used as a rapid molecular weight screening procedure for determining the average molecular weight range and polydispersity of polygalacturonic acid intended for use in pharmaceutical formulations. A TosoHaas G3000PWXL HPLC column, 50 mM phosphate buffer (pH approximately 6.9) mobile phase, and refractive index detection can be applied.

Interpretation and Analysis: A molecular weight calibration curve is linear for polysaccharide standards of 180 to 100,000 Da with a 0.999 coefficient of correlation. The method has been employed to screen commercially available polygalacturonic acid raw materials for average molecular weight data (Mn, Mw, and Mp) and polydispersity (Mw/Mn).

The use of high-performance size exclusion chromatography (HPSEC) as a molecular weight screening technique for polygalacturonic acid for use in pharmaceutical applications. White GW, Katona T, Zodda JP. J Pharm Biomed Anal 1999 Sep; 20 (6), pp. 905–12.

STEM CELLS

Stem Cell Migration: Brain/[Parkinson's Disease Research]
Gadolinium Rhodamine Dextran (GRID)/ Ex Vivo Stem Cell Labeling

Background Information: The ability to track stem cell transplants in the brain by *in vivo* neuroimaging will improve current understanding about of how these cells mediate functional recovery after neural transplantation. One major challenge for the development and refinement of stem cell transplantation is to map the spatial distribution and rate of *in situ* migration.

Application: Tracking transplanted stem cells in ischemia-damaged hippocampus by MRI.

Implementation: Assessment of stem cell migration applying *in vitro* labeling of populations with the novel bifunctional contrast agent GRID prior to transplantation. Detection is achieved applying both MRI and fluorescence microscopy, or with PKH26, visible exclusively under fluorescence microscopy. At different time points following engraftment, brains are evaluated by both histology and *ex vivo* MRI.

Animal Model: Rat (ischemic hippocampus).

Interpretation and Analysis: Transplanted stem cells can be identified by MRI only if prelabeled with GRID, whereas fluorescence microscopy can detect transplanted cells using either labeling reagent. Distribution of GRID-labeled stem cells established by applying MRI corresponds to results using fluorescence microscopy. Stem cells and their migration can therefore be reliably identified applying GRID-enhanced MRI methodologies.

Tracking transplanted stem cell migration using bifunctional, contrast agent-enhanced, magnetic resonance imaging. Modo M, Cash D, Mellodew K, Williams SC, Fraser SE, Meade TJ, Price J, Hodges H. Neuroimage 2002 Oct; 17 (2), pp. 803–11.

Stem Cell Migration (Mesenchymal)/Mesenchymal Stem Cells/Lymphocytes/Oligodendrocyte Progenitor CG-4 Cells/Cervical Carcinoma/Lymphocytes/[Iron Load: *Ex Vivo*]
Transfection Agents/Ferumoxides/MION-46L/ [Prussian Blue]

Cellular/Molecular Mechanism of Action: Transfection agents incorporate foreign DNA (oligonucleotide) sequences into the genome of "host" cell populations in a manner that allows or facilitates gene expression (e.g., DNA → messenger RNA (mRNA) → biological protein) and gene amplification (e.g., mitosis).

Application: Transfection labeling of mammalian and stem cell populations.

Route of Administration: Intended for intravascular applications.

Semi-Synthetic Methods: Transfection agents are incubated with ferumoxides and MION-46L in cell culture medium at various concentrations. Different viable cell types are subsequently incubated 2–48 hours with 25 μg of iron per milliliter of combined transfection agent/superparamagnetic iron oxide (TA/SPIO). Cellular labeling effectiveness can be assessed with T_2 relaxometry, MRI of labeled cell suspensions, and Prussian blue staining for iron assessment.

Cellular Model: Human mesenchymal stem cells, mouse lymphocytes, rat oligodendrocyte progenitor CG-4 cells, and human cervical carcinoma cells.

Implementation: Evaluation of the proliferation and viability of mesenchymal stem cells and human cervical carcinoma cells labeled with a combination of TAs and ferumoxides.

Interpretation and Analysis: When ferumoxides-TA or MION-46L-TA are utilized, intracytoplasmic particles stained with Prussian blue stain can be detected in all cell types with a labeling efficiency of nearly 100%. Limited or no uptake can be observed for cells incubated with ferumoxides or MION-46L alone. For TA-SPIO-labeled cells, MR images and relaxometry analysis reveal a 50–90% decrease in signal intensity and a more than 40-fold increase in T_2s. Cell viability varies from 103.7% ± 9 to 123.0% ± 9 compared with control cell viability at 9 days, while cell proliferation is not affected by endosomal incorporation of SPIO nanoparticles. Iron concentrations vary with ferumoxides-TA combinations and cells with a maximum of 30.1 pg ± 3.7 of iron per cell for labeled mesenchymal stem cells. Therefore, in summary, magnetic labeling of mammalian cells aided by the use of ferumoxides and TAs is possible and may enable cellular MRI and tracking in experimental and clinical settings.

Clinically applicable labeling of mammalian and stem cells by combining superparamagnetic iron oxides and transfection agents. Frank JA, Miller BR, Arbab AS, Zywicke HA, Jordan EK, Lewis BK, Bryant LH Jr, Bulte JW. Radiology 2003 Aug; 228 (2), pp. 480–7.

Stem Cells (Embryonic)/Migration within the Central Nervous System (CNS)/Lesion Traffic of Stem Cells
MRI Agent Cell Labeling/Lipofection Cell Labeling Methodology

Background Information: *In vivo* monitoring of stem cells after grafting is essential for a better understanding of their migrational dynamics and differentiation processes and of their regeneration potential. Migration of endogenous or grafted stem cells and neurons has been described in vertebrate brain, both under normal conditions from the subventricular zone along the rostral migratory stream and under pathophysiological conditions, such as degeneration or focal cerebral ischemia. However, such relied on invasive analysis of brain sections applied in combination with appropriate staining techniques.

Application: Embryonic stem cells could theoretically function as a selective delivery modality for diagnostic and therapeutic pharmaceutical agents. The concept of labeling stem cell–like populations could also function as a methodology for monitoring the therapeutic or diagnostic infiltration of desirable cell types into specific lesions (e.g., CNS ischemic foci or Parkinson's disease).

Route of Administration: Variable, but most frequently will be infused intravascularly or by direct injection into pathologic lesions.

Implementation: Mapping the *in vivo* dynamics of cell migration over a prolonged period of time. Embryonic stem (ES) cells constitutively expressing green fluorescent protein (GFP) can be labeled with a lipofection procedure employing an MRI contrast agent. Contrast agent–labeled cells are then implanted into the CNS.

Animal Model: Focal cerebral ischemia are induced 2 weeks before implantation of ES cells into the healthy, contralateral hemisphere (rats).

Interpretation and Analysis: Magnetic resonance imaging at 78-μm isotropic spatial resolution permits the observation of implanted cells with high contrast against host tissue background that can be confirmed by GFP detection profiles. During a 3-week period, cells migrate along the corpus callosum into the ventricular walls and massively populate the border zone of damaged brain tissue on the hemisphere opposite to the implantation sites. Embryonic stem cells have high migrational dynamics and are attracted to induced cerebral lesion areas in a relatively selective manner. Techniques employing labeled ES cells are ideally suited for the noninvasive imaging of cell migration, engraftment, and morphological differentiation at high spatial and temporal resolution.

Monitoring of implanted stem cell migration *in vivo*: A highly resolved *in vivo* magnetic resonance imaging investigation of experimental stroke in rat. Hoehn M, Kustermann E, Blunk J, Wiedermann D, Trapp T, Wecker S, Focking M, Arnold H, Hescheler J, Fleischmann BK, Schwindt W, Buhrle C. Proc Natl Acad Sci U S A 2002 Dec 10; 99 (25), pp. 16267–72. Epub 2002 Nov 20.

18 Nuclear Planar Scintigraphy

GENERAL

Artifact Reduction and Imaging Enhancement/ Target to Background Ratio Enhancement/ Acceleration of Liberated Nucleotide Excretion/ Neoplastic Disease: Carcinoembryonic Antigen (CEA)
$CaNa_2EDTA$/[^{111}Indium] ([^{111}In])-Anti-CEA MAb/ [^{90}Y]-Anti-CEA Monoclonal Antibody (Mab)/ Diethylenetriamine Pentaacetic Acid (DTPA) Chelate-Labeled Peptides

Cellular/Molecular Mechanism of Action: $CaNa_2EDTA$ is an organic compound that is capable of chelating the "free" forms of various elemental ions like [^{111}In] and [^{90}Y].
Application: The chelation group $CaNa_2EDTA$ can be applied to bind [^{111}In] and promote [^{90}Y] liberation from DTPA chelation groups for the purpose of generating scintigraphic images of relatively high specificity, selectivity, lesion resolution, and superior target to background ratios.
Semi-Synthetic Methods: Labeling of murine MAb F33-104 with binding avidity for CEA using the diethylenetriamine pentaacetic-anhydride method for the introduction of DTPA chelations groups capable of binding [^{111}In] and [^{90}Y] radioisotopes.
Implementation: Evaluation of murine [^{111}In]-DTPA and [^{90}Y]-DTPA-labeled anti-CEA MAb to determine the influence of $CaNa_2EDTA$ on the *in vitro* loss of radioactivity in serum. Complementary investigations can involve delineation of the effects of $CaNa_2EDTA$, administered before and after [^{111}In]-MAb or [^{90}Y]-MAb, on the biodistribution of radioactive isotopes. Formulations of 0.9% NaCl can be administered as a control in both *in vitro* and *in vivo* investigations.

Animal Model: Murine (nude mice bearing human colon adenocarcinoma LS 180 tumors expressing CEA or negative reference controls consisting of human pulmonary carcinoma PC 9 tumor expressing no CEA).
Interpretation and Analysis: $CaNa_2EDTA$ does not cause any decrease in levels of radioactivity of radiolabeled MAbs. Pre- and posttreatment with $CaNa_2EDTA$ reduces radioctivity in both specific and non-specific tumors at 72 hours after [^{111}In]-MAb injection, resulting in an increase of the specific tumor-to-non-specific-tumor radioactivity ratio. The levels of hepatic and renal radioactivity are also subsequently decreased by $CaNa_2EDTA$. On the other hand, $CaNa_2EDTA$ pre- and posttreatment reduces levels of bony, hepatic, and renal radioactivity at 24, 48, and 72 hours, respectively, after [^{90}Y]-MAb injection, although it has no effect on tumor radioactivity. Therefore, pre- and posttreatment with $CaNa_2EDTA$ would be of great use in humans who undergo radioimmunodiagnostics (RID) or radioimmunotherapy (RIT) with [^{111}In]-MAb and [^{90}Y]-MAb accompanied by disassociation of the labeled radionuclides.

$CaNa_2EDTA$ for improvement of radioimmunodetection and radioimmunotherapy with [^{111}In] and [^{90}Y]-DTPA-anti-CEA MAbs in nude mice bearing human colorectal cancer. Watanabe N, Oriuchi N, Endo K, Inoue T, Kuroki M, Matsuoka Y, Tanada S, Murata H, Kim EE, Sasaki Y. J Nucl Med 2000 Feb; 41 (2), pp. 337–44.

Interference/Incompatible Combinations
[Gd]-Diethylenetriamine Pentaacetic Acid (DTPA) and Gallium-67 ([^{67}Ga])-Citrate

Background Information: Previously, it has been reported that administration of the paramagnetic contrast agent [Gd]-DTPA (gadopentate) prior to [^{67}Ga]-citrate can lead to poor quality scans, characterized by pronounced bone uptake and a loss of tumor avidity. Suggestions that have been offered to account for the occurrence of such profiles include *in vivo* dissociation or the presence of free DTPA in formulations.
Route of Administration: Commercial gadopentate and gadodiamide contrast agents (intravascular); [^{67}Ga]-citrate (intravenous).
Dosage: Commercial gadopentate and gadodiamide contrast agents ($\leq$ 5 mmol/kg).
Implementation: Determine the extent that gadopentate and gadodiamide contrast agents influence 4 hours later the bone uptake and tumor avidity for [^{67}Ga] preparations. Biodistribution profiles are then determined at 24 hours postinjection. Formulations of [Gd]-DTPA containing excess Gd or DTPA are also evaluated for comparison. The model is verified by identical studies using inactive [Ga^{+3}] or [Fe^{+3}] at 0.1 mmol/kg. The effects of [Gd^{+3}] or the DTPA ligand at this dose can also be determined.

Animal Model: Murine (mature female BALB/c mice).
Interpretation and Analysis: Administration of [Gd]-DTPA does not produce marked changes in [^{67}Ga] biodistribution. Minor changes occur after 0. 1mmol/kg [Gd^{+3}] or the DTPA ligand, but cannot account for observed scan changes. Inactive [Ga^{+3}] or [Fe^{+3}] causes a marked reduction of [^{67}Ga] uptake in all tissues except bone, leading to greatly increased total bone:total soft tissue ratios. Therefore, [Gd]-DTPA or its constituents do not significantly alter the biodistribution of [^{67}Ga]-citrate (e.g., murine model). Extrapolating these findings to the human situation suggests that the previously reported scan changes may have been the result of other undetermined factors.

The effect of gadolinium-based MRI contrast agents on the biodistribution of [^{67}Ga]. Baker RJ. Nucl Med Commun 2002 Feb; 23 (2), pp. 139–45.

AMYLOID AND AMYLOIDOSIS

Amyloid/Arthropathy and Hepatic Amyloidosis (Avian Model)
[^{99m}Technetium] ([^{99m}Tc])-MAMA-Chrysamine G (CG)/ Chrysamine G/Congo Red

Cellular/Molecular Mechanism of Action: The radiopharmaceutical [^{99m}Tc]-MAMA-CG is a radiolabeled chrysamine G analogue of Congo red, which is a staining reagent used to selectively detect amyloid deposits present within histopathology tissue sections.

Application: Utilization as a non-invasive diagnostic probe for the *in vivo* detection of amyloid deposits within organ systems.
Considerations: Systemic amyloidosis is currently diagnosed histologically using Congo red staining or *in vivo* using [^{123}I]-labeled serum amyloid P component ([^{123}I]-SAP)for scintigraphic imaging.
Animal Model: Avian (chicken)
Interpretation and Analysis: Scintigraphic imaging *in vivo* with [^{99m}Tc]-MAMA-CG performed in animal models with spontaneous joint amyloidosis display images of radiopharmaceutical uptake in amyloid-loaded organs (liver, joints) at $\geq$ 10 minutes postinjection. Scintigraphic imaging with [^{123}I]-SAP also detects binding to hepatic amyloid deposits. However, up to 11 hours after injection no radioactivity can be appreciated in amyloid positive joints. Autoradiography (*in vitro*) performed on sections of joints with *Enterococcus faecalis*-induced amyloid arthropathy (chjAA) reveals a failure of [^{99m}Tc]-MAMA-CG to bind significantly to amyloid deposits in the presence of 10 μM Congo red reagent. The specificity of [^{99m}Tc]-MAMA-CG localization has also been established by the absence of *in vitro* and *in vivo* binding of [^{99m}Tc]-MAMA-CG to organs devoid of amyloid deposits. Acute toxic reactions with [^{99m}Tc]-MAMA-CG have not been observed (avian).

Evaluation of [^{99m}Tc]-MAMA-chrysamine G as an *in vivo* probe for amyloidosis. Dezutter NA, Landman WJ, Jager PL, de Groot TJ, Dupont PJ, Tooten PC, Zekarias B, Gruys E, Verbruggen AM. Amyloid 2001 Sep; 8 (3), pp. 202–14.

Amyloid Beta (Abeta) Plaque Deposits: Central Nervous System (CNS)/Alzheimer's Disease (AD) *[^{123}I/^{125}I]IMPY/6-Iodo-2-(4′-Dimethylamino-) Phenyl-Imidazo[1,2-a]Pyridine/Thioflavin Dye*

Background Information: Development of small molecular probes for *in vivo* labeling and detection of beta-amyloid (abeta) plaques in patients of AD is of significant scientific interest, and it may also assist the development of drugs targeting abeta plaques for treatment of AD.
Application: Detection and evaluation of amyloid plaque deposits.
Route of Administration: [^{125}I]IMPY (intravascular).
Semi-Synthetic Methods: A novel probe, [^{123}I/^{125}I]IMPY, 6-iodo-2-(4′-dimethylamino-)phenyl-imidazo[1,2-a]pyridine, can be prepared using an iododestannylation reaction catalyzed by hydrogen peroxide.
Implementation: Characterization of amyloid plaque deposits (*in vivo*).
Animal Model: Murine (transgenic mice model (Tg2576) engineered to produce excess amyloid plaques within the brain).
Interpretation and Analysis: The modified thioflavin-T derivative displays good binding affinity for preformed synthetic abeta$_{40}$ aggregates in solution ($K_i = 15 \pm 5$ nM) and shows selective plaque labeling on postmortem AD brain sections. Biodistribution of [^{125}I]IMPY exhibits excellent brain uptake (2.9% initial dose/brain at 2 minutes) and fast washout (0.2% initial dose/brain at 60 minutes). These properties are highly desirable for amyloid plaque imaging agents. *Ex vivo* autoradiograms of brain sections of Tg2576 mice obtained 4 hours after an intravenous injection of [^{125}I]IMPY clearly display distinct plaque labeling with low background levels of activity. When the same brain section is stained with the fluorescent dye thioflavin-S, the same abeta plaques show prominent fluorescent labeling consistent with results observed from autoradiogram analyses. Radioiodinated IMPY demonstrates desirable characteristics for *in vivo* labeling of abeta plaques, and it may be useful as a molecular imaging agent to study amyloidogenesis in the brain of living AD patients.

IMPY: An improved thioflavin-T derivative for *in vivo* labeling of beta-amyloid plaques. Kung MP, Hou C, Zhuang ZP, Zhang B, Skovronsky D, Trojanowski JQ, Lee VM, Kung HF. Brain Res 2002 Nov 29; 956 (2), pp. 202–10.

Amyloid Beta (Abeta) Deposits: Central Nervous System (CNS)/Alzheimer's Disease (AD)/ Vascular Amyloid Deposits *[^{99m}Technetium] ([^{99m}Tc])-MAMA-CG/Chrysamine G*

Cellular/Molecular Mechanism of Action: Chrysamine G (CG), an analogue of Congo red, is known to bind *in vitro* to the beta-amyloid protein (abeta 10-43) and to homogenates of several regions of the brains of AD patients.
Application: Detection of beta-amyloid deposits similar to those recognized in the CNS of cases suffering from AD.
Route of Administration: Intravascular.
Semi-Synthetic Methods: Synthesis of a conjugate of 2-(acetamido)-CG with a bis-S-trityl protected monoamide-monoaminedithiol (MAMA-Tr$_2$) tetraligand that is efficiently deprotected and [^{99m}Tc] labeled with a 75% yield to obtain [^{99m}Tc]-MAMA-CG.
Implementation: Evaluation of hepatobiliary clearance and brain uptake and selective detection of beta-amyloid deposits similar to those found in the CNS in conditions of AD.
Animal Model: Murine
Interpretation and Analysis: In mice, [^{99m}Tc]-MAMA-CG is cleared mainly by the hepatobiliary system, resulting in a fast blood clearance. Brain uptake of [^{99m}Tc]-MAMA-CG is low. Co-injection with the blood-pool tracer iodine-125 human serum albumin ([^{125}I]-HSA) demonstrates a brain/blood activity ratio for [^{99m}Tc]-MAMA-CG that is significantly higher than that for [^{125}I]-HSA (t test for dependent samples, $p < 0.02$), indicating the ability of [^{99m}Tc]-MAMA-CG to cross the blood-brain barrier. *In vitro* autoradiography reveals a pronounced binding of [^{99m}Tc]-MAMA-CG to beta-amyloid deposits in autopsy sections of the parietal and occipital cortex of an AD patient as compared with controls. Adding 10 μM Congo red during incubation displaces the binding of [^{99m}Tc]-MAMA-CG. Congo red staining and autoradiography both identify the same lesions. [^{99m}Tc]-MAMA-CG seems to bind selectively to beta-amyloid deposition in human brain parenchyma and blood vessels *in vitro* and thus might be a lead compound for further development of a useful tracer agent for the *in vivo* diagnosis of AD.

[^{99m}Tc]-MAMA-chrysamine G, a probe for beta-amyloid protein of Alzheimer's disease. Dezutter NA, Dom RJ, de Groot TJ, Bormans GM, Verbruggen AM. Eur J Nucl Med 1999 Nov; 26 (11), pp. 1392–9.

Amyloid Deposit Detection *[^{123}I]-Labeled Serum Amyloid P Component: Human vs Murine*

Cellular/Molecular Mechanism of Action: Amyloid P component binds to amyloid A deposits.
Application: Utilization as a non-invasive diagnostic probe for the *in vivo* detection of amyloid A deposits within organ systems.
Route of Administration: [^{123}I]-labeled serum amyloid P component (intravenous).
Interpretation and Analysis: Highly specific, high-resolution scintigraphic images of amyloid-laden organs in mice with experimentally-induced amyloid A protein (AA) amyloidosis can be ob-

tained with [^{123}I]-labeled serum amyloid P component. Interestingly, a much higher proportion (up to 40%) of the injected dose of heterologous human amyloid P localizes within amyloid deposits and is retained there than occurs with isologous mouse amyloid P, indicating that human amyloid P binds more avidly to mouse AA fibrils than does mouse amyloid P. Specificity of amyloid P localization has been established based on the observation of a failure of the related proteins, human C-reactive protein and Limulus C-reactive protein, to deposit significantly in amyloid and by the absence of human amyloid P deposition in non-amyloidotic organs. However, only partial correlation is observed between the quantity of amyloid P localized and 2 independent estimates, histology examination and RIA analyses for amyloid A of the amount of amyloid in particular organs. Uncertainty exists as to which of the 3 methods most accurately reflects the extent or clinical significance of amyloid deposition. However, the *in vivo* localization of radiolabeled serum amyloid P, detectable and quantifiable by gamma camera imaging, is apparently extremely sensitive. Based on the ability of serum amyloid P to function in this capacity, it may have potential applicability as a specific targeting agent for diagnostic and even therapeutic purposes in clinical amyloidosis.

Specific localization and imaging of amyloid deposits *in vivo* using [^{123}I]-labeled serum amyloid P component. Hawkins PN, Myers MJ, Epenetos AA, Caspi D, Pepys MB. J Exp Med 1988 Mar 1; 167 (3), pp. 903–13.

Amyloid: Familial Amyloid Polyneuropathy/ Hepatic Posttransplant Renal Amyloid Deposition *[^{123}I]-Labeled Serum Amyloid P Component*

Cellular/Molecular Mechanism of Action: Serum amyloid P component (SAP) recognizes and physically binds to amyloid deposits.

Background Information: Familial amyloid polyneuropathy (FAP) associated with transthyretin (TTR) mutations is the commonest type of hereditary amyloidosis. Plasma TTR is produced almost exclusively in the liver. Orthotopic liver transplantation is the only available treatment, although the clinical outcome varies. Serum amyloid P component scintigraphy is a method for identifying and quantitatively monitoring amyloid deposits *in vivo*, but it has not previously been used to study the outcome of visceral amyloid deposits in FAP following liver transplantation.

Application: Detection and characterization of amyloid deposits analogous to those associated with Alzheimer's disease and other conditions.

Route of Administration: Intravascular.

Implementation: Evaluation of amyloid lesions applying whole-body scintigraphy following [^{123}I]-SAP injection in conditions of amyloid polyneuropathy, asymptomatic gene carriers, and postorthotopic liver transplantation.

Human Model: Cases of familial amyloid polyneuropathy (n = 17) associated with the expression of TTR Met30 in addition to asymptomatic gene carriers (n = 5). Follow-up studies of interest can include general cases (n = 10), some of whom have undergone orthotopic liver transplantation 1–5 years beforehand (n = 8/10 cases).

Interpretation and Analysis: An abnormal uptake of [^{123}I]-SAP can be appreciated in the vast majority of cases with FAP including the (i) kidneys (n = 17); (ii) spleen (n = 5/17); and (iii) adrenal gland (n = 3/17). Renal amyloid deposits can also be appreciated in some asymptomatic carriers (n = 3/5). Follow-up studies 1–5 years after liver transplantation show that there is substantial regression of visceral amyloid deposits in some cases (n = 2/10 compared to n = 3/10 modest improvement; n = 2 unchanged amyloid deposits). Therefore, [^{123}I]-SAP scintigraphy can identify unsuspected visceral amyloid cases with FAP due to TTR Met30. The universal presence of renal amyloid probably underlies the high frequency of renal failure that occurs in FAP following liver transplantation. The variable capacity of patients to mobilize amyloid deposits following liver transplantation may contribute to their long-term clinical outcome.

Serum amyloid P component scintigraphy in familial amyloid polyneuropathy: Regression of visceral amyloid following liver transplantation. Rydh A, Suhr O, Hietala SO, Ahlström KR, Pepys MB, Hawkins PN. Eur J Nucl Med 1998 Jul; 25 (7), pp. 709–13.

Amyloidosis of β_2-Microglobulin Origin *[^{111}Indium] ([^{111}In])-Recombinant Human β_2mH5 (Rhβ_2mH5)*

Cellular/Molecular Mechanism of Action: β_2-microglobulin recognizes and physically (chemically) binds to amyloid composed of β_2-microglobulin (β_2m) deposits.

Background Information: Amyloid of β_2m origin can be diagnosed using [^{131}I]-radiolabeled-β_2m scintigraphy in patients with uremia and hemodialysis treatment. As the tracer β_2m is isolated from another case, it carries the common risks, including viral infections such as hepatitis B and C and human immunodeficiency virus (HIV), which are associated with human plasma products.

Application: The *in vitro* production of recombinant human β_2m yields a protein fraction that is devoid of any potential risk of exposing recipients to various viral pathogens. Purified protein fractions can then be radiolabeled for the purpose of detecting and characterizing various conditions of amyloidosis.

Route of Administration: [^{111}In]-rhβ_2mH5 (intravenous).

Semi-Synthetic Methods: β_2m-type amyloid can be produced *in vitro* as rhβ_2m in *Escherichia coli* populations. The expression vector pASK40DeltaLβ_2m(His)5 can be used because it contains a C-terminal (His)5-tag for purification via immobilized metal ion affinity chromatography (IMAC). Size exclusion chromatography on a Superose 12 column can be used as a 2nd purification step. The isolated rhβ_2mH5 reacts in an immunochemically identical manner to native human β_2m, and can be identified as a single band of approximately 11.8 kDa when analyzed by Western blot immunodetection methodologies. Additionally, fractions can be visualized as a single spot in 2-dimensional gel electrophoresis (2D-PAGE: expected molecular mass = 12,415.8 Da). Uniformity can be further proven by crystallization and N-terminal amino-acid sequence analysis.

Implementation: Evaluation of β_2m-amyloidosis conditions applying sterile preparations of rhβ_2mH5 radiolabeled with [^{111}In] to produce [^{111}In]-rhβ_2mH5.

Human Model: Cases managed with hemodialysis (± known β_2m-amyloidosis).

Interpretation and Analysis: The [^{111}In]-rhβ_2mH5 tracer localizes specifically to particular areas known to contain amyloid. Rhβ_2mH5 preparations therefore are suitable for the *in vivo* detection of deposits of β_2m-type amyloid contained within organs and fulfill the requirements of a tracer for common use. Finally, the use of [^{111}In] instead of [^{131}I] reduces the radioactive load and results in higher lesion resolution.

Production of recombinant human β_2-microglobulin for scintigraphic diagnosis of amyloidosis in uremia and hemodialysis. Linke RP, Schäeffer J, Gielow P, Lindner P, Lottspeich F, Plückthun A, Weiss EH. Eur J Biochem 2000 Feb; 267 (3), pp. 627–33.

APOPTOSIS

Apoptosis
[^{99m}Technetium] ([^{99m}Tc])-Hydrazinonicotinamido (HYNIC)-Annexin V

Background Information: [^{99m}Tc]-HYNIC-annexin V is a novel tracer for *in vivo* imaging of cellular apoptosis.
Route of Administration: [^{99m}Tc]-HYNIC-annexin V (intravenous).
Dosage: [^{99m}Tc]-HYNIC-annexin V (250 MBq).
Implementation: Imaging is initiated with a transmission scan using a [^{57}Co]-flood source to obtain a map of the regional "thickness" of the volunteer. [^{99m}Tc]-HYNIC-annexin V is then injected directly, followed by a 30-minute dynamic study. Whole-body scans are obtained at about 30 minutes, 3 hours, 6 hours, and 24 hours after administration. Organ uptake is determined after correction for background, scatter, and attenuation. The MIRDOSE3.1 program can be used to calculate organ-absorbed doses and effective dose. Signs of adverse effects can be detected by monitoring renal and liver function, hematology, blood coagulation, and vital signs (blood pressure, pulse, respiration rate, temperature, and electrocardiogram).
Human Model: Healthy male volunteers (n = 6).
Interpretation and Analysis: The kidneys accumulate 49.7 ± 8.1 percent injected dose (%ID) at 3 hours after injection; the liver, 13.1 ± 1.0 %ID; the red marrow, 9.2 ± 1.8 %ID; and the spleen, 4.6 ± 1.6 %ID. More than 90% of the blood activity is cleared with a half-life of 24 ± 3 minutes. The biologic half-life of the activity registered over the total body is long (69 ± 7 hours). Excretion of the activity is almost exclusively through the urine (22.5 ± 3.5 %ID at 24 hours), and hardly any activity is seen in the bowel or feces. Absorbed doses are found to be 196 ± 31 μGy/MBq for the kidneys, 41 ± 12 μGy/MBq for the spleen, 16.9 ± 1.3 μGy/MBq for the liver, and 8.4 ± 0.9 μGy/MBq for the red marrow. The effective dose is approximately 11.0 ± 0.8 μSv/MBq, or 2.8 ± 0.2 mSv for the average injected activity of 250 MBq. No adverse effects are usually observed. Therefore, [^{99m}Tc]-HYNIC-annexin V is a safe radiopharmaceutical, having a favorable biodistribution for imaging of apoptosis in the abdominal cavity as well as the thoracic region with an acceptable radiation dose.
Instrumentation: A dual-head gamma camera was used to acquire conjugate anterior and posterior views.

Safety, biodistribution, and dosimetry of [^{99m}Tc]-HYNIC-annexin V, a novel human recombinant annexin V for human application. Kemerink GJ, Liu X, Kieffer D, Ceyssens S, Mortelmans L, Verbruggen AM, Steinmetz ND, Vanderheyden JL, Green AM, Verbeke K. J Nucl Med 2003 Jun; 44 (6), pp. 947–52.

Apoptosis/Hepatic and Thymic Phenomenon
[^{99m}Technetium] ([^{99m}Tc])-HYNIC-Annexin V

Cellular/Molecular Mechanism of Action: Annexin V, a 32-kDa endogenous human protein, has a high affinity for membrane-bound phosphatidylserine.
Background Information: Either inadequate or excessive apoptosis (programmed cell death) is associated with many diseases. A method for imaging apoptosis *in vivo*, rather than requiring histologic evaluation of tissue, could assist with therapeutic decision-making requirements associated with these disorders. Programmed cell death is associated with a well-choreographed series of events resulting in the cessation of normal cell function and the ultimate disappearance of the cell. One component of apoptosis is signaling adjacent cells that this cell is committing suicide by externalizing phosphatidylserine to the outer leaflet of the cell membrane.
Application: Technetium-^{99m} HYNIC-annexin V can be utilized for the *in vivo* detection and quantification of apoptosis phenomenon in tissues and organ systems. Dexamethasone can be applied to induce thymic apoptosis.
Route of Administration: [^{99m}Tc]-HYNIC-annexin V (intravenous).
Dosage: Dexamethasone (1–20 mg/kg) 5 hours prior to [^{99m}Tc]-HYNIC-annexin.
Semi-Synthetic Methods: Covalent coupling of annexin V with the bifunctional hydrazinonicotinamide reagent (HYNIC) for the preparation of [^{99m}Tc]-HYNIC-annexin V.
Implementation: Detection of apoptosis phenomenon in the liver and thymus. Biodistribution analysis of [^{99m}Tc]-HYNIC-annexin V is performed (in rats) 10–180 minutes following intravenous injection. Delineation of the degree of apoptosis required for *in vivo* localization of [^{99m}Tc]-annexin V (mice) can be determined with the aid of dexamethasone administered 5 hours prior to [^{99m}Tc]-HYNIC-annexin V. The thymus is then excised 1 hour after radiolabeled HYNIC-annexin V injection; thymocytes isolated, incubated with Hoechst 33342 followed by propidium iodide, and analyzed on a fluorescence-activated cell sorter. Each sorted cell population is counted in a scintillation counter. Evaluation of [^{99m}Tc]-HYNIC-annexin V as a tracer for external radionuclide imaging of apoptotic cell death can be performed by radionuclide imaging 1 hour following injection of anti-Fas IgG in Fas-defective mice (lpr/lpr mice) and wild-type mice.
Animal Model: Rat and mouse.
Interpretation and Analysis: Biodistribution analysis of [^{99m}Tc]-HYNIC-annexin V in rats reveals a blood clearance half-time of less than 10 minutes. The kidneys have the highest concentration of radioactivity at any particular time point. Studies in the mouse thymus have demonstrated a 40-fold increase in [^{99m}Tc]-HYNIC-annexin V concentration in apoptotic thymocytes compared with viable cell populations. A correlation value of r = 0.78 can be appreciated between radioactivity and flow cytometric and histologic evidence of apoptosis. Imaging studies in lpr/lpr and wild-type mice show a substantial increase in activity within the liver of wild-type mice treated with anti-Fas, while there is no significant change, irrespective of anti-Fas administration, in lpr/lpr mice. Excellent imaging of hepatic apoptosis can be obtained in wild-type mice 30 minutes after injection of [^{99m}Tc]-HYNIC-annexin V. Imaging results are consistent with histologic analysis.

Technetium-^{99m} HYNIC-annexin V: A potential radiopharmaceutical for the *in vivo* detection of apoptosis. Ohtsuki K, Akashi K, Aoka Y, Blankenberg FG, Kopiwoda S, Tait JF, Strauss HW. Eur J Nucl Med 1999 Oct; 26 (10), pp. 1251–8.

Apoptosis: Neoplastic Disease States/ Chemotherapeutic-Induced
[^{99m}Technetium] ([^{99m}Tc])-Annexin V/ [Cyclophosphamide]/TUNEL Tissue Analysis

Cellular/Molecular Mechanism of Action: Annexin V is a human protein that possesses a high affinity for phosphatidylserine.
Application: Annexin V can be labeled with [^{99m}Tc] to detect *in vivo* apoptosis phenomenon.
Route of Administration: [^{99m}Tc]-annexin V (intravenous).
Semi-Synthetic Methods: Radiolabeling of annexin V with [^{99m}Tc] (specific activity, 3.0 MBq/μg protein).
Implementation: Evaluate the effectiveness of [^{99m}Tc]-annexin V for determining the degree of apoptosis induced in inoculated allogenic hepatoma lesions 20 hours after the 1st dose of chemotherapy (e.g., cyclophosphamide). Radioactivity within specific tissues is

then determined 6 hours after [^{99m}Tc]-annexin V administration. Tumor uptake of [^{14}C]-iodoanitpyrine can be employed as an indicator or marker for tumor blood flow. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) of tissue harvested at necropsy is then performed to detect evidence of tumor apoptosis phenomenon.

Animal Model: Rat (inoculated with allogenic engraphed hepatoma cells [KDH-8] in the left calf muscle, followed 11 days later by randomized administration of a single dose of cyclophosphamide [150 mg/kg intraperitoneally]. Reference controls include rats not receiving doses of chemotherapeutic agent).

Interpretation and Analysis: Cyclophosphamide treatment significantly increases tumor uptake (percentage activity of injected dose per gram of tissue after normalization to the animal's weight [%ID/g/kg] of [^{99m}Tc]-annexin V, 0.070 ± 0.007 %ID/g/kg for treated rats and 0.046 ± 0.009 %ID/g/kg for controls, $p < 0.001$). [^{14}C]-Iodoantipyrine uptake is similar between treated and untreated groups. The number of TUNEL-positive cells in the tumor is significantly larger in treated rats (297.70 ± 50.34 cells/mm^2) than in control rats (168.45 ± 23.60 cells/mm^2, $p < 0.001$). Tumor uptake of [^{99m}Tc]-annexin V correlates with the number of TUNEL-positive cells within tumor specimens ($r = 0.712$; $p < 0.001$). Tumor uptake of [^{99m}Tc]-annexin V is significantly increased by a single dose of cyclophosphamide treatment, and the increase is concordant with the number of TUNEL-positive cells detected within tumors. Such observations suggest the utility of [^{99m}Tc]-annexin V as a non-invasive means for assessing tumor response, although further testing, including clinical evaluation, is required.

Detection of apoptotic tumor response *in vivo* after a single dose of chemotherapy with [^{99m}Tc]-annexin V. Mochizuki T, Kuge Y, Zhao S, Tsukamoto E, Hosokawa M, Strauss HW, Blankenberg FG, Tait JF, Tamaki N. J Nucl Med 2003 Jan; 44 (1), pp. 92–7.

Apoptosis and Oncosis: Cardiac Myocardial Cells/ Apoptosis: Doxorubicin-Induced/Oncosis: Isoproterenol-Induced
[^{111}Indium] ([^{111}In])-Antimyosin Antibody/ [^{99m}Technetium] ([^{99m}Tc])-Glucaric Acid

Background Information: Non-invasive imaging techniques would be highly desirable to differentiate oncotic from apoptotic cell death.

Application: [^{99m}Tc]-glucaric acid can be applied to detect oncotic myocardial cell death. Conversely, radiolabeled antimyosin is capable of detecting both oncotic and apoptotic types of myocardial cell death.

Route of Administration: [^{111}In]-antimyosin and [^{99m}Tc]-glucaric acid (intravenous).

Implementation: Evaluation of the ability for [^{111}In]-antimyosin antibody and [^{99m}Tc]-glucaric acid to be applied together in a manner that allows detection of and differentiation between doxorubicin-induced myocardial apoptosis and isoproterenol-induced myocardial oncosis. Scanning electron microscopy, DNA laddering, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL), [^{111}In]-antimyosin antibody, and [^{99m}Tc]-glucaric acid can be collectively utilized *in vitro* and *in vivo* to detect doxorubicin-induced apoptosis or isoproterenol-induced myocardial oncosis.

***Ex Vivo* Model:** Cultured rat H9C2 embryonic cardiocytes. In this context, myocardial cell apoptosis can be induced by serial exposure of H9C2 embryonic cardiocytes to diluted doxorubicin formulations.

Animal Model: Rats (CD1 strain for *in vivo* investigations). Myocardial apoptosis can be induced *in vivo* in rats by doxorubicin administration. Related investigations can employ heat or subcutaneous isoproterenol administration to induce myocardial oncosis.

Interpretation and Analysis: Scanning electron microscopy, DNA laddering, and TUNEL staining of H9C2 cardiocytes treated with doxorubicin all show cell death by apoptosis. Rat hearts treated with doxorubicin (10 and 20 mg/kg) are DNA ladder-positive and localize significantly greater [^{111}In]-antimyosin antibody (mean + standard deviation, 0.1942 ± 0.0150 percent injected dose per gram [%ID/g] and 0.1825 ± 0.0238 %ID/g, respectively) than normal hearts (0.1154 ± 0.0270 %ID/g, $p < 0.05$). Increases in myocardial [^{99m}Tc]-glucaric acid activity cannot be observed in rat hearts after 6, 12, and 24 hours of doxorubicin injection (0.0311 ± 0.0066 %ID/g, 0.0356 ± 0.007 %ID/g, and 0.0368 ± 0.0047 %ID/g, respectively; control hearts, 0.0352 ± 0.0099 %ID/g; p = not significant). Alternatively, [^{99m}Tc]-glucaric acid uptake is significantly greater in isoproterenol-induced oncotic hearts (0.1256 ± 0.1023 %ID/g) than in controls ($p < 0.0001$). [^{99m}Tc]-glucaric acid is avid only for the oncotic myocardium. Antimyosin, on the other hand, is positive in both oncotic and apoptotic myocardium.

Indium 111 antimyosin and [^{99m}Tc] glucaric acid for non-invasive identification of oncotic and apoptotic myocardial necrosis. Khaw BA, Silva JD, Petrov A, Hartner W. J Nucl Cardiol 2002 Sep–Oct; 9 (5), pp. 471–81.

GENE EXPRESSION: *IN VIVO* DETECTION

Gene Expression/Herpes Simplex Virus Type I Thymidine Kinase: HSV1-tk(+) Reporter Gene/ [Oncogenes]/[Hereditary Disorders]
[^{123}I]-2′-Fluoro-2′-Deoxy-1-Beta-D-Arabinofuranosyluracil/[^{125}I]-2′-Fluoro-2′-Deoxy-1-Beta-D-Arabinofuranosyluracil

Cellular/Molecular Mechanism of Action: The molecular agent 2′-fluoro-2′-deoxy-5-iodo-1-beta-D-arabinofuranosyluracil has been designed to function as a substrate for HSV1-tk biochemical activity.

Background Information: Previous studies have shown that the HSV1-tk gene, in combination with appropriate radiolabeled substrates (e.g., [I*]-2′-fluoro-2′-deoxy-5-iodo-1-beta-D-arabinofuranosyluracil, or I*-FIAU, where the asterisk indicates the use of any radioactive iodine isotopes), can be used as a reporter gene detection modality for monitoring *in vivo* gene transfer and expression.

Application: Potential gene expression imaging. In this context, [^{123}I]-2′-fluoro-2′-deoxy-1-beta-D-arabinofuranosyluracil and [^{125}I]-2′-fluoro-2′-deoxy-1-beta-D-arabinofuranosyluracil can be applied as radiolabeled substrates for HSV1-tk gene expression. Note: The *in vivo* detection of HSV1-tk gene expression utilizing [^{123}I]-2′-fluoro-2′-deoxy-1-beta-D-arabinofuranosyluracil and [^{125}I]-2′-fluoro-2′-deoxy-1-beta-D-arabinofuranosyluracil represents a model feasibility study designed to determine optimum conditions and methodologies that can be utilized for the *in vivo* detection of altered expression of other unrelated genetic sequences (e.g., oncogenes, hereditary disorders).

Route of Administration: [^{123}I]-2′-fluoro-2′-deoxy-1-beta-D-arabinofuranosyluracil and [^{125}I]-2′-fluoro-2′-deoxy-1-beta-D-arabinofuranosyluracil (intravenous).

Semi-Synthetic Methods: The molecular agent 2′-fluoro-2′-deoxy-1-beta-D-arabinofuranosyluracil (FAU) can be radioiodinated (e.g., [^{123}I] or [^{125}I]) using the iodogen method. High-performance liquid chromatography purification results in a yield of product with

a high specific activity and radiochemical purity for both tracers ([^{123}I]-FIAU and [^{125}I]-FIAU).

Implementation: Evaluate the early kinetic profiles of [^{123}I]- or [^{125}I]-labeled 2′-fluoro-2′-deoxy-5-iodo-1-beta-D-arabinofuranosyluracil (I*-FIAU) and delineate the potential utilization of [^{123}I]-labeled FIAU for *in vivo* imaging for detecting gene expression events. Fibrosarcoma CMS-5 cells can be transduced *in vitro* with the retroviral vector STK containing the HSV1-tk gene. Complementary investigations can involve determination of the biodistribution profiles for radiopharmaceuticals applying gamma camera imaging at 0.5, 1, 2, and 4 hours postinjection. In addition, the genomic DNA of the tumors can be isolated for measurement of accumulated activity resulting from the [^{125}I]-FIAU incorporation.

Animal Model: Murine (fibrosarcoma cells are transduced *in vitro* with the retroviral vector STK containing the HSV1-tk gene. BALB/c mice can be inoculated subcutaneously with HSV1-tk(+) and tk(−) cells into both flanks).

Interpretation and Analysis: Biodistribution studies 0.5 hours postinjection reveal tumor/blood and tumor/muscle ratios of 3.8 and 7.2, respectively, for the HSV1-tk(+) tumors, and 0.6 and 1.2, respectively, for negative control tumors. Fast renal elimination of the tracer from the body results in rapidly increasing tumor/blood and tumor/muscle ratios that reach values of 32 and 88 at 4 hours postinjection, respectively. Tracer clearance from blood is biexponential, with an initial half-life of 0.6 hours followed by a half-life of 4.6 hours. The tracer half-life in herpes simplex viral thymidine kinase-expressing tumors is 35.7 hours. The highest activity accumulation (20.3% ± 5.7 %ID/g) in HSV1-tk(+) tumors is observed 1 hour postinjection. At that time, 46% of the total activity found in HSV1-tk(+) tumors is incorporated within genomic DNA. Planar gamma camera imaging reveals distinct tracer accumulation as early as 0.5 hours postinjection, with an increase in contrast over time. Such findings suggest that sufficient tumor/background ratios for *in vivo* HSV1-tk expression imaging with [^{123}I]FIAU are reached as early as 1 hour postinjection.

In vivo imaging of herpes simplex virus Type 1 thymidine kinase gene expression: Early kinetics of radiolabeled FIAU. Haubner R, Avril N, Hantzopoulos PA, Gansbacher B, Schwaiger M. Eur J Nucl Med 2000 Mar; 27 (3), pp. 283–91.

Gene Expression: Thymidine Kinase Expression/ Neoplastic Disease: DNA Synthesis/Cellular Proliferation Rate
5-[^{125}I]Iodo-4′-Thio-2′-Deoxyuridine (ITdU)/ 5-[^{125}I]-Iodo-2′-Deoxyuridine (IUdR)

Cellular/Molecular Mechanism of Action: ITdU is a metabolically stable nucleoside that can function as a substrate for cellular thymidine kinase biochemical activity.

Application: Radioiodinated forms of ITdU and IUdR can be applied to detect elevations in DNA synthesis in cell poplulations that positively express thymidine kinase activity. In this capacity, both ITdU and IUdR can serve as indicator molecules for measuring alterations in cellular proliferation (especially relevant to the characterization of neoplastic disease states).

Route of Administration: Usually ITdU or IUdR would most frequently be administered intravascularly. Theoretically, it may be possible to effectively image lesions if the agents are administered intrathecally for central nervous system (CNS) tumors, by direct injection into tumors, or into the arterial network supplying a suspected neoplastic lesion.

Semi-Synthetic Methods: Radiolabeling of ITdU and 5-iodo-1-(4-thio-beta-D-arabinofuranosyl)uracil (ITAU) with [^{125}I] can be performed utilizing a destannylation reaction of the trimethylstannyl precursor of each nucleoside. The products can be isolated in high yields and with a > 99% level of radiochemical purity.

Implementation: Evaluation of ITdU and ITAU for their ability to detect and characterize cellular proliferation rates as a function of thymidine kinase expression.

***Ex Vivo* Model:** Thymidine kinase-expressing L-M cells rather than a thymidine kinase-deficient mutant L-M (TK$^-$) cells.

Interpretation and Analysis: [^{125}I]-ITdU is effectively phosphorylated by cytosolic nucleoside kinases and specifically incorporated into thymidine kinase-expressing L-M cells to a far greater extent than into thymidine kinase-deficient mutant L-M (TK$^-$) cells. In addition, *in vitro* cell metabolism studies employing [^{125}I]-ITdU reveal that [^{125}I]-ITdU is effectively and specifically incorporated into a DNA fraction (> 90% at 60 minutes). Therefore, IUdR or [^{125}I]-ITdU appears to be an effective marker for the detection of DNA synthesis. Conversely, [^{125}I]-ITAU is neither remarkably phosphorylated by cytosolic nucleoside kinases nor notably incorporated into L-M cells relative to L-M (TK$^-$) cells. [^{125}I]-ITdU and [^{125}I]-ITAU show a higher resistance to phosphorolytic cleavage by recombinant thymidine phosphorylase than does [^{125}I]-IUdR. Furthermore, biodistribution of [^{125}I]-ITdU and [^{125}I]-ITAU displays better *in vivo* radioiodination stability than does [^{125}I]-IUdR preparations. [^{125}I]-ITdU also displays a significantly higher uptake in proliferating organs (thymus, spleen, small intestine, and bone) than in non-proliferating organs (brain, muscle, liver, and lung), as does [^{125}I]-IUdR, at 18 hours postinjection. As indicated by *in vitro* studies, [^{125}I]-ITAU does not show any significant uptake in proliferating organs. Therefore, radioiodinated ITdU is potentially useful as a proliferation-imaging agent, and further studies should clarify the usefulness of this compound as a nuclear medicine and tumor-imaging agent.

Rationale of 5-[^{125}I]-iodo-4′-thio-2′-deoxyuridine as a potential iodinated proliferation marker. Toyohara J, Hayashi A, Sato M, Tanaka H, Haraguchi K, Yoshimura Y, Yonekura Y, Fujibayashi Y. J Nucl Med 2002 Sep; 43 (9), pp. 1218–26.

Gene Expression and *In Vivo* Quantification: Antisense Gene Targeting/Huntingtin RNA Genetic Targeting for Huntington's Disease (HD)/Central Nervous System (CNS) Macromolecule Delivery: Transferrin Receptor Complexes
[^{125}I]- Peptide Nucleic Acid (PNA)/8D3/ [^{125}I]-PNA-[Antitransferrin Receptor IgG]

Cellular/Molecular Mechanism of Action: The PNA agent is a 16-mer peptide nucleic acid that is the antisense strand corresponding to the segment around the methionine initiation codon of the huntingtin gene of HD. In this context, it recognizes and physically binds to huntingtin RNA (HD exon-1 messenger RNA).

Background Information: Disease-specific genes of unknown function can be imaged *in vivo* with antisense radiopharmaceuticals, providing the transcellular transport of these molecules is enabled with drug-targeting technology.

Route of Administration: [^{125}I]-PNA/8D3 conjugate (intravenous).

Semi-Synthetic Methods: Biotinylation of PNA. Covalent cross-linking strepavidin and rat 8D3 monoclonal antibody (MAb) to the mouse transferrin receptor (TfR). The latter moiety contains a tyrosine residue that can facilitate [^{125}I] radioiodination. The reformulated PNA antisense radiopharmaceutical conjugated to 8D3 MAb is designated [^{125}I]-PNA/8D3.

Implementation: Evaluation of biotinylated PNA that can be rapidly captured by conjugates of covalently cross-linked strepavidin

and [antimouse transferrin receptor IgG]. Complementary *in vivo* studies in mice can be used to determine the biodistribution of the PNA analogue[^{125}I]-PNA/8D3 through harvest of tissue/organ systems 6 hours postadministration for frozen sectioning of the brain and quantitative autoradiography.

Animal Model: Murine (littermate control mice or littermate R6/2 transgenic mice that express the exon 1 of the human HD gene for HD).

Interpretation and Analysis: Despite covalent cross-linking of the PNA analog, [^{125}I]-PNA/8D3 to MAb, it can gain access to endogenous transferrin transport pathways at both the blood-brain barrier and the brain cell membrane, thereby undergoing both import from the blood to the brain and export from the brain to the blood through the transferin receptor complexes (TfR). In this manner, preparations are able to hybridize with "target" huntingtin RNA based on analyses from both cell-free translation assays and ribonuclease protection assays.

Approximately a 3-fold increase in sequestration *in vivo* can be appreciated for antisense [^{125}I]-PNA/8D3 radiopharmaceutical conjugates in the brains of HD transgenic mice. Such observations are consistent with the selective expression of the HD exon-1 messenger RNA in these animals. Therefore, gene expression *in vivo* can be quantitated with antisense radiopharmaceuticals, providing these molecules are reformulated with drug-targeting technology. Drug targeting enables access of the antisense agent to endogenous transport pathways, which permits passage across cellular barriers that separate blood and intracellular compartments of target tissues.

Imaging gene expression in the brain *in vivo* in a transgenic mouse model of Huntington's disease with an antisense radiopharmaceutical and drug-targeting technology. Lee HJ, Boado RJ, Braasch DA, Corey DR, Pardridge WM. J Nucl Med 2002 Jul; 43 (7), pp. 948–56.

CARDIOVASCULAR: CARDIAC

Cardiovascular: Angina (Coronary Arteriogram) *[^{201}Thallium] ([^{201}Tl])/Dipyridamole Stress Test*

Cellular/Molecular Mechanism of Action: Influences the metabolism and transport of adenosine and adenosine nucleotides. In this context, dipyridamol suppresses intracellular infux of adenosine that is released from myocardial tissues that have been deprived of adequate oxygen.

Physiological Mechanism of Action: Dipyridamole promotes diminished coronary artery vascular resistance, elevated coronary artery blood flow, and increased coronary artery oxygen delivery. One of the most prominent influences of dipyridamole in this regard is its effect on the smaller diameter vascular bed networks supplied by the coronary artery that have minimal effect in regions of ischemia because these same vessels tend to already be maximally dilated.

Induces coronary artery vasodilation due to the promotion of intracellular adenosine influx.

Application: Evaluation of subjects affected by effort-induced angina using the following tests: ECG stress test, stress [^{201}Tl] scan, coronary arteriography; [^{201}Tl] scan, coronary arteriography, hemodynamic, echo 2D, and ecgraphic monitoring during the dipyridamole test (D).

Implementation: Evaluation of cardiac-associated angina conditions induced by stress tests, utilizing dipyridamole-induced vasodilation and scintigraphic image analyses detected with the aid of [^{201}Tl] radionuclide.

Human Model: Cases of cardiac-associated angina. Based on coronary arteriography results, cases can be divided into groups classified as Case Type A (n = 10 patients with significant stenoses ≥ 50%) or Case Type B (n = 9 subjects with no significant stenosis).

Interpretation and Analysis: Case Type A disease states can result in positive stress ECG and scintigraphy images (e.g., n = 9 cases). The dipyridamole test can induce angor and ecgraphic changes (e.g., n = 5 cases) and left ventricle wall motion disorders (e.g., n = 4 cases). Usually, [^{201}Tl] scans are positive in the vast majority of Case Type A subjects (e.g., n = 9/9). Case Type B disease states can be associated with ecgraphic changes in a relatively small number of cases (e.g., n = 2/9), and approximately 2/3 will demonstrate positive [^{201}Tl] scan results (e.g., n = 6/9). In Case Type B subjects, dipyridamole can produce angor (e.g., n = 2/9); ecgraphic changes (e.g., n = 1/9); left ventricle wall motion disorders (e.g., n = 1); and similar [^{201}Tl] defects as previously detected. In both case type groups, identical coronary arteriography findings can be observed immediately before and during dipyridamole infusion. Therefore, dipyridamole confirms provocative tests for ischemia. The same ischemic pattern observed with stress and dipyridamole scintigraphy in patients with no significant coronary stenosis suggests as pathogenetic mechanism the regional lack of dilatory reserve.

Instrumentation: Coronary arteriography; [^{201}Tl] scan, coronary arteriography, hemodynamic, echo 2D, ecgraphic monitoring.

Dipyridamole test and myocardial scintigraphy with Tl-201 in the diagnosis of myocardial ischemic pathology. Caputo V, De Nardo D, Antolini M, Caiazza F, Pitucco G. Radiol Med (Torino) 1987 May; 73 (5), pp. 390–3.

Cardiovascular: Angiotensin II Receptors (Myocardial/Adrenal Cortex)/ Angiotensin II Receptor TH-142177 Occupancy/ Angiotensin II Receptor Losartan Occupancy *[^{125}I]Sar1,Ile8-AII*

Physiological Mechanism of Action: [^{125}I]Sar1,Ile8-AII is a radiolabeled analogue of angiotensin II that recognizes and physically binds to antiogensin II receptor complexes.

Application: Detection and characterization of tissue/organ angiotensin II receptor complexes and determination of ligand receptor occupancy.

Implementation: Evaluation of myocardial, adrenal cortex, and cerebral cortex angiotensin II (AII) receptors at 2, 8, and 24 hours after oral administration of TH-142177 and losartan using the radioligand binding agent [^{125}I]Sar1,Ile8-AII.

Plasma concentrations of both drugs and metabolites in rats can be measured using validated high-performance liquid chromatography (HPLC) assays. Further, systolic blood pressure (SBP) in conscious renal hypertensive rats treated orally with TH-142177 and losartan can be measured by using a tail cuff plethysmographic method.

Animal Model: Rat.

Interpretation and Analysis: Oral administration of TH-142177 (1.8 and 5.5 μmol/kg) and losartan (6.5 and 21.7 μmol/kg) is associated with dose-dependent decreases in [^{125}I]Sar1,Ile8-AII binding sites (Bmax) in the myocardium and adrenal cortex. The extent of receptor occupancy by both drugs is maximal in the adrenal cortex and myocardium at 2 hours and 8 hours, respectively. Furthermore, receptor occupancy is more sustained in the myocardium than in the adrenal cortex. The *ex vivo* binding affinity of TH-142177 for AII receptors in these tissues is roughly 3 times higher than that of losartan. Also, cerebral cortical [^{125}I]Sar1,Ile8-AII binding is significantly reduced by the oral administration of losartan but not by TH-142177. The time course of AII receptor occupancy by both drugs in

adrenal cortex appears to be in parallel with that of their plasma concentrations, while the time course within the myocardium correlates with that of their hypotensive effects rather than plasma concentrations.

Therefore, TH-142177 produces a relatively selective and sustained *ex vivo* occupancy of AII receptors in myocardium and adrenal cortex with approximately 3 times greater potency than losartan. Its time course of myocardial receptor occupancy is in parallel with that of hypotensive effect rather than plasma concentration.

Receptor occupancy in myocardium, adrenal cortex, and brain by TH-142177, a novel AT1 receptor antagonist in rats, in relation to its plasma concentration and hypotensive effect. Nozawa Y, Miyake H, Yamada S, Kimura R. Pharm Res 1998 Jun; 15 (6), pp. 911–7.

Cardiovascular: Apoptosis and Oncosis (Myocardial Cells)/Apoptosis: Doxorubicin-Induced/ Oncosis: Isoproterenol-Induced
[^{111}Indium] ([^{111}In])-Antimyosin Antibody/ [^{99m}Technetium] ([^{99m}Tc])-Glucaric Acid

Background Information: Non-invasive imaging techniques would be highly desirable for differentiating oncotic from apoptotic cell death.

Application: [^{99m}Tc]-glucaric acid can be applied to detect oncotic myocardial cell death. Conversely, radiolabeled antimyosin is capable of detecting both oncotic and apoptotic types of myocardial cell death.

Route of Administration: [^{111}In]-antimyosin and [^{99m}Tc]-glucaric acid (intravenous).

Implementation: Evaluation of the ability for [^{111}In]-antimyosin antibody and [^{99m}Tc]-glucaric acid to be applied together in a manner that allows the detection of and differentiation between doxorubicin-induced myocardial apoptosis and isoproterenol-induced myocardial oncosis. Scanning electron microscopy, DNA laddering, TUNEL staining, [^{111}In]-antimyosin antibody, and [^{99m}Tc]-glucaric acid can be collectively utilized *in vitro* and *in vivo* to detect doxorubicin-induced apoptosis or isoproterenol-induced myocardial oncosis.

***Ex Vivo* Model:** Cultured rat H9C2 embryonic cardiocytes. Myocardial cell apoptosis can be induced by exposure of H9C2 embryonic cardiocytes to serial doxorubicin dilutions.

Animal Model: Rats (CD1 strain for *in vivo* investigations). Myocardial apoptosis can be induced *in vivo* in rats by doxorubicin administration. Related investigations can employ heat or subcutaneous isoproterenol administration to induce myocardial oncosis.

Interpretation and Analysis: Scanning electron microscopy, DNA laddering, and TUNEL staining of H9C2 cardiocytes treated with doxorubicin all showed cell death by apoptosis. Rat hearts treated with doxorubicin (10 and 20 mg/kg) are DNA ladder-positive and localized significantly greater [^{111}In]-antimyosin antibody (mean ± standard deviation, 0.1942 ± 0.0150 percent injected dose per gram [%ID/g] and 0.1825 ± 0.0238 %ID/g, respectively) than normal hearts (0.1154 ± 0.0270 %ID/g, $p < 0.05$). Increases in myocardial [^{99m}Tc]-glucaric acid activity cannot be observed in rat hearts after 6, 12, and 24 hours of doxorubicin injection (0.0311 ± 0.0066 %ID/g, 0.0356 ± 0.007 %ID/g, and 0.0368 ± 0.0047 %ID/g, respectively; control hearts, 0.0352 ± 0.0099 %ID/g; p = not significant). Alternatively, [^{99m}Tc]-glucaric acid uptake is significantly greater in isoproterenol-induced oncotic hearts (0.1256 ± 0.1023 %ID/g) than in controls ($p < 0.0001$). [^{99m}Tc]-glucaric acid is avid only for the oncotic myocardium. Antimyosin, on the other hand, is positive for both oncotic and apoptotic myocardium.

Indium 111 antimyosin and [^{99m}Tc] glucaric acid for non-invasive identification of oncotic and apoptotic myocardial necrosis. Khaw BA, Silva JD, Petrov A, Hartner W. J Nucl Cardiol 2002 Sep–Oct; 9 (5), pp. 471–81.

Cardiovascular: Atherosclerosis
[^{125}I]-Cholesteryl Iopanoate (CI)/ Acetylated Low-Density Lipoprotein (AcLDL)

Cellular/Molecular Mechanism of Action: Radiolabeled cholesterol ester analogue CI/AcLDL conjugate is passively assimilated into vascular cholesterol plaques (e.g., hydrophobic environment and hydrogen ion binding molecular interactions).

Application: Detection of atherosclerosis plaques responsible for disease states including stroke, aneurysm, and myocardial infarction. The agent [^{125}I]-CI/AcLDL is capable of detecting early-stage intravascular cholesterol plaques and the monitoring of therapeutic pharmaceutical efficacy. The early detection of atherosclerotic plaques is considered to be crucial for successful prevention and/or therapeutic and dietary intervention of cardiovascular disease (CVD).

Route of Administration: Intravenous.

Dosage: 2 μCi/kg (rabbits).

Considerations: Incorporation of CI into AcLDL results in the selective localization of CI within atherosclerotic plaque lesions. Radiolabeled CI/AcLDL can potentially to be used as a probe for visualizing early atherosclerotic lesions using scintigraphy-based imaging modalities.

Interpretation and Analysis: The agent [^{125}I]-CI/AcLDL is more sensitive than many other diagnostic methodologies that can only detect abnormalities after they have reached an advanced stage. The injected drug is cleared from blood following a 2-compartment model. Radioactivity in the atherosclerotic aorta can be about 8 times higher than that in normal aorta, suggesting that the proposed diagnostic probe is selectively taken up by the atherosclerotic lesion. The autoradiography and staining confirm that the localization of the proposed probe is superimposed with the atherosclerotic lesion site.

Instrumentation: Computer-integrated scintigraphy.

Radiolabeled cholesteryl iopanoate/acetylated low-density lipoprotein as a potential probe for visualization of early atherosclerotic lesions in rabbits. Xiao W, Wang L, Scott T, Counsell RE, Liu H. Pharm Res 1999 Mar; 16 (3), pp. 420–6.

Cardiovascular: Brugada Syndrome (Arryhythmia)/ Presynaptic Sympathetic Innervation Dysfunction
[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is an analogue of the adrenergic neurotransmitter norepinephrine.

Background Information: Patients with Brugada syndrome present with characteristic ECG abnormalities (atypical right bundle-branch block and ST-segment elevation) and life-threatening ventricular tachyarrhythmias despite structurally normal hearts. Involvement of the autonomic nervous system is suggested by the occurrence of ventricular tachyarrhythmias and sudden death at rest or during sleep and by changes of typical ECG signs under pharmacological modulation of the myocardial autonomic tone.

Application: Characterization of presynaptic sympathetic innervation to the heart in conditions of Brugada syndrome.

Route of Administration: [^{123}I]-MIBG (intravenous).

Implementation: Evaluation of the presynaptic cardiac neuronal reuptake of norepinephrine (uptake 1) in cases of Brugada syndrome and age-matched control subjects using the norepinephrine

analogue [^{123}I]-MIBG in concert with single photon emission computed tomography (SPECT) and quantitative 33-segment bull's-eye analysis.

Human Model: Brugada syndrome (n = 17; n = 10 age-matched controls).

Interpretation and Analysis: Regionally reduced [^{123}I]-MIBG uptake is present in almost half of all cases with Brugada syndrome (e.g., n = 8/17 or 47%) but not in reference controls. Quantitative analysis shows segmental reduction of [^{123}I]-MIBG uptake in the inferior and septal left ventricular wall in patients with Brugada syndrome compare with control subjects ($p < 0.05$). No correlation can be appreciated between the findings from [^{123}I]-MIBG-SPECT and clinical characteristics of study patients. Abnormal [^{123}I]-MIBG uptake in Brugada syndrome cases indicates presynaptic sympathetic dysfunction exists within the heart. These findings may have potential impact on the pathophysiology and arrhythmogenesis in cases with Brugada syndrome. Future quantitative investigations of the presynaptic and postsynaptic sympathetic and parasympathetic branches of the cardiac autonomic nervous system may clarify whether these observations represent a primary adrenergic dysfunction or an imbalance between sympathetic and parasympathetic innervation of the heart.

Cardiac autonomic dysfunction in Brugada syndrome. Wichter T, Matheja P, Eckardt L, Kies P, Schäfers K, Schulze-Bahr E, Haverkamp W, Borggrefe M, Schober O, Breithardt G, Schäfers M. Circulation 2002 Feb 12; 105 (6), pp. 702–6.

Cardiovascular: Cardiomyopathy (Dilated)/ Left Ventricular Ejection Fraction (LVEF)/ Predict Improved Cardiac Sympathetic Nerve Activity/Predict Improved Cardiac Function/ Predict Improved Symptoms Posttreatment for Dilated Cardiomyopathy

Dobutamine/[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG functions as an analogue of norepinephrine.

Physiological Mechanism of Action: Dobutamine exerts adrenergic/sympathomimetic properties.

Route of Administration: Dobutamine (intravascular).

Dosage: Dobutamine (5, 10, and 15 μg/kg/min).

Implementation: Measure LVEF at rest and in response to gradient increases in the dose of dobutamine infused before therapy in cases of dilated cardiomyopathy (DCM) according to protocols for dobutamine gated blood pool scintigraphy (DOB-GBP). Examinations are performed before and after 1 year of therapy. The heart/mediastinum (H/M) count ratio and total defect score (TDS) are determined for [^{123}I]-MIBG images from anterior planar image and single photon emission computed tomography (SPECT) images. Left ventricular ejection fraction and left ventricular end-diastolic dimension (LVDd) are determined by echocardiography.

Human Model: Cases of dilated cardiomyopathy (n = 22).

Interpretation and Analysis: After 1 year of treatment, echocardiographic LVEF improves > 5% (e.g., n = 11/22 cases). Before treatment, TDS, H/M, LVEF, and LVDd are similar in both groups. However, there is a greater increase in the LVEF during dobutamine infusion in cases displaying improvement compared to those that do not improve ($21 \pm 8\%$ vs $9 \pm 3\%$, $p < 0.001$). If a critical value of 15% for the DeltaLVEF is used to predict the improvement in LVEF after treatment, sensitivity is 91% and specificity is 82%. The TDS, H/M ratio, LVDd, and New York Heart Association functional class improves in cases demonstrating a post–1 year response that is greater than comparable values for cases that do not demonstrate a response. Dobutamine gated blood pool scintigraphy, therefore, can be used to predict improved cardiac sympathetic nerve activity, cardiac function, and symptoms after treatment in patients with DCM.

Dobutamine gated blood pool scintigraphy predicts the improvement of cardiac sympathetic nerve activity, cardiac function, and symptoms after treatment in patients with dilated cardiomyopathy. Kasama S, Toyama T, Hoshizaki H, Oshima S, Taniguchi K, Suzuki T, Kurabayashi M. Chest 2002 Aug; 122 (2), pp. 542–8.

Cardiovascular: Cardiomyopathy/Myocardial Ischemia/Angina Pectoris

[^{99m}Technetium] ([^{99m}Tc])-Methoxy-Isobutyl-Isonitrile (MIBI) and Coronary Angiography/[^{99m}Tc]-MIBI and Trans-Esophogeal Pacing

Application: Detection of alterations in the metabolic/biochemical capacity and function of the myocardium that can become substantially altered during episodes of profound ischemia.

Route of Administration: [^{99m}Tc]-MIBI (intravenous) applied in combination with a maximal stress test (phases of rest, stress, and tranesphogeal atrial pacing).

Considerations: Newly developed radioactive agents with physical and biological properties more favorable than those of [^{201}thallium] include MIBI labeled with [^{99m}Tc]. Such diagnostic agents permit performing simultaneous perfusion and function studies in cases of ischemic cardiopathy.

Trans-esophageal atrial pacing (TAP) techniques have evolved as an alternative provocative test for detecting ischemia.

Interpretation and Analysis: The functional evaluation of myocardial perfusion during stress utilizing [^{99m}Tc]-MIBI scintigraphic imaging in combination with TAP reveals that it is has approximately an 85% level of correlation with [^{99m}Tc]-MIBI scintigraphic imaging applied in concert with coronary angiography evaluations in detecting reversible and irreversible defects. Therefore, TAP can serve as a valid and sensitive provocative test for myocardial ischemia when combined with myocardial [^{99m}Tc]-MIBI scintigraphy.

Diagnosis of coronary artery disease with [^{99m}Tc]-methoxy isobutyl isonitrile and transesophageal pacing. Santomauro M, Cuocolo A, Celentano L, Ferraro S, Pace L, Pappone C, Maddalena G, Salvatore M, Chiariello M. Angiology 1992 Oct; 43 (10), pp. 818–25.

Cardiovascular: Cardiomyopathy/Dilated Cardiomyopathy (DCM)/Heart/Mediastinum Ratio: Prognostic Indicator/[Congestive Heart Failure]

[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is an analogue of the neurotransmitter norepinephrine that can recognize and physically bind to adrenergic receptor complexes.

Physiological Mechanism of Action: [^{123}I]-MIBG is a reliable marker for the detection of cardiac adrenergic neuronal damage in heart failure.

Application: Evaluation of the potential for repeated measurements of cardiac [^{123}I]-MIBG imaging parameters before and after optimized treatment to predict the prognosis of congestive heart failure (CHF) resulting from DCM.

Route of Administration: [^{123}I]-MIBG (intravascular).

Implementation: Assessment of DCM with [^{123}I]-MIBG and meaurement of neurohumoral factor concentrations at baseline and after 6 months of optimized treatment. Cox proportional hazards

analysis can then be performed to assess various parameters before and after treatment.

Human Model: Dilated cardiomyopathy (n = 85 cases with left ventricular ejection fraction (LVEF) of less than 45%).

Interpretation and Analysis: A relatively large number of cases will experience complications (e.g., n = 12 deaths; n = 11 hospitalizations) over a 2-year mean follow-up period. Although there is no difference between the baseline heart/mediastinum (H/M) ratio measured by MIBG between survivors and non-survivors, the H/M ratio is significantly decreased in non-survivors after 6 months. Multivariate analysis reveals that a high plasma concentration of brain natriuretic peptide levels after 6 months (p = 0.0049) and absolute changes in the H/M ratio (p = 0.0046) are independent predictors of mortality. Comparison of the H/M ratio on MIBG imaging before and after optimized additional treatment provides useful information for predicting mortality and is independent of clinical and neurohumoral factors previously shown to be associated with poor prognosis in patients with DCM.

Prognostic value of repeated [^{123}I]-metaiodobenzylguanidine imaging in patients with dilated cardiomyopathy with congestive heart failure before and after optimized treatments-comparison with neurohumoral factors. Matsui T, Tsutamoto T, Maeda K, Kusukawa J, Kinoshita M. Circ J 2002 Jun; 66 (6), pp. 537–43.

Cardiovascular: Cardiomyopathy (Dilated) (DCM)/ β-Blocker Response
[^{201}Thallium] ([^{201}Tl])/[[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)]

Background Information: Metaiodobenzylguanidine is a reliable marker for detecting cardiac adrenergic neuronal damage in heart failure.

Application: Determination of the degree of response to β-blocker therapeutic intervention in conditions of dilated cardiomyopathy.

Route of Administration: Intravenous.

Implementation: Evaluation (co-prediction) of the efficacy of β-blocker therapy in conditions of dilated cardiomyopathy utilizing [^{201}Tl myocardial scintigraphy and [^{123}I]-MIBG myocardial scintigraphy performed before and after the initiation of therapeutic intervention.

Human Model: Cases of dilated cardiomyopathy (n = 47 cases with [^{201}Tl] and [^{123}I]-MIBG scans performed prior to β-blocker therapy). Simple categorization of cases by type can include the following scheme: (i) Classification Group A: cardiac function becomes improved; and (ii) Classification Group B: cardiac function remains unchanged.

[^{201}Tl] extent score (ES) schemes that can be employed for quantitating myocardial damage involve the use of the statistical threshold values of statistical mean-2SD (ES-2) and mean-3SD (ES-3). The ES difference between ES-2 and ES-3 is calculated, and according to ES and ES difference, DCM cases can be classified into 3 groups, including (i) mild-defect type (mild-type); (ii) moderate-defect type (moderate-type); and (iii) severe-defect type (severe-type). The H/M MIBG uptake ratio can be evaluated, and the % washout ratio of myocardial MIBG obtained from these data.

Interpretation and Analysis: For assessment of cases affected by dilated cardiomyopathy, the following patient categorization scheme can be adopted: (i) Group A (n = 18 mild-type, n = 14 moderate-type, n = 1 severe-type cases); and (ii) Group B (n = 5 mild-type, n = 4 moderate-type, n = 5 severe-type cases). A significant degree of correlation can be observed between the defect type detected with [^{201}Tl] and response to β-blocker therapy (p = 0.0090). Both H/M MIBG uptake ratios and washout ratio are not significantly different between the 2 groups. Therefore, [^{201}Tl] may be useful for predicting the response to β-blocker therapy in DCM conditions.

Use of thallium-201 myocardial scintigraphy for the prediction of the response to β-blocker therapy in patients with dilated cardiomyopathy. Hara Y, Hamada M, Ohtsuka T, Ogimoto A, Saeki H, Suzuki J, Matsunaka T, Nakata S, Shigematsu Y. Circ J 2002 Dec; 66 (12), pp. 1139–43.

Cardiovascular: Coronary Spasms/Vascular Spasm Detection and Localization
[^{123}I]-15-(p-Iodophenyl)-3-R,S-Methyl Pentadecanoic Acid/[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)

Cellular/Molecular Mechanism of Action: Metaiodobenzylguanidine functions as an analogue of norepinephrine.

Background Information: Although various non-invasive methods have been used to detect vasospasm, none of them are sensitive enough for cases with sporadic attacks. Because abnormal fatty acid metabolism and cardiac adrenergic neuronal damage are observed in ischemic myocardium, [^{123}I]-15-(*p*-iodophenyl)-3-(R,S)-methylpentadecanoicacid ([^{123}I]-BMIPP) and [^{123}I]-MIBG have been proposed as useful tracers for detection of myocardial damage.

Application: [^{123}I]-BMIPP and [^{123}I]-MIBG scintigraphy are highly accurate and non-invasive techniques for determining the presence and location of vasospasm.

Route of Administration: [^{123}I]-BMIPP and [^{123}I]-MIBG are both given intravenously.

Implementation: Evaluation of the relationship between the coronary vasospastic regions, abnormal left ventricular regional wall motion, fatty acid metabolism, and sympathetic nerve functions and their changes during treatment in cases with vasospastic angina. Conditions can be assessed utilizing comparative analysis of (i) territorial regions of the vasospasm-induced coronary artery; (ii) wall motion determined by left ventriculography; and (iii) BMIPP and MIBG uptake.

Human Model: Vasospastic angina (n = 50, of which n = 25 Type A cases with clinically documented vasospasm; n = 25 Type B cases with vasospasm induced by ergonovine provocation; and n = 25 Type C controls with chest pain but normal coronary arteries without ergonovine provocation of spasm). After 6 months, during-treatment vasospasm is reinduced by ergonovine provocation in Type A cases (e.g., n = 6) and not reinduced in Type B cases (n = 10). Reevaluation of Type A conditions following medical intervention (n = 16/25).

Interpretation and Analysis: Regions exhibiting a positive reaction to the ergonovine provocation can be observed in the (i) right coronary artery (e.g., n = 41 cases); (ii) left anterior descending artery (n = 33 cases); and (iii) left circumflex artery (n = 21 cases). Provocation can (i) occur in multiple vessels (e.g., n = 29 or 58%); and/or (ii) promote reduction in wall motion (n = 19 or 38%). Sensitivity and specificity for the identification of vasospastic angina is 86% (n = 43/50 cases) and 88% (n = 22/25 cases), respectively, for BMIPP scintigraphy and 100% (n = 50/50 cases) and 56% (n = 14/25 controls), respectively, for MIBG scintigraphy. In regions exhibiting reductions in left ventricular wall motion, BMIPP or MIBG uptake is decreased. The sensitivity and specificity for determination of vasospasm-induced coronary arteries is 71% (n = 67/95 arteries) and 95% (n = 71/75 arteries), respectively, for BMIPP scintigraphy and 96% (n = 91/95 arteries) and 55% (n = 41/75 arteries), respectively, for MIBG scintigraphy. Reinduced vasospasm (ergonovine provocation) after 6 months during treatment reveals improvements in decreased BMIPP, and MIBG uptake is lower in Type A (25% ± 4% and 16% ± 4%, respectively) than in Type B

(69% ± 4% and 50% ± 3%, respectively; both $p < 0.01$). Regions in which vasospasm is reinduced exhibit decreases in BMIPP and MIBG uptake. Abnormal fatty acid metabolism and cardiac sympathetic denervation are observed more frequently than wall motion abnormalities in the vasospastic region in cases with vasospastic angina. BMIPP and MIBG scintigraphy are highly accurate and noninvasive techniques for determining the presence and location of vasospasm.

Myocardial sympathetic denervation, fatty acid metabolism, and left ventricular wall motion in vasospastic angina. Watanabe K, Takahashi T, Miyajima S, Hirokawa Y, Tanabe N, Kato K, Kodama M, Aizawa Y, Tazawa S, Inoue M. J Nucl Med 2002 Nov; 43 (11), pp. 1476–81.

Cardiovascular: Endocarditis (Septic/Bacterial)/ Platelet GP IIb/IIIa Receptor Expression Postactivation
[^{99m}Technetium] ([^{99m}Tc])-DMP444

Cellular/Molecular Mechanism of Action: The agent DMP444 recognizes GP IIb/IIIa receptors expressed on the exterior surface membrane of activated platelets.
Background Information: Bacterial endocarditis is an important clinical problem that may result in persistent bacteremia and irreversible cardiac damage. The condition is also characterized by the aggregation of activated platelets, fibrin, and bacteria.
Application: Scintigraphic detection and characterization of bacterial endocarditis conditions.
Route of Administration: [^{99m}Tc]-DMP444 (37 MBq/kg).
Dosage: [^{99m}Tc]-DMP444 (intravenous).
Implementation: Evaluation of the expression of GP IIb/IIIa receptors by activated platelets utilizing [^{99m}Tc]-labeled DMP444 as a high-affinity antagonist of activated platelet GP IIb/IIIa receptor complexes. One day following intervention (bacterial challenge), [^{99m}Tc]-DMP444 (37 MBq/kg) or [^{99m}Tc]-IgG (37 MBq/kg non-specific control agent) is injected intravascularly. Images are obtained up to 4 hours postinjection. Samples are then obtained for tissue counting, microbiology, and histology.

Animal Model: Canine (n = 7 beagles [11–15 kg] with catheterization of their left ventricle through the right carotid artery; n = 5 injected with bacteria; n = 5/7 given [^{99m}Tc]-DMP444; n = 2/7 given [^{99m}Tc]-IgG; n = 2 complete sham procedure). One hour following catheterization, a dose of 5×10^7 colony-forming units of *Staphylococcus aureus* bacteria is injected (e.g., intracardiac). Half an hour later, catheters are removed.
Interpretation and Analysis: From 1 to 2 hours postinjection onward, there is clear focal accumulation of DMP444 in the aortic valve region when endocarditis lesions are present, and the accumulation increases with time. The non-infected and the [^{99m}Tc]-IgG injected models show only persistent blood-pool activity without evidence of a focal cardiac abnormality. At 4 hours postinjection, the *in vivo* valve-to-blood-pool ratios are 1.87 ± 0.18 in endocarditis, 1.01 ± 0.05 in non-infected controls, and 1.09 ± 0.02 in [^{99m}Tc]-IgG injected dogs ($p < 0.05$). Targeting of activated platelets with the [^{99m}Tc]-labeled GP IIb/IIIa antagonist DMP444 allows a final diagnosis of experimental bacterial endocarditis within 4 hours that is associated with a high, specific, and rapid *in vivo* uptake of imaging agent.

Scintigraphic detection of acute experimental endocarditis with the technetium-99m-labeled glycoprotein IIb/IIIa receptor antagonist DMP444. Oyen WJ, Boerman OC, Brouwers FM, Barrett JA, Verheugt FW, Ruiter DJ, Corstens FH, van der Meer JW. Eur J Nucl Med 2000 Apr; 27 (4), pp. 392–9.

Cardiovascular: Heart Failure (Chronic)/Adrenergic Receptor Complexes/Metaiodobenzylguanidine (MIBG) Washout Rate Changes/Pharmaceutical Efficacy Evaluation
[^{123}I]-MIBG

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG functions as a norepinephrine analog.
Background Information: Previous studies have not investigated the efficacy of angiotensin II (AII) receptor antagonists against cardiac sympathetic overactivity in patients with chronic heart failure (CHF) using [^{123}I]-MIBG myocardial imaging.
Application: [^{123}I]-MIBG is a reliable marker for the detection of cardiac adrenergic neuronal damage in conditions of cardiac failure.
Route of Administration: Intravascular.
Implementation: Evaluation of congestive heart failure (CHF) conditions therapeutically managed with an AII receptor antagonist (losartan or candesartan). Image evaluation is performed with MIBG and echocardiograms before and then 6 months after administration in concert with the assessment of neurohumoral parameters.

Human Model: Cases of congestive heart failure patients (n = 34) with fractional shortening of the left ventricular (LV) diameter ≤ 25% or LV ejection fraction ≤ 45% in echocardiogram images.
Interpretation and Analysis: Metaiodobenzylguanidine imaging reveals that the antagonist does not significantly change the heart/mediastinum ratio. However, the washout rate falls significantly (from 32.6% ± 7.6% to 28.2% ± 7.5%; $p < 0.001$). No significant changes occur in LV diameter, fractional shortening, or LV ejection fraction. Circulating atrial (ANP) and brain natriuretic peptides (BNP) and aldosterone fall significantly. Changes in MIBG washout rate correlate positively with changes in BNP ($r = 0.35$, $p < 0.05$). In cases also being treated with angiotensin-converting enzyme (ACE) inhibitors (e.g., n = 19), the MIBG washout rate also falls significantly with AII antagonists, as does BNP and aldosterone. Decreases in MIBG washout and BNP in cases with CHF induced by the AII receptor antagonists suggests the efficacy of these agents in modifying cardiac sympathetic function and neurohumoral factors, even with ACE inhibition. Combination therapy with AII receptor antagonists and ACE inhibitors appears effective for CHF.

Effects of angiotensin II receptor antagonists on [^{123}I]metaiodobenzylguanidine myocardial imaging findings and neurohumoral factors in chronic heart failure. Shinohara H, Fukuda N, Soeki T, Sakabe K, Onose Y, Tamura Y. Heart Vessels 2002 Dec; 17 (2), pp. 47–52.

Cardiovascular: Heart Failure (Congestive)/ Renal Failure: Acute/Endothelin (ET) Receptor Expression: Antagonist Receptor Occupancy/ ET Receptor Antagonist Dosage (Individualized Design of Treatment Schemes)
[^{125}I]ET-1/BQ-123/BQ-788/L-749,329

Cellular/Molecular Mechanism of Action: The agents BQ-123 and BQ-788 bind to and block ETA and ETB type ET receptor complexes and are therefore classified as receptor antagonists. Similarly, the non-peptide antagonist L-749,329 has a relatively balanced affinity for each of the 2 ET receptor subtypes.
Physiological Mechanism of Action: Endothelin is 1 of the most potent vasoconstrictor agents identified to date.
Background Information: Endothelin has been implicated in various disease conditions, including acute renal failure and congestive heart failure. Such observations imply that these disease states can possibly be managed with ET receptor antagonists. However,

establishing the dosages for these antagonists may be difficult because no convenient physiologic indicator of action exists, and because of complexities in receptor function. Two receptor subtypes have been identified for which selective antagonists have been reported (e.g., BQ-123 for the ETA receptor and BQ-788 for the ETB receptor). Of the 3 natural peptide hormones (ET-1, ET-2, and ET-3), ET-1 exhibits high affinity for both subtypes of receptor.

Application: Characterization of the relative expression of 2 endothelium receptor subtypes in a manner that provides information that can be utilized to develop individualized dosages for ET receptor antagonists and facilitate management of various disease states, including acute renal failure and congestive heart failure.

Implementation: Evaluation of the *in vivo* biodistribution of [^{125}I]ET-1 with and without the presence of selective peptide antagonists (BQ-123, BQ-788), and a non-peptide antagonist (L-749,329). The agent [^{125}I]ET-1 possesses binding avidity for ET receptor complexes.

Animal Model: Rat.

Animal Model: Primate (rhesus monkey).

Interpretation and Analysis: The agents BQ-123, BQ-788, and L-749,329 inhibit [^{125}I]ET-1 binding consistent with physical interaction with a single receptor site. However, the sum of inhibition by the selective antagonist is greater than 100% (as defined by inhibition with L-749,329), which suggests (i) lower in vivo selectivity than determined in vitro, and/or (ii) receptor subtype interactions. The latter explanation is supported, in part, by in vitro autoradiographic studies as well as studies in isolated tissues and cell preparations. Synthesized ET-1 labeled with [^{123}I] facilitates the acquisition of images reflecting receptor distribution in 2 in vivo animal models. Therefore, it is possible to visualize, via planar, non-invasive imaging, the occupancy of ET receptor by antagonists in both the kidney and lung. [^{123}I]ET-1 can therefore be used to determine clinically appropriate dosages for antagonist needed for receptor saturation.

Radioiodinated endothelin-1: A radiotracer for imaging endothelin receptor distribution and occupancy. Gibson RE, Fioravanti C, Francis B, Burns HD. Nucl Med Biol 1999 Feb; 26 (2), pp. 193–9.

Cardiovascular: Inflammation/Induced Myocardial Ischemia/Neutrophil Leukotriene B_4 (LTB_4) Receptors
[^{99m}Technetium] ([^{99m}Tc])-RP517/[F]-RP517

Cellular/Molecular Mechanism of Action: In whole blood, [F]-RP517 (500 nmol/L) preferentially labels neutrophil LTB_4 receptor complexes.

Application: [^{99m}Tc]-RP517 is an LTB_4 receptor antagonist that has been developed for imaging acute inflammation or infection. A unique property of [^{99m}Tc]-RP517 is its ability to label white blood cells *in vivo* after intravenous injection.

Route of Administration: [^{99m}Tc]-RP517 (intravenous).

Implementation: Evaluate the relative [^{99m}Tc]-RP517 binding to human leukocyte subtypes and the [^{99m}Tc]-RP517 uptake pattern in myocardium with induced inflammatory lesions. One approach to assessing such conditions can involve delineation of RP517 binding to neutrophil populations *in vitro* within whole blood, and *in vivo* within the intravascular compartment. This can be achieved through the use of fluorescence-activated cell sorter analysis performed on whole human blood samples (n = 2) and isolated neutrophils (n = 4) with a fluorescent analogue of [^{99m}Tc]-RP517 referred to as [F]-RP517. Evaluation strategies *in vivo* can entail administration of [^{99m}Tc]-RP517 to determine its biodistribution within the myocardium before and after coronary occlusion. To facilitate *ex vivo* imaging of the myocardium, the left ventricle can be divided into 72 segments for flow and [^{99m}Tc]-RP517 uptake analyses.

Animal Model: Canine (n = 9 dogs, anesthetized, open-chest surgical exposure before coronary occlusion [90 minutes] and reperfusion [120 minutes]; or n = 3 dogs, before intramyocardial TNF-α administration). In this context, myocardium inflammation can be induced by either coronary occlusion and reperfusion or administration by injection of tumor necrosis factor-alpha (TNF-α) preparations.

Interpretation and Analysis: In whole blood, [F]-RP517 (500 nmol/L) preferentially labels neutrophils. In isolated neutrophil populations, [F]-RP517 (10 nmol/L) binding is inhibited by 44% in the presence of LTB4 (400 nmol/L). In the analysis of *ex vivo* cardiac samples, an inverse exponential relationship can be detected between [^{99m}Tc]-RP517 uptake and occlusion flow (r = 0.73). In the same 15 segments, [^{99m}Tc]-RP517 uptake is highly correlated with neutrophil myeloperoxidase enzyme levels (r = 0.91). *Ex vivo* images reveal tracer uptake in the reperfused area (ischemic to normal count ratio = 2.7 ± 0.2). Therefore, it can be concluded that RP517 binds to the neutrophil LTB4 receptor complex after intravenous injection. After reperfusion, [^{99m}Tc]-RP517 uptake correlates with myeloperoxidase levels and can be observed on *ex vivo* images, indicating that this tracer may have potential as an imaging agent for detecting inflammatory conditions.

Assessment of myocardial inflammation produced by experimental coronary occlusion and reperfusion with [^{99m}Tc]-RP517, a new leukotriene B_4 receptor antagonist that preferentially labels neutrophils *in vivo*. Riou LM, Ruiz M, Sullivan GW, Linden J, Leong-Poi H, Lindner JR, Harris TD, Beller GA, Glover DK. Circulation 2002 Jul 30; 106 (5), pp. 592–8.

Cardiovascular: Myocardial Hypoxia/Myocardial Oxygen Availability/Myocardial Intracellular Energy Level/Myocardial Mitochondrial Redox State
[^{99m}Technetium] ([^{99m}Tc])-Nitroimidazole

Physiological Mechanism of Action: Nitroimidazole is preferentially internalized and retained by hypoxic cardiac myocardium.

Background Information: The agent [^{99m}Tc]-labeled nitroimidazole is preferentially taken up and retained by hypoxic cardiac myocardium.

Application: Nitroimidazole is an agent that can potentially detect and characterize myocardial hypoxia associated with conditions such as cardiac ischemic injury.

Route of Administration: Nitroheterocycle in a research scenario can be applied in isolated perfused (*ex vivo*) hearts but in a clinical environment it would in all probability be given intravascularly.

Implementation: Evaluation of the influence of O_2 or N_2 equilibrated cell-free medium perfused into hearts on the uptake of infused nitroheterocycle and its subsequent washout from myocardial tissues.

***Ex Vivo* Model:** Rat (isolated perfused hearts).

Interpretation and Analysis: Nitroheterocycle displays biphasic kinetic washout patterns in isolated perfused hearts (rat). For both uptake and washout, the early phase is very rapid, whereas the late phase is much slower. The amount of radioactivity retained after 40 minutes of clearance is about 2-fold greater in hypoxic hearts than in normoxic hearts. Cardiac myocytes and mitochondria isolated from hearts also accumulate nitroheterocycle. Association of the compound with heart cells is inversely related to the level of available oxygen and is independent of intracellular energy levels or mitochondrial redox state in the presence of oxygen. The results indicate that [^{99m}Tc]-labeled nitroimidazole may serve as a sensitive marker for detecting hypoxic myocardium.

The agent [^{99m}Tc]-labeled nitroimidazole is preferentially taken up and retained by hypoxic cardiac myocardium.

A novel [^{99m}technetium]-labeled nitroheterocycle capable of identification of hypoxia in heart. Rumsey WL, Cyr JE, Raju N, Narra RK. Biochem Biophys Res Commun 1993 Jun 30; 193 (3), pp. 1239–46.

Cardiovascular: Myocardial Infarction
Antimyosin Monoclonal Antibody (MAb)

Application: Non-invasive *in vivo* detection of acute myocardial infarction.
Route of Administration: Antimyosin MAb (intravenous).
Dosage: Antimyosin MAb.
Design and Validation: Antimyosin antibody preparations have been developed as a diagnostic imaging platform for the *in vivo* detection of acute myocardial infarction. Antimyosin antibody can detect the presence of an assortment of other disease states affecting the heart where the hallmark of the pathogenesis of the condition includes myocyte necrosis. Consistent with this concept, antimyosin antibody has been used as a non-invasive imaging agent for the diagnosis of acute myocarditis, heart transplant rejection, drug-induced cardiotoxicity, and other cardiomyopathies.

Clinical experience gained with 1st-generation antimyosin monoclonal antibody preparations has served as a foundation for the design of 2nd-generation monoclonal antibodies such as antifibrin and antiplatelet immunoglobulins used in the *in vivo* diagnosis of deep venous thrombosis affecting the upper limbs, pulmonary embolism, and antiatherosclerotic lesion-specific antibody for diagnosis of metabolically active lesions. Potential molecular candidates for use in the design of a class of 3rd-generation monoclonal antibodies include ultrasmall antigen-binding immunoglobulin fragments or synthetic peptides and negative charge-modified antibodies.
Interpretation and Analysis: Positive accumulation of antimyosin MAb with a region of the myocardium indicates the presence of some type of injury to the heart (e.g., ischemia/ischemic necrosis). Antimyosin preparations have also been used clinically for non-invasive diagnosis of acute myocarditis, heart transplant rejection, drug-induced cardiotoxicity, and other cardiomyopathies.

Antibody imaging in the evaluation of cardiovascular diseases. Khaw BA, Narula J. J Nucl Cardiol 1994 Sep–Oct; 1 (5 Pt 1), pp. 457–76.

Cardiovascular: Myocardial Infarction/Myocardial Salvage as a Function of Infarct Location/ Myocardial Salvage with AMP579 plus Percutaneous Transluminal Coronary Angioplasty (PTCA) Therapy
^{99m}Technetium ([^{99m}Tc])-SestaMIBI/AMP579

Cellular/Molecular Mechanism of Action: AMP579 is a mixed adenosine agonist with both A1 and A2 properties.
Background Information: Evidence suggests that myocardial ischemic preconditioning and reperfusion injury may be mediated by adenosine A1 and A2 receptors. In animal models of acute myocardial infarction (MI), AMP579 reduces infarct size at serum levels of 15 to 24 ng/mL.
Application: Predictive indicators for myocardial salvage as a function of infarct location.
Route of Administration: AMP579 (6-hour infusion).
Dosage: AMP579 (multiple including 60-mcg/kg).
Considerations: Placebo-controlled studies can be employed.
Implementation: Evaluation of the influence of 3 different AMP579 doses infused continuously over a 6-hour period. The primary end point is final MI size measured by [^{99m}Tc]-sestaMIBI scanning at 120 to 216 hours after PTCA. Secondary end points include (i) myocardial salvage and salvage index values established at the same time interval (case subset subjected to baseline [^{99m}Tc]-sestaMIBI scan); (ii) left ventricular ejection fraction and heart failure at 4 to 6 weeks; (iii) duration of hospitalization; and (iv) cardiac events at 4 weeks and 6 months.
Human Model: Cases undergoing primary PTCA after acute ST-segment elevation MI (n = 311).
Interpretation and Analysis: Final infarct size does not differ among case groups receiving either AMP579 or placebo pertaining to either anterior myocardial infarction or non-anterior myocardial infarction. In conditions of anterior myocardial infarction, success of median myocardial salvage is increasingly higher in cases receiving ascending dosages of AMP579 plus PTCA. Serum levels approaching concentrations that reduce infarct size in animal models can be achieved only with dosages of 60-mcg/kg. In summary, AMP579 is safe at the doses tested, but it does not reduce infarct size. There is a trend toward greater myocardial salvage in treated patients with anterior MI.

A randomized, double-blinded, placebo-controlled, dose-ranging study measuring the effect of an adenosine agonist on infarct size reduction in patients undergoing primary percutaneous transluminal coronary angioplasty: The ADMIRE (AmP579 Delivery for Myocardial Infarction REduction) study. Kopecky SL, Aviles RJ, Bell MR, Lobl JK, Tipping D, Frommell G, Ramsey K, Holland AE, Midei M, Jain A, Kellett M, Gibbons RJ. Am Heart J 2003 Jul; 146 (1), pp. 146–52.

Cardiovascular: Myocardial Infarction or Ischemia/ Lipid Metabolism: Differentiation Parameter
[^{123}I]-15-(p-Iodophenyl)-3-(R,S)-Methylpentadecanoicacid ([^{123}I]-BMIPP)/Non-Radioactive Colored Microspheres

Cellular/Molecular Mechanism of Action: [^{123}I]-BMIPP is a radiolabeled lipid analogue that is perceived by cells within the body as an endogenous lipid molecule.
Background Information: The tracer [^{123}I]-BMIPP has been examined for its ability to reflect myocardial lipid metabolism. Studies in mice indicate that myocardial BMIPP uptake correlates with adenosine triphosphate (ATP) content. Details, however, of myocardial accumulation in the ischemic period with either infarct or ischemia are not well documented.
Application: [^{123}I]-BMIPP can be utilized to detect and characterize myocardial lipid metabolism in a manner that can potentially be used as a parameter to differentiate myocardial ischemia from myocardial infarction.
Route of Administration: [^{123}I]-BMIPP (intravascular).
Implementation: Evaluation of myocardial regional wall motion applying echocardiography in the short-axial view from the epicardium. Tissue blood flow can be calculated using non-radioactive colored microspheres. Changes in blood glucose levels, lipid levels, and lactate extraction can be examined in blood collected from the aorta and great cardiac vein (GCV). The ATP concentration and [^{123}I]-BMIPP count are determined by high-performance liquid chromatography and gamma counter data, respectively.
Animal Model: Canine (n = 16 adult mongrel dogs). The occluded left anterior descending artery (LAD) alone is reperfused to create an ischemic region, and the 1st diagonal branch of the LAD is kept occluded to make the infarct lesion.
Interpretation and Analysis: Two hours after reperfusion, blood flow decreases to 20% ± 5% in infarct regions and 64% ± 9% in ischemic areas ($p < 0.05$), despite comparable (similar)

wall-motion reduction (32% ± 5% and 42% ± 12% in the infarct and ischemic areas, respectively). BMIPP content and ATP concentration display parallel reductions: 40% ± 7% and 75% ± 4% ($p < 0.05$) of BMIPP and 32% ± 9% and 69% ± 7% ($p < 0.05$) of ATP in infarct and ischemic regions, respectively. Non-esterified fatty acid extraction, defined as "flow × ([artery] − [GCV])," decreases to 87% ± 5.6% during occlusion and 75% ± 20.1% 2 hours after reperfusion compared to control values. BMIPP uptake correlates well with lipid metabolism and tissue ATP levels and may prove useful in differentiating myocardial infarction from ischemia in the acute phase of ischemic episodes.

Evaluation of myocardial viability with iodine-123-BMIPP in a canine model. Nohara R, Okuda K, Ogino M, Hosokawa R, Tamaki N, Konishi J, Fujibayashi Y, Yonekura Y, Fujita M, Sasayama S. J Nucl Med 1996 Aug; 37 (8), pp. 1403–7.

Cardiovascular: Myocardial Infarction/Sympathetic (Adrenergic) Neuronal Dysfunction and Receptor Expression Alterations/QT-RR Slope and Metaiodobenzylguanidine (MIBG) Heart/Mediastinum Ratio/QT Dispersion and MIBG Heart/Mediastinum Ratio
[^{123}I]-MIBG

Cellular/Molecular Mechanism of Action: Metaiodobenzylguanidine is an analogue of norepinephrine and recognizes and physically binds to sympathetic/adrenergic receptor complexes.
Background Information: Cardiac [^{123}I]-MIBG imaging is widely used to assess cardiac sympathetic neuronal function. However, physiologic significance of impaired cardiac MIBG uptake has not been fully elucidated.
Application: Detection and characterization of sympathetic adrenergic receptor expression as a parameter that can function as a means for classifying different cardiovascular disease states (e.g., myocardial infarction).
Route of Administration: [^{123}I]-MIBG (intravascular).
Implementation: Evaluate the influences of abnormal cardiac sympathetic neuronal function on heart rate variability (HRV) and ventricular repolarization process in conditions of myocardial infarction. Classification of myocardial infarction can be into various group types based on heart/mediastinum (H/M) ratios established by MIBG scintigraphy. Holter recording and a standard 12-lead electrocardiography can be used for the evaluation of HRV, QT-RR relation, and QT dispersion. The groups are (i) MIBG Classification Scheme I: globally decreased MIBG uptake (H/M < 1.5; n = 10 cases); (ii) MIBG Classification Scheme II: decreased MIBG uptake (H/M ≥ 1.5; n = 19 cases); and (iii) Normal Reference Control Scheme III: (n = 17 cases).

Human Model: Cases of myocardial infarction (n = 29) classified into 2 groups by an H/M ratio established by MIBG scintigraphy.
Interpretation and Analysis: Low, high, and total frequency components are decreased in MIBG Classification Schemes I and II compared to reference controls. Reduction of frequency domain measurements are more severe in Scheme I than in Scheme II cases, but the differences do not reach statistically significant levels. Circadian variation of frequency domain measures disappear in Scheme I cases. The slope of QT-RR relation is significantly greater in group I than in group II or group III. QT dispersion is also greater in group I (64 ± 25 msec) than in group II (43 ± 19 msec) and group III (28 ± 9 msec). Therefore, conditions of sympathetic neuronal dysfunction inferred from globally impaired cardiac MIBG uptake have an altered capacity to modulate ventricular repolarization process as well as displaying decreased HRV.

Modulation of ventricular repolarization and RR interval is altered in patients with globally impaired cardiac [^{123}I]-MIBG uptake. Yoshida N, Nozawa T, Igawa A, Fujii N, Kato B, Mizumaki K, Fujiki A, Asanoi H, Seto H, Inoue H. Ann Noninvasive Electrocardiol 2001 Jan; 6 (1), pp. 55–63.

Cardiovascular: Myocardial Injury (Early)
[^{111}Indium] ([^{111}In])-Antimyosin IgG

Cellular/Molecular Mechanism of Action: Antimyosin recognizes and physically binds to cardiac myosin.
Application: [^{111}In]-antimyosin scintigraphy is very sensitive in detecting myocardial damage similar to that associated with cumulative dose levels of doxorubicin.
Implementation: Evaluate various cumulative anthracycline dose levels applying [^{111}In]-antimyosin scintigraphy in order to identify the early stages of severe cardiac injury and increased long-term risk of cardiac dysfunction. Cases receiving chemotherapeutic agents are monitored at baseline, low (2 cycles), middle (4 cycles), and high (6 cycles) cumulative dose based on the elucidation of [^{111}In]-antimyosin 48-hour heart-to-lung ratios (HLR), left ventricle ejection fractions (LVEF), and peak filling rates (PFR).

Human Model: Cases receiving standard doses of 60 to 75 mg/m doxorubicin or 90 to 112.5 mg/m epirubicin.
Interpretation and Analysis: At low cumulative dose levels only HLR is significantly increased ($p = 0.0001$); at middle dose heart-to-lung-ratio ($p < 0.0001$) and LVEF ($p = 0.0054$), but not PFR, significantly change; and at high dose HLR ($p < 0.0001$), LVEF ($P = 0.0001$), and PFR ($p = 0.033$) all change significantly. Concerning individual results, heart-to-lung-ratios become abnormal in a large percentage of cases (n = 18/24 [75%] at low, n = 22/24 [92%] at middle, and n = 24/24 [100%] at high cumulative doses). Alternatively, LVEF and PFR remain within normal limits in essentially all exposed cases. Myocyte damage appears to precede left ventricle systolic and diastolic dysfunction in anthracycline treatment. [^{111}In]-antimyosin scintigraphy is very sensitive in detecting myocardial damage after cumulative dose levels even as low as 120–150 mg/m doxorubicin or 180–225 mg/m epirubicin. Myocyte damage appears to precede left ventricle systolic and diastolic dysfunction in anthracycline treatment.

High sensitivity of radiolabeled antimyosin scintigraphy in assessing anthracycline-related early myocyte damage preceding cardiac dysfunction. Valdés Olmos RA, Carrió I, Hoefnagel CA, Estorch M, ten Bokkel Huinink WW, López-Pousa J, Dalesio O. Nucl Med Commun 2002 Sep; 23 (9), pp. 871–7.

Hypercholesterolemia in minipigs impairs left ventricular response to stress: Association with decreased coronary flow reserve and reduced capillary density. Theilmeier G, Verhamme P, Dymarkowski S, Beck H, Bernar H, Lox M, Janssens S, Herregods MC, Verbeken E, Collen D, Plate K, Flameng W, Holvoet P. Circulation 2002 Aug 27; 106 (9), pp. 1140–6.

Cardiovascular: Myocardial Injury/Early Detection of Myocyte Apoptosis/Oncotic and Apoptotic Myocardium Detection
[^{111}Indium] ([^{111}In])-Antimyosin Antibody/ [^{99m}Technetium] ([^{99m}Tc])-Glucaric Acid

Cellular/Molecular Mechanism of Action: Antimyosin antibody detects and physically binds to cardiac myosin.
Background Information: Non-invasive imaging techniques would be highly desirable for differentiating between oncotic cell death from apoptotic cell death. [^{111}In]-antimyosin and [^{99m}Tc]-glu-

caric acid have been used to assess such parameters in myocardial cell populations.

Application: Detection and characterization of myocardial (myocyte) injury and damage.

Implementation: Evaluation of the effect of doxorubicin exposure on embryonic cardiocytes and rats in a manner that induces myocardial apoptosis. Acute myocardial oncosis can be induced by heat or subcutaneous isoproterenol administration. Scanning electron microscopy, DNA laddering, TUNEL staining, [^{111}In]-antimyosin antibody, and [^{99m}Tc]-glucaric acid can be utilized to demonstrate *in vitro* and *in vivo* doxorubicin-induced apoptosis or isoproterenol-induced myocardial oncosis.

Animal Model: Rats (CD1 strain).

Animal Model: Cultured H9C2 rat embryonic cardiocytes.

Interpretation and Analysis: Scanning electron microscopy, DNA laddering, and TUNEL staining of H9C2 cardiocytes treated with doxorubicin all demonstrate evidence of cell death by apoptosis. Rat hearts treated with doxorubicin (10 and 20 mg/kg) are DNA ladder-positive and localize (accumulate) significantly greater amounts of [^{111}In]-antimyosin antibody (mean ± standard deviation, 0.1942 ± 0.0150 percent injected dose per gram [%ID/g] and 0.1825 ± 0.0238 %ID/g, respectively) than normal hearts (0.1154 ± 0.0270 %ID/g, $p < .05$). No increase in myocardial [^{99m}Tc]-glucaric acid activity is observed in hearts after 6, 12, and 24 hours of doxorubicin injection (0.0311 ± 0.0066 %ID/g, 0.0356 ± 0.007 %ID/g, and 0.0368 ± 0.0047 %ID/g, respectively; control hearts, 0.0352 ± 0.0099 %ID/g; p = not significant). [^{99m}Tc]-glucaric acid uptake is significantly greater in isoproterenol-induced oncotic hearts (0.1256 ± 0.1023 %ID/g) than in controls ($p < 0.0001$). [^{99m}Tc]-glucaric acid is avid only for the oncotic myocardium. Antimyosin, on the other hand, is positive for both oncotic and apoptotic myocardium.

Indium 111 antimyosin and [^{99m}Tc] glucaric acid for non-invasive identification of oncotic and apoptotic myocardial necrosis. Khaw BA, Silva JD, Petrov A, Hartner W. J Nucl Cardiol 2002 Sep–Oct; 9 (5), pp. 471–81.

Cardiovascular: Myocardial Ischemia
Dithiosemicarbazone (DTS) Derivatives/[Cu]-DTS/[Tc]-ATSM(2)

Background Information: Dithiosemicarbazone derivatives radiolabeled with divalent copper (^{62}Cu, ^{64}Cu) have demonstrated effectiveness as agents for detecting tissue ischemia. Development of analogous derivatives labeled with the more accessible [^{99m}technetium] ([^{99m}Tc]) would be highly desirable.

Application: Detection and characterization of tissue ischemia.

Route of Administration: Intravascular.

Semi-Synthetic Methods: Radiolabeling of various synthesized DTS derivatives using a macromolecular Sn(II)-complex under an anaerobic condition at pH 3.4–4.5 in concert with stabilization with ascorbate solution at pH 6.7–7.0. Characterization of [Tc]-DTS derivatives can be achieved applying various analytical methods (thin layer chromatography [TLC], high-performance liquid chromatography [HPLC], electrophoresis [EP], paper chromatography [PC]).

Implementation: Evaluation of the the capacity for radiolabeled DTS derivatives to detect regions of myocardial ischemia.

Animal Model: Murine and rat (myocardial left anterior descent [LAD] coronary artery occlusion model).

Interpretation and Analysis: The DTSs [Tc]-ATSE, [Tc]-ATSM, and [Tc]-ATSM(2) have distinctive characteristics, with the latter preparations displaying high myocardium uptake in regions of ischemia in LAD rat myocardium models. The [Cu]-DTS mimetic agent [Tc]-ATSM(2) may have potential as an ischemia-damaged myocardium imaging agent.

The development of [^{99m}Tc]-analogue of Cu-DTS as an agent for imaging hypoxia. Horiuchi K, Tsukamoto T, Saito M, Nakayama M, Fujibayashi Y, Saji H. Nucl Med Biol 2000 May; 27 (4), pp. 391–9.

Cardiovascular: Myocardial Ischemia (Acute)/ Prediction Analysis: High vs Low Risk Assessment
[^{99m}Technetium] ([^{99m}Tc])-SestaMIBI

Background Information: In evaluating conditions of non-diagnostic (initial clinical) or ECG findings suggestive of acute cardiac ischemia, continuous 12-lead ECG monitoring increases the detection of diagnostic ECG findings, including ST-segment elevation, in cases awaiting hospital admission. Cardiac markers, particularly the troponins, show great promise for the detection of a wide spectrum of acute coronary syndromes in emergency departments, including cases with minimal myocardial damage that have a high risk for short-term death and non-fatal acute myocardial infarction. Accelerated diagnostic protocols using serial testing with cardiac markers, ECGs, and then provocative testing over a 14-hour period are feasible to perform, safe, and cost-effective.

Application: [^{99m}Tc]-sestaMIBI can be applied to risk stratify low-moderate risk cases into lower and higher risk groups for cardiac events.

Route of Administration: Intravascular.

Implementation: Correlation of resting [^{99m}Tc]-sestaMIBI scans with case history and other diagnostic marker parameters (e.g., 12-lead ECG monitoring; assay of troponin concentrations).

Interpretation and Analysis: Non-stress, at-rest scanning with [^{99m}Tc]-sestaMIBI is able to risk stratify low-moderate risk cases into lower and higher risk groups for cardiac events. Caveats to such diagnostic schemes include the reduced sensitivity of scanning cases that are pain free and the need for follow-up exercise scans for patients free of perfusion defects at rest.

Diagnostic testing of the emergency department patient with chest pain. Zalenski RJ, Shamsa FH. Curr Opin Cardiol 1998 Jul; 13 (4), pp. 248–53.

Cardiovascular: Myocardial Ischemia/Angina Pectoris/Cardiomyopathy
[^{99m}Technetium] ([^{99m}Tc])-Methoxy-Isobutyl-Isonitrile (MIBI) and Trans-Esophogeal Pacing/ [^{99m}Tc]-MIBI and Coronary Angiography

Application: Detection of alterations in myocardial metabolic/biochemical capacity and function that become substantially altered during episodes of profound ischemia.

Route of Administration: [^{99m}Tc]-MIBI (intravenous) applied in combination with a maximal stress test (phases of rest, stress, and tranesphogeal atrial pacing).

Considerations: [^{99m}Tc]-MIBI is a radioactive agent that has been developed with physical and biological properties more favorable than those of [^{201}thallium]. Such diagnostic agents permit performing simultaneous perfusion and function studies in cases of ischemic cardiopathy.

Trans-esophageal atrial pacing (TAP) techniques have evolved as an alternative provocative test of ischemia.

Interpretation and Analysis: The functional evaluation of myocardial perfusion during stress stimuli utilizing [^{99m}Tc]-MIBI scintigraphic imaging in combination with TAP reveals that it has approximately an 85% level of correlation with [^{99m}Tc]-MIBI scintigraphic imaging applied in concert with coronary angiography evaluations

pertaining to the detection of reversible and irreversible defects. Therefore, transesophogeal pacing can serve as a valid and sensitive provocative test for myocardial ischemia when combined with myocardial [^{99m}Tc]-MIBI scintigraphy.

Diagnosis of coronary artery disease with [^{99m}Tc]-methoxy isobutyl isonitrile and transesophageal pacing. Santomauro M, Cuocolo A, Celentano L, Ferraro S, Pace L, Pappone C, Maddalena G, Salvatore M, Chiariello M. Angiology 1992 Oct; 43 (10), pp. 818–25.

Cardiovascular/Myocardial Necrosis: Acute (Carbon Monoxide–Induced)
[^{99m}Technetium] ([^{99m}Tc])-Glucarate/ [^{99m}Tc]-Methoxy-Isobutyl-Isonitrile (MIBI) Alternative

Cellular/Molecular Mechanism of Action: The fundamental myocellular uptake mechanism involves passive distribution across plasma and mitochondrial membranes, and at equilibrium, sestaMIBI is sequestered within the mitochondria in part due to the large negative mitochondrial trans-membrane potential.

Background Information: Cellular and subcellular uptake studies have shown that [^{99m}Tc]-glucarate localizes in necrotic tissues binding primarily to the nucleoprotein subfractions and to a lesser extent to the DNA fractions. Glucarate is taken up rapidly by necrotic myocardium and is cleared relatively rapidly from the circulation, resulting in early, high target-to-background ratios. Reverse distribution into blood stream is stopped by the high membrane potential of the cardiac cells.

Application: Imaging and diagnosis of acute myocardial necrosis ([^{99m}Tc]-glucarate).

Route of Administration: Intravenous.

Considerations: Scintigraphic changes and metabolic abnormalities associated with acute carbon monoxide poisoning can be attributed to tissue necrosis or transitory ischemia.

Implementation: Evaluation of myocardial ischemia.

Interpretation and Analysis: Detection of positive image profiles implies the presence of myocardial ischemia. The blood clearance of [^{99m}Tc]-MIBI is rapid and biexponential, reflected as an initial fast phase followed by a 2nd slower phase. [^{99m}Tc]-MIBI seems to detect accurately the extension of the early necrosis (unreversible) of the myocardium. In acute myocardial infarction studies, [^{99m}Tc]-glucarate (Amiscan) sensitivity is 100% when administered within 9 hours of the onset of chest pain. Preliminary scintigraphic scans with [^{99m}Tc]-glucarate in conditions of acute carbon monoxide poisoning have confirmed that some cases of acute CO poisoning have typical myocardial necrosis. Alternatively, some cases have hibernating and/or stunned myocardium.

Instrumentation: Computer-integrated scintigraphy.

New possibilities in scintigraphy detection of carbon monoxide cardiotoxicity. Pach J, Hubalewska-Hola A, Szybinski Z, Pach D. Przegl Lek 2001; 58 (4), pp. 182–4.

Cardiovascular/Myocardial Necrosis: Acute/Deep Venous Thrombosis
[^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)-Antimyosin-Fab/[^{99m}Technetium] ([^{99m}Tc)]-Antifibrin Fab

Cellular/Molecular Mechanism of Action: Immunoglobulin forms a complex with specific (unique) antigens on the exterior surface of necrotic cells, blood clots, and cellular components of the circulatory system. Besides their level of uniqueness, these antigenic sites are virtually universal pertaining to their expression by cardiovascular tissues/cells across different segments of the human population.

Application: Cardiovascular disorders, including acute myocardial necrosis and deep venous thrombosis.

Considerations: Represents an accurate, safe, non-invasive diagnostic modality. In contrast to tumor markers present on the surface of malignant cells, the epitope for these antibodies is less prone to "shedding" phenomenon. Examples in this regard include the utilization of the 2 immunoscintigraphic diagnostic agents specifically designed for the assessment of cardiovascular disorders, including [^{111}In]-DTPA-antimyosin-Fab fragment for detection of myocardial necrosis/death, and identification of acute venous thrombosis with [^{99m}Tc]-antifibrin Fab′ (T2G1s).

Cardiovascular applications: Current status of immunoscintigraphy in the detection of myocardial necrosis using antimyosin (R11D10) and deep venous thrombosis using antifibrin (T2G1s). Manspeaker P, Weisman HF, Schaible TF. Semin Nucl Med 1993 Apr; 23 (2), pp. 133–47.

Cardiovascular/Myocardial Oncotic Changes/ Myocardium Apoptotic Changes
[^{99m}Technetium] ([^{99m}Tc])-Glucaric Acid/ [^{111}Indium] ([^{111}In])-Antimyosin

Application: The imaging agent [^{99m}Tc]-glucaric acid possesses binding avidity only for the oncotic myocardium. Alternatively, [^{111}In]-antimyosin is positive for both oncotic and apoptotic myocardium changes.

Animal Model: Doxorubicin can be applied to induce apoptosis in cultured rat embryonic cardiocytes (H9C2) and myocardial apoptosis CD1 rats. Acute myocardial oncosis can be induced by heat or subcutaneous isoproterenol administration.

Laboratory Methodologies: Scanning electron microscopy, DNA laddering, TUNEL staining, [^{111}In]-antimyosin antibody, and [^{99m}Tc]-glucaric acid can be applied to demonstrate *in vitro* and *in vivo* doxorubicin-induced apoptosis or isoproterenol-induced myocardial oncosis.

Considerations: Non-invasive imaging techniques would be highly desirable to differentiate oncotic from apoptotic cell death.

Interpretation and Analysis: Scanning electron microscopy, DNA laddering, and TUNEL staining of H9C2 cardiocytes treated with doxorubicin display apoptotic cell death. Rat hearts treated with doxorubicin (10 and 20 mg/kg) are DNA ladder-positive and localize significantly greater [^{111}In]-antimyosin antibody (mean + standard deviation, 0.1942 ± 0.0150 percent injected dose per gram [%ID/g] and 0.1825 ± 0.0238 %ID/g, respectively) than normal hearts (0.1154 ± 0.0270 %ID/g, $p < 0.05$). No increases in myocardial [^{99m}Tc]-glucaric acid activity are observed in rat hearts after 6, 12, and 24 hours of doxorubicin injection (0.0311 ± 0.0066 %ID/g, 0.0356 ± 0.007 %ID/g, and 0.0368 ± 0.0047 %ID/g, respectively; control hearts, 0.0352 ± 0.0099 %ID/g; p = not significant). The uptake of [^{99m}Tc]-glucaric acid is significantly greater in isoproterenol-induced oncotic hearts (0.1256 ± 0.1023 %ID/g) than in controls ($p < 0.0001$).

Indium 111 antimyosin and [^{99m}Tc] glucaric acid for non-invasive identification of oncotic and apoptotic myocardial necrosis. Khaw BA, Silva JD, Petrov A, Hartner W. J Nucl Cardiol 2002 Sep–Oct; 9 (5), pp. 471–81. (Comment in: J Nucl Cardiol 2002 Sep–Oct; 9 (5), pp. 554–60.)

Cardiovascular: Myocardial Perfusion (Kawasaki Disease [KD])/Myocardial Perfusion/Regional Hypoperfusion
[^{99m}Technetium] ([^{99m}Tc])-Tetrofosmin (TF)/ Dipyridamole

Physiological Mechanism of Action: Dipyridamole promotes vasodilator activity.

Background Information: The coronary artery abnormalities in KD often cause myocardial ischemia. Previous publications have described the use of ^{201}thallium myocardial perfusion imaging to determine the extent of ischemia in KD conditions. The technetium perfusion agents offer better resolution and may offer additional information about ventricular function in such conditions.
Application: Tetrofosmin myocardial perfusion imaging can detect regional areas of myocardial hypoperfusion (e.g., KD of children).
Implementation: Evaluation of myocardial perfusion in cases of KD applying [^{99m}Tc]-TF in conjunction with performing a dipyridamole vasodilator stress test. Significant coronary artery stenosis is classified as being ≥ a 75% reduction in luminal diameter. After administration of high-dose dipyridamole infusion (0.70 mg/kg), [^{99m}Tc]-TF is injected, followed by a 2nd [^{99m}Tc]-TF injection at rest 4 hours later. Single photon emission computed tomography (SPECT) images are obtained more than 30 minutes after TF injection.

Human Model: Kawasaki disease (n = 86 cases, 11.5 ± 6.4 years; n = 20 age-matched controls without heart disease). Dipyridamole (high-dose = 0.70 mg/kg); [^{99m}Tc]-TF (1st dose = 74–370 MBq; 2nd dose = 148–740 MBq).
Interpretation and Analysis: Significant coronary artery stenosis; coronary aneurysm without stenosis; and an absence of coronary lesions can be appreciated in a considerable number of cases (e.g., n = 20/86; n = 37/86; and n = 29/86, respectively). Regional myocardial hypoperfusion can be observed in a number of cases that have coronary artery stenosis of 75% or greater (n = 18/86; sensitivity 90%; and specificity 100%). A small percentage of cases do not demonstrate ischemic changes in coronary artery stenosis (n = 2) and some of these cases have good collateral blood flow (n = 1 of 2).

Myocardial ischemia in Kawasaki disease: Evaluation with dipyridamole stress [^{99m}technetium] tetrofosmin scintigraphy. Fukuda T, Ishibashi M, Yokoyama T, Otaki M, Shinohara T, Nakamura Y, Miyake T, Kudoh T, Oku H. J Nucl Cardiol 2002 Nov–Dec; 9 (6), pp. 632–7.

Cardiovascular: Myocardial Perfusion/Influence Hyperlipidemia and Hyperemia on "Washout" Imaging Kinetics
[^{99m}Technetium] ([^{99m}Tc]) Bis(N-Ethoxy, N-Ethyldithiocarbamato)Nitrido

Background Information: *Bis*(N-ethoxy,N-ethyldithiocarbamato)nitrido technetium (V) ([^{99m}Tc]N-NOET) is a myocardial perfusion imaging agent demonstrating significant redistribution that has been utilized in phase III clinical trials. Previous studies have suggested that [^{99m}Tc]N-NOET is bound intravascularly.
Route of Administration: [^{99m}Tc]N-NOET (isolated perfused rat heart bolus; intracoronary artery in dogs).
Dosage: [^{99m}Tc]N-NOET (1.5 MBq in isolated perfused rat heart; 0.75 MBq *in vivo* in dogs).
Implementation: Determine whether modifications in the vascular compartment would provide further insights into the mechanisms of [^{99m}Tc]N-NOET myocardial washout and redistribution. One approach to achieving this objective can entail the evaluation of [^{99m}Tc]N-NOET cardiac washout characteristics in *ex vivo* isolated perfused hearts in the absence or presence of bovine serum albumin (BSA, 0.03%) with or without bound lipids. The intrinsic myocardial washout of the tracer can also be determined *in vivo* following intracoronary bolus injection of the tracer before and after (i) hyperlipidemia induced by intravenous administration of 20% intralipids (300 ml) (n = 3); or (ii) hyperemia induced by intravenous infusion of the adenosine A_{2A} receptor agonist ATL-146e (0.3 μg/kg/min; n = 6).

***Ex Vivo* Model:** Isolated purfused rat hearts (n = 15; n = 6 without albumin; n = 5 also given bound lipid; n = 4 also without bound lipid).

Animal Model: Canine (n = 6 dogs; n = 3 also given intralipids).
Interpretation and Analysis: In isolated hearts, there is no significant myocardial washout of [^{99m}Tc]N-NOET with Krebs-Henseleit buffer. Addition of BSA without bound lipids results in a significant cardiac washout of the tracer ($p < 0.001$ by repeated measures ANOVA). The presence of lipids bound to BSA further accelerates the washout rate of [^{99m}Tc]N-NOET (half-life [$t_{1/2}$], 431.5 ± 23.2 minutes vs 242.9 ± 63.2 minutes; $p < 0.05$). *In vivo* in dogs, intralipid administration significantly increases the intrinsic washout rate of [^{99m}Tc]N-NOET ($t_{1/2}$, 108.0 ± 23.9 minutes vs 51.8 ± 11.8 minutes; $p < 0.05$). In addition, vasodilatation with ATL-146e results in a 4.9-fold increase in coronary flow ($p < 0.05$ vs baseline) and a significantly faster intrinsic [^{99m}Tc]N-NOET myocardial washout ($t_{1/2}$, 81.1 ± 12.1 minutes vs 40.7 ± 7.3 minutes; $p < 0.05$). Therefore, myocardial washout kinetics of [^{99m}Tc]N-NOET is affected by a variety of intravascular factors that support the hypothesis that the tracer is most likely localized on the vascular endothelium. The potential impact of variations in circulating lipid levels on clinical imaging with [^{99m}Tc]N-NOET remains to be fully delineated.

Effects of increased lipid concentration and hyperemic blood flow on the intrinsic myocardial washout kinetics of [^{99m}Tc]N-NOET. Riou LM, Unger S, Toufektsian MC, Ruiz M, Watson DD, Beller GA, Glover DK. J Nucl Med 2003 Jul; 44 (7), pp. 1092–8.

Myocardial Perfusion
[^{201}Thallium] ([^{201}Tl])

Application: Diagnostic imaging for assessment of myocardial perfusion for the purpose of detecting myocardial ischemia and myocardial infarction.
Route of Administration: [^{201}Tl] (intravenous).
Dosage: [^{201}Tl].
Considerations: Measurement of organ-specific uptake applying a gamma-well counter for assessment of internal distribution, combined with the application of autoradiograms, recognizes myocardial [^{201}Tl] accumulation within 5 minutes after injection. Maximum accumulation is achieved at 30 minutes and gradually decreases thereafter.
Interpretation and Analysis: Myocardial scans reliably detect both acute myocardial infarction and transient myocardial ischemia of angina pectoris. In human adults, lesions of myocardial infarction can be clearly detected, but in children it can be difficult to obtain sufficient information due to small heart size and high pulse rate. Judging from the nature of such findings, it has been proposed that it is possible to obtain much clearer images by using a data analyzer. Some clinicians believe that although [^{201}Tl] reliably indicates perfusion defects within the myocardium, its diagnostic use may need to be reserved to clarify such diagnostic problems in cases with coronary artery disease that cannot be satisfactorily explained by conventional diagnostic modalities.
Instrumentation: Computer-integrated scintillation camera.

Cardiovascular: Myocardial Sodium-Potassium ATPase (Na^+/K^+-ATPase)
[^{125}I]-Digoxin/[^{125}I]-Digoxin-Iodohistamine(bis(O-Carboxymethyloxime))

Cellular/Molecular Mechanism of Action: Cardiac glycosides recognize and physically bind to membrane-associated myocardial Na^+/K^+-ATPase complexes.

Background Information: Cardiac glycosides have a high binding avidity for myocardial Na^+/K^+-ATPase complexes.
Implementation: Evaluation of radioiodinated digoxin (cardiac glycoside) derivatives as potential candidates for myocardial imaging with particular emphasis on non-invasive monitoring of cardiac glycoside therapy.

Animal Model: Canine (cardiac scintigraphy).
Interpretation and Analysis: One radioiodinated digoxin derivative, [^{125}I]-digoxin-iodohistamine(*bis*(O-carboxymethyloxime)), achieves a high level of accumulation within the myocardium and has similar binding avidity for Na^+/K^+-ATPase as digoxin itself and behaves in an analogous manner pertaining to ouabain displacement as indicated during the course of *in vivo* and *in vitro* investigations. Based on these results, [^{123}I] labeling of digoxin-histamine(*bis*(O-carboxymethyloxime)) and imaging in a dog demonstrate uptake in the myocardium.

High myocardial accumulation of radioiodinated digoxin derivative: A possible Na^+/K^+-ATPase imaging agent. Fujibayashi Y, Takemura Y, Matsumoto K, Wada K, Yonekura Y, Konishi J, Yokoyama A. J Nucl Med 1992 Apr; 33 (4), pp. 545–9.

Cardiovascular: Sympathetic (Adrenergic) Innervation/Myocardial Infarction (MI)/ Heterogeneous Cardiac Sympathetic Innervation
[^{131}I]-Metaiodobenzylguanidine ([^{131}I]-MIBG)/ [^{125}I]-Iodocyanopindolol ([^{125}I]-ICYP)

Cellular/Molecular Mechanism of Action: Metaiodobenzylguanidine is a norepinephrine analogue that recognizes and physically binds to adrenergic receptor complexes. Iodocyanopindolol is a β-adrenoreceptor ligand.
Application: Detection and characterization of myocardial infarction.
Route of Administration: [^{131}I]-MIBG and [^{125}I]-ICYP are both administered intravenously.
Implementation: Evaluation of cardiac neuronal function and β-receptor expression utilizing a dual-tracer method employing [^{131}I]-MIBG and [^{125}I]-ICYP in heart failure after MI.

Animal Model: Rat (heart failure after induced MI).
Interpretation and Analysis: In conditions of MI (rat), left ventricular (LV) systolic function is decreased, and LV dimension and right ventricular (RV) mass increase gradually. Accumulations of [^{131}I]-MIBG within non-infarcted LV (remote region) and RV decrease by 15% at 1 week compared with sham-operated controls. These same accumulations are restored by 71% and 56%, respectively, at 24 weeks compared with age-matched sham controls, despite sustained depletion of myocardial norepinephrine contents within these anatomical regions. Accumulations of [^{125}I]-ICYP in the remote region and in the RV do not decrease during any stage. Myocardial MIBG distribution is heterogeneous at 1 week and lower in the peri-infarcted region than in the remote region, associated with reduced ICYP accumulation in the peri-infarcted region. At week 12, the heterogeneous distribution of both isotopes disappears. Thus cardiac sympathetic neuronal alteration appears to be coupled with downregulation of β receptors in heart failure after MI (rat). The abnormal adrenergic signaling occurs heterogeneously in terms of ventricular distribution and time course after myocardial infarction.

Heterogeneous cardiac sympathetic innervation in heart failure after myocardial infarction of rats. Igawa A, Nozawa T, Yoshida N, Fujii N, Inoue M, Tazawa S, Asanoi H, Inoue H. Am J Physiol Heart Circ Physiol 2000 Apr; 278 (4), pp. H1134–41.

Cardiovascular: Sympathetic (Adrenergic) Innervation (Denervation)/Ischemia-Related Sympathetic Denervation/Myocardial Sympathoexcitatory Response/[Balloon Coronary Occlusion]
[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is a norepinephrine analogue that recognizes and physically binds to adrenergic receptor complexes.
Background Information: Alterations of cardiac sympathetic nerve function could modulate sympathetic reflexes originating from areas of ischemic injury.
Application: Detection and characterization of cardiac sympathetic denervation.
Route of Administration: [^{123}I]-MIBG (intravenous).
Implementation: Evaluate conditions of angina pectoris as a function of quantified baseline cardiac sympathetic denervation corresponding to anatomical regions of ischemia utilizing [^{123}I]-MIBG. Complementary diagnostic procedures can include establishing the nature of transient changes in sympathetic activity during balloon coronary occlusion (BCO) by wavelet analysis of RR interval variability. The research objective of the referenced study was to elucidate the influence of cardiac sympathetic denervation on the sympathoexcitatory response to acute myocardial ischemia during BCO.

Human Model: Cases suffering from angina pectoris ($n = 23$) subjected to BCO.
Interpretation and Analysis: Balloon coronary occlusion results in a transient augmentation of low-frequency (LF; 0.04–0.14 Hz) spectral components of RR interval variability in approximately 30% of cases (e.g., $n = 4/12$) with cardiac denervation, and in greater than two-thirds of cases without denervation (e.g., $n = 8/11$; $p < 0.01$ by the chi^2 test). Consequently, increases in LF components are significantly less during BCO in cases with cardiac denervation (34%) than in those without denervation (273%: interaction: $p < 0.05$). In some cases with severe ischemia provoked by a fall of $\geq 10\%$ in the left ventricular ejection fraction, LF components increase by 506% during BCO, regardless of the condition of cardiac denervation ($n = 7/23$ cases). Conversely, in conditions of mild ischemia provoked by a fall of $< 10\%$ in the ejection fraction, the changes of LF components during BCO are significantly less in cases with denervation than in those without denervation (84 vs 344%, $p < 0.05$). Such observations suggest that if provoked ischemia is not severe, cardiac sympathetic denervation could prevent ischemia-induced sympathoexcitation.

Cardiac sympathetic denervation modulates the sympathoexcitatory response to acute myocardial ischemia. Joho S, Asanoi H, Takagawa J, Kameyama T, Hirai T, Nozawa T, Umeno K, Shimizu M, Seto H, Inoue H. J Am Coll Cardiol 2002 Feb 6; 39 (3), pp. 436–42.

Cardiovascular: Sympathetic (Adrenergic) Receptor Expression in Heart Failure/ Clinical Variables in Risk Stratification
[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is a norepinephrine analogue that recognizes and physically binds to adrenergic receptor complexes.
Background Information: Both myocardial [^{123}I]-MIBG uptake and plasma norepinephrine are markers of sympathetic activation in heart failure that have been shown to be of value in predicting a poorer prognosis. However, these observations have been noted

before treatment with β-blockers became part of standard clinical practice.

Application: Both myocardial [^{123}I]-MIBG uptake and plasma norepinephrine are markers of sympathetic activation in heart failure conditions.

Implementation: Evaluation of conditions of chronic heart failure over a mean follow-up period of 36 months. Potential prognostic parameters are obtained before initiation of β-blocker therapy.

Human Model: Cases of chronic heart failure (n = 58) identified according to the established guidelines (New York Heart Association functional Class II and III, ejection fraction < 35%; 53% ischemic cardiomyopathy).

Interpretation and Analysis: During the observation period, cases may display cardiovascular complications (e.g., n = 17/58 or 29.3%, death or heart transplantation). Multivariate Cox regression analysis, plasma norepinephrine, peak oxygen consumption, end-diastolic volume as measured by echocardiography, and exercise performance during bicycling and walking have prognostic significance in cases with heart failure treated with β-blockers in addition to angiotensin-converting enzyme inhibitors. In both uni- and multivariate analysis, the heart/mediastinum ratio of [^{123}I]-MIBG uptake does not correlate with cardiovascular mortality.

Cardiac [^{123}I]-MIBG imaging and clinical variables in risk stratification in patients with heart failure treated with β-blockers. de Milliano PA, Tijssen JG, van Eck-Smit BL, Lie KI. Nucl Med Commun 2002 Jun; 23 (6), pp. 513–9.

Cardiovascular: Sympathetic (Adrenergic) Receptor Expression/β-blocker Therapeutic Response (Dilated Cardiomyopathy [DCM])
[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)/[^{201}Thallium] ([^{201}Tl])

Application: Determination of the degree of response to β-blocker therapeutic intervention in conditions of dilated cardiomyopathy.

Route of Administration: Intravenous.

Implementation: Evaluation (co-prediction) of the efficacy of β-blocker therapy in conditions of dilated cardiomyopathy utilizing thallium-201 [^{201}Tl] myocardial scintigraphy and [^{123}I]-MIBG myocardial scintigraphy performed before and after initiation of therapeutic intervention.

Human Model: Cases of dilated cardiomyopathy (n = 47 cases with [^{201}Tl] and [^{123}I]-MIBG scans performed prior to β-blocker therapy).

[^{201}Tl] extent score (ES) schemes that can be employed for quantitating myocardial damage involve the use of statistical threshold values of statistical mean-2SD (ES-2) and mean-3SD (ES-3). The ES difference between ES-2 and ES-3 is calculated, and according to ES and ES difference, DCM cases can be classified into 3 groups including (i) mild-defect type (mild-type); (ii) moderate-defect type (moderate-type); and (iii) severe-defect type (severe-type). The heart/mediastinum (H/M) MIBG uptake ratio can be evaluated, and the percent washout ratio of myocardial MIBG obtained from these data.

Interpretation and Analysis: In clinical investigations assessing cases of dilated cardiomyopathy (e.g., n = 47), conditions can be categorized according to the following classification schemes: Group A (n = 18 mild-type, n = 14 moderate-type, n = 1 severe-type cases) and Group B (n = 5 mild-type, n = 4 moderate-type, n = 5 severe-type cases). A significant relation can be observed between the defect type on [^{201}Tl] and the response to β-blocker therapy ($p = 0.0090$). Both H/M MIBG uptake ratios and washout ratios are not significantly different between each of the groups. Therefore, [^{201}Tl] may be useful for predicting the response to β-blocker therapy in DCM conditions.

Use of thallium-201 myocardial scintigraphy for the prediction of the response to β-blocker therapy in patients with dilated cardiomyopathy. Hara Y, Hamada M, Ohtsuka T, Ogimoto A, Saeki H, Suzuki J, Matsunaka T, Nakata S, Shigematsu Y. Circ J 2002 Dec; 66 (12), pp. 1139–43.

Cardiovascular: Sympathetic (Adrenergic) Receptor Expression Profiles/Congestive Heart Disease: Heart and Lung/[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)/[^{201}Thallium] ([^{201}Tl]) Ratio vs Left Ventricular Ejection Fraction
[^{123}I]-MIBG)/[^{201}Tl]/Cardiac [^{123}I]-MIBG Washout/[^{123}I]-MIBG (Adrenergic Activity)/[^{201}Tl] (Perfusion) Ratio/[^{123}I]-MIBG:[^{201}Tl] vs Plasma Norepinephrine

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is a radioiodinated analogue of norepinephrine that recognizes and binds to sympathetic/adrenergic receptor complexes. The radionucleotide [^{201}Tl] per se does not exert a true biological effect.

Application: [^{123}I]-MIBG can be utilized to detect and characterize tissue/organ sympathetic/adrenergic receptor expression profiles. The radionuclide [^{201}Tl] can serve as a tracer molecule suitable for measuring vascular perfusion parameters. Simultaneous use of both of these agents can facilitate assessment of adrenergic activity and perfusion in various organs in conditions of heart failure.

Route of Administration: [^{123}I]-MIBG and [^{201}Tl] (intravascular).

Implementation: Simultaneous assessment of regional adrenergic activity and vascular perfusion with [^{123}I]-MIBG and [^{201}Tl] in various organs. In order to reduce crosstalk from [^{201}Tl] into the [^{123}I] energy window to less than 5%, doses of [^{123}I]-MIBG that are more than 5 times greater than that of [^{201}Tl] are administered following [^{201}Tl] scintigraphy.

Human Model: Cases suffering from congestive heart failure (CHF) (n = 13; n = 13 without heart failure).

Interpretation and Analysis: Regional uptake of [^{201}Tl] (%dose) becomes significantly increased in the heart (left ventricle) and lung (both $p < 0.01$) in conditions of CHF. Increases in global cardiac uptake may be related to left ventricular enlargement. Patterns of regional uptake for [^{123}I]-MIBG (%dose) at 15 minutes are similar to those of [^{201}Tl], suggesting that early [^{123}I]-MIBG uptake could in part depend on regional perfusion in both case group types. At 3 hours, regional uptake of [^{123}I]-MIBG is significantly increased within the heart, lung, and kidney (all $p < 0.01$) in conditions of CHF. Delayed [^{123}I]-MIBG uptake indicates the degree of neuronal accumulation of the tracer, and therefore reflects adrenergic activity. Interestingly, ratios of cardiac [^{123}I]-MIBG (adrenergic activity) to [^{201}Tl] (unit of perfusion) decreases significantly in the heart ($p < 0.01$) but increases significantly in the kidney ($p < 0.01$) in conditions of CHF compared to negative reference controls. Cardiac [^{123}I]-MIBG washout is also significantly increased with congestive heart failure. Moreover, the cardiac [^{123}I]-MIBG:[^{201}Tl] ratio negatively correlates with plasma norepinephrine concentrations ($r = -0.74$, $p < 0.01$), but positively correlates with left ventricular ejection fraction values ($r = 0.60$, $p < 0.01$). These data suggest that there may be impairment of both the neuronal uptake function and the vesicular storage function in the failing heart, and an increment in neuronal uptake function in the kidneys in conditions of congestive heart failure. It is therefore suggested that dual-tracer scintigraphy can function as a useful non-invasive method for the simultaneous assessment of adrenergic activity and perfusion in various organs in conditions of cardiac failure.

Simultaneous assessment of regional adrenergic activity and perfusion with [^{123}I]-MIBG and [^{201}Tl] in congestive heart failure. Seto H, Shimizu M, Nozawa T, Yoshida N, Inagaki S, Kageyama M, Kakishita M. Nucl Med Commun 1996 Mar; 17 (3), pp. 225–30.

Cardiovascular: Sympathetic (Adrenergic) Receptor Expression/Cardiac Arrythmia: Brugada Syndrome/ Sympathetic Innervation Dysfunction (Presynaptic) *[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)*

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is an analogue of the adrenergic neurotransmitter norepinephrine.

Background Information: Patients with Brugada syndrome present with characteristic ECG abnormalities (atypical right bundle-branch block and ST-segment elevation) and life-threatening ventricular tachyarrhythmias despite structurally normal hearts. Involvement of the autonomic nervous system is suspected by the occurrence of ventricular tachyarrhythmias and sudden death at rest or during sleep and by changes of typical ECG signs under pharmacological modulation of the myocardial autonomic tone.

Application: Characterization of presynaptic sympathetic innervation to the heart in conditions of Brugada syndrome.

Route of Administration: [^{123}I]-MIBG (intravenous).

Implementation: Evaluation of the presynaptic cardiac neuronal reuptake of norepinephrine (uptake 1) in conditions of Brugada syndrome and age-matched control subjects using the norepinephrine analogue [^{123}I]-MIBG in concert with single photon emission computed tomography (SPECT) and quantitative 33-segment bull's-eye analysis.

Human Model: Brugada syndrome ($n = 17$; $n = 10$ age-matched controls).

Interpretation and Analysis: Regionally reduced [^{123}I]-MIBG uptake is present in almost half of all cases with Brugada syndrome (e.g., $n = 8/17$ or 47%) but not in reference controls. Quantitative analysis shows segmental reduction of [^{123}I]-MIBG uptake in the inferior and septal left ventricular wall in patients with Brugada syndrome compare with control subjects ($p < 0.05$). No correlation can be appreciated between the findings from [^{123}I]-MIBG-SPECT and clinical characteristics of study patients. Abnormal [^{123}I]-MIBG uptake in Brugada syndrome cases indicates presynaptic sympathetic dysfunction exists within the heart. These findings may have potential impact on the pathophysiology and arrhythmogenesis in cases with Brugada syndrome. Future quantitative investigations of the presynaptic and postsynaptic sympathetic and parasympathetic branches of the cardiac autonomic nervous system may clarify whether these observations represent a primary adrenergic dysfunction or an imbalance between sympathetic and parasympathetic innervation of the heart.

Cardiac autonomic dysfunction in Brugada syndrome. Wichter T, Matheja P, Eckardt L, Kies P, Schäfers K, Schulze-Bahr E, Haverkamp W, Borggrefe M, Schober O, Breithardt G, Schäfers M. Circulation 2002 Feb 12; 105 (6), pp. 702–6.

Cardiovascular: Sympathetic (Adrenergic)/ Salt Sensitivity and Regulation/Cardiac Neuronal Function: Late Phase Deterioration/Adrenergic β-Receptor Downregulation *[^{131}I]-Metaiodobenzylguanidine ([^{131}I]-MIBG)/ [^{125}I]-Cyanopindolol ([^{125}I]-ICYP)*

Cellular/Molecular Mechanism of Action: [^{131}I]-MIBG is an analogue of norepinephrine and detects and physically binds to adrenergic/sympathetic receptor complexes. [^{125}I]-ICYP also recognizes and binds to sympathetic/adrenergic receptor complexes.

Background Information: Heart failure is associated with sympathetic nervous system activation and downregulation of β receptors. However, the coupling between cardiac sympathetic neuronal function and β receptors during the development of hypertensive heart failure is not clear.

Application: [^{131}I]-MIBG and [^{125}I]-ICYP (intravascular) can be utilized to evaluate and profile adrenergic/sympathetic adrenergic receptor expression.

Route of Administration: [^{131}I]-MIBG and [^{125}I]-ICYP (intravascular).

Implementation: Evaluation of cardiac neuronal function and β receptors utilizing a dual-tracer method employing [^{131}I]-MIBG and [^{125}I]-ICYP under *in vivo* conditions of salt-sensitivity and salt-resistance.

Animal Model: Rats (Dahl salt-sensitive [DS] and salt-resistant [DR]) fed an 8% NaCl diet after the age of 6 weeks. Blood pressure is raised to $>$ 200 mm Hg at 12 weeks in DS rats and remains elevated until 18 weeks, but only slightly in DR rats. Left ventricular (LV) function of DS rats is preserved at 12 weeks but is deteriorated by 18 weeks.

Interpretation and Analysis: Despite a 56% reduction of cardiac norepinephrine content at 12 weeks in salt-sensitive *in vivo* models, neither MIBG nor ICYP uptakes in salt-sensitive *in vivo* models is different from salt-resistant *in vivo* models. At 18 weeks, both MIBG and ICYP uptakes decrease by 52% and 39%, respectively, in association with 71% reductions in cardiac norepinephrine in salt-sensitive *in vivo* models. MIBG uptake of the LV is homogeneous at 6 weeks but lower in the LV endocardial regions at 18 weeks in salt-sensitive *in vivo* models. Cardiac sympathetic neuronal function, therefore, is relatively preserved at the compensated, hypertrophic stage in salt-sensitive *in vivo* models but deteriorates in association with β-receptor downregulation at the failing stage. The cardiac neuronal dysfunction occurs heterogeneously. A combination of scintigraphic portrayal of β receptors with MIBG should provide valuable information regarding sympathetic nerve signaling in living hearts.

Dual-tracer assessment of coupling between cardiac sympathetic neuronal function and downregulation of β receptors during development of hypertensive heart failure of rats. Nozawa T, Igawa A, Yoshida N, Maeda M, Inoue M, Yamamura Y, Asanoi H, Inoue H. Circulation 1998 Jun 16; 97 (23), pp. 2359–67.

Cardiovascular: Sympathetic (Adrenergic) Tone/ Congestive Heart Failure (CHF)/Cardiac Sympathetic Nervous System Function/ Predictive Response to Carvedilol Therapy *[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)*

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is perceived as an analogue of norepinephrine.

Background Information: Carvedilol treatment reduces the mortality rate in conditions of CHF. It is not known whether carvedilol treatment is effective in heart failure cases with substantial cardiac sympathetic nerve dysfunction.

Application: Assessment of cardiac adrenergic/sympathomimetic tone in various cardiovascular conditions (e.g., CHF), and functions as a probe for evaluating the efficacy of cardiovascular therapeutic agents.

Route of Administration: Intravenous.

Implementation: Evaluate the effect of chronic carvedilol treatment in patients with cardiac sympathetic nerve dysfunction of vary-

ing severity. A viable approach to achieving such an objective can involve assessment of sympathetic nerve function before and after 7.2 ± 2.7 months of carvedilol treatment applying [^{123}I]-MIBG imaging modalities, radionuclide ventriculography, and transmyocardial norepinephrine sampling.

Human Model: Congestive heart failure patients with idiopathic cardiomyopathy (n = 22).

Interpretation and Analysis: Cases of relatively advanced impairment of cardiac sympathetic nerve function, as manifested by a baseline [^{123}I]-MIBG ratio lower than 1.40, have a statistically significant improvement in [^{123}I] heart/mediastinum (H/M) ratio with carvedilol treatment (1.26 ± 0.12 to 1.39 ± 0.20 at $p = 0.004$). Of cases with a baseline [^{123}I]-MIBG ratio lower than 1.40 (e.g., n = 10), a large percentage have an increase in the H/M ratio with carvedilol treatment (n = 9/10 cases). Left ventricular ejection fraction increases from 25.4% ± 7.8% to 37.3% ± 14.7% ($p < 0.001$), with no difference between cases with relatively advanced vs relatively preserved cardiac sympathetic nerve function. Most cases of CHF show a favorable response in left ventricular function to carvedilol treatment, regardless of the baseline level of cardiac sympathetic nervous system function, as assessed by neuronal [^{123}I]-MIBG imaging. Cases with relatively advanced impairment of baseline [^{123}I]-MIBG uptake are most likely to show evidence of improved cardiac sympathetic nervous system function in response to carvedilol therapy.

Carvedilol improves left ventricular function in heart failure patients with idiopathic dilated cardiomyopathy and a wide range of sympathetic nervous system function as measured by iodine 123 metaiodobenzylguanidine. Gerson MC, Craft LL, McGuire N, Suresh DP, Abraham WT, Wagoner LE. J Nucl Cardiol 2002 Nov–Dec; 9 (6), pp. 608–15.

Cardiovascular: Ventricular (Left) Myocardium (Isolated Non-Compaction)/Anatomical and Pathophysiology Parameters/Computed Tomography
^{201}Thallium ([^{201}Tl])/Echocardiography/ Magnetic Resonance Imaging (MRI)/ Computed Tomography (CT)

Application: Detection and characterization of the anatomical features and pathophysiology of isolated non-compaction of the left ventricular myocardium.

Route of Administration: Intravenous.

Implementation: Evaluate the feasibility of ultrafast CT and MRI for anatomical and pathophysiological diagnosis of isolated non-compaction of the left ventricular myocardium (INVM) compared with other imaging modalities including thallium myocardial imaging.

Human Model: Six patients, 3 sets of siblings, ranging in age from 13 to 18 years, were included in this study.

Interpretation and Analysis: Echocardiograms (2-dimensional) reveal numerous prominent trabeculations and deep intertrabecular recesses in 1 or more ventricular wall segments (e.g., n = 6/6 cases). [^{201}Tl] myocardial imaging can detect regions of hypoperfusion corresponding to zones where non-compacted ventricular myocardium is localized. Ultrafast CT shows early defects of varying degrees and rate enhancement of the non-compacted ventricular myocardium, implying fibrosis within these regions. Magnetic resonance imaging detects inner zones of non-compacted myocardium distinguishable from thin outer zones of compacted myocardium. T_2-weighted imaging reveals high-intensity areas at the apex of the left ventricle, suggesting the presence of disturbed microcirculation due to fibrosis, thrombus formation, and hypokinesis. Cine MRI reveals hypokinesis of the non-compacted ventricular wall during the cardiac cycle. Ultrafast CT and MRI provide high-resolution imaging of non-compacted myocardium, and also pathophysiological details regarding this rare disease.

Isolated non-compaction of the ventricular myocardium: ultrafast computed tomography and magnetic resonance imaging. Hamamichi Y, Ichida F, Hashimoto I, Uese KH, Miyawaki T, Tsukano S, Ono Y, Echigo S, Kamiya T. Int J Cardiovasc Imaging 2001 Aug; 17 (4), pp. 305–14.

Cardiovascular: Ventricular (Left) Dysfunction/ Skeletal Muscle Anaerobic Threshold/Exercise Intolerance
[^{201}Thallium] ([^{201}Tl])

Cellular/Molecular Mechanism of Action: ^{201}Thallium is a radioactive isotope that emits photons and does not per se exert any biological effect.

Background Information: Decreased blood flow to working muscles makes an important contribution to exercise intolerance in patients with chronic heart failure.

Application: Radioactive tracer/marker system that can be applied for the detection of alterations in muscle metabolism.

Route of Administration: [^{201}Tl].

Implementation: Determine if maldistribution of skeletal muscle blood flow is closely related to exercise intolerance in conditions of cardiac dysfunction. One approach to determining the implications of this consideration involves performing whole-body thallium scintigraphy during 1-leg exercise challenge in cases of left ventricular (LV) dysfunction. Blood flow distribution to the exercising and resting legs is quantified by expressing regional thallium counts as a percentage of the whole-body counts at rest, at the level of anaerobic threshold, and at peak exercise.

Human Model: Left ventricular dysfunction (LV ejection fraction < 45%; n = 11 cases).

Interpretation and Analysis: At anaerobic threshold, thallium activity of exercising muscle increases from 4.2 ± 0.7% to 14.0 ± 2.5% ($p < 0.05$) in the thigh and from 1.7 ± 0.3 to 4.1% ± 0.9% ($p < 0.05$) in the calf, compared to resting values. Consequently, the ratio of thallium activity between exercising and resting legs increases to 2.7 ± 0.7 ($p < 0.05$) in the thigh and to 2.3 ± 0.7 ($p < 0.05$) in the calf. When plotted as a function of anaerobic threshold, thallium activity of the exercising thigh ($r = 0.78$, $p < 0.05$) and the thallium ratio between exercising and resting thigh ($r = 0.69$, $p < 0.05$) declines with a reduction in exercise tolerance. These correlations are not observed in lower limb musculature. Whole-body thallium scintigraphy demonstrates a maldistribution of leg blood flow in cases with reduced aerobic exercise capacity, suggesting that this abnormality could play an important role in exercising intolerance in such conditions.

Quantitative evaluation of blood flow distribution to exercising and resting skeletal muscles in patients with cardiac dysfunction using whole-body thallium-201 scintigraphy. Wada O, Asanoi H, Miyagi K, Ishizaka S, Kameyama T, Ishise H, Seto H, Inoue H. Clin Cardiol 1997 Sep; 20 (9), pp. 785–90.

Vascular "Blood-Pool"
[^{99m}Technetium] ([^{99m}Tc])-Human Serum Albumin (HSA)/[Kit Preparation Variability]

Physiological Mechanism of Action: Human serum albumin is a protein found in the intravascular compartment that maintains plasmas oncotic pressure and serves as a source of reserve protein/amino acids that can be metabolized during starvation.

Application: Endogenous protein marker system/tracer molecule that can potentially be used to characterize various parameters associated with the size of the intravascular compartment, angiography procedures, and regional tissue/organ perfusion.

Semi-Synthetic Methods: Evaluation of the suitability of 5 different commercially available kits that provide HSA labeled with [^{99m}Tc] for cardiac blood-pool imaging. Four of the kit preparations are produced using a 1-step process employing stannous chloride as the reducing agent; the 5th is based on an electrolytic reduction. Other methods for labeling have also been evaluated (electrolytic method).

Implementation: Radiochemical purity of preparations can be assessed by precipitation with trichloroacetic acid and by gel filtration on a Biogel P4 column. Additional analyses can entail measurement of radioactivity clearance from the blood at frequent time intervals after intravenous injection.

Human Model: Cases in normal health (n = 6/kit preparation).

Interpretation and Analysis: The labeling efficiency of 1-step kits varies between 73 and 93% compared with 94 and 98% for electrolytically labeled albumin. The blood clearance for all 1-step kits is significantly faster than that obtained for the radiopharmaceuticals prepared by the electrolytic method. Radiolabeling of HSA with [^{99m}Tc] by the electrolytic method therefore appears to be the preferred method.

An evaluation of 6 kits of [^{99m}technetium] human serum albumin injection for cardiac blood-pool imaging. Millar AM, Hannan WJ, Sapru RP, Muir AL. Eur J Nucl Med 1979 Apr 1; 4 (2), pp. 91–4.

Vascular: Endothelial Inflammation (Acute)/ Pulmonary Injury: Bleomycin-Induced/LFA-1α *[^{111}Indium] ([^{111}In])-Anti-ICAM-1 Monoclonal Antibody (MAb)*

Cellular/Molecular Mechanism of Action: The antibody component of [^{111}In]-anti-ICAM-1 monoclonal IgG antibody recognizes and physically binds to intracellular adhesion molecule-1 complexes expressed on the exterior surface membrane of "activated" endothelial cells.

Application: Detection of endothelial injury and acute vascular inflammatory responses, including bleomycin-induced pulmonary injury.

Route of Administration: [^{111}In]-anti-ICAM-1 monoclonal IgG antibody (intravascular/intravenous).

Implementation: Evaluation of alterations in endothelial intracellular adhesion molecule expression following bleomycin-induced pulmonary injury employing [^{111}In]-anti-ICAM-1 monoclonal IgG antibody (^{111}In*aICAM-1) compared to [^{111}In]-labeled normal mouse IgG (^{111}In*nmIgG). Tissue samples are harvested 24 hours following image acquisition and their radioactivity measured. Lung tissue can be cryostat-sectioned to detect the presence of ICAM-1 by immunofluorescence, and the upregulation of LFA-1α identified in blood polymorphonuclear leukocytes (PMNs) using fluorescence-activated cell-sorter (FACS) analysis.

Animal Model: Rat (lung injury induced by intratracheal bleomycin injection 4 or 24 hours before humane sacrifice).

Interpretation and Analysis: [^{111}In]-anti-ICAM-1 monoclonal IgG antibody.

The percent injected dose/organ in lungs both at 4 and 24 hours postbleomycin increases significantly with the application of [^{111}In]-anti-ICAM-1 monoclonal IgG antibody in contrast to values in either uninjured control states or the administration of [^{111}In]*nmIgG. At 4 and 24 hours postinjury, the target-to-blood (T/B) ratio is 8/1 and 6/1, respectively. For [^{111}In]*nmIgG, the T/B ratio at 4 hours is 0.5/1 and 0.4/1 at 24 hours. [^{111}In]-anti-ICAM-1 monoclonal IgG antibody preparations administered postbleomycin-induced pulmonary injury produce 4- and 24-hour images that can easily be distinguished from uninjured reference controls. All images with [^{111}In]*nmIgG show only cardiac blood-pool and liver activity with little lung activity. Lung ICAM-1 immunofluorescence intensity increases in the bleomycin-treated pulmonary samples compared to uninjured lungs. Expression of LFA-1α on PMNs increases 19% and 210% at 4 hours and 24 hours postinjury, respectively, compared to control values. Biodistribution and imaging data, therefore, are capable of demonstrating that [^{111}In]-anti-ICAM-1 monoclonal IgG antibody is capable of detecting early acute bleomycin-induced lung injury. Immunofluorescence and FACS data suggest that [^{111}In]-anti-ICAM-1 monoclonal IgG antibody uptake is a specific process. Monoclonal antibody preparations that possess such attributes have potential as an early radionuclide detector of acute inflammation.

Early detection of bleomycin-induced lung injury in rat using indium-111-labeled antibody directed against intercellular adhesion molecule-1. Weiner RE, Sasso DE, Gionfriddo MA, Syrbu SI, Smilowitz HM, Vento J, Thrall RS. J Nucl Med 1998 Apr; 39 (4), pp. 723–8.

Vascular Endothelium: $\alpha_V\beta_3$ Integrin Complexes *[^{99m}Technetium] ([^{99m}Tc])-(HYNIC)-Arg-Gly-Asp/ [^{99m}Tc]-(HYNIC -RGD-4C)*

Cellular/Molecular Mechanism of Action: The peptide amino acid residues, Arg-Gly-Asp (RGD), in vitronectin and fibronectin possess binding affinity for cell membrane–associated $\alpha_V\beta_3$ integrin complexes.

Background Information: Three amino acid residues, Arg-Gly-Asp (RGD), in vitronectin and fibronectin show affinity for $\alpha_V\beta_3$ integrins expressed in vascular endothelial cells. That tumor growth can upregulate the expression of these integrins on tumor cells for invasion and metastasis and in tissue neovasculature suggests the potential of developing radiolabeled RGD peptides as antagonists of $\alpha_V\beta_3$ integrins for broad spectrum tumor-specific imaging. The polypeptide RGD-4C, which contains 4 cysteine residues for cyclization, has shown preferential localization on integrins at sites of tumor angiogenesis.

Application: Detection and characterization of neoplastic cell populations/tumors that express relatively high $\alpha_V\beta_3$ integrin membrane densities.

Route of Administration: Polypeptides RGD-4C and RGE (Arg-Gly-Glu)-4C (intravascular).

Semi-Synthetic Methods: The polypeptide RGD-4C and control RGE (Arg-Gly-Glu)-4C are conjugated with 6-hydrazinopyridine-3-carboxylic acid (HYNIC) to facilitate [^{99m}Tc] radiolabeling. Separation of conjugated products from residual unreacted molecular components can be achieved by C18 chromatography (e.g., Sep-Pak cartridge) applied in combination with 20% methanol solvent. Each of the peptides can be radiolabeled using tricine. For cell binding studies, both [^{99m}Tc] peptides can be further purified by SE HPLC. High specific radioactivity of labeled cyclized RGD/E of about 20 Ci/μmol can be achieved. Both [^{99m}Tc] complexes are stable in the labeling solution for 24 hours at room temperature.

Implementation: Evaluation of the relative binding avidities of RGD-4C and RGE (Arg-Gly-Glu)-4C peptides for endothelial cell membrane preparations. Determination of such properties can be established by exposure of HUVE to RGD-4C or RGE over a 1-hour incubation period (4°C) at concentrations in the picomolar to nanomolar range. Specificity can be approximated by determining at

what concentration native cyclized RGD blocks 50% of the [^{99m}Tc]-labeled RGD binding to cell preparations.

***Ex Vivo* Model:** Human (umbilical vein endothelium or HUVE).

Interpretation and Analysis: In the HUVE cell binding-avidity analyses, cell accumulation of [^{99m}Tc] in the case of RGD can be as much as 16 times greater than the RGE control peptide. Specificity analyses reveal that 7 nM of native cyclized RGD blocked 50% binding of [^{99m}Tc]-labeled RGD to cells. The binding percentage of [^{99m}Tc]-labeled RGD to purified $\alpha_V\beta_3$ integrin protein, as determined by SE HPLC, increases with integrin concentration, while [^{99m}Tc]-labeled RGE displays no binding. The association constant for [^{99m}Tc]-RGD is rather modest at 7×10^6 M^{-1}. In nude mouse tumor models employing both human renal adenocarcinoma (ACHN) and human colon cancer cell lines (LS174T) explants, there is no statistical difference in [^{99m}Tc]-labeled RGD/E accumulation. In conclusion, possibly because of limited numbers of $\alpha_V\beta_3$ integrin receptors per tumor cell and low binding affinity, radiolabeled RGD peptides may have limitations as tumor-imaging agents.

In vitro and *in vivo* evaluation of a [^{99m}technetium]-labeled cyclic RGD peptide as a specific marker of $\alpha_V\beta_3$ integrin for tumor imaging. Su ZF, Liu G, Gupta S, Zhu Z, Rusckowski M, Hnatowich DJ. Bioconjug Chem 2002 May–Jun; 13 (3), pp. 561–70.

Vascular Endotheluim/Cardiac Washout Evaluation/ Lipid Administration Influence *[^{99m}Technetium] ([^{99m}Tc])-N-NOET*

Background Information: *Bis*(*N*-ethoxy,*N*-ethyldithiocarbamato)nitrido technetium or [^{99m}Tc]-*N*-NOET is a myocardial perfusion imaging agent that demonstrates significant systemic redistribution. Previous studies have suggested that [^{99m}Tc]-*N*-NOET is bound intravascularly. The agent has been evaluated in phase III clinical trials.

Application: [^{99m}Tc]-*N*-NOET possesses an affinity for vascular endothelium.

Dosage: [^{99m}Tc]-*N*-NOET (1.5 MBq/*ex vivo* rat heart preparations; 0.75 MBq for canine intracoronary artery injection).

Implementation: Determination of the influence of modifications in the vascular compartment on mechanisms of [^{99m}Tc]-*N*-NOET myocardial washout and redistribution. Cardiac washout studies with [^{99m}Tc]-*N*-NOET can be performed *ex vivo* utilizing isolated hearts after bolus injection (1.5 MBq) in the absence or presence of bovine serum albumin (BSA, 0.03%) with or without bound lipids. The intrinsic myocardial washout of the tracer can also be studied *in vivo* after intracoronary bolus injection of the tracer (0.75 MBq) before and after hyperlipidemia induced by intravenous administration of 300 mL of 20% intralipids (n = 3) or hyperemia induced by intravenous infusion of the adenosine A_{2A} receptor agonist ATL-146e (0.3 μg/kg/min; n = 6).

***Ex Vivo* Model:** Rat hearts (n = 15 isolated and perfused; n = 9/15 with BSA; n = 5 bound lipids; n = 4 without bound lipids).

Animal Model: Canine (n = 6 dogs, intracoronary artery injection).

Interpretation and Analysis: In isolated hearts, there is no significant myocardial washout of [^{99m}Tc]-*N*-NOET with Krebs-Henseleit buffer. Addition of BSA without bound lipids results in a significant cardiac washout of the tracer ($p < 0.001$ by repeated measures ANOVA). The presence of lipids bound to BSA further accelerates the washout rate of [^{99m}Tc]-*N*-NOET (half-life [$t_{1/2}$] 431.5 ± 23.2 minutes vs 242.9 ± 63.2 minutes; $p < 0.05$). Intralipid administration *in vivo* significantly increases the intrinsic washout rate of [^{99m}Tc]-*N*-NOET (canine $t_{1/2}$ 108.0 ± 23.9 minutes vs 51.8 ± 11.8 minutes; $p < 0.05$). In addition, vasodilatation with ATL-146e results in a 4.9-fold increase in coronary flow ($p < 0.05$ vs baseline) and a significantly faster intrinsic [^{99m}Tc]-*N*-NOET myocardial washout ($t_{1/2}$, 81.1 ± 12.1 minutes vs 40.7 ± 7.3 minutes; $p < 0.05$). The myocardial washout kinetics of [^{99m}Tc]-*N*-NOET is affected by a variety of intravascular factors, supporting the hypothesis that the tracer is most likely localized on the vascular endothelium. The potential impact of variations in circulating lipid levels among cases on clinical imaging with [^{99m}Tc]-*N*-NOET requires further investigation.

Effects of increased lipid concentration and hyperemic blood flow on the intrinsic myocardial washout kinetics of [^{99m}Tc]-*N*-NOET. Riou LM, Unger S, Toufektsian MC, Ruiz M, Watson DD, Beller GA, Glover DK. J Nucl Med 2003 Jul; 44 (7), pp. 1092–8.

Vascular Hypertension: Renal Artery Stenosis (RAS)/ Renal Ischemia/[Renal Artery Stenosis > 50%] *[^{99m}Technetium] ([^{99m}Tc])-Mercaptoacetyltriglycine/ Captopril*

Cellular/Molecular Mechanism of Action: Captopril inhibits the biochemical activity of angiotensin-converting enzyme (ACE inhibitor).

Physiological Mechanism of Action: Angiotensin in its most active form promotes increased vascular tone.

Application: Enhanced detection can characterize RAS associated with peripheral vascular hypertension.

Route of Administration: Captopril (oral).

Dosage: Captopril (50 mg).

Implementation: Evaluation of vascular hypertension utilizing renal scintigraphic imaging facilitated by the application of [^{99m}Tc]-mercaptoacetyltriglycine ([^{99m}Tc]-MAG3) 1 hour after captopril premedication. Baseline studies are obtained only for those cases showing abnormal findings in provocative studies in concert with renal arteriography procedures.

Human Model: Cases with vascular hypertension (e.g., n = 82).

Interpretation and Analysis: Sensitivity and specificity for the detection of RAS > 50% are approximately 89% and 91%, respectively. After captopril administration, tracer parenchymal transit time increases significantly in ischemic kidneys (334 ± 93 seconds in baseline conditions vs 468 ± 96 seconds after captopril, $p < 0.001$) but not in kidneys without RAS or RAS < 50% (243 ± 46 seconds vs 271 ± 95 seconds, p = ns). False-positive responses are mostly bilateral and associated with marked decreases in blood pressure. [^{99m}Tc]-MAG3 is an effective compound for detecting RAS ≥ 50% with captopril renal scintigraphy. Performing the provocative test as a 1st step procedure considerably reduces the number of scintigraphic studies required.

Use of technetium-^{99m}-MAG3 for renal scintigraphy after angiotensin-converting enzyme inhibition. Dondi M, Monetti N, Fanti S, Marchetta F, Corbelli C, Zagni P, De Fabritis A, Losinno F, Levorato M, Zuccalá. J Nucl Med 1991 Mar; 32 (3), pp. 424–8.

Vascular Hypertension: Renal Artery Stenosis (RAS) *[^{99m}Technetium] ([^{99m}Tc])-Diethylenetriamine Pentaacetic Acid (DTPA)/Captopril*

Cellular/Molecular Mechanism of Action: Captopril inhibits the biochemical activity of angiotensin-converting enzyme (ACE inhibitor).

Physiological Mechanism of Action: Angiotensin in its most active form promotes increased vascular tone.

Application: Enhanced detection and characterization of renal perfusion applying scintigraphic imaging techniques. Such a diagnostic approach can be particularly beneficial pertaining to conditions of RAS.
Route of Administration: Captopril (oral).
Dosage: Captopril (50 mg).
Implementation: Evaluation of vascular hypertensive cases applying conventional renal scintigraphy followed 2 or 3 days later by captopril-enhanced renal scintigraphy, performed 1 hour after premedication with captopril. Renal arteriography is performed within 15 to 30 days to further evaluate renal vascular integrity.

Human Model: Cases of peripheral vascular hypertension (n = 105).
Interpretation and Analysis: In the population of cases evaluated, approximately 50% will have no evidence of RAS (e.g., n = 55/105); while slightly less than one-third will have unilateral disease (e.g., n = 29/105) and close to 20% will have bilateral involvement (e.g., n = 21/105).

A large percentage of cases evaluated can be diagnosed applying a provocative test when they have at least 1 renal artery affected by stenosis of 50% or greater (e.g., n = 34/37). In conditions where stenosis does not exist (e.g., n = 55) or stenosis < 50% of the lumen diameter (n = 13), a very small number of cases will be classified as false-positives (e.g., n = 2). The sensitivity of the procedure is therefore approximately 92% while the specificity approaches 97%. In a single kidney determined to have stenosis of greater than 50%, the sensitivity of scintigraphy after captopril administration is 94%, with a 98% level of specificity. Captopril enhances renal scintigraphy and its application has therefore been suggested as the 1st test to be performed in hypertensive conditions referred for renal scintigraphic studies. Only those cases with equivocal results require a baseline study for better assessment.

Evaluation of hypertensive patients by means of captopril-enhanced renal scintigraphy with technetium-^{99m} DTPA. Dondi M, Franchi R, Levorato M, Zuccalà A, Gaggi R, Mirelli M, Stella A, Marchetta F, Losinno F, Monetti N. J Nucl Med 1989 May; 30 (5), pp. 615–21.

Vascular Integrity: Renal/Glomerulus/Chronic Allograft/Transplant Rejection
[^{99m}Technetium] ([^{99m}Tc])-Acid Fibroblast Growth Factor 1/[^{99m}Tc]-MAG3

Background Information: Acidic fibroblast growth factor (FGF-1) functions as a potent hormonal inducer of wound repair mechanisms *in vivo*. In addition, the involvement of FGF-1 in a number of pathophysiological conditions, including chronic human renal allograft rejection, has been described. Consequently, there is an increasing need to monitor FGF-1 pharmacokinetics and distribution for both therapeutic and diagnostic opportunities.
Route of Administration: [^{99m}Tc]-MAG3 and [^{99m}Tc]-labeled FGF-1 (intravenous).
Implementation: Evaluation of renal function facilitated by the application of dynamic [^{99m}Tc]-MAG3 imaging and subsequent image analysis of [^{99m}Tc]-labeled FGF-1 (^{99m}Tc-FGF-1) biodistrubution profiles. Complementary validation investigations entail performing microautoradiography of harvested kidneys to facilitate determination of [^{99m}Tc]-FGF-1 compartmental localization.

Animal Model: Rat (sham-operated, syngeneic renal transplanted, and allogeneic renal transplanted rats).
Interpretation and Analysis: Allogeneic renal transplants appear grossly abnormal on [^{99m}Tc]-MAG3 renal scans. In scenarios of this type, imaging analysis detects a significant reduction in [^{99m}Tc]-FGF-1 renal binding/uptake, in contrast to renal binding in the sham-operated and syngeneic renal transplanted scenarios, which are not significantly different. Both groups of renal transplants show a redistribution of FGF-1 to the glomerular compartment. Therefore, [^{99m}Tc]-FGF-1 serves as a radiotracer agent that can effectively target and measure *in vivo* growth factor expression. Reduced renal binding of [^{99m}Tc]-FGF-1 in the allogeneic transplanted kidney correlates with decreases in blood flow. Unique glomerular targeting of [^{99m}Tc]-FGF-1 in the transplanted kidney provides additional evidence supporting a role for this growth factor in the pathogenesis of chronic rejection phenomenon.
Instrumentation: Anger gamma camera.

Glomerular targeting of acidic fibroblast growth factor-1 in renal transplanted rats. Zinn KR, Kelpke S, Akhi K, Viera L, Chaudhuri TR, Thompson JA. Transplantation 2002 May 15; 73 (9), pp. 1447–54.

Vascular Lesions: Balloon Denudation/ Atherosclerotic Arterial Wall Lesions
[^{99m}Technetium] ([^{99m}Tc])-Labeled Endothelin Derivative

Implementation: Evaluation of vascular integrity applying angiogram procedures in combination with scintigraphic imaging facilitated by the injection of [^{99m}Tc]-labeled endothelin derivative. The aorta is then dissected for autoradiography analysis and further evaluated applying sudan-III-staining, morphometry, and immunohistology.

Animal Model: Rabbits (e.g., n = 32) with induction of neointima of different cellularity and various severity levels of stenosis using balloon denudation. Following intervention procedures, distinct (specific) dietary regimens are employed in concert with the administration of test pharmaceutical agents.
Interpretation and Analysis: Induced lesions can be detected *in vivo* (whole-body scintigram) in the vast majority of cases 15 minutes after the injection of [^{99m}Tc]-labeled endothelin derivative. Autoradiography reveals a strong relationship between tracer accumulation and sudan-III-staining of lesions. Accumulation of the endothelin derivative correlates with the number of neointimal smooth muscle cells (SMC) but not with the number of medial SMC, neointimal macrophages, and neointimal area. The *in vivo* imaging of atherosclerosis with an endothelin derivative appears to be a feasible method of detecting and characterizing atherosclerotic arterial wall lesions at early stages.

[^{99m}Tc]-labeled endothelin derivative for imaging of experimentally induced atherosclerosis. Tepe G, Duda SH, Meding J, Brehme U, Ritter J, Hanke H, Hilger CS, Claussen CD, Dinkelborg LM. Atherosclerosis 2001 Aug; 157 (2), pp. 383–92.

Vascular Perfusion and Anatomy (Radioangiographic) Analyses
[^{99m}Technetium] ([^{99m}Tc])-Dextran 70

Application: Characterization of vascular anatomy and evaluation of organ/tissue perfusion parameters.
Route of Administration: [^{99m}Tc]-Dextran 70 (intravenous push).
Dosage: [^{99m}Tc]-Dextran 70 (185 megabecquerels).
Semi-Synthetic Methods: Dextran 70 can be injection-labeled with [^{99m}Tc] by using readily available pharmaceutical components. The radiopharmaceutical can be prepared by mixing 10 mg (0.17 mL) of dextran 70 in sodium chloride with 0.33 mL of 0.9% sodium chloride injection in a 1-mL syringe. This solution is then added to a sterile, pyrogen-free vial containing stannous chloride, and the steps

repeated until 5 vials have been prepared. The contents of each vial are then mixed by swirling until the solid phase has been dissolved. The mixture is incubated for 5 minutes at 22°C, then 1.48 gigabecquerels of sodium pertechnetate [^{99m}Tc] injection in a volume of 0.5 mL is added to each vial. The final mixture is incubated for 15 minutes at 22°C and then stored at room temperature. Thin-layer chromatography is performed after 0, 3, and 6 hours of storage to assess radiochemical purity. Immediately after preparation, a mean ± standard deviation (SD) of 99.0 ± 1.0% of the [^{99m}Tc] is bound to dextran 70. Mean ± SD binding is 98.1 ± 3.7% and 95.8 ± 7.5% at 3 and 6 hours, respectively.

Implementation: Evaluation of the biodistribution profile for [^{99m}Tc]-dextran 70 to determine its suitability to function as an agent for performing radioangiography procedures and assessement of vascular perfusion parameters. Following systemic administration, scintigraphic images are acquired immediately over the anterior chest and then at 1, 2, and 24 hours postinjection.

Human Model: Cases of normal health (n = 5 male volunteers).

Interpretation and Analysis: Scintigraphy analyses yield high-contrast images of the cardiac blood pool with little uptake of the radionuclide within the lungs.

Preparation of dextran 70 injection labeled with [^{99m}technetium] for use as a cardiac blood-pool imaging agent. Dansereau RN, Line BR. Am J Hosp Pharm 1994 Nov 15; 51 (22), pp. 2797–800.

Vascular Perfusion/Hepatic Metastatic Lesions
EOE-13/[^{99m}Technetium] ([^{99m}Tc])-Macroaggregated Albumin (MAA)

Cellular/Molecular Mechanism of Action: Macroaggregated albumin is a biological preparation that is passively retained within the intravascular compartment for a prolonged period of time following intravenous injection due to its relatively large molecular weight.

Application: Assessment of organ and tissue vascular perfusion in a manner that can detect hepatic metastatic lesions.

Route of Administration: Intravenous (catheter infusion at a rate of 0.66 ml/min).

Implementation: Evaluation of hepatic perfusion patterns applying both nuclear scintigraphy and computed tomography (CT) imaging methodologies.

Interpretation and Analysis: Computed tomography with EOE-13 yields more precise anatomic detail and is unique in demonstrating the perfusion status of each hepatic metastasis. Further evaluation of EOE-13 for this purpose is warranted.

Instrumentation: Computer-integrated nuclear medicine scintigraphy camera imaging (CT with EOE-13 used as a validating procedure).

Assessment of perfusion patterns during hepatic artery infusion chemotherapy: EOE-13 CT and ^{99m}Tc-MAA scintigraphy. Miller DL, Schneider PD, Gianola FJ, Willis M, Vermess M, Doppman JL. AJR Am J Roentgenol 1984 Oct; 143 (4), pp. 827–31.

Vascular Perfusion/Iohexol (Non-Ionic) and Iothalamate (Ionic) Contrast Media
[^{99m}Technetium] ([^{99m}Tc])-Macroaggregated Albumin (MAA)/[^{201}Thallium] ([^{201}Tl])

Cellular/Molecular Mechanism of Action: Both [^{99m}Tc]-MAA albumin and [^{201}Tl] per se do not exert a biological function. Macroaggregated albumin instead functions as a passive tracer marker agent applicable for the evaluation of body fluid compartments.

Background Information: Cardiac output is effectively redistributed to each part of the body by regional changes in vascular resistance. The side effects of iodinated contrast media in different organs have been well documented in animal experiments and human clinical studies. However, no simultaneous assessment of regional blood flow change has been described for different organ systems throughout the body.

Application: [^{201}Tl] distribution reflects the fractional distribution of cardiac output and parallels regional blood flow. Macroaggregated albumin instead functions as a passive tracer marker agent applicable for the evaluation of body fluid compartments.

Route of Administration: [^{201}Tl] and [^{99m}Tc]-MAA (intravenous).

Implementation: Evaluation of the effect of contrast media on the regional distribution of [^{201}Tl] in individual organ systems as an estimate of *in vivo* regional blood flow changes using a [^{201}Tl] double-dose method. Contrast media that can be employed include non-ionic (Iohexol) and ionic (Iothalamate).

Animal Model: Rabbit.

Interpretation and Analysis: Distribution of [^{201}Tl] and [^{99m}Tc]-MAA is similar in the heart, spleen, kidneys, and leg muscles in the control animals (r = 0.99). Regional distribution of [^{201}Tl] significantly increases in heart and leg muscle, but decreases in the liver when non-ionic (Iohexol) and ionic (Iothalamate) contrast medium is administered, compared to negative reference controls. There are no significant changes within the kidneys or intestine in case groups receiving either non-ionic (Iohexol) or ionic (Iothalamate). Intravenous infusion of contrast media causes uneven redistribution of regional blood flow in the heart, leg muscle, and liver. These changes are more prominent with the administration of ionic contrast medium.

Effect of ionic and non-ionic contrast media on regional distribution of thallium-201 in rabbits. Seto H, Kageyama M, Shimizu M, Nagayoshi T, Futatsuya R, Kakishita M. Radiat Med 1995 Jan–Feb; 13 (1), pp. 19–22.

Vascular Thromboses: Arterial/Antiplatelet Therapy: Pharmaceutical Efficacy Evaluation
[^{111}Indium] ([^{111}In]) Platelets

Cellular/Molecular Mechanism of Action: The radioisotope [^{111}In] can be used as a tracer agent that emits photon energy. Platelets within the intravascular compartment accumulate at anatomical (e.g., intravascular) sites of platelet aggregation and fibrin monomer polymerization.

Application: Preparations of [^{111}In] platelets can be utilized for the detection of intravascular thrombosis and evaluation of the efficacy of antithrombotic pharmaceutical agents. Aorta (arterial) thromboses are composed of a higher percentage of platelets in contrast to venous thromboses that are composed predominantly of polymerized fibrin monomer (± cross-linking).

Implementation: Combined application of [^{111}In] platelet scintigraphy (PSG) and B-mode ultrasonography (US) for the evaluation of the short-term-effect antiplatelet agents [e.g., 4-cyano-5,5-bis(methoxyphenyl)-4-pentenoic acid (E5510)]. After 8 weeks, [^{111}In]-PSG and US can then be repeated. Platelet deposition is then assessed visually and semi-quantitatively using a platelet accumulation index. Such procedures can then be applied to evaluate the efficacy of antiplatelet or antithrombosis agents.

Human Model: Cases demonstrating platelet deposition at either the carotid bifurcation or abdominal aorta (e.g., n = 15) detected by [^{111}In] platelet scintigraphy. Half of affected cases are administered antiplatelet therapy (e.g., E5510 at 4 mg/day.)

Interpretation and Analysis: Visual analysis reveals that a high percentage of cases demonstrate no evidence of platelet deposition after the administration of effective antithrombotic therapies (e.g., n = 7/8), while essentially none are changed without the benefit of antithrombotic intervention. The platelet accumulation index of vessels with platelet deposition is significantly reduced after delayed antithrombotic treatment (6.0 ± 7.1% vs 12.4 ± 3.9%. $p < 0.01$), while there are no significant changes in vessels without platelet deposition (2.9 ± 3.0% vs 2.9 ± 4.1%). In cases with early-phase antithrombotic therapy, vessels rarely demonstrate any change (8.1 ± 6.4% vs 8.9 ± 7.3%). However, there is no significant reduction of carotid plaque size in either group. Short-term E5510 therapy inhibits platelet deposition in active atherothrombotic lesions, and the *in vivo* combination of PSG and US can be useful for evaluating the effectiveness of antithrombotic pharmaceuticals.

Effect of E5510, a novel antiplatelet agent, on platelet deposition in atherothrombotic lesions: Evaluation by [^{111}In] platelet scintigraphy. Moriwaki H, Matsumoto M, Handa N, Hashikawa K, Hori M, Nishimura T. Nucl Med Commun 2000 Nov; 21 (11), pp. 1051–8.

Vascular Thromboses: Arterial/Hemostatic Stimuli and Devices
[^{111}Indium] ([^{111}In])-Tropolone-Platelets/ [^{99m}Technetium] ([^{99m}Tc])-Hexamethylpropyleneamine Oxime (HMPAO) Platelets

Physiological Mechanism of Action: The function of normal platelets includes maintenance of the integrity of endothelial surfaces and active participation in thrombogenic responses. Thrombi formed within arterial structures have a much higher platelet content than do thrombi found within the venous vasculature.

Background Information: Because the photons emitted from [^{111}In] and ^{99m}Tc are of a high energy [^{111}In, 173 and 247 keV α, $T_{1/2}$ = 2.8 days; ^{99m}Tc, 140 keV α, $T_{1/2}$ = 6.05 hours], lesions located at significant tissue depths can be detected within the thoracic and abdominal cavities. Radiolabeled platelets can also be applied to document the antiplatelet activity of non-steroidal anti-inflammatory drugs (NSAIDS) and to monitor intravascular platelet kinetics in the diagnosis of consumptive disease conditions such as immune-mediated thrombocytopenia (ITP) and disseminated intravascular coagulation (DIC).

Application: Diagnosis and serial evaluation of arterial thrombosis lesions within the pulmonary vein, cerebral hemispheres, and other arterial structures of interest. Radiolabeled platelets can also be utilized to characterize platelet intravascular half-life and assess the potential thrombogenic properties of foreign materials.

Semi-Synthetic Methods: Radiolabeling of platelets with [^{111}In]-tropolone (detergent-free) and [^{99m}Tc]-HMPAO following their isolation and partial purification from fresh whole blood.

Implementation: Evaluation of the platelet thrombogenicity of a hemodialyzer assessed through the application of [^{99m}Tc] platelets and [^{111}In] platelets. Platelets are 1st isolated from whole blood, then radiolabeled with [^{111}In] and [^{99m}Tc]. Following hemodialysis procedures, hollow-fiber dialyzer (HFD) radioactivity is measured with an ionization chamber and imaged with a gamma camera. The radioactivity of hollow fiber samples taken from the top, middle, and bottom of the dialyzer is determined with a gamma counter.

Animal/Human Model: Normal beagle dogs, Yorkshire pigs, and human volunteers. Hemodialysis is performed with an HFD in a flow loop, the temperature of which is maintained at 37°C, with flow rates of 7, 150, and 270 mL/min.

Interpretation and Analysis: Canine platelets are more thrombogenic than porcine and human platelets. The adhesivity of porcine platelets to the biomaterial (cellulose-acetate) of the dialyzer approximates that of human platelets. The [^{99m}Tc] label underestimates thrombus formation ($p < 0.01$). The dynamic processes of thrombosis and embolization from the hemodialyzer result in a large standard deviation around the mean values of the adherent thrombus. In spite of this limitation of the dynamic pathology, the quantitation of comparative thrombogenicity with [^{111}In]- and [^{99m}Tc]-labeled platelets suggests that both radionuclides may be used to measure device-induced thrombogenicity and may provide an estimation of prosthesis-induced thrombogenicity of human platelets by extrapolating from animal studies.

Quantitation of thrombogenicity of hemodialyzer with technetium-99m and indium-111-labeled platelets. Dewanjee MK, Kapadvanjwala M, Ruzius K, Serafini AN, Zilleruelo GE, Sfakianakis GN. Nucl Med Biol 1993 Jul; 20 (5), pp. 579–87.

Vascular Thrombosis: Arterial and Venous/Deep Vein Thrombosis/Pulmonary Embolism/ Arterial Thromboembolic Disorders/Cerebral Vascular Stroke/Coronary Artery Thrombotic Disorders/Peripheral Artery Thromboses
Platelet GPIIb/IIIa Receptor Antagonist/ [^{99m}Technetium] ([^{99m}Tc])-DMP728/ [^{3}H]-DMP728

Cellular/Molecular Mechanism of Action: The agent DMP728 functions as a GPIIb/IIIa receptor antagonist on the exterior surface membrane of platelets.

Physiological Mechanism of Action: Venous thrombi is composed predominantly of fibrin monomer or polymer in constrast to arterial thrombi, which have a much higher platelet content.

Background Information: Either venous or arterial thrombosis is a potentially life-threatening event and many diagnostic modalities are inadequate for diagnostic purposes and for determining the morphology of an evolving thrombus. Thus development of a non-invasive imaging agent that can detect clot location represents a critical need in nuclear diagnostic medicine.

Application: Detection and characterization of intravascular venous and arterial thromboses.

Implementation: Evaluation of artificially (*in vitro*) created venous and arterial type thrombi using the potent radiolabeled GPIIb/IIIa receptor antagonist [^{3}H]-DMP728. Complementary investigations can involve the *in vivo* application of [^{99m}Tc]-DMP728 to determine the clot/blood distribution ratio (e.g., target:background ratio). In addition, non-invasive single photon emission computed tomography (SPECT) of venous clots in deep vein thrombosis can be performed. Thrombin inhibitors can also be evaluated in the same context.

Animal Model: Canine (dogs with mixed arterial/venous or venous clots within arteriovenous shunts and in venous clot foci and deep vein thrombi).

Interpretation and Analysis: Radiolabeled preparations of the GPIIb/IIIa receptor antagonist DMP728 in the form of [^{3}H]-DMP728 and [^{99m}Tc]-DMP728 have been found to retain marked binding avidity for platelet-associated GPIIb/IIIa receptor complexes. DMP728 demonstrates a relatively greater affinity for activated than for unactivated human platelets, which may be essential for attaining an optimal thrombus/blood (target/background) distribution ratio and the optimal detection of small clots (i.e., greater sensitivity). Therefore, [^{99m}Tc]-GPIIb/IIIa receptor antagonists have potential utility in the diagnosis of venous clots in deep vein thrombosis, pulmonary embolism, and arterial thromboembolic disorders,

including stroke and coronary and peripheral artery thrombotic disorders. Thrombin inhibitors do not appear to be as effective in this same context.

Novel technetium-99m-labeled platelet GPIIb/IIIa receptor antagonists as potential imaging agents for venous and arterial thrombosis. Mousa SA, Bozarth JM, Edwards S, Carroll T, Barrett J. Coron Artery Dis 1998; 9 (2–3), pp. 131–41.

Vascular Thromboses: Deep Vein/[Neoplastic Disease States]
[^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)-Fibrinogen/(DTPA Cyclic Anhydride)

Physiological Mechanism of Action: The agent is actively incorporated into intravascular fibrin clots. Initially, the radiolabeled fibrinogen is converted to fibrin by thrombin with greatest level of biochemical activity located on the external surface of fibrin clots. Monomers of fibrin are then covalently cross-linked with adjoining strands by the enzymatic action of coagulation factor XIIIa*.

Background Information: Albumin can be applied as a biological protein for optimizing semi-synthetic conjugation strategies employing DTPA chelation groups. Such methodologies can subsequently serve as an experimental foundation for ultimately designing analogues and techniques for covalently conjugating DTPA (DTPA cyclic anhydride) to selective targeting modalities including immunoglobulin (e.g., monoclonal Fab′ fragment) or fibrinogen/fibrin peptides.

Application: Detection and monitoring of intravascular thromboemboli formation analogous to those that may develop within the pulmonary artery (pulmonary thromboembolism) and iliac vessels of the limbs (deep vein thrombosis).

Considerations: Isotopes that emit low energy photons (^{125}I, 35 keV, $T_{1/2}$ = 60.2 days) are only suitable for imaging superficially located vascular thromboses such as those residing in the iliac veins. Vascular lesions located at greater tissue depths can be more appropriately imaged with isotopes that emit higher energy photons [^{123}I], 159 keV α 83% emission, $T_{1/2}$ = 13.3 hours; [^{124}I] $T_{1/2}$ = 4.2 days); [^{125}I] 27-35 keV α, 60.2 days). Traditionally, [^{131}I] is used for radiation therapy due to its beta emitting characteristics (^{131}I, $T_{1/2}$ = 61 hours; and 364 keV α, $T_{1/2}$ = 8.06 days).

Semi-Synthetic Methods: The DTPA cyclic anhydride chelation group can be covalently conjugated to human fibrinogen. Optimum DTPA cyclic anhydride labeling conditions (e.g., pH, DTPA:fibrinogen labeling ratios) can be established by evaluating the ablity of this biological protein to function as a substrate for thrombin (fibrinogen → fibrin monomer). In an *in vivo* environment, the viability or integrity of fibrinogen in the DTPA-labeled fibrinogen conjugate can be conveniently evaluated by determining its intravascular $T_{1/2}$ compared to native fibrinogen and its ability to be incorporated into intravascular thrombi (e.g., percent of fibrin clot-associated radioactivity).

Implementation: Diethylenetriamine pentaacetic acid cyclic anhydride labeling of human fibrinogen can be used to detect intravascular thrombi, while conjugation to monoclonal antibody fractions could facilitate the identification of a variety of pathological disease states. A relevant example in this regard includes a wide variety of neoplastic conditions and certain septic disease states. Various radioisotopes including [^{111}In] can potentially be chelated with functional DTPA groups.

Animal Model: Canine (*in vivo* pharmacokinetic profiles and thrombogenic catheter-induced intravascular fibrin clots).

Interpretation and Analysis: Clotting times for DTPA:fibrinogen fractions labeled with DTPA cyclic anhydride remain identical to that of the native protein whether the anhydride-to-protein molar ratio is 1:1 or 5:1. During the course of canine *in vivo* studies, the 130-hour blood clearance profiles for [^{125}I]DTPA-fibrinogen and [^{111}In]DTPA-fibrinogen closely correspond to reference controls for both 1:1 or 5:1 coupling ratios. In a canine model employing a thrombogenic catheter, the clot-to-blood ratios for the 2 radiotracers are essentially identical and display the same level of experimental error. Scintigraphic imaging of a jugular vein catheter at 24 hours postinjection of 1:1-coupled canine DTPA-fibrinogen labeled with [^{111}In] clearly detects clotting along the length of the catheter. Therefore, fibrinogen, coupled to DTPA, retains its viability, behaving in a manner analogous to radioiodinated fibrinogen *in vivo*, and [^{111}In]-labeled fibrinogen looks promising as a clinical diagnostic agent.

Evaluation of the viability of In-111-labeled DTPA coupled to fibrinogen. Layne WW, Hnatowich DJ, Doherty PW, Childs RL, Lanteigne D, Ansell J. J Nucl Med 1982 Jul; 23 (7), pp. 627–30.

Vascular Thrombosis: Deep Vein (Venography)/ Thrombosis of the Iliac Vein and Inferior Vena Cava
[^{99m}Technetium] ([^{99m}Tc])-Red Blood Cells (RBC)

Application: The accurate diagnosis of deep vein thrombosis (DVT) is essential in order to prevent associated complications, in addition to the selection and timing of appropriate therapeutic intervention. Doppler ultrasound (DUS), contrast venography, and intravenous radionuclide venography have been used for many years to detect DVT. However, obtaining venous access in the foot for injection of contrast agent can be difficult.

Route of Administration: [^{99m}Tc] (intravenous).

Implementation: Evaluation of venous structures by venography applying [^{99m}Tc] radionuclide injected subcutaneously for *in vivo* labeling of red blood cells. Comparative analyses can involve the simultaneous application of DUS procedures.

Human Model: Cases of normal health subjected to subcutaneous radionuclide venography and ultrasound (n = 60 with n = 120 lower extremity images).

Interpretation and Analysis: The concordance rate is approximately 94% within the femoral veins and 95% in the popliteal veins. Subcutaneous radionuclide venography in some studies has detected 10 iliac vein thromboses (n = 10) and inferior vena cava thromboses (n = 2) that could not be detected by DUS. Subcutaneous radionuclide venography is a useful alternative method for DVT detection. It is particularly valuable for evaluating DVT in the iliac veins and in the inferior vena cava.

Subcutaneous whole-body radionuclide venography using [^{99m}Tc] *in vivo* tagged red blood cells. Yang DC, Ratani RS, Kalani J, Joyce Chen YW, Huang YF, Magzadeh P, Ilkhanizadeh R. Clin Nucl Med 2001 Jun; 26 (6), pp. 491–4.

CENTRAL NERVOUS SYSTEM: NEUROLOGY

Amyloid Beta (Abeta) Deposits: Alzheimer's Disease (AD)/Abeta 10-43 Peptide
Chrysamine G (CG)/[^{99m}Technetium] ([^{99m}Tc])-MAMA-CG

Cellular/Molecular Mechanism of Action: Chrysamine G, an analogue of Congo red, is known to bind *in vitro* to the beta-amyloid protein (abeta 10-43) and to homogenates of several regions of the brain of AD patients.

Application: Detection of beta-amyloid deposits similar to those recognized in the central nervous system (CNS) of AD cases.

Route of Administration: Intravascular.
Semi-Synthetic Methods: Synthesis of a conjugate of 2-(acetamido)-CG with a *bis-S*-trityl protected monoamide-monoaminedithiol (MAMA-Tr(2)) tetraligand that is efficiently deprotected and labeled at a 75% yield with [^{99m}Tc] to obtain [^{99m}Tc]-MAMA-CG.
Implementation: Evaluation of hepatobiliary clearance and brain uptake and selective detection of beta-amyloid deposits similar to those found within the CNS in AD conditions.
Interpretation and Analysis: The agent [^{99m}Tc]-MAMA-CG is cleared mainly by the hepatobiliary system (mice), resulting in a fast blood clearance. Brain uptake of [^{99m}Tc]-MAMA-CG is low. Co-injection with the blood-pool tracer iodine-125 human serum albumin ([^{125}I]-HSA) reveals a brain/blood activity ratio for [^{99m}Tc]-MAMA-CG that is significantly higher than that for [^{125}I]-HSA (t test for dependent samples, $p < 0.02$), indicating the ability of [^{99m}Tc]-MAMA-CG to cross the blood-brain barrier. *In vitro* autoradiography demonstrates pronounced binding of [^{99m}Tc]-MAMA-CG to beta-amyloid deposits in autopsy sections of the parietal and occipital cortex in conditions of AD that are in contrast to reference controls. Adding 10 μM Congo red during incubation displaces the binding of [^{99m}Tc]-MAMA-CG. Congo red staining and autoradiography identifies the same lesions. [^{99m}Tc]-MAMA-CG seems to bind selectively to beta-amyloid deposition in human brain parenchyma and blood vessels *in vitro* and thus might be a lead compound for further development that can be utilized as a useful tracer agent for the *in vivo* diagnosis of AD.

[^{99m}Tc]-MAMA-chrysamine G, a probe for beta-amyloid protein of Alzheimer's disease. Dezutter NA, Dom RJ, de Groot TJ, Bormans GM, Verbruggen AM. Eur J Nucl Med 1999 Nov; 26 (11), pp. 1392–9.

Amyloid Beta (Abeta) Deposits/Amyloid Angiopathy (AA)/(Alzheimer's Disease [AD]) *[^{99m}Technetium] ([^{99m}Tc])-Anti-Abeta Fab*

Cellular/Molecular Mechanism of Action: Murine antihuman abeta amyloid monoclonal antibody recognizes and binds to abeta amyloid deposits.
Application: Detection of amyloid deposits *in vivo* within the central nervous system (CNS) vasculature analogous to those found in AD conditions.
Production and Semi-Synthetic Methods: Extracts of abeta amyloid can be purified for the purpose of producing monoclonal antibody preparations. Appropriateness of the antigenic recognition capabilities of anti-(abeta) monoclonal antibodies involves screening for effectiveness in visualizing AA and neuritic plaques in post-mortem AD brain sections. In an effort to minimize non-specific binding of anti-(abeta) mononclonal antibody to membrane-associated Fc receptors expressed by the mononuclear phagocytic system within the spleen and liver, preparations are enzymatically treated and extracts of Fab fragment purified.
Implementation: Evaluation of preparations of intact anti-(abeta) amyloid monoclonal antibody (± [^{99m}Tc] label: dimercaptide ligand methodology); and anti-(abeta) amyloid Fab fragment (± [^{99m}Tc] label: dimercaptide ligand methodology).
Interpretation and Analysis: Modified and radiolabeled Fab fragments retain activity and specificity toward amyloid-laden blood vessels and neuritic plaques. Highly specific murine monoclonal antibodies can be prepared that possess all the characteristics and properties necessary for the development of effective *in vivo* diagnostic imaging agents. Toxicity studies performed in rats have demonstrated the safety of such preparations. Biodistribution studies in mice have validated the desirability of [^{99m}Tc]-anti-(abeta) Fab fragment as an imaging agent. Advantages associated with the use of [^{99m}Tc]-anti-(abeta) Fab fragment include higher target-to-background ratios compared to radiolabeled IgG monoclonal antibody preparations; non-invasive diagnostic properties; and assessment of the contribution of AA to the pathophysiology and clinical profiles of AD conditions.
Instrumentation: Laboratory facilities for the preparation of abeta amyloid extractions; production of monoclonal antibody fractions; production of monoclonal antibody Fab fractions; and semi-synthesis of [^{99m}Tc]-anti-abeta Fab preparations. Some suppliers of immunology-oriented reagents offer preparations of anti-abeta monoclonal antibody and/or anti-abeta Fab monoclonal antibody.

Development of a monoclonal antibody specific for beta/A4 amyloid in Alzheimer's disease brain for application to *in vivo* imaging of amyloid angiopathy. Majocha RE, Reno JM, Friedland RP, VanHaight C, Lyle LR, Marotta CA. J Nucl Med 1992 Dec; 33 (12), pp. 2184–9.

Development of an anti-abeta monoclonal antibody for *in vivo* imaging of amyloid angiopathy in Alzheimer's disease. Friedland RP, Majocha RE, Reno JM, Lyle LR, Marotta CA. Mol Neurobiol 1994 Aug–Dec; 9 (1–3), pp. 107–13.

Amyloid Beta (Abeta) Plaques/Alzheimer's Disease (AD) *[^{125}I]Abeta 1-40-8D3 Bifunctional Conjugate*

Cellular/Molecular Mechanism of Action: The abeta 1-40-8D3 conjugate is a bifunctional molecular complex. The rat monoclonal antibody 8D3 possesses binding avidity for mouse transferrin receptor (TfR) complexes.
Application: [^{125}I]abeta 1-40 represents a biopharmaceutical platform that could potentially be used to image brain abeta amyloid plaque lesions in AD. One factor that limits the applicability of [^{125}I]abeta 1-40 for such purposes is its limited ability to traverse across an intact blood-brain barrier. Conjugation of various "targeting" modalities represents 1 strategy that can be applied to overcome this particular obstacle.
Route of Administration: Abeta 1-40 and abeta 1-40-8D3 conjugate (intravenous).
Semi-Synthetic Methods: Covalent conjugation of abeta 1-40 to rat 8D3 monoclonal antibody (antimouse transferin receptor) in a manner that creates a bifunctional molecular complex.
Implementation: Delineation of the relative ability of radiolabeled unconjugated abeta 1-40 and abeta 1-40-8D3 conjugate to penetrate the blood-brain barrier can be achieved by performing brain scans 6 hours following administration. Immunocytochemical analyses can additionally be performed to document selective uptake within abeta plaques.
Animal Model: App SW/Psen1 double-transgenic and littermate control mice.
Interpretation and Analysis: Immunocytochemical analysis reveals abundant abeta immunoreactive plaques in the brains of App SW/Psen1 transgenic mice and a selective retention of radioactivity in the brains of these mice at 6 hours after intravenous administration of [^{125}I]-abeta 1-40-8D3 conjugate. Conversely, there is no selective sequestration of either conjugate in control littermate mouse brain or for unconjugated abeta 1-40 in transgenic mouse brain. The blood-brain barrier transport of [^{125}I]-abeta 1-40 in a transgenic mouse model is enabled by conjugation to rat 8D3 monoclonal antibody within selective binding avidity for mouse transferrin receptor complexes. Therefore, it is possible *in vivo* to image the abeta amyloid burden within the brain utilizing an amyloid imaging agent, provided the molecule is conjugated to a blood-brain barrier drug-targeting system.

Imaging brain amyloid of Alzheimer's disease *in vivo* in transgenic mice with an abeta peptide radiopharmaceutical. Lee HJ, Zhang Y, Zhu C, Duff K, Pardridge WM. J Cereb Blood Flow Metab 2002 Feb; 22 (2), pp. 223–31.

Amyloid Beta (Abeta) Plaque Deposits/Alzheimer's Disease (AD)
[^{123}I/^{125}I]IMPY, 6-Iodo-2-(4′-Dimethylamino-)Phenyl-Imidazo[1,2-a]Pyridine/Thioflavin

Background Information: Development of small molecular probes for *in vivo* labeling and detection of beta-amyloid (abeta) plaques in patients of AD is of significant scientific interest, and it may also assist in the development of schemes for the pharmaceutical targeting of abeta plaques relevant to AD conditions.
Route of Administration: [^{125}I]IMPY (intravenous).
Semi-Synthetic Methods: A novel probe, [^{123}I/^{125}I]IMPY, 6-iodo-2-(4′-dimethylamino-)phenyl-imidazo[1,2-a]pyridine, can be prepared using an iododestannylation reaction catalyzed by hydrogen peroxide.
Implementation: Evaluation of *in vivo* plaque labeling strategies.

Animal Model: Mice (normal) and a transgenic mouse model (Tg2576) engineered to produce excess amyloid plaques within the brain.
Interpretation and Analysis: The modified thioflavin-T derivative displays a good binding affinity for preformed synthetic abeta40 aggregates in solution ($K_i = 15 \pm 5$ nM) and shows selective plaque labeling on postmortem AD brain sections. Biodistribution of [^{125}I]IMPY exhibits excellent brain uptake (2.9% initial dose/brain at 2 minutes) and fast washout (0.2% initial dose/brain at 60 minutes). These properties are highly desirable for amyloid plaque imaging agents. *Ex vivo* autoradiograms of brain sections of the Tg2576 mouse obtained 4 hours after an intravenous injection of [^{125}I]IMPY clearly display distinct plaque labeling with low background activity. When the same brain sections are stained with the fluorescent dye thioflavin-S, the same abeta plaques show prominent fluorescent labeling consistent with results observed from autoradiography analyses. Radioiodinated IMPY demonstrates desirable characteristics for the *in vivo* labeling of abeta plaques and it may be useful as a molecular imaging agent to study amyloidogenesis within the brain of living AD patients.

IMPY: An improved thioflavin-T derivative for *in vivo* labeling of beta-amyloid plaques. Kung MP, Hou C, Zhuang ZP, Zhang B, Skovronsky D, Trojanowski JQ, Lee VM, Kung HF. Brain Res 2002 Nov 29; 956 (2), pp. 202–10.

Amyloid Deposition/Familial Amyloid Polyneuropathy (FAP)/Hepatic Posttransplant Renal Amyloid Deposition
[^{123}I]-Serum Amyloid P Component (SAP)

Cellular/Molecular Mechanism of Action: Serum amyloid P component recognizes and physically binds to amyloid deposits.
Background Information: Familial amyloid polyneuropathy associated with transthyretin (TTR) mutations is the commonest type of hereditary amyloidosis. Plasma TTR is produced almost exclusively in the liver and orthotopic liver transplantation is the only available treatment, although the clinical outcome varies. Serum amyloid P component scintigraphy is a method for identifying and quantitatively monitoring amyloid deposits *in vivo*, but it has not extensively been used to study the outcome of visceral amyloid deposits in FAP following liver transplantation.
Route of Administration: Intravascular.
Implementation: Evaluate the effectiveness of injected [^{123}I]-labeled SAP to detect and characterize amyloid lesions *in vivo* applying whole-body scintigraphy techniques.

Human Model: Cases of FAP (n = 17) associated with TTR Met30 in addition to asymptomatic gene carriers (n = 5). Follow-up studies performed in affected cases (n = 10), some of whom have undergone orthotopic liver transplantation 1–5 years beforehand (n = 8/10 cases).
Interpretation and Analysis: An abnormal uptake of [^{123}I]-SAP can be appreciated in the vast majority of cases with FAP, including the (i) kidneys (n = 17); (ii) spleen (n = 5/17); and (iii) adrenal gland (n = 3/17). Renal amyloid deposits can also be appreciated in some asymptomatic carriers (n = 3/5). Follow-up studies 1–5 years after liver transplantation show that there is substantial regression of the visceral amyloid deposits in some cases (n = 2/10 compared to n = 3/10 modest improvement; n = 2 unchanged amyloid deposits). Therefore [^{123}I]-SAP scintigraphy can identify unsuspected visceral amyloid cases with FAP due to TTR Met30. The universal presence of renal amyloid probably underlies the high frequency of renal failure that occurs in FAP following liver transplantation. The variable capacity of patients to mobilize amyloid deposits following liver transplantation may contribute to their long-term clinical outcome.

Serum amyloid P component scintigraphy in familial amyloid polyneuropathy: Regression of visceral amyloid following liver transplantation. Rydh A, Suhr O, Hietala SO, Ahlström KR, Pepys MB, Hawkins PN. Eur J Nucl Med 1998 Jul; 25 (7), pp. 709–13.

Cerebral Cortex Dysfunction/Drug-Resistant Focal Epilepsy/Accuracy of Amobarbital Target Delivery/Temporary Neurologic Deficits/Risk Assessment of Postsurgical Motor Deficits Before Functional Hemispherectomy/Language Area Identification
Amobarital (Sodium) Wada Test/[^{99m}Technetium] ([^{99m}Tc])-Hexamethylpropyleneamine Oxime (HMPAO)/Electrical Stimulation Mapping

Cellular/Molecular Mechanism of Action: Amobarbital is a barbiturate.
Physiological Mechanism of Action: Amobarbital suppresses neurologic function.
Application: Analyze the role of selective middle cerebral artery (MCA) Wada tests in the presurgical workup of patients with drug-resistant focal epilepsies.
Route of Administration: Amobarbital is administered selectively into arterial branches supplying specific regions of the cerebral cortex using a catheter introduced into the carotid artery.
Implementation: Evaluation of cerebral cortex function in conditions of (i) motor cortex cerebral hemisphere lesions (connatal MCA infarct, unilateral cortical dysplasia, Rasmussen encephalitis); (ii) circumscribed neoplastic or non-neoplastic lesions adjacent to the motor cortex or classical regions; and (iii) sleep electrical status elepticus. Sodium amobarbital and [^{99m}Tc]-HMPAO are simultaneously injected through a microcatheter into the distal M1 segment, the inferior MCA trunk, or the MCA branches in order to identify eloquent cortex.

Human Model: Cases of cerebral cortex dysfunction including (i) hemispheric lesions involving the motor cortex (n = 9 cases, of which n = 5/9 connatal MCA infarct, n = 3/9 unilateral cortical dysplasia, n = 1/9 Rasmussen encephalitis); (ii) circumscribed neoplastic or non-neoplastic lesions adjacent to the motor cortex or classic language areas (n = 5 cases); and (iii) purely electrophysiologic

analyses for conditions of electrical status epilepticus in sleep (n = 2 cases). Example Total Wada tests performed = 20.

Interpretation and Analysis: Co-registered single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI; n = 18) reveal that sodium amobarbital does not reach the target area in some procedures (e.g., n = 3/20). Temporary neurologic deficits can occur in slightly more than half of total Wada procedures performed (e.g., n = 12/20). Cases subjected to surgical intervention require the use of several different surgical approaches, including functional hemispherectomy (n = 3); partial or extended lesionectomy (n = 4); anterior temporal lobectomy (n = 1); and multiple subpial transsections (n = 3). Seizure freedom (Engel Class I) can be achieved in a modest percentage of cases (e.g., n = 5/16 cases). The MCA Wada tests can therefore contribute to risk assessment concerning postsurgical motor deficits before functional hemispherectomy. Other indications are less clear. The identification of language areas is targeted primarily by electrical-stimulation mapping after subdural grid implantations, and selective MCA Wada tests in electrical status epilepticus in sleep (ESES) patients have yet to be validated in large patient groups.

Selective middle cerebral artery Wada tests as a part of presurgical evaluation in patients with drug-resistant epilepsies. Urbach H, Von Oertzen J, Klemm E, Koenig R, Linke DB, Kurthen M, Schramm J, Elger CE. Epilepsia 2002 Oct; 43 (10), pp. 1217–23.

Dopamine Transporter (DAT): Central Nervous System (CNS)/Attention Deficit Hyperactivity Disorder (ADHD)/Parkinson's Disease (PD) *[^{99m}Technetium] ([^{99m}Tc])-Based Tropane Ligand/ FLUORATEC*

Cellular/Molecular Mechanism of Action: Detects and physically binds to the DAT, located presynaptically on dopamine neurons.

Background Information: In ADHD, DAT density levels are elevated, while in PD these levels are depleted. The depletion of DAT levels also corresponds with the loss of dopamine.

Application: Characterization of the density of DAT complex expression within the central nervous system. Dopamine transporters are located presynaptically on dopamine neurons and in concept can function as a biological marker for PD and ADHD.

Semi-Synthetic Methods: Design and synthesis of 2nd-generation [^{99m}Tc]-based tropane ligands (e.g., N-[(2-((3′-N′-propyl-(1′′R)-3′′alpha-(4-fluorophenyl)tropane-2′′beta-1-propanoyl)(2-mercaptoethyl)amino)acetyl)-2-aminoethanethiolato]technetium(V) oxide).

Implementation: Evaluation of the biology and single photo emission computed tomography (SPECT) imaging potential of [^{99m}Tc]-based tropane ligands based on their potent and selective binding avidity for DAT complexes.

Animal Model: Non-human primates.

Interpretation and Analysis: [^{99m}Tc]-based tropane ligands possess improved selectivity and biological stability. Such qualities facilitate sufficient entry of the agent into the brain and label DAT complexes to provide a quantitative measure of DAT densities (non-human primates). The agent N-[(2-((3′-N′-propyl-(1′′R)-3′′alpha-(4-fluorophenyl)tropane-2′′beta-1-propanoyl)(2-mercaptoethyl) amino)acetyl)-2-aminoethanethiolato]technetium(V) oxide, also known as FLUORATEC, functions as a DAT imaging agent that is a representative [^{99m}Tc]-based tropane ligand.

A 2nd-generation [^{99m}technetium] single photon emission computed tomography agent that provides *in vivo* images of the dopamine transporter in primate brain. Meltzer PC, Blundell P, Zona T, Yang L, Huang H, Bonab AA, Livni E, Fischman A, Madras BK. J Med Chem 2003 Jul 31; 46 (16), pp. 3483–96.

Huntington's Disease (HD)/Huntingtin RNA Genetic Targeting/Antisense Gene Targeting: *In Vivo* Gene Expression Quantification/Central Nervous System (CNS) Macromolecule Delivery: Transferrin Receptor Complexes *[^{125}I]-Peptide Nucleic Acid (PNA)-[Antitransferrin Receptor IgG]*

Cellular/Molecular Mechanism of Action: The agent is a 16-mer PNA that has an antisense strand that corresponds to the segment around the methionine initiation codon of the huntingtin gene of HD. In this context, it recognizes and physically binds to huntingtin RNA (HD exon-1 messenger RNA).

Background Information: Disease-specific genes of unknown function can be imaged *in vivo* with antisense radiopharmaceuticals. One of the critical limiting features of such biotechnology is the potential to apply drug-targeting strategies that enhance transcellular transport.

Route of Administration: [^{125}I]-PNA/8D3 conjugate (intravenous).

Semi-Synthetic Methods: Biotinylation of PNA performed in concert with the covalent cross-linking of strepavidin and rat 8D3 monoclonal antibody (MAb) to the mouse transferrin receptor (TfR). The latter moiety contains a tyrosine residue that can facilitate [^{125}I] radioiodination. The reformulated PNA antisense radiopharmaceutical conjugated to 8D3 MAb is designated [^{125}I]-PNA/8D3.

Implementation: Evaluation of biotinylated PNA that can be rapidly captured by conjugates of covalently cross-linked strepavidin and [antimouse transferrin receptor IgG]. Complementary *in vivo* studies in mice can be used to determine the biodistriubtion of the PNA analogue [^{125}I]-PNA/8D3 through harvest of tissue/organ systems 6 hours postadministration for frozen sectioning of the brain and quantitative autoradiography.

Animal Model: Murine (littermate control mice or R6/2 transgenic mice that express the exon 1 of the human HD gene for Huntington's disease).

Interpretation and Analysis: Despite covalent cross-linking of the PNA analog, [^{125}I]-PNA/8D3 to MAb, it can gain access to endogenous transferrin transport pathways at both the blood-brain barrier and the brain cell membrane and undergoes both importation from the blood to the brain and exportation from the brain to the blood through TfR. In this manner, it is able to hybridize with "target" huntingtin RNA based on analysis from both cell-free translation assays and ribonuclease protection assays.

Approximately a 3-fold increase in sequestration *in vivo* can be appreciated for antisense [^{125}I]-PNA/8D3 radiopharmaceutical conjugates in the brains of HD transgenic mice. Such observations are consistent with the selective expression of the HD exon-1 messenger RNA in these animals. Therefore, gene expression *in vivo* can be quantitated with antisense radiopharmaceuticals, providing these molecules are reformulated with drug-targeting technology. Drug targeting enables access of the antisense agent to endogenous transport pathways, which permits passage across the cellular barriers that separate blood and intracellular compartments of target tissues.

Imaging gene expression in the brain *in vivo* in a transgenic mouse model of Huntington's disease with an antisense radiopharmaceutical and drug-targeting technology. Lee HJ, Boado RJ, Braasch DA, Corey DR, Pardridge WM. J Nucl Med 2002 Jul; 43 (7), pp. 948–56.

Hypercapnia/Cerebral Blood Flow Reserves *[^{125}I]-HIPDM*

Application: Detection and characterization of regional alterations in cerebral blood flow and central nervous system (CNS) hypercapnia.
Route of Administration: [^{125}I]-HIPDM (intravascular).
Implementation: Evaluation of normocapnic, hypercapnic, and hyperoxic states utilizing a "conscious-state" 30-minute brain uptake scintigraphic imaging strategy employing [^{125}I]-HIPDM preparations.

Animal Model: Rat (normocapnic rats, n = 8; hypercapnic rats, n = 12; hyperoxic rats, n = 6).
Interpretation and Analysis: A mean 41.2% higher uptake is found in the brains of hypercapnic animals ($p \leq 0.01$). In the 3 groups of rats, brain HIPDM uptake has a negative correlation with body weight ($p \leq 0.001$) and a positive correlation with arterial pCO_2 ($p \leq 0.01$), when adjusted for body weight. Such findings indicate that HIPDM uptake with hypercapnia may be used as a provocative test for measuring cerebral blood flow reserves.

Comparison of [^{125}I]HIPDM and [^{125}I]iodoantipyrine in quantifying regional cerebral blood flow in rats. Albright RE Jr, Friedman AH, Fram EK, Harbury OL, Molter BA, Skatoff JH, Harris CC, Coleman RE, Drayer BP. Stroke 1988 Nov; 19 (11), pp. 1404–10.

Experimental increase in brain HIPDM uptake by hypercapnia. Karatzas ND, Sfakianakis GN, Pappas D, Duncan R, Heal A, Serafini A, Kung HF. J Nucl Med 1988 Oct; 29 (10), pp. 1675–82.

Hypocampus/Neuropeptide Receptor Distribution *RF-Amide/[^{125}I]-RFRP-3/[^{125}I]-Neuropeptide FF (NPFF)*

Cellular/Molecular Mechanism of Action: RF-amide (28 amino acid residues) can be isolated from the bovine hypothalamus.
Application: Characterization of the *in vivo* distribution and expression density of neuropeptide distribution.
Implementation: Determination of the tissue distribution of RFRP-3.

Animal Model: Rat.
Interpretation and Analysis: The tissue distribution of RFRP-3 using a sandwich enzyme immunoassay has revealed that the peptide is found in highest concentration within the hypothalamus. In binding assays, [^{125}I]-labeled RFRP-3 becomes bound to OT7T022 with a high level of affinity, but its binding affinity for HLWAR77 is low. Alternatively, [^{125}I]-labeled NPFF binds to both OT7T022 and HLWAR77 with high affinity. Serial deletion in the N-terminal portions of RFRP-3 and NPFF reveals that 4 C-terminal amino acid residues (PQRFamide), which are common between the 2 peptides, comprise a core sequence responsible for binding with the receptor complexes. In contrast, if 3 amino acid residues (PNL in RFRP-3 and LFQ in NPFF) are added to the N terminus of PQRFamide, substantial changes in agonistic properties are observed for RFRP-3 (OT7T022 cell line) and NPFF (HLWAR77 cell line).

Molecular properties of endogenous RFamide-related peptide-3 and its interaction with receptors. Yoshida H, Habata Y, Hosoya M, Kawamata Y, Kitada C, Hinuma S. Biochim Biophys Acta 2003 Feb 17; 1593 (2–3), pp. 151–7.

Parkinson's Disease (PD)/Attention Deficit Hyperactivity Disorder (ADHD)/Dopamine Transporter (DAT) *[^{99m}Technetium] ([^{99m}Tc])-Based Tropane Ligand/ FLUORATEC*

Cellular/Molecular Mechanism of Action: Interacts with DAT complexes located presynaptically on dopamine neurons.
Background Information: In ADHD, DAT density levels are elevated, while in PD these levels are depleted. The depletion of DAT levels also corresponds with the loss of dopamine.
Application: Characterization of the density of DAT complex expression within the central nervous system. Dopamine transporters are located presynaptically on dopamine neurons and therefore can function as a biological marker for PD and ADHD.
Semi-Synthetic Methods: Design and synthesis of 2nd-generation [^{99m}Tc]-based tropane ligands [e.g., N-[(2-((3′-N′-propyl-(1′′R)-3′′alpha-(4-fluorophenyl)tropane-2′′beta-1-propanoyl)(2-mercaptoethyl)amino)acetyl)-2-aminoethanethiolato]technetium(V) oxide].
Implementation: Evaluation of the biology and single photon emission computed tomography (SPECT) imaging potential of [^{99m}Tc]-based tropane ligands as a function of their potent and selective binding to DAT complexes.

Animal Model: Non-human primates.
Interpretation and Analysis: [^{99m}Tc]-based tropane ligands possess improved selectivity and biological stability characteristics that facilitate transfer of the agent into the brain and label DAT complexes. A sufficient amount of agents enter the brain to provide a quantitative measurement of DAT densities (non-human primates). The agent N-[(2-((3′-N′-propyl-(1′′R)-3′′alpha-(4-fluorophenyl) tropane-2′′beta-1-propanoyl)(2-mercaptoethyl)amino)acetyl)-2-aminoethanethiolato]technetium(V) oxide, also known as FLUORATEC, can therefore function as a DAT imaging agent that is a representative [^{99m}Tc]-based tropane ligand.

A 2nd-generation [^{99m}technetium] single photon emission computed tomography agent that provides *in vivo* images of the dopamine transporter in primate brain. Meltzer PC, Blundell P, Zona T, Yang L, Huang H, Bonab AA, Livni E, Fischman A, Madras BK. J Med Chem 2003 Jul 31; 46 (16), pp. 3483–96.

Somatostatin Receptor (SR) Expressing Neoplasia/ Meningiomas: High-Density SR Expression/ Astrocytoma (High-Grade): High-Density SR Expression/Craniopharyngioma: High-Density SR Expression/Mammary Carcinoma/Lymphoma/ Monitoring SR Expression during Therapy *[^{111}Indium] ([^{111}In])-Pentetreotide*

Cellular/Molecular Mechanism of Action: Pentetreotide recognizes and physically binds to SR complexes.
Background Information: Somatostatin receptors are surface markers that are characteristic of not only APUDomas associated with neuroendocrine neoplasias but also malignancies without neuroendocrine properties. The somatostatin analogue pentetreotide can be labeled with [^{111}In] for the *in vivo* imaging of SR-positive lesions (OctreoScan 111, Mallinckrodt Medical BV).
Application: Detection, characterization, and monitoring the growth and therapeutic response of (neoplastic) cell populations that express SR complexes.
Dosage: [^{111}In]-pentetreotide (111 MBq; 3 mCi).
Implementation: Evaluation of SR-specific scintigraphy as a clinical tool for tissue characterization in correlation with histological and radiological examinations. Scintigraphy can be focused toward and performed in cancer types without neuroendocrine tissue expression (e.g., brain, breast tumors, and lymphomas). Scintigraphic imaging can be performed in order to establish comparisons at 6 and 22 hours after intravenous application of [^{111}In]-pentetreotide.

Human Model: Cases of neoplasia without neuroendocrine tissue expression (n = 6 brain; n = 9 breast tumors; n = 5 lymphomas).

Interpretation and Analysis: In conditions of breast cancer, primary tumors can be visualized in a high percentage of cases (e.g., n = 9/9 or 100% and n = 4 palpable axillary lymph nodes).

Some cases with negative axillary node scans and impalpable nodes can have positive biopsy findings (e.g., n = 3/9) and may be accompanied by mediastinal lymph node involvement (e.g., n = 2/3). So far the role of SR-positive breast cancer scans remains unknown. It is tempting to speculate that in resected women who are histologically and scintigraphically SR positive, it might be of value in the early detection of symptom-free recurrences. High densities of SR are present within both meningiomas, high-grade astrocytoma and craniopharyngioma. Differentiation of low- and high-grade astrocytomas cannot be successfully achieved because both grades showed intense radioactivity uptake, even though high-grade tumors lack SR. The latter might be due to disruption of the blood-brain barrier and poor radioactivity washout observed in high-grade astrocytomas. Often, a large percentage of lymphomas can be detected due to the presence of activated lymphocytes and macrophages that express SR at a sufficient density (e.g., n = 5/5). In conclusion, SR scintigraphy in non-neuroendocrine malignancies does not seem to be reliable for an initial tumor staging but is rather more suitable for tissue characterization and extremely useful for monitoring changes of SR expression after treatment.

Receptor scintigraphy of non-neuroendocrine cancers with In-111 pentetreotide. Limouris GS, Rassidakis A, Kondi-Paphiti A, Triantafyllou N, Manetou A, Gennatas K, Vitoratos N, Gregoriou O, Stavraka A, Vlahos L. Hybridoma 1997 Feb; 16 (1), pp. 133–7.

AUTONOMIC NERVOUS SYSTEM

Parasympathetic Nervous System/Acetylcholine Cardiac Muscarinic Receptors/Central Nervous Sytem Muscarinic Acetylcholinergic Receptors (mAChR)/Carcinoma of the Colon
[^{123}I]-PIP/[^{125}I]-PIP

Cellular/Molecular Mechanism of Action: The agent IPIP functions as a ligand that physically binds to mAChR.
Background Information: Prior to approval for *in vivo* use in humans, organ doses for radioiodinated IPIP ligands must 1st be evaluated.
Application: Radioiodinated IPIP ligands can be applied *in vivo* for imaging mAChR using modalities like single photon emission computed tomography (SPECT).
Implementation: Identify the most optimum mAChR ligand-binding properties of 4 different IPIP sterioisomers through evaluation of their biodistribution pattern. A biokinetic model can be developed for the Z-(S)-IPIP stereoisomer for estimating the internal absorbed dose in humans based on extrapolation from an appropriate rat model.

Animal Model: Rat (IPIP biokinetic model).
Interpretation and Analysis: The thyroid gland following the administration of radioiodinated PIP is a critical organ at risk for innocent radiation exposure. The absorbed dose is estimated to be 2.4 mGy/MBq for both males and females, when labeled with [^{123}I]. Even when blocked, the thyroid is still the critical organ at risk, even with a 90% dose reduction. The heart and brain receive the next highest doses in both males and females. Effective dose estimates for the use of pure [^{123}I]-PIP in humans are 0.16 mSv/MBq for males and 0.14 mSv/MBq for females. Biodistribution studies indicate that the Z-(S)-IPIP stereoisomer shows the most promise as a successful agent for imaging muscarinic receptor sites in the heart and brain. IPIP can potentially be applied as a therapeutic radiopharmaceutical for certain colon carcinomas where muscarinic receptor sites are expressed in the tumor cells. These results provide preliminary data for use of IPIP in clinical studies on humans.

In vivo biodistribution of [^{125}I]-PIP and internal dosimetry of [^{123}I]-PIP radioiodinated agents selective to the muscarinic acetylcholinergic receptor complex. Breeden WK 3rd, Hamby DM, Carey JE Jr, Eckerman KF, McPherson DW, Knapp FF Jr. Med Phys 2000 Apr; 27 (4), pp. 778–86.

Parasympathetic/Acethylcholine Nictotinic $\alpha_4\beta_2$ Receptor Complexes/Neurodegenerative Disease/ Tobacco Dependency
(S)-5-[^{123}I]Iodo-3-(2-Azetidinylmethoxy)Pyridine

Cellular/Molecular Mechanism of Action: (*S*)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine selectively binds to $\alpha_4\beta_2$ subtype nicotinic acetylcholine receptor complexes.
Application: The iodinated analogue (*S*)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine of A-85380 can be used for the specific detection and characterization of $\alpha_4\beta_2$ subtype nicotinic acetylcholine receptor complexes in neurodegenerative disease states, including conditions associated with tobacco dependency.
Route of Administration: (*S*)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine (intravenous).
Implementation: Accurate measurement of (*S*)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine plasma concentrations in order to determine its capacity to function as a "tracer" molecule for *in vivo* quantification of $\alpha_4\beta_2$ subtype nicotinic acetylcholine receptor complexes.

Human Model: Non-human and human primates.
Interpretation and Analysis: In human or non-human primate whole blood as well as in plasma, the parent compound is only stable for approximately 5 minutes, while substantial decomposition occurs after this time period. Alternatively, the radioligand is stable in the injection solution and in protein-free (> 30 KMW) plasma ultrafiltrate for at least 18 hours. To preserve the parent compound in plasma the radioactive plasma must be mixed with equal volumes of acetonitrile within 5 minutes after its collection or, alternatively, radioactive blood should be collected and mixed with sodium azide (3 mg/ml blood). The *in vivo* metabolism of [^{123}I]5-IA generates 2 distinct components including (i) a radiometabolite that is less lipophilic than the parent compound; and (ii) a polar radiometabolite that is not free radioiodide because of the absence of radioactivity accumulation in the thyroid.

Measurement of plasma metabolites of (*S*)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine (5-IA-85380), a nicotinic acetylcholine receptor imaging agent, in non-human primates. Zoghbi SS, Tamagnan G, Baldwin MF, Al-Tikriti MS, Amici L, Seibyl JP, Innis RB. Nucl Med Biol 2001 Jan; 28 (1), pp. 91–6.

Sympathetic Cardiac Adrenergic Receptor Expression Profiles/Congestive Heart Disease
[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)/ [^{201}Thallium] ([^{201}Tl]) Cardiac/[^{123}I]-MIBG Washout/ [^{123}I]-MIBG (Adrenergic Activity)/[^{201}Tl] (Perfusion) Ratio/[^{123}I]-MIBG: [^{201}Tl] vs Plasma Norepinephrine/ [^{123}I]-MIBG/[^{201}Tl] Ratio vs Left Ventricular Ejection Fraction (LVEF)

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is a radioiodinated analogue of norepinephrine that recognizes and binds to sympathetic/adrenergic receptor complexes. The radionucleotide [^{201}Tl] per se does not exert a true biological effect.

Application: [^{123}I]-MIBG can be utilized to detect and characterize tissue/organ sympathetic/adrenergic receptor expression profiles. The radionuclide [^{201}Tl] can serve as a tracer molecule suitable for the measurement of vascular perfusion parameters. Simultaneous use of both of these agents can assess adrenergic activity and perfusion in various organs in heart failure conditions.
Route of Administration: [^{123}I]-MIBG and [^{201}Tl] (intravascular).
Implementation: Simultaneous assessment of regional adrenergic activity and vascular perfusion with [^{123}I]-MIBG and [^{201}Tl] in various organ systems. In order to reduce crosstalk from [^{201}Tl] into the [^{123}I] energy window to less than 5%, doses of [^{123}I]-MIBG can be administered that are 5 times greater than that of [^{201}Tl] following [^{201}Tl] scintigraphy.

Human Model: Cases suffering from congestive heart failure (CHF, n = 13; n = 13 without heart failure).
Interpretation and Analysis: Regional uptake of [^{201}Tl] (%dose) becomes significantly increased in the heart (left ventricle) and lung (both $p < 0.01$) in conditions of CHF. Increases in global cardiac uptake may be related to left ventricle enlargement. Patterns of regional uptake for [^{123}I]-MIBG (%dose) at 15 minutes are similar to those of [^{201}Tl], suggesting that early [^{123}I]-MIBG uptake could in part depend on regional perfusion in both case group types. At 3 hours, regional uptake of [^{123}I]-MIBG is significantly increased in the heart, lung, and kidney (all $p < 0.01$) in conditions of CHF. Delayed [^{123}I]-MIBG uptake indicates the degree of neuronal accumulation of the tracer, and therefore reflects adrenergic activity. Interestingly, cardiac [^{123}I]-MIBG (adrenergic activity) to [^{201}Tl] (perfusion parameters) ratios decrease significantly in the heart ($p < 0.01$) but increase significantly in the kidney ($p < 0.01$) in conditions of CHF compared to negative reference controls. Cardiac [^{123}I]-MIBG washout is also significantly increased with CHF. Moreover, the cardiac [^{123}I]-MIBG:[^{201}Tl] ratio negatively correlates with plasma norepinephrine concentrations ($r = -0.74$, $p < 0.01$) but positively correlates with LVEF ($r = 0.60$, $p < 0.01$). Such data suggests that there may be impairment of both the neuronal uptake function and the vesicular storage function in the failing heart and an increment in neuronal uptake function in the kidneys in conditions of CHF. It is therefore suggested that dual-tracer scintigraphy can function as a useful non-invasive method for the simultaneous assessment of adrenergic activity and perfusion in various organs in conditions of heart failure.

Simultaneous assessment of regional adrenergic activity and perfusion with [^{123}I]-MIBG and [^{201}Tl] in congestive heart failure. Seto H, Shimizu M, Nozawa T, Yoshida N, Inagaki S, Kageyama M, Kakishita M. Nucl Med Commun 1996 Mar; 17 (3), pp. 225–30.

Sympathetic: Cardiac/Cardiomyopathy: Dilated/ Heart/Mediastinum (H/M) Ratio: Prognostic Indicator for Mortality
[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is an analogue of the neurotransmitter norepinephrine that can recognize and physically bind to adrenergic receptor complexes.
Application: Evaluation of the potential for repeated measurements of cardiac [^{123}I]-MIBG imaging parameters before and after optimized treatments to be useful for predicting the prognosis of congestive heart failure (CHF) resulting from dilated cardiomyopathy (DCM).
Route of Administration: [^{123}I]-MIBG (intravascular).
Implementation: Assessment of DCM with [^{123}I]-MIBG and measurement of neurohumoral factor concentrations at baseline and after 6 months of optimized treatments. Cox proportional hazards analysis can be performed to assess the various parameters before and after treatment.

Human Model: Dilated cardiomyopathy (n = 85 cases with left ventricular ejection fraction (LVEF) of less than 45%).
Interpretation and Analysis: A relatively large number of cases will experience complications (e.g., n = 12 death; n = 11 hospitalization) over a 2-year mean follow-up period. Although there is no difference between the baseline H/M ratio measured by MIBG between survivors and non-survivors, the H/M ratio is significantly decreased in non-survivors after 6 months. Multivariate analysis reveals that a high plasma concentration of brain natriuretic peptide levels after 6 months ($p = 0.0049$) and absolute changes in the H/M ratio ($p = 0.0046$) are independent predictors of mortality. Comparison of the H/M ratio on MIBG imaging before and after optimized additional treatment provides useful information for predicting mortality and is independent of clinical and neurohumoral factors previously shown to be associated with poor prognosis in patients with DCM.

Prognostic value of repeated [^{123}I]-metaiodobenzylguanidine imaging in patients with dilated cardiomyopathy with congestive heart failure before and after optimized treatments—Comparison with neurohumoral factors. Matsui T, Tsutamoto T, Maeda K, Kusukawa J, Kinoshita M. Circ J 2002 Jun; 66 (6), pp. 537–43.

Sympathetic Cardiac Denervation and Ischemia/ Myocardial Sympathoexcitatory Response/ [Balloon Coronary Occlusion (BCO)]
[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is a norepinephrine analogue that recognizes and physically binds to adrenergic receptor complexes.
Background Information: Alterations of cardiac sympathetic nerve function could modulate sympathetic reflexes originating from the ischemic area.
Application: Detection and characterization of cardiac sympathetic denervation.
Route of Administration: [^{123}I]-MIBG (intravenous).
Implementation: Evaluate conditions of angina pectoris as a function of quantified baseline measurements of cardiac sympathetic denervation associated with anatomical regions of ischemia utilizing [^{123}I]-MIBG. Complementary diagnostic procedures can include establishing the nature of transient changes in sympathetic activity during BCO by wavelet analysis of RR interval variability.

Human Model: Cases with angina pectoris (n = 23) subjected to BCO. The research objective of the referenced study was to elucidate the influence of cardiac sympathetic denervation on the sympathoexcitatory response to acute myocardial ischemia during BCO.
Interpretation and Analysis: Balloon coronary occlusion results in a transient augmentation of low-frequency (LF; 0.04–0.14 Hz) spectral components of RR interval variability in approximately 30% of cases (e.g., n = 4/12) with cardiac denervation, and in greater than two-thirds of cases without denervation (e.g., n = 8/11; $p < 0.01$ by the chi^2 test). Consequently, increases in LF components are significantly less during BCO in cases with cardiac denervation (34%) than in those without denervation (273%: interaction: $p < 0.05$). In some cases with severe ischemia provoked by a fall of > 10% in the left ventricular ejection fraction, LF components increase by 506% during BCO, regardless of the condition of cardiac denervation (n = 7/23 cases). Conversely, in conditions of mild ischemia provoked by a fall of < 10% in the ejection fraction, the changes of

LF components during BCO are significantly less in cases with denervation than in those without denervation (84 vs 344%, $p < 0.05$). Such observations suggest that if the provoked ischemia is not severe, cardiac sympathetic denervation could prevent ischemia-induced sympathoexcitation.

Cardiac sympathetic denervation modulates the sympathoexcitatory response to acute myocardial ischemia. Joho S, Asanoi H, Takagawa J, Kameyama T, Hirai T, Nozawa T, Umeno K, Shimizu M, Seto H, Inoue H. J Am Coll Cardiol 2002 Feb 6; 39 (3), pp. 436–42.

Sympathetic Cardiac Denervation/Myocardial Infarction (MI)/Heterogeneous Cardiac Sympathetic Innervation
[^{131}I]-Metaiodobenzylguanidine ([^{131}I]-MIBG)/ [^{125}I]-Iodocyanopindolol ([^{125}I]-ICYP)

Cellular/Molecular Mechanism of Action: Metaiodobenzylguanidine is a norepinephrine analogue that recognizes and physically binds to adrenergic receptor complexes. Iodocyanopindolol is a β-adrenoreceptor ligand.
Application: Detection and characterization of MI.
Route of Administration: [^{131}I]-MIBG and [^{125}I]-ICYP are both administered intravenously.
Implementation: Evaluation of cardiac neuronal function and β receptor utilizing a dual-tracer method of [^{131}I]-MIBG and [^{125}I]-ICYP in rat heart failure after MI.
Animal Model: Rat (MI).
Interpretation and Analysis: In conditions of MI, left ventricular (LV) systolic function is decreased, and LV dimension and right ventricular (RV) mass increase gradually (rat). Accumulations of [^{131}I]-MIBG within non-infarcted LV (remote region) and RV decrease by 15% at 1 week compared with controls (e.g., sham-operated rats). These same accumulations are restored by 71% and 56%, respectively, at 24 weeks compared with age-matched sham rats despite sustained depletion of myocardial norepinephrine content within these anatomical regions. Accumulations of [^{125}I]-ICYP in the remote region and in the RV do not decrease during any stage. Myocardial MIBG distribution is heterogeneous at 1 week and lower in the peri-infarcted region than in the remote region. These profiles are accompanied by reduced ICYP accumulation in the peri-infarcted region. At week 12, the heterogeneous distribution of both isotopes disappears. Thus cardiac sympathetic neuronal alterations appear to be coupled with downregulation of β receptors in rat heart failure after MI. The abnormal adrenergic signaling occurs heterogeneously in terms of ventricular distribution and time course after MI.

Heterogeneous cardiac sympathetic innervation in heart failure after myocardial infarction of rats. Igawa A, Nozawa T, Yoshida N, Fujii N, Inoue M, Tazawa S, Asanoi H, Inoue H. Am J Physiol Heart Circ Physiol 2000 Apr; 278 (4), pp. H1134–41.

Sympathetic: Cardiac Failure/Clinical Variables in Risk Stratification
[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is a norepinephrine analogue that recognizes and physically binds to adrenergic receptor complexes.
Background Information: Both myocardial [^{123}I]-MIBG uptake and plasma norepinephrine are markers of sympathetic activation in heart failure and have been shown to indicate a poorer prognosis. However, these observations have been noted before treatment with β-blockers became part of standard clinical practice.
Application: Both myocardial [^{123}I]-MIBG uptake and plasma norepinephrine are markers of sympathetic activation in heart failure conditions.
Implementation: Evaluation of chronic heart failure (CHF) cases over a mean follow-up period of 36 months. Potential prognostic parameters are obtained before initiation of β-blocker therapy.
Human Model: Cases of CHF (n = 58) recognized according to established guidelines (New York Heart Association functional Class II and III, ejection fraction < 35%; 53% ischemic cardiomyopathy).
Interpretation and Analysis: During the observation period, cases may display cardiovascular complications (e.g., n = 17/58 or 29.3%, death or heart transplantation). Multivariate Cox regression analysis, plasma norepinephrine, peak oxygen consumption, end-diastolic volume as measured by echocardiography, and exercise performance during bicycling and walking have prognostic significance in conditions of heart failure treated with β-blockers in addition to angiotensin-converting enzyme inhibitors. In both uni- and multivariate analysis, the heart/mediastinum ratio of [^{123}I]-MIBG uptake does not correlate with cardiovascular mortality.

Cardiac [^{123}I]-MIBG imaging and clinical variables in risk stratification in patients with heart failure treated with β-blockers. de Milliano PA, Tijssen JG, van Eck-Smit BL, Lie KI. Nucl Med Commun 2002 Jun; 23 (6), pp. 513–9.

Sympathetic: Cardiac Infarct/Adrenergic Receptor Expression/Sympathetic Neuronal Dysfunction/ QT-RR Slope and Metaiodobenzylguanidine (MIBG) Heart: Mediastinum (H/M) Ratio/QT Dispersion and MIBG H/M Ratio
[^{123}I]-MIBG

Cellular/Molecular Mechanism of Action: Metaiodobenzylguanidine is an analogue of norepinephrine that recognizes and physically binds to sympathetic/adrenergic receptor complexes.
Background Information: Cardiac [^{123}I]-MIBG imaging is widely used to assess cardiac sympathetic neuronal function. However, physiologic significance of impaired cardiac MIBG uptake has not been fully elucidated.
Application: Detection and characterization of sympathetic adrenergic receptor expression as a means of classifying different cardiovascular disease states (e.g., myocardial infarction).
Route of Administration: [^{123}I]-MIBG (intravascular).
Implementation: Evaluation of the influences of abnormal cardiac sympathetic neuronal function on heart rate variability (HRV) and ventricular repolarization process. In this context, conditions of myocardial infarction can be classified into groups based on H/M ratio established by MIBG scintigraphy. Holter recordings and a standard 12-lead electrocardiography can be used for evaluation of HRV, QT-RR relation, and QT dispersion. MIBG Classification Scheme I: globally decreased MIBG uptake (H/M < 1.5; n = 10 cases); MIBG Classification Scheme II: decreased MIBG uptake (H/M ≥ 1.5; n = 19 cases); and Normal Reference Control Scheme III: (n = 17 cases).
Human Model: Cases of myocardial infarction (n = 29) classified into 2 groups by an H/M ratio established by MIBG scintigraphy.
Interpretation and Analysis: Low, high, and total frequency components are decreased in MIBG Classification Schemes I and II compared to reference controls. The reductions of these frequency domain measures are more severe in Scheme I than in Scheme II cases, but the differences do not reach statistically significant levels. Circadian variation of frequency domain measures disappears in

Scheme I cases. The slope of the QT-RR relationship is significantly greater in group I than in groups II and III. QT dispersion is also greater in group I (64 ± 25 msec) than in group II (43 ± 19 msec) and group III (28 ± 9 msec). Therefore, in conditions of sympathetic neuronal dysfunction inferred from globally impaired cardiac MIBG uptake, there are alterations in the modulation of ventricular repolarization process as well as decreased HRV.

Modulation of ventricular repolarization and RR interval is altered in patients with globally impaired cardiac [^{123}I]-MIBG uptake. Yoshida N, Nozawa T, Igawa A, Fujii N, Kato B, Mizumaki K, Fujiki A, Asanoi H, Seto H, Inoue H. Ann Noninvasive Electrocardiol 2001 Jan; 6 (1), pp. 55–63.

Sympathetic: Cardiovascular/Salt Sensitivity and Adrenergic Regulation/Hypertrophic Stage in Salt-Sensitive *In Vivo* Models/Cardiac Neuronal Function: Late Phase Deterioration/Adrenergic β-Receptor Downregulation
[^{131}I]-Metaiodobenzylguanidine ([^{131}I]-MIBG)/ [^{125}I]-Cyanopindolol ([^{125}I]-ICYP)

Cellular/Molecular Mechanism of Action: [^{131}I]-MIBG is an analogue of norepinephrine and detects and physically binds to adrenergic/sympathetic receptor complexes. [^{125}I]-ICYP also recognizes and binds to sympathetic/adrenergic receptor complexes.
Background Information: Heart failure is associated with activation of the sympathetic nervous system and downregulation of β-receptor complexes. However, the coupling between cardiac sympathetic neuronal function and β receptors during development of hypertensive heart failure is not clear.
Application: [^{131}I]-MIBG and [^{125}I]-ICYP (intravascular) can be utilized to evaluate adrenergic/sympathetic adrenergic receptor expression profiles.
Route of Administration: [^{131}I]-MIBG and [^{125}I]-ICYP (intravascular).
Implementation: Evaluation of cardiac neuronal function and β receptors utilizing a dual-tracer method employing [^{131}I]-MIBG and [^{125}I]-ICYP under *in vivo* conditions of salt-sensitivity and salt-resistance.

Animal Model: Rats (Dahl salt-sensitive [DS] and salt-resistant [DR]). Rats are fed an 8% NaCl diet after the age of 6 weeks. Blood pressure is raised to > 200 mm Hg at 12 weeks in DS rats and is maintained at an elevated level until 18 weeks, but is only slightly in DR rats. Left ventricular (LV) function of DS rats is preserved at 12 weeks but deteriorates by 18 weeks.
Interpretation and Analysis: Despite a 56% reduction of cardiac norepinephrine content at 12 weeks in salt-sensitive *in vivo* models, neither MIBG nor ICYP uptake in salt-sensitive *in vivo* models is different from salt-resistant *in vivo* models. At 18 weeks, both MIBG and ICYP uptakes decrease by 52% and 39%, respectively, in association with 71% reductions in cardiac norepinephrine in salt-sensitive *in vivo* models. MIBG uptake of the LV is homogeneous at 6 weeks but lower in the LV endocardial regions at 18 weeks in salt-sensitive *in vivo* models. Cardiac sympathetic neuronal function therefore is relatively preserved at the compensated, hypertrophic stage in salt-sensitive *in vivo* models but deteriorates when associated with β-receptor downregulation at the failing stage. The cardiac neuronal dysfunction occurs heterogeneously. A combination of scintigraphic portrayal of β receptors with MIBG should provide valuable information regarding sympathetic nerve signaling in living hearts.

Dual-tracer assessment of coupling between cardiac sympathetic neuronal function and downregulation of β receptors during development of hypertensive heart failure of rats. Nozawa T, Igawa A, Yoshida N, Maeda M, Inoue M, Yamamura Y, Asanoi H, Inoue H. Circulation 1998 Jun 16; 97 (23), pp. 2359–67.

Trauma-Induced Neurochemical Changes
[^{3}H]Glutamate/[^{3}H]MK801

Background Information: Trauma is the leading cause of death in individuals between the ages of 1 and 44 years. In cases of severe head injury mortality can reach as high as 35–70%. Despite this fact, there has been little progress in the development of effective pharmacological agents to protect brain-injured patients. To date, there is little data on the mechanisms involved in neuronal cellular insult after severe head injury, especially in humans. Glutamate acts both as a primary excitatory neurotransmitter and a potential neurotoxin within the mammalian brain. Evidence indicates that hyperactivity of the glutamate system contributes to neuronal death in brain trauma. Also, in animal models of neurotrauma, neural injury is followed by development of gliosis, which has been linked to the severity of brain injury.
Implementation: Evaluate the glutamate system in brain trauma as a function of [^{3}H]glutamate and [^{3}H]MK801 (a non-competitive N-methyl-D-aspartate [NMDA]-receptor antagonist) binding and [^{3}H]glutamate uptake assays in human cerebral cortex preparations obtained from severely brain-injured and control victims. Investigation of gliosis phenomenon following brain injury can be determined by performing glial fibrillary acidic protein (GFAP) immunohistochemistry.
Interpretation and Analysis: There were no significant differences in [^{3}H]glutamate binding (affinity or density of sites) between the control and head-injured groups. Conversely, cerebral cortical [^{3}H]MK801 binding analysis reveals both a significant increase in the density of sites (Bmax) and a decrease in the dissociation constant (Kd) in groups of head-injury cases when compared to reference controls. There were no significant differences in [^{3}H]glutamate uptake between groups. Injury brains present with an increase number of GFAP-positive astrocytes and a more intense GFAP reaction in comparison to control brains. In the context of traumatic brain injury, results from previous research represent evidence that encourages further investigation into compounds capable of selectively modulating NMDA receptor subtype in humans, which can also serve as an approach for therapeutically manipulating glial cell responses following brain trauma.

Neurochemical characterization of traumatic brain injury in humans. Regner A, Alves LB, Chemale I, Costa MS, Friedman G, Achaval M, Leal L, Emanuelli T. J Neurotrauma 2001 Aug; 18 (8), pp. 783–92.

ENDOCRINE SYSTEM

Adrenal Cortex Hypertrophy
Radioiodinated Cholesterol ([^{123}I]/[^{124}I])/ [^{131}I]-6-Beta-Iodomethyl-19-Norcholesterol.

Cellular/Molecular Mechanism of Action: Radiolabeled cholesterol preferentially accumulates within the mass of the hypertrophied adrenal cortex, where it is perceived as a precursor for steroid hormone synthesis (e.g., cortisol).
Background Information: The adrenal gland consists of cortical and medullary components, both of which may be functionally imaged using radiopharmaceuticals designed to take advantage of the specialized physiology of each tissue.
Diagnostic Application: Radiolabeled cholesterol derivatives are taken up by normal and abnormal adrenal cortex and provide a map of adrenocortical function in Cushing's syndrome, hyperaldosteronism, and hyperadrenogenism.

Route of Administration: Intravenous injection.

Considerations: Radiolabeled cholesterol-based hormones have also been applied for various diagnostic purposes (e.g., radiolabeled estrone sulfate). Radiolabeled metaiodobenzylguanidine (MIBG) is a tracer of Type I uptake and intracellular storage capacity for catecholamines in sympathomedullary tissues and tumors derived from this body system. This permits the location of pheochromocytomas of all types (benign and malignant, intra- and extra-adrenal, sporadic and familial) as well as neuroblastomas in all stages. Other neuroendocrine tumors with Type I uptake capacity may also be imaged. In suitable cases preliminary data suggests the administration of large doses of MIBG may permit internal radiotherapy of otherwise untreatable neuroendocrine tumors.

Implementation: Adrenal and total body scintigraphs performed with [^{131}I]-6-beta-iodomethyl-19-norcholesterol. Results can be compared with roentgenographic findings and liver, bone, and total body [^{67}Ga]-citrate scintigraphs.

Human Model: Cases with a history of a prior resection of adrenal cortical carcinoma (e.g. n = 5).

Interpretation and Analysis: Abnormal lesions are detected as adrenal glands with elevated levels of imaging agent accumulation in addition to irregularities in adrenal gland size and/or shape. Quantification of uptake shows strong correlation with biochemical parameters of adrenocortical hyperfunction. In cases of incidentally discovered euadrenal masses, scintigraphy may help to distinguish between benign, non-functional cortical adenomas and destructive, potentially malignant lesions.

Metastatic lesions can be detected with radiolabeled cholesterol (e.g., n = 4/5 cases, including 3 liver metastases, 2 bone metastases, and 1 lung metastasis). Such lesions may also be demonstrated by one or more other diagnostic modalities. In some cases (e.g., n = 1/5) all initial findings may be negative despite subsequent development of disseminated disease (e.g., brain metastases). The 6-methyl-analog of iodocholesterol makes it possible to detect metastatic adrenocortical carcinoma with total body scans. Whether or not this agent is "tumor specific" and will be of significant clinical utility will have to be determined more fully in a larger series of patients.

Detection of metastatic adrenal carcinoma using [^{131}I]-6-β-iodomethyl-19-norcholesterol total body scans. Seabold HJ, Haynie TP, DeAsis DN, Samaan NA, Glenn HJ, Jahns MF. J Clin Endocrinol Metab 1977 Oct; 45 (4), pp. 788–97.

Radiochemical diagnosis of adrenal disease. Shapiro B, Fig LM, Gross MD, Khafagi F. Crit Rev Clin Lab Sci 1989; 27 (3), pp. 265–98.

Adrenal Gland: Function and Structure
[^{131}I]-6-β-Iodomethyl-Norcholesterol/ [^{75}Se]-Selenomethyl-Norcholesterol/ [^{131}I]-, [^{123}I]-, and [^{124}I]-Metaiodobenzylguanidine (MIBG)/[^{18}F]-Metafluoro-Benzyl-Guanidine/ [^{123}I]-tyr-Octreotide/[^{111}In]-Diethylenetriamine Pentaacetic Acid (DTPA)-Octreotide/[^{111}In]-DOTA-Octreotide/[^{18}F]-2-Fluoro-d-Deoxyglucose/ Metyrapone/Tyrosine Analogues/Low-Density Lipoprotein (LDL) Receptors/Bombesin/Vasoactive Intestinal Polypeptide/Cholecystokinin/Opiate Peptides/[^{11}C]-Acetate

Background Information: Over the last 30 years nuclear medicine imaging of the adrenal gland and its lesions has been achieved by exploitation of a number of physiological characteristics associated with this organ. By seeking and utilizing features that are quantitatively or qualitatively different from those of the adjacent tissues, it is possible to functionally characterize the adrenal gland and associated disease states. In most instances lesions affecting both the cortex and the medulla retain the basic physiology related to their original tissue type. Diagnostic strategies widely used in clinical practice include assessment of (i) radiolabeled cholesterol analog uptake and storage facilitated by LDL receptors and cholesterol ester storage pools in the adrenal cortex utilizing the agents [^{131}I]-6-β-iodomethyl-norcholesterol or [^{75}Se]-selenomethyl-norcholesterol; (ii) catecholamine Type I presynaptic, uptake mechanisms, and intracellular granule uptake and storage processes in the adrenal medulla and extra-adrenal paraganglia utilizing [^{131}I]-, [^{123}I]-, and [^{124}I]-MIBG and [^{18}F]-metafluoro-benzyl-guanidine; (iii) cell surface receptor binding of peptides/neurotransmitters/modulators such as those for the family of 5 subtypes of somatostatin receptors ([^{123}I]-tyr-octreotide, [^{111}In]-DTPA-octreotide, [^{111}In]-DOTA-octreotide, and many others); (iv) increases in tumor glycolysis, particularly in the most malignant adrenal-associated cell types, utilizing [^{18}F]-2-fluoro-d-deoxyglucose, that can be expected to detect certain neoplastic lesions such as malignant pheochromocytomas (particularly the minority of cases that are not detected with MIBG) and adrenal incidentalomas (particularly with known extra-adrenal malignancies).

There are a variety of adrenal tissue characteristics that have the potential for being exploited for various purposes that are not currently employed for clinical uses, but nevertheless have potential as imaging agents. These include (i) inhibitors of adrenal cortical steroid hormone synthesis enzymes (e.g., radiolabeled analogues of metyrapone); (ii) radiolabeled lipoproteins that bind to adrenocortical LDL receptors; (iii) inhibitors of catecholamine biosynthesis enzymes (e.g., radiolabeled analogues of tyrosine and related amino acids); and (iv) cell surface receptors for various peptides and hormones that may be overexpressed by adrenal cortical or adrenal medullary tumors (e.g., radiolabeled analogues of adenocorticotropic hormone (ACTH) for adrenocortical cells of zona fasciculata or zona glomerulosa origin; neurotransmitter/hormone message peptides binding to cell surface receptors such as bombesin, vasoactive intestinal polypeptide, cholecystokinin, and opiate peptides). The adrenal cortex can also synthesize cholesterol from acetate, and preliminary studies with [^{11}C]-acetate positron emission tomography have shown interesting results.

Application: Detection and characterization of the adrenal gland function and dysfunction.

Functional scintigraphy of the adrenal gland. Rubello D, Bui C, Casara D, Gross MD, Fig LM, Shapiro B. Eur J Endocrinol 2002 Jul; 147 (1), pp. 13–28.

Thyroid Gland
Technetium Pertechnetate [^{99m}TcO$_4$]

Cellular/Molecular Mechanism of Action: Radioisotopes accumulate within the parenchymal tissues of the thyroid gland. Abnormal thyroid lesions frequently accumulate [^{99m}Tc] pertechnetate at relatively high tissue densities (activity/gram tissue).

Diagnostic Application: Diagnosis of hyperthyroidism due to adenomatous hyperplasia or certain neoplastic conditions. It is of particular value in detecting metastatic lesions or in cases suspected of suffering an abnormality but that do not display obvious thyroid gland enlargement (thoracic thyroid gland).

Route of Administration: Intravenous [^{99m}Tc]-pertechnetate.

Implementation: Evaluate the accuracy of fine-needle aspiration (FNA) cytology and thyroid scintigraphy (TS) in patients with solitary thyroid nodules. Prior to surgery, cases undergo FNA cytology (e.g., n = 657 cases) and [^{99m}Tc]-pertechnetate scintigraphic imaging (n = 496/657 or 75.5%).

Human Model: Retrospectively review of a series of 657 consecutive patients (n = 531 or 80.8% women and n = 126 or 19.2% men, median age 45 years, range 16–81 years) with solitary thyroid nodules.

Interpretation and Analysis: Histopathology analysis reveals the presence of a high percentage of benign nodules (e.g., n = 533 or 81.1%), including follicular adenomas (e.g., n = 251 or 38.2%) and thyroid carcinomas (e.g., n = 124 or 18.9%). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy in the detection of thyroid cancer is approximately 95.8%, 21.1%, 22.6%, 95.5%, and 35.7% for TS, and 81.4%, 99.4%, 97.1%, 95.8%, and 96.0% for FNA cytology, respectively. The presence of thyroid cancer may be confirmed in approximately 22.6% of cases examined with a "cold" nodule (e.g., n = 92/407); in about 4.5% of cases with normal or increased uptake on TS (e.g., n = 4/89); in approximately 97.1% of cases with smears suggesting malignancy (e.g., n = 101/104); and in about 0.6% of cases with smears suggestive of a benign lesion (e.g., n = 3/533). In conclusion, the specificity of TS is low and this technique should be abandoned as a routine test in patients with non-toxic thyroid nodules. However, a more careful evaluation should be suggested for patients with cold thyroid nodules and an FNA cytology that reads follicular neoplasm.

An advantage of [^{99m}Tc] is that in the normal individual, the intensity of uptake by the salivary and thyroid gland are approximately equal. Conversely, the intensity of [^{99m}Tc] uptake is much greater within the thyroid gland of cases with hyperthyroidism compared to that of the salivary gland.

Usefulness of [^{99m}Tc]-pertechnetate scintigraphy and fine-needle aspiration cytology in patients with solitary thyroid nodules and thyroid cancer. Lumachi F, Varotto L, Borsato S, Tregnaghi A, Zucchetta P, Marzola MC, Cecchin D, Bui F. Anticancer Res 2004 Jul–Aug; 24 (4), pp. 2531–4.

Thyroid Gland Function and Anatomical Configuration/Differentiated Thyroid Cancer (DTC)/Hyperthyroidism/Prediction of Response to Therapy

Radioiodine/[^{123}I]/[^{131}I]

Cellular/Molecular Mechanism of Action: Radioiodine is assimilated by the thyroid gland for synthetic processes in a manner essentially identical to non-radioactive iodine.

Diagnostic Application: Radioactive iodine [^{131}I] plays a major role in the diagnosis and management of DTC; however, data on the use of the [^{123}I] isotope in DTC are limited. Measurement of serum thyroglobulin (Tg) levels and [^{131}I] whole-body scintigraphy (WBS) can be used in monitoring conditions of DTC. Radioiodine can be applied to aid in the characterization of other abnormalities affecting the thyroid gland, including hyperthyoidism, hypothyroidism, and goiter.

Route of Administration: [^{123}I] (intravenous).

Dosage: [^{123}I] (185 MBq). Feline hyperthyroid: 4 mCi of radioiodine.

Considerations: Of differentiated thyroid cancer scans with elevated Tg and negative pretreatment scans, some posttreatment scans can remain negative, with a small proportion of posttreatment scans showing thyroid bed uptake, or they can display posttreatment scans with lung uptake in cases that had computed tomography (CT) scans of the chest showing only bronchiectasis and/or lung scarring without evidence of lung metastases.

Implementation #1: Evaluate the significance of persistent [^{131}I] uptake in the thyroid bed in patients with DTC following surgery and/or radioactive iodine ablation. [^{99m}Tc] thyroid scintigraphy (TS) and [^{131}I] thyroid uptake (IU) are also performed to determine their clinical impact on case management.

Implementation #2: Evaluate by comparison 238 diagnostic whole-body scans performed 24 hours after oral ingestion of 185–555 MBq [^{123}I] with their corresponding [^{131}I] posttherapy whole-body scans obtained 4–5 days after [^{131}I] therapy. Scans can be employed in 3 clinical situations: with the 1st [^{131}I] therapy, with the 2nd [^{131}I] therapy, and in cases of elevated Tg and negative diagnostic scan.

Implementation #3: In veterinary medicine radioiodine has been applied to evaluate hyperthyroid cats for pretreatment factors that would predict response to radioiodine therapy. Cats can be treated with an intravenous bolus of radioiodine and follow-up serum thyroxine levels evaluated at 1 week and 1, 3, 6, and 12 months posttherapy. Complementary investigations can include the evaluation of methimazole pretreatment to determine the effect of this drug on therapy response.

Human Model #1: Sixty-two non-metastatic patients (14 men, 48 women) with a mean age of 44 years (range: 16–75) who have undergone surgical thyroidectomy for DTC evaluated prospectively. All patients have undergone technetium and iodine scintigraphy (IS). Serum Tg levels are measured in all patients, (IU available for n = 36).

Human Model #2: Differentiated thyroid carcinoma cases (e.g., n = 238) yielding approximately 177 pairs of thyroid scintigraphic images. Following a 2nd [^{131}I] administration, 34 pairs are obtained.

Animal Model: Hyperthyroid cats (e.g., n = 193) based on elevated serum thyroxine levels and/or elevated thyroid to salivary gland ratios on thyroid scintigraphy. Metrimazole pretreatment evaluation (n = 98 cats) with administration discontinued ≥ days before radioiodine therapy (e.g., n = 58 cats) or < 5 days (e.g., n = 31 cats), or unknown number of days off methimazole (e.g., n = 9 cats).

Interpretation and Analysis: In human cases of DTC that have undergone surgical thyroidectomy, Tg values are in the range of 0.2 to 24 ng/ml (median: 0.2 ng/ml) when cases are in the hypothyroid state. [^{131}I] WBS can detect residual tissue in the neck in 48% of cases (e.g., n = 30); however, TS may be positive in only 19% of cases (e.g., n = 12). [^{131}I] uptake in the thyroid bed ranges from 0 to 14% (median: 0.1%). A large percentage of cases with positive IS and negative TS have uptake values ≤ 0.3% ($p < 0.00001$ for n = 12/13 or 93.2%). When IU values are ≤ 0.3%, about 54% of cases will not have any uptake in the thyroid bed on TS or IS, whereas when the IU is > 0.3%, approximately 80% of cases will have neck uptake on both TS and IS ($p < 0.00001$). Such findings demonstrate that the concordance of IS and TS depends on the IU level after suspension of replacement therapy. Measurements of IU and TS are of considerable value in evaluating patient response to therapy and will substantially reduce the need for repetitive radioiodine scans and unnecessary treatment doses in patients with undetectable Tg values.

In another study of cases affected by DTC, paired images of the thyroid acquired with the first [^{131}I] administration show complete concordance between pretreatment and posttreatment scans in most instances (e.g., n = 166 pairs with a concordance rate = 93.8%). Some posttreatment scans may show more foci in the thyroid bed than the pretreatment scans, but no evidence of uptake in new areas (e.g., n = 6). Only a small number of posttreatment scans reveal foci in new locations (e.g., n = 5 with n = 3/5 in cervical lymph nodes (CLN), n = 1/5 in the lung, n = 1/5 new bone metastasis). Following a 2nd [^{131}I] administration yielding 34 pairs of images, analysis also reveals complete concordance at a relatively high level (e.g., n = 28 pairs and concordance rate = 82.4%). A relatively small number of discordant pairs have additional foci in areas that are already positive

on pretreatment scans (e.g., n = 5; n = 1 posttreatment scan detecting new bone metastasis in a different site from the bone metastases that were seen on its corresponding pretreatment scan).

Slightly greater that 50% of paired scans in cases with elevated Tg and negative pretreatment scans will have posttreatment scans that remain negative (e.g., n = 15/27), while others may show uptake in the thyroid bed (e.g., n = 6). Additionally, posttreatment scans may show lung uptake in cases whose computed tomography scans of the chest show only bronchiectasis (e.g., n = 2/3 cases) and lung scarring (e.g., n = 1/3) in conditions without evidence of lung metastases. A small number of posttreatment scans (e.g., n = 3) show definite uptake (in thyroid bed, thyroid bed and lung, and CLN) compared with their corresponding pretreatment scans initially reported negative but retrospectively may be considered to have faint uptake. In pretreatment scans with a [^{123}I] diagnostic activity of 185 MBq, results reveal complete concordance in a large number of pairs (e.g. n = 54/56). A small number of posttreatment scans show additional uptake (e.g., n = 2; with n = 1 in the bone, n = 1 in CLN). Such findings suggest that pretreatment scanning using [^{123}I] is highly comparable to [^{131}I] posttreatment scanning and that [^{123}I] is an excellent diagnostic agent in DTC.

In hyperthyroid cats, there is a significant relationship between pretreatment thyroxine values and posttreatment thyroxine values at all of the follow-up time points ($p < 0.001$). There is also a relationship between thyroid to salivary gland technetium scan ratio results and serum thyroxine values at pretreatment and at 1 week posttreatment ($p = 0.02, 0.005$, respectively). A greater scan ratio is associated with higher thyroxine levels at those time points, but not at 1, 3, 6, or 12 months posttherapy. There is no difference in response to radioiodine based upon when methimazole was discontinued ($p = 0.70$).

[^{123}I] isotope as a diagnostic agent in the follow-up of patients with differentiated thyroid cancer: Comparison with post-[^{131}I] therapy whole-body scanning. Alzahrani AS. J Clin Endocrinol Metab, 2001 Nov; 86 (11), pp. 5294–300.

Predictors of response to radioiodine therapy in hyperthyroid cats. Chun R, Garrett LD, Sargeant J, Sherman A, Hoskinson JJ. Vet Radiol Ultrasound 2002 Nov–Dec; 43 (6), pp. 587–91.

Value of technetium scintigraphy and iodine uptake measurement during follow-up of differentiated thyroid cancer. Caglar M, Tuncel M, Alpar R. Ann Nucl Med 2004 Sep; 18 (6), pp. 479–82.

Thyroid Nodules (Solitary)/Thyrotoxicosis *[^{99m}TcO^{-4}]/[^{123}I]/[^{201}T1]*

Background Information: Palpable thyroid nodules occur in 4 to 7% of the population, but nodules found incidentally on ultrasonography suggest a prevalence of 19 to 67%. The majority of thyroid nodules are asymptomatic. Because about 5% of all palpable nodules are found to be malignant, the main objective of evaluating thyroid nodules is to exclude malignancy. Laboratory evaluation, including a thyroid-stimulating hormone test, can help differentiate a thyrotoxic nodule from a euthyroid nodule. In euthyroid patients with a nodule, fine-needle aspiration should be performed, and radionuclide scanning should be reserved for patients with indeterminate cytology or thyrotoxicosis. Insufficient specimens from fine-needle aspiration is less of a problem when ultrasound guidance is used. Surgery is the primary treatment for malignant lesions, and the extent of surgery depends on the extent and type of disease. Ablation by postoperative radioactive iodine is done for high-risk patients—identified as those with metastatic or residual disease. While suppressive therapy with thyroxine is frequently used postoperatively for malignant lesions, its use for management of benign solitary thyroid nodules remains controversial.

Nuclear medicine has a distinct role to play in otolaryngological practice. Accurate diagnosis of endocrine conditions is now possible using precise *in vitro* hormone measurement. Specific clinical questions can be answered using *in vivo* investigations. [^{99m}TcO$_4^-$]/[^{123}I] scintigraphy is used to evaluate thyrotoxicosis and solitary thyroid nodules. [^{99m}Tc]/[^{201}T1] subtraction scanning is of value in the preoperative localization of parathyroid adenomas, and [^{99m}Tc] is particularly useful in assessing salivary and lachrymal gland function and drainage. [^{99m}Tc]- methylene diphosphonate (MDP) bone scanning is useful in the evaluation of osteomyelitis, temporomandibular joint dysfunction, bone graft viability, and some facial fractures. The role of radioisotopes in the management of differentiated and medullary carcinoma of the thyroid is now well established. Although there are many other agents available to image head and neck cancer, few can actually achieve the required diagnostic sensitivity and specificity. The introduction of monoclonal antibodies into routine imaging has been hampered by distinct practical problems, and the search is now on for more sensitive non-specific diagnostic agents. It is now possible to evaluate new [^{99m}Tc]-labeled tumor-imaging agents using animal tumor model systems, and the use of radioactivity in all aspects of otolaryngological research adds an extra quantitive dimension. Together with single photon emission computed tomography (SPECT), and the introduction of positron emission tomography (PET) to image the physiology of normal tissues and tumors, the use of radionuclide investigations can lead only to an increase in ear, nose, and throat (ENT) diagnostic sensitivity and specificity and, subsequently, to an overall improvement in the way head and neck cancer is diagnosed, staged, and treated.

Application: Detection and differentiation between thyrotoxicosis and solitary thyroid nodules by scintigraphic imaging.

Nuclear medicine in otolaryngology. Watkinson JC. Clin Otolaryngol 1990 Oct; 15 (5), pp. 457–69.

Thyroid nodules. Welker MJ, Orlov D. Am Fam Physician 2003 Feb 1; 67 (3), pp. 559–66.

GASTROINTESTINAL SYSTEM

Gastroesophageal Reflux/Gastric Motility *[^{99m}Technetium] ([^{99m}Tc])-Hydroxy Ethyl Starch*

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-hydroxy ethyl starch functions as a passive radioactive marker when mixed in a "meal" for the purpose of assessing gastroesophageal reflux while performing gastric motility studies.

Application: [^{99m}Tc]-hydroxy ethyl starch is an agent that is suitable for studies assessing gastroesophageal reflux and gastric motility.

Semi-Synthetic Methods: A reduction method based on stannous chloride has been developed for preparing hydroxy ethyl starch kits developed to characterize conditions of gastrointestinal reflux and gastric motility function. Verification of the consistency of radiolabeling reveals that high labeling efficiencies can consistently be achieved (> 95% when prepared at neutral pH) and products remain stable under conditions simulating the gastric environment.

Implementation: Evaluation and validation of [^{99m}Tc]-hydroxy ethyl starch as a liquid phase and solid phase gastric motility imaging radiotracer. Gastroesophageal reflux, liquid phase, and solid phase studies can then be conducted *in vivo* to assess the radiotracer stability.

Human Model: Case volunteers (n = 13).

Interpretation and Analysis: No absorption of any radioactivity from the gastrointestinal tract can be detected and suggests that [^{99m}Tc]-hydroxy ethyl starch is an agent that is suitable for gastroesophageal reflux and gastric motility studies. It is available in kit form and is a more "physiologically appropriate" agent compared to [^{99m}Tc]-sulphur colloid when used to prepare solid radioactive meals. [^{99m}Tc]-hydroxy ethyl starch represents a true carbohydrate meal and, unlike [^{99m}Tc]-sulphur colloid, is easy to prepare and can be conveniently optimized to produce a standardized vegetarian meal.

Development and validation of hydroxy ethyl starch kits for instant use in gastroesophageal reflux and gastric motility studies. Singh AK, Bhatnagar A, Singh T, Shankar LR. Nucl Med Commun 2002 Apr; 23 (4), pp. 373–7.

Inflammation/Absorption of the Intestine/ Intestinal Lesions: Non-Steroidal Anti-Inflammatory Drug (NSAID)-Associated/Intestinal Lesions: Crohn's Ileitis
[^{111}Indium] ([^{111}In]) Leukocytes/Tauro 23 [^{75}Se]-Selena-25-Homocholic Acid/ [^{58}Co]-Labeled Cyanocobalamine

Cellular/Molecular Mechanism of Action: The agent tauro 23 [^{75}Se]-selena-25-homocholic acid is a bile acid analog, while [^{58}Co]-labeled cyanocobalamine is an analogue of vitamin B_{12}. Radiolabeled [^{111}In] leukocytes behave *in vivo* like endogenous leukocytes of the same cell type.

Application: The agents tauro 23 [^{75}Se]-selena-25-homocholic acid and [^{58}Co]-labeled cyanocobalamine can be applied to evaluate intestinal absorption. Conversely, [^{111}In] leukocytes can be used to detect foci of septic and non-septic inflammation.

Route of Administration: Intravenous.

Implementation: Evaluation of residual inflammatory lesions following discontinuation of NSAIDs using [^{111}In] leukocyte scintigraphy imaging methodologies.

Human Model #1: Cases of rheumatoid arthritis (n = 90) and osteoarthritis (n = 7).

Human Model #2: Cases treated with NSAIDs (n = 19); healthy controls (n = 20); and Crohn's ileitis (n = 13).

Interpretation and Analysis: Using a [^{111}In] leukocyte imaging technique reveals that cases of rheumatoid arthritis and osteoarthritis administered NSAIDs are accompanied by the development of gastrointestinal lesions. More specifically, approximately two-thirds of all cases evaluated on long-term treatment display inflammatory lesions that may persist for up to 16 months following discontinuation. The prevalence and magnitude of intestinal inflammation is unrelated to the type and dose of non-steroidal drugs and previous or concomitant 2nd-line drug treatment. There is a significant inverse correlation ($r = -0.29$, $p < 0.05$) between fecal [^{111}In] excretion and hemoglobin levels in cases treated with NSAIDs. The kinetics of fecal [^{111}In] excretion in cases treated with NSAIDs is almost identical to that of cases with small bowel Crohn's disease. Small bowel radiological examination of cases on NSAIDs reveals that approximately one-sixth have asymptomatic ileal disease with ulceration and strictures (e.g., n = 3/18 cases). Evaluation of cases on anti-inflammatory agents, normal healthy controls, and cases of Crohn's ileitis with tauro 23 [^{75}Se]-selena-25-homocholic acid and [^{58}Co]-labeled cyanocobalamine reveals several abnormalities. On day 4, more than half the cases with rheumatoid arthritis have evidence of bile acid malabsorption, but the ileal dysfunction is much milder than seen in patients with Crohn's ileitis.

Non-steroidal anti-inflammatory drug–induced intestinal inflammation in humans. Bjarnason I, Zanelli G, Smith T, Prouse P, Williams P, Smethurst P, Delacey G, Gumpel MJ, Levi AJ. Gastroenterology 1987 Sep; 93 (3), pp. 480–9.

Lymphangiectasia: Intestinal
[^{99m}Technetium] ([^{99m}Tc])-Dextran

Background Information: Intestinal lymphangiectasia is a well-recognized complication of the Fontan procedure, occurring in up to 24% of cases. Because of the loss of chylous fluid into the gut lumen, protein-losing enteropathy results, as well as lymphopenia and hypogammaglobulinemia. In some cases, dilated lymphatics in the intestinal serosa or mesentery also rupture, causing chylous ascites. Standard medical and cardiac surgical interventions are generally ineffective and the condition is frequently fatal.

Application: Detection and characterization of protein-losing enteropathy.

Considerations: Condition is refractory to all standard medical therapies (e.g., dietary modifications, diuretics, corticosteroid therapy, albumin infusions, octreotide, heparin, bowel rest, and parenteral nutrition). Cardiac surgery to optimize hemodynamic status is also ineffective (continued loss of large volumes of pleural and ascitic fluid).

Implementation: Evaluation of intractable and life-threatening chylous ascites and chylothorax associated with intestinal lymphangiectasia and protein-losing enteropathy after a Fontan procedure for tricuspid atresia. One strategy includes [^{99m}Tc]-dextran scintigraphy performed to assess the extent of intestinal protein loss and the potential for surgical intervention. Scintigraphy can localize protein loss from the proximal jejunum and subsequent segmental resection can be effective. Postoperatively, ascites and pleural effusions can subsequently resolve without evidence of short bowel syndrome. Growth may ultimately increase and be accompanied by the onset of puberty if delayed. A mild intestinal protein loss may persist that may require diuretic therapy. Ascites or pleural effusions may be absent and cases remain asymptomatic for > 2 years postsurgery. Intestinal lymphangiectasia post-Fontan procedures has traditionally been ascribed to hemodynamic factors such as raised systemic venous pressure, which would predispose to a generalized intestinal lesion. However, scintigraphy can demonstrate a localized, surgically correctible lesion.

Successful resection of localized intestinal lymphangiectasia post-Fontan: Role of [^{99m}technetium]-dextran scintigraphy. Connor FL, Angelides S, Gibson M, Larden DW, Roman MR, Jones O, Currie BG, Day AS, Bohane TD. Pediatrics 2003 Sep; 112 (3 Pt 1), pp. e242–7.

Pancreatic Function and Disease
[^{125}I]-L-3 Iodo-α-Methyl Tyrosine ([^{125}I]-IMT)

Cellular/Molecular Mechanism of Action: [^{125}I]-IMT.

Considerations: The difficulty of a reliable diagnosis of pancreatic diseases by scintiscanning is due in part to the lack of adequate radiopharmaceuticals. The agent [^{125}I]-IMT has been previously applied for the purpose of diagnosing different kinds of brain tumors. Preliminary *in vivo* trials suggest that [^{125}I]-IMT can be used as a diagnostic agent for detecting pancreatic disease and can be produced using either [^{123}I] or [^{125}I] radioisotopes.

Experimental: L-α-methyl tyrosine can be labeled with [^{125}I] using I-/I03 and afterward purified by an anionic exchange resin. The labeling yield obtained is high (96.0 ± 0.5%) when the incubation time of 15 minutes is applied and no statistically significant difference can be appreciated with 1-hour incubation periods.
Interpretation and Analysis: Biodistribution studies (mice) show that the percentage of activity concentration within the pancreas is 34.24 ± 14.03% at 15 minutes postinjection and remains constant for 30 minutes. The pancreas/liver ratio 15 minutes after the injection of [^{125}I]-IMT approaches 12.22 ± 3.59 and remains constant for an additional 45 minutes.
Instrumentation: Computer-integrated nuclear medicine scintigraphic camera.

New methodology for pancreatic scintiscanning imaging using [^{125}I]-L-3 iodo-α-methyl tyrosine. Zubillaga MB, Bocciot JR, Gentile A, Mitta AE, Nicolini JO, Caro RA. Acta Physiol Pharmacol Ther Latinoam 1996; 46 (2), pp. 83–9.

Sepsis/Appendicitis/Inflammatory Bowel Disease (IBD)/Osteomyelitis/Soft Tissue Sepsis/Fever of Unknown Origin
[^{99m}Technetium] ([^{99m}Tc])-Hexamethylpropyleneamine Oxime (HMPAO)-Labeled Leukocytes

Cellular/Molecular Mechanism of Action: The preparation [^{99m}Tc]-HMPAO functions as a radiolabeling agent for leukocytes. Neutrophils are the cell type most widely used for labeling procedures of this nature. Radiolabeled neutrophils preferentially localize at sites of inflammation and sepsis in response to chemotactic stimuli.
Background Information: [^{99m}Tc]-HMPAO-labeled leukocytes are well established for their use in investigating inflammatory disease states. Their kinetics and normal distribution are similar to those of [^{111}In]-labeled leukocytes except for non-specific activity in urine, kidneys, gallbladder, and bowel, which results from the elution of secondary form of [^{99m}Tc]-labeled HMPAO complexes.
Application: Identification of septic and inflammatory foci (e.g., appendicitis).
Considerations: Diagnostic imaging with [^{99m}Tc]-HMPAO-labeled leukocytes is valuable for the detection of appendicitis and other intra-abdominal septic/inflammatory conditions. In 1 *in vivo* trial, 8 scans were interpreted as negative for acute appendicitis but were alternatively found to be positive for other conditions, including diverticulitis (n = 5 cases), neoplastic disease (n = 1 case), tuboovarian abscessation (n = 1 case), and small intestinal infarction (n = 1 case).
Interpretation and Analysis: In *in vivo* clinical trials, the sensitivity of [^{99m}Tc]-HMPAO leukocyte scans is 87%, with a specificity rate of 92% and an overall accuracy rate of 89% for the detection of septic/inflammatory foci.

The principal clinical indications for [^{99m}Tc]-HMPAO leukocytes include IBD, osteomyelitis, soft tissue sepsis, and, to a lesser extent, occult fever. The superior resolution and count density of [^{99m}Tc] places [^{99m}Tc]-HMPAO leukocytes at an advantage over [^{111}In] leukocytes in IBD, especially for the identification of small bowel involvement in patients with Crohn's disease. However, quantification of disease activity is more difficult than with [^{111}In]. [^{99m}Tc]-HMPAO leukocytes are indicated for most forms of acute soft tissue and abdominal sepsis, although when compared with [^{111}In], it may be more difficult to demonstrate communication between an abdominal abscess and bowel lumen. Chronic osteomyelitis, including infected joint prostheses, is better characterized with [^{111}In]-labeled leukocytes. Occult fever and fever of unknown origin (FUO) are more controversial. There is still a place for [^{67}Ga] in FUO, of which there is a wide spectrum of causes. Occult fever implies a pyogenic cause for an undiagnosed fever and should probably be imaged with [^{111}In] leukocytes. With the advances being made in other imaging modalities and in interventional radiology, there is a clear need for radionuclide agents that can be used for whole-body screening in cases with undiagnosed fever. Such agents may include [^{18}F]-fluorodeoxy-glucose and radiolabeled monoclonal antibodies to endothelial adhesion molecules activated at the foci of inflammation.

The role of radiolabeled leukocyte imaging in the management of patients with acute appendicitis. Kipper SL. Q J Nucl Med 1999 Mar; 43 (1), pp. 83–92.

The utility of [^{99m}Tc]-HMPAO leukocytes for imaging infection. Peters AM. Semin Nucl Med 1994 Apr; 24 (2), pp. 110–27.

Sepsis/Appendicitis
[^{99m}Technetium] ([^{99m}Tc])-Labeled Anti-CD15 IgM Immunoglobulin

Cellular/Molecular Mechanism of Action: Anti-CD15 immunoglobulin M monoclonal antibody recognizes and binds to CD15 complexes expressed on the exterior surface membrane of polymorphonuclear neutrophils. The difference between IgG and IgM immunoglobulin is that IgM is the principal immunoglobulin produced after the 1st exposure to an antigen, while IgG is the principal immunoglobulin produced after 2nd exposure to the same antigen. IgM is composed of a pentomer of IgG-like molecules and generally has less specific antigen-binding properties than does IgG. In addition, IgM has a higher propensity to generate multimolecular antigen/antibody complexes.
Application: Detection and monitoring of septic foci and lesions.
Route of Administration: Anti-CD15 immunoglobulin M monoclonal antibody (LeuTech: intravenous).
Dosage: Anti-CD15 immunoglobulin M monoclonal antibody (LeuTech: 10–20 mCi of [^{99m}Tc]).
Semi-Synthetic Methods: Preparations of anti-CD15 immunoglobulin M monoclonal antibody (e.g., LeuTech) avidly bind to circulating and sequestered human polymorphonuclear neutrophils *in vivo,* eliminating the need for *in vitro* cell labeling and the risks of blood handling.
Implementation: Evaluation of the safety and efficacy of anti-CD15 immunoglobulin M monoclonal antibody (LeuTech) imaging in cases of suspected acute appendicitis. An effective procedure includes the acquisition of serial dynamic and static planar images for up to 3 hours after intravenous administration. Scans are then interpreted as either positive or negative for acute appendicitis or other intra-abdominal (septic) infection. The institutional diagnosis is validated by surgery and histopathology of the appendix, results from other diagnostic studies, or 2-week clinical follow-up.

Human Model: Cases presenting with equivocal clinical signs (e.g., n = 99).
Interpretation and Analysis: Scans are usually positive in a very high percentage of cases of suspected appendicitis with validation of the condition at surgery (e.g., n = 39/40; sensitivity 98%). Conversely, images are negative for appendicitis in a large percentage of cases found not to have appendicitis (e.g., n = 49/58, specificity = 84%). Accuracy, positive predictive, and negative predictive values are approximately 90%, 81%, and 98%, respectively. In cases with appendicitis and positive scans more than 50% of the images are positive at 4 minutes, and essentially all images are positive by 1 hour. Mean time of 1st positive image is about 15 minutes. Serious adverse reactions are generally rare. Imaging with anti-CD15 immunoglobulin M monoclonal antibody (LeuTech) is considered a highly sensitive diagnostic agent for detecting appendicitis in equivocal

cases. Advantages of this agent over the other currently used radiotracers include greater convenience and shorter time to diagnosis, particularly with respect to the expedient nature with which acute appendicitis can be visualized on scintigraphic images.

Scintigraphic determination of equivocal appendicitis. Rypins EB, Kipper SL. Am Surg 2000 Sep; 66 (9), pp. 891–5.

HEMATOLOGY

Aluminum Overload/Toxicity/Hemodialysis/ Hematopoeitin/Iron Absorption: Iron Deficiency *Desferrioxamine/Deferoxamine/[^{59}Fe]*

Cellular/Molecular Mechanism of Action: Desferrioxamine and deferoxamine are metallic ion chelating agents. Desferrioxamine can induce elevations in basal plasma aluminum concentrations.

Background: The response to recombinant human erythropoietin (rHuEpo) is determined primarily by the availability of iron. In contrast to intravenous iron, oral iron supplementation is often insufficient for an optimal response.

Application: Estimation of iron and total iron binding capacity.

Implementation: Evaluation of iron absorption and the effects of iron status, aluminium status, and inflammation. In this context, iron mucosal uptake after 24 hours, iron retention after 2 weeks, and mucosal transfer of iron are determined with a whole-body counter using an oral dose of [^{59}Fe]. Iron absorption is measured once without and once after the ingestion of 2 g aluminium hydroxide.

Human Model: Cases on chronic hemodialysis and maintenance rHuEpo therapy (n = 19).

Interpretation and Analysis: On the basis of transferrin saturation, 2 dialysis case groups can be distinguished: cases with a functional iron deficiency (n = 9), and iron-replete cases (n = 10). In the iron-deficient dialysis case group, mucosal uptake, mucosal transfer, and iron retention are 49.9% ± 29.4, 0.73% ± 0.29, and 41.6% ± 32.2, being significantly lower than in a non-uremic iron deficient population ($p < 0.01$, $p < 0.05$, $p < 0.01$, respectively). In iron-replete dialysis cases, mucosal uptake, mucosal transfer, and iron retention are 20.0 ± 12.3, 0.59 ± 0.18, and 11.1 ± 6.7, mucosal uptake and iron retention being lower than in a normal iron-replete population ($p < 0.0005$ and $p < 0.003$ respectively). Dialysis cases with high C-reactive protein (CRP) values show lower iron absorption. Iron absorption data correlates significantly with transferrin saturation and CRP in the iron-deficient group, and with serum ferritin in the iron-replete group. Iron absorption decreases after an aluminium hydroxide challenge in the iron-deficient cases to the lower levels of the iron-replete subjects. Body aluminium stores, estimated by the desferrioxamine test, do not correlate with parameters of iron absorption. The absorption of iron in dialysis patients is decreased by hemodialysis, which may, at least in part, be due to inflammation. Aluminium ingestion further reduces absorption in functional iron-deficient patients.

Iron absorption in erythropoietin-treated hemodialysis patients: Effects of iron availability, inflammation, and aluminium. Kooistra MP, Niemantsverdriet EC, van Es A, Mol-Beermann NM, Struyvenberg A, Marx JJ. Nephrol Dial Transplant 1998 Jan; 13 (1), pp. 82–8.

Hepatic Function *[^{99m}Technetium] ([^{99m}Tc])-Diethylenetriamine Pentaacetic Acid-Galactosyl Human Serum Albumin*

Cellular/Molecular Mechanism of Action: Diethylenetriamine pentaacetic acid (DTPA) generally functions as a chelating agent, including heavy metal ion isotopes like [^{111}In]. [^{99m}Tc]-galactosyl human serum albumin (GSA) is removed from the intravascular compartment by viable hepatocytes.

Application: Characterization of hepatic function without collection of blood samples for *in vitro* sample analysis.

Route of Administration: Intravenous.

Implementation: Delineate the clinical utility of a graphical method for estimating liver uptake and blood retention of [^{99m}Tc]-GSA, using dynamic single photon emission computed tomography (SPECT) analysis. When considering the kinetics of [^{99m}Tc]-GSA, if it is assumed that (i) [^{99m}Tc]-GSA distributes only between blood and liver, and (ii) no metabolism of [^{99m}Tc]-GSA occurs during the observation period, a plot of liver counts vs cardiac blood-pool counts should, theoretically, be a straight line. From the slope and y-intercept of a regression line, coefficients for converting count based liver and blood-pool data to the percent injected dose (%ID) can be calculated. Applicability of the method can be delineated for dynamic SPECT data from cases of hepatic dysfunction. To validate this method, plasma concentrations (%ID/ml plasma) at 6, 15, and 30 minutes after the injection are estimated and compared with measured values. To investigate the clinical significance of the % liver uptake, the value obtained by this method is compared with results from conventional liver function tests, including serum albumin, the hepaplastin test, prothrombin time, and indocyanine green clearance.

Human Model: Hepatic disease (n = 30 cases with hepatic disease).

Interpretation and Analysis: In every data set, a plot of liver counts to cardiac blood-pool counts can be represented (fitted) well by a straight line ($p < 0.00001$). Plasma concentrations estimated by this method show good correlation with measured values at 6, 15, and 30 minutes after injection ($r = 0.748$, 0.838, 0.875, respectively; $p < 0.0001$). The liver uptake determined by the method shows good correlation with results from conventional hepatic function tests ($p < 0.002$). The described graphical method could provide an accurate estimate of %ID of [^{99m}Tc]-GSA in blood without the need for blood sampling. The liver uptake determined by this method could be a simple but useful quantitative indicator of hepatic function.

Estimation of fractional liver uptake and blood retention of [^{99m}Tc]-DTPA-galactosyl human serum albumin: An application of a simple graphical method to dynamic SPECT. Shuke N, Aburano T, Okizaki A, Zhao C, Nakajima K, Yokoyama K, Kinuya S, Watanabe N, Michigishi T, Tonami N. Nucl Med Commun 2003 May; 24 (5), pp. 503–11.

Hepatic Function *[^{99m}Technetium] ([^{99m}Tc])-Galactosyl-Neoglycoalbumin*

Application: Assess hepatic function.

Route of Administration: [^{99m}Tc]-galactosyl neoglycoalbumin (intravenous).

Dosage: [^{99m}Tc]-galactosyl neoglycoalbumin (150 mBq at a rate of 3.5 mg [50 nmol; 1 ml]).

Implementation: Simulation of [^{99m}Tc]-galactosyl neoglycoalbumin kinetics allows quantification of galactosyl neoglycoalbumin binding to (human) hepatic binding protein. In conditions of (i) viral hepatitis A, hepatitis B, non-A/b hepatitis with increases in hepatic binding protein concentration (up to 3 times the initial concentration), binding rate constant, and hepatic blood flow; and (ii) hepatitis B and cytomegalovirus infection, [^{99m}Tc]-galactosyl neoglycoalbumin can be a valuable agent used in conjuction with clinical pathology parameters to assess the return of hepatic function. In monitoring the prolonged course of such disease states, it can be noted that binding protein increases from 0.41 ± 0.11 μmol/L after the onset of acute hepatitis (n = 12) to 0.78 ± 0.21 μmol/L after 6 months of follow-up (n = 10 cases; $p < 0.001$). During this period, binding rate

constant (72.4 ± 12.6 vs 82 ± 11.5 μmol/L/sec; $p < 0.05$) and hepatic blood flow increases (0.027 ± 0.0051 vs 0.031 ± 0.0083 L/sec; $p < 0.05$). Hepatic binding protein concentration correlates highly with actual laboratory test results for liver function ($r = 0.98$; $p = 0.0001$).

Interpretation and Analysis: The liver is the exclusive site of trace uptake for [^{99m}Tc]-galactosyl neoglycoalbumin administered intravenously. Scintigraphic evaluation of functional liver cell mass using the receptor-tracer [^{99m}Tc]-galactosyl neoglycoalbumin can provide an *in vivo* diagnostic strategy for quantifying liver function and assessing liver morphology. In addition, research findings suggest that changes in hepatic binding protein-receptor concentration are likely to occur *in vivo.*

Liver function in acute viral hepatitis as determined by a hepatocyte-specific ligand: [^{99m}Tc]-galactosyl-neoglycoalbumin. Virgolini I, Müller C, Höbart J, Scheithauer W, Angelberger P, Bergmann H, O'Grady J, Sinzinger H. Hepatology 1992 Apr; 15 (4), pp. 593–8.

Hepatic Function
[^{123}I], [^{125}I], or [^{131}I] Radioiodinated Chylomicrons

Cellular/Molecular Mechanism of Action: Chylomicrons are rapidly assimilated and metabolized by the liver (hepatocytes). Cholesteryl is a normal constituent of the hydrophobic core of chylomicrons.

Application: Cholesteryl iopanoate (II), is an iodinated analogue of a normal constituent of the hydrophobic core of chylomicrons that mimics the biology and physiology of chylomicron assimilation and metabolism by the liver.

Route of Administration: Intravenous.

Synthetic Chemistry and Analysis: Cholesteryl iopanoate (II) is synthesized from cholesterol and iopanoic acid (I) and then subsequently radiohalogenated (e.g., [^{123}I], [^{125}I], or [^{131}I]).

Diagnostic Interpretation and Implications: Intravenous administration of cholesteryl iopanoate (II) in physiological saline results in the appearance of approximately 31% of the dose in the liver at 0.5 hours postinjection. Prior incorporation of cholesteryl iopanoate (II) into chylomicrons results in an almost 3-fold (87%) increase in the liver accumulation of cholesteryl iopanoate (II) during the same time period. A more gradual appearance of II in steroid-secreting tissues is consistent with the association of cholesteryl iopanoate (II) with high-density lipoproteins following administration.

Potential tumor- or organ-imaging agents XXIV: Chylomicron remnants as carriers for hepatographic agents. Damle NS, Seevers RH, Schwendner SW, Counsell RE. J Pharm Sci 1983 Aug; 72 (8), pp. 898–901.

Hepatic Function: Cirrhosis and Chronic Liver Disease
[^{99m}Technetium] ([^{99m}Tc])-Phytate Colloid

Cellular/Molecular Mechanism of Action: Colloid preparations are assimilated internally by perfused Kupffer cells within the hepatic sinusoids.

Background Information: Accurate quantitative determination of liver function is critical in cirrhotic conditions in order to predict an outcome, particularly in cases undergoing hepatic resection or non-hepatic surgery. As colloid uptake by perfused Kupffer cells is proportional to perfused hepatocyte mass, quantitative liver-spleen scan may be used as an index of perfused hepatocyte mass.

Application: Non-invasive quantitative test for assessment of hepatic function and severity of liver disease in cirrhotic patients.

Route of Administration: Intravenous.

Implementation: Evaluate hepatic function in cirrhotic conditions utilizing [^{99m}Tc]-phytate colloid uptake by the liver as a quantitative test assessed by single photon emission computed tomography (SPECT). In this context, quantitative SPECT can be used to measure liver volume as a function of quantitative colloid uptake by the liver and percentage of injected dose/ml of liver tissue.

Human Model: ($n = 75$ cirrhosis cases; $n = 52$ non-cirrhosis cases with chronic liver disease; $n = 36$ cases without hepatic disease).

Interpretation and Analysis: Although liver volume is similar among the 3 groups, the cirrhotic cases have significantly lower total quantitative uptake and quantitative uptake/ml compared to non-cirrhosis cases with chronic liver disease and cases without hepatic disease ($p < 0.001$). Quantitative liver uptake in cirrhotic conditions is highly correlated with Child-Pugh score values ($r = 0.64$, $p < 0.0001$) and with indocyanine green retention at 15 minutes ($r = 0.84$, $p < 0.0001$). Quantitative SPECT of the liver may be an additionally useful, non-invasive quantitative test for assessment of hepatic function and severity of liver disease in cirrhotic conditions.

Quantitative liver-spleen scan using single photon emission computerized tomography (SPECT) for assessment of hepatic function in cirrhotic patients. Zuckerman E, Slobodin G, Sabo E, Yeshurun D, Naschitz JE, Groshar D. J Hepatol 2003 Sep; 39 (3), pp. 326–32.

Hepatic Function: Jaundice/Infant Biliary Atresia/ Hepatic Jaundice in Frail and Compromised Patients
[^{131}I]-Rose Bengal (Fecal Excretion Test)/ [^{99m}Technetium] ([^{99m}Tc])-Pyridoxylidene Glutamate (PG)

Cellular/Molecular Mechanism of Action: Pyridoxylidene glutamate and rose bengal are both removed from the plasma by viable hepatocytes.

Application: Assess postoperative bile drainage with repeat scans in infants with biliary atresia. Scintigraphic imaging with [^{99m}Tc]-PG may also be useful as a screening test in difficult cases of jaundice, especially in frail patients, and cases with complications.

Route of Administration: Intravenous.

Implementation: Evaluation of hepatic function in cases of systemic jaundice applying [^{99m}Tc]-PG for nuclear scan imaging.

Human Model: Cases of systemic jaundice ($n = 24$ adults with mean total bilirubin = 255 μmol/l; $n = 11$ infants with prolonged obstructive jaundice and mean total bilirubin = 165 μmol/l).

Interpretation and Analysis: Absence of radioactivity in the gastrointestinal tract is interpreted as complete biliary obstruction and assumed to be the cause of jaundice (e.g., $n = 10/24$ adult; and $n = 6$ infant jaundice cases). Using this criterion, occlusion or patency of the bile ducts can be correctly determined in a high percentage of cases (e.g., $n = 21/24$ adults; and $n = 6/11$ infants). A low percentage of jaundice cases display either false-negative gut images (e.g., $n = 1/21$ adults; and $n = 2/11$ infants) or inconclusive results (e.g., $n = 2/24$ adult; and $n = 1/11$ infants). Scans are unable to show details about the site of obstruction or pathology but the technique is simple, atraumatic, and safely performed in cases with serious complications (e.g., renal failure, coagulation defects, septicemia). In infants, [^{99m}Tc]-PG scans compare well with the [^{131}I]-rose bengal fecal excretion tests and with liver biopsies in evaluations of prolonged obstructive jaundice.

Hepatobiliary imaging: The use of [^{99m}Tc]-pyridoxylidene glutamate scanning in jaundiced adults and infants. Jenner RE, Howard ER, Clarke MB, Barrett JJ. Br J Radiol 1978 Nov; 51 (611), pp. 862–6.

Hepatic/Hepatic Contrast Agent: Backdiffusion Phenomenon
Diethyl Iminodiacetic Analogues (Diethyl IDA)/ Mebrofenin

Background Information: The kinetics of organic anions is well described and backdiffusion from hepatocyte to plasma is accepted phenomenon. Although iminodiacetic (IDA) analogues, as organic anions, should also show bidirectional transport between hepatocyte and plasma, this has not been directly demonstrated.
Application: Hepatic contrast image-enhancing agent for magnetic resonance imaging modalities.
Route of Administration: Diethyl iminodiacetic analogues (diethyl IDA; intravascular); mebrofenin (intravascular).
Implementation: Evaluate backdiffusion characteristics of hepatic contrast agents into the intravascular compartment and quantify the corresponding fractional rate constant. Kinetics of diethyl IDA can be characterized through the analysis of femoral artery and hepatic venous samples collected following the injection of tracer preparations into a (i) peripheral vein, or (ii) hepatic artery or portal vein. Arterial time-concentration curves can also be compared between peripheral venous and either hepatic arterial or portal venous injections (canine). Complementary findings can be obtained by generating time-activity curves recorded from regions-of-interest (ROIs) over the cardiac blood-pool and peripheral hepatic parenchyma during routine IDA hepatobiliary imaging with diethyl IDA or mebrofenin and fractional rate constants for clearance of IDA from the hepatocyte compared to compartmental and deconvolution analyses (e.g., n = 30 cases).
Animal Model: Anesthetized dogs (e.g., n = 3).
Human Model: Hepatobilliary imaging with diethyl IDA (n = 30 cases).
Interpretation and Analysis: Following peripheral injection, there is an early arteriovenous concentration gradient that develops across the liver indicating a hepatocyte extraction fraction of 0.9 (dog #1), 0.8 (dog #2), and 0.6 (dog #3). The net extraction fraction decreases exponentially over a 40-minute period. Time-concentration curves for the hepatic vein and femoral artery are virtually superimposed following intrahepatic injections. Peripheral arterial curves, however, have different shapes according to whether injections are delivered intrahepatic or peripherally, and are consistent with the occurrence of significant backdiffusion. In human clinical cases, the blood disappearance curves can be fitted as the sum of 2 exponentials and the liver curves as the difference of 2 exponentials (rate constants = alpha1h and alpha2h). Based on compartmental analysis of the blood curves, the sum of the fractional rate constants of tracer movement from hepatocyte to bile canaliculus (k_{32}) and to plasma (k_{12}) is similar to and correlates with the rate constant, alpha, for the hepatocyte impulse response function ($r = 0.62$, $n = 30$, $p < 0.001$). In contrast, alpha1h and alpha2h are respectively clearly greater and smaller than alpha. Moreover, neither of these hepatic rate constants correlates with alpha. Diffusion of IDA from hepatocyte into the blood is significant and even in the presence of normal liver function accounts for about 50% of IDA transport out of the hepatocyte. It should be taken into account in pharmacokinetic studies based on either compartmental or deconvolution analysis.

Bidirectional transport of iminodiacetic organic anion analogues between plasma and hepatocyte. Peters AM, Myers MJ, Mohammadtaghi S, Mubashar M, Mathie RT. Eur J Nucl Med 1998 Jul; 25 (7), pp. 766–73.

IMMUNE SYSTEM AND INFLAMMATORY (SEPTIC/ NON-SEPTIC) RESPONSES

Refer to the neoplastic disease chapter (chapter 12) in Section II for references related to primary and metastatic neoplastic disease states and evaluation of sentinel lymph nodes.

Abscess Formation/Non-Septic Inflammatory Responses
[^{99m}Technetium] ([^{99m}Tc])-Interleukin-8 (IL-8)

Cellular/Molecular Mechanism of Action: Interleukin-8 recognizes and binds to IL-8 receptor complexes expressed on the exterior surface membrane of neutrophil and monocyte populations.
Physiological Mechanism of Action: Interleukin-8 can function as a neutrophil chemotactic agent, but this has little relevance to the use of this agent as an *in vivo* diagnostic agent in the field of nuclear medicine.
Route of Administration: [^{99m}Tc]-IL-8 (intravenous).
Implementation: Evaluation of the specificity of [^{99m}Tc]-IL-8 for detecting focal non-septic abscess lesions as a function of biodistribution patterns and pharmacokinetics profiles. Complementary analyses include determination of the interaction of [^{99m}Tc]-IL-8 with blood cells in circulation, which can be achieved through characterization of the radiolabel in circulating leukocytes, red blood cells, and plasma. The *in vivo* kinetics of [^{99m}Tc]-IL-8 can be studied by quantitative analysis of whole-body images acquired between 0 and 6 hours postinjection. The results of this analysis (*in vivo* biodistribution) can be validated by *ex vivo* counting of radioactivity in dissected tissues.
Animal Model: Rabbit (turpentine-induced abscesses in neutropenic and normal rabbits).
Interpretation and Analysis: Abscess uptake (percentage of injected dose per gram of tissue [%ID/g] ± standard error of measurement [SEM]) in immunocompetent scenarios (0.41 ± 0.05) is 10 times higher than in neutropenic conditions (0.038 ± 0.014), demonstrating target specificity for [^{99m}Tc]-IL-8 uptake. Abscess-to-muscle ratios ± SEM are also 10 times higher (110 ± 10 vs 10 ± 5). Lung and spleen uptake is 3-fold less with conditions of neutropenia compared to normal reference controls. The blood clearance of the radiolabel in conditions of neutropenia is similar to that in normal reference controls. In the vascular compartment, most of the [^{99m}Tc]-IL-8 (70%) is found in the plasma. Less than one-third is associated with red blood cells, and only a very low percentage (< 2.5%) is associated with white blood cell populations. Image analysis reveals a gradual increase in abscess uptake over time up to > 15 %ID, which can be confirmed by *ex vivo* gamma counting of infected muscle. The highest increase in abscess uptake is observed > 2 hours postinjection when most of [^{99m}Tc]-IL-8 has cleared from the blood, suggesting specific neutrophil-mediated accumulation of [^{99m}Tc]-IL-8 within abscesses. Furthermore, region-of-interest (ROI) analysis reveals that gradual accumulation of [^{99m}Tc]-IL-8 in abscesses is accompanied by a simultaneous clearance of activity from the lungs, suggesting that neutrophil-associated [^{99m}Tc]-IL-8 is initially trapped within the lungs but migrates to abscesses at a later time point (e.g., favoring neutrophil-bound transportation from the lungs to abscesses). Substantial support is given for the hypothesis that [^{99m}Tc]-IL-8 localizes within abscesses, primarily due to physical binding to peripheral neutrophils. Accumulation in abscesses is highly specific and a neutrophil-driven process. As assessed through *in vivo* and *ex vivo* analyses, the total fraction that accumulates in the inflamed tissue is extremely high (up to > 15 %ID) compared with that of other agents used for imaging infection and inflammation.

Kinetics of [^{99m}Tc]-labeled interleukin-8 in experimental inflammation and infection. Rennen HJ, Boerman OC, Oyen WJ, Corstens FH. J Nucl Med 2003 Sep; 44 (9), pp. 1502–9.

Abscess Formation/Septic Disease
[^{99m}Technetium] ([^{99m}Tc])-HYNIC-C5adR/ [^{99m}Tc]-HYNIC-C5a

Cellular/Molecular Mechanism of Action: Complement fraction C5a and its natural metabolite C5adR recognize and bind to receptor complexes expressed on the exterior surface membrane of neutrophil and monocyte populations.

Application: The complement anaphylatoxin C5a and its natural metabolite C5a des Arg(74) (C5adR) are involved in several stages of the inflammatory process. Both act on a common receptor expressed on different cell types, including neutrophils and monocytes. The receptor binding affinity of C5a is in the nanomolar range and exceeds that of C5adR by 1 to 2 orders of magnitude. The biological potency of C5a is considerably higher than that of C5adR.

Semi-Synthetic Methods: Labeling of proteins can be achieved utilizing [^{99m}Tc] facilitated by initial conjugation with the chelating agent hydrazinonicotinamide (HYNIC).

Implementation: Evaluation of intramuscular septic abscesses induced by bacterial injection utilizing [^{99m}Tc]-HYNIC-C5a and [^{99m}Tc]-HYNIC-C5adR as imaging agents. Biodistribution of the radiolabel can be determined by gamma camera imaging and by counting dissected tissues at 5 hours postinjection.

Interpretation and Analysis: Complement fractions [^{99m}Tc]-HYNIC-C5a and [^{99m}Tc]-HYNIC-C5adR display *in vivo* abscess uptake values of 0.12 and 0.025 %ID/g, abscess/muscle ratios of 76 and 14, abscess/blood ratios of 9.1 and 2.6, and region-of-interest (ROI)-derived target-to-background ratios of 5.9 and 2.1, respectively, at 5 hours postinjection. Therefore, [^{99m}Tc]-HYNIC-C5a provides superior capabilities as an agent for *in vivo* imaging of foci of sepsis (e.g., abscess formation). However, C5a is a very bioactive protein, a quality that impedes its clinical use as an infection imaging agent. The naturally occurring partial agonist C5adR has less biological effect but shows suboptimal imaging characteristics. Therefore, adequate localization of a receptor binding ligand with affinities for the receptor in the nanomolar range is required.

[^{99m}Tc]-labeled C5a and C5a des Arg74 for infection imaging. Rennen HJ, Oyen WJ, Cain SA, Monk PN, Corstens FH, Boerman OC. Nucl Med Biol 2003 Apr; 30 (3), pp. 267–72.

Abscess Formation/Septic Disease
[^{99m}Technetium] ([^{99m}Tc])-HYNIC-Interleukin-8 (IL-8)

Cellular/Molecular Mechanism of Action: Interleukin-8 recognizes and binds to IL-8 receptor complexes expressed on the exterior surface membrane of neutrophil and monocyte populations.

Background Information: Previous investigations have demonstrated that IL-8 can be labeled with [^{99m}Tc] using hydrazinonicotinamide (HYNIC) as bifunctional coupling agent and tricine as a coligand. Such preparations possess excellent characteristics for imaging infections (e.g., rabbit model of soft-tissue infection).

Route of Administration: [^{99m}Tc]-HYNIC-IL-8 (intravenous).

Semi-Synthetic Methods: Propylaldehyde hydrazone formulation of HYNIC can be introduced into stabilized HYNIC-IL-8 and [^{99m}Tc]-HYNIC-IL-8 prepared using 5 different coligand formulations. In this context, HYNIC-conjugated IL-8 can be radiolabeled with [^{99m}Tc] in the presence of either (i) tricine; (ii) ethylenediaminediacetic acid (EDDA); (iii) tricine and trisodium triphenylphosphinetrisulfonate (TPPTS); (iv) tricine and nicotinic acid (NIC); or (v) tricine and isonicotinic acid (ISONIC). These preparations can be characterized *in vitro* by (i) reverse-phase high-pressure liquid chromatography (RP-HPLC) analysis; (ii) measurement of octanol/water partition coefficient parameters; (iii) delineation of molecular stability; and (iv) receptor affinity analysis (ligand binding assays).

Implementation: Evaluation of the *in vivo* biodistribution of [^{99m}Tc]-HYNIC-IL-8 in conditions of induced localized sepsis (*Escherichia coli*–induced soft-tissue infection), which can be assessed by gamma camera imaging and counting of tissue samples at 6 hours postinjection.

Animal Model: Rabbit (*E. coli*–induced soft-tissue infection).

Interpretation and Analysis: Specific activity (MBq/μg) is highest for ISONIC (up to 80) > TPPTS (40) > tricine (15) > EDDA (7). RP-HPLC and octanol/water partition coefficients show a shift toward higher lipophilicity for TPPTS preparations. The leukocyte receptor binding fractions are around 40–55% for all preparations except for TPPTS, which displays predominantly nonspecific binding. All preparations are stabile in serum, but the stability in phosphate buffered saline (PBS) is highest for NIC and TPPTS > EDDA > ISONIC > tricine. The *in vivo* biodistribution shows highest abscess/muscle ratios for NIC and ISONIC (> 200) > EDDA and tricine (approximately 100) > TPPTS (< 40). Gamma camera imaging rapidly visualize abscess from 2 hours postinjection onward for all formulations. The abscess/background (A/B) at 6 hours postinjection for ISONIC is significantly higher ($p < 0.05$) than that of tricine and the A/B of TPPTS is significantly lower ($p < 0.05$). IL-8 can be rapidly and easily labeled with [^{99m}Tc] using HYNIC as a chelator group when applied in combination with various coligands. The most desirable infection-imaging characteristics can be realized with formulations using nicotinic acid/tricine as coligand system, which provides the features of high specific activity and high *in vitro* stability with high abscess/muscle ratios (> 200) and high abscess/background ratios (> 20). Protein doses administered are as low as 70 ng/kg body weight so side effects are not to be expected in the human system. This paves the way for infection-imaging studies in patients.

Instrumentation: Gamma camera imaging.

Effects of coligand variation on the *in vivo* characteristics of [^{99m}Tc]-labeled interleukin-8 in detection of infection. Rennen HJ, van Eerd JE, Oyen WJ, Corstens FH, Edwards DS, Boerman OC. Bioconjug Chem 2002 Mar–Apr; 13 (2), pp. 370–7.

Sepsis Abscess Formation/ Bacterial (*Escherichia Coli*)-Induced Abscesses/ Comparison of Radioiodination Methods/ Plasma Kinetic Profiles: $T_{1/2}$
[^{123}I]-Interleukin-8 (IL-8)

Background Information: Several small receptor-binding agents have been tested for imaging foci infection and inflammation. The potential of chemotactic peptides and of interleukins is promising and superior to that of conventional agents.

Application: Detect and characterize conditions of septic and non-septic inflammation.

Route of Administration: [^{123}I]-IL-8 (intravenous).

Dosage: [^{123}I]-IL-8 (18.5 MBq).

Semi-Synthetic Methods: Radiolabeling of biological protein fractions with radioiodine ([^{123}I], [^{124}I], [^{125}I], [^{127}I]) facilitated by the use of Bolton-Hunter reagent.

Implementation: Evaluate the potential of IL-8 to image infection (septic lesions). Similar to other biological protein fractions, IL-8 can be radiolabeled by several techniques, including the use of

[^{123}I] in concert with Bolton-Hunter reagent. Imaging of septic abscesses can be performed 24 hours postinduction with [^{123}I]-IL-8 preparations. Gamma camera images are obtained at 5 minutes and at 1, 4, and 8 hours after injection. Biodistribution is determined 8 hours after injection. Gamma camera imaging is then performed at 5 minutes and at 1, 4, and 8 hours postinjection with biodistribution profiles established at 8 hours postinjection.

Animal Model: Rabbits (*E. coli*-induced abscesses in the left thigh muscle).

Interpretation and Analysis: Radioiodinated [^{123}I]-IL-8 is rapidly cleared from the blood. Accumulation of [^{123}I]-IL-8 in abscesses is visible as early as 1 hour postinjection. The highest abscess uptake is obtained 4 hours after injection (2.6 ± percent injected dose [%ID]), whereas [^{123}I]-IL-8 rapidly clears from all other tissues. Approximate increases in abscess-to-background ratios are 13.0 ± 0.7 (8 hours postinjection), as determined by quantification of images. In tissue biodistribution evaluations (8 hours postinjection), abscess uptake is 0.057 ± 0.011 %ID/g with abscess-to-contralateral muscle ratios of 114.7 ± 23.0. The radioiodination method clearly affects the *in vivo* biodistribution of IL-8 because IL-8 iodinated using the Iodo-Gen method clears significantly slower from the blood and most other organs, resulting in poor visualization of the abscess. The superior characteristics of radioiodinated IL-8 using the Bolton-Hunter method include high abscess uptake and rapid background clearance within a few hours. These features make IL-8 a promising agent for imaging infection and inflammation.

Radiolabeled interleukin-8: Specific scintigraphic detection of infection within a few hours. van der Laken CJ, Boerman OC, Oyen WJ, van de Ven MT, van der Meer JW, Corstens FH. J Nucl Med 2000 Mar; 41 (3), pp. 463–9.

Abscess Formation: Septic Inflammatory Disease
[^{111}Indium] ([^{111}In])-DPC11870-11/ Leukotriene B_4 (LTB_4) Receptor-Binding Ligands

Cellular/Molecular Mechanism of Action: The agent DPC11870-11 is an antagonist of LTB_4 at its respective receptor complex.

Background Information: Several radiolabeled chemotactic peptides have been tested for their suitability to detect regions or foci of infection and inflammation. Leukotriene B_4 receptor-binding ligands can be useful agents for revealing neutrophil infiltrations because the LTB_4 receptor is abundantly expressed on neutrophils after an inflammatory stimulus.

Route of Administration: [^{111}In]-DPC11870-11 (intravenous).

Implementation: Evaluate the *in vivo* and *in vitro* characteristics of a hydrophilic [^{111}In]-labeled LTB_4 antagonist for detecting septic foci applying scintigraphic imaging techniques. The LTB_4 antagonist DPC11870-11 can be radiolabeled with [^{111}In] to produce an imaging agent for detecting infection and inflammation. Complementary investigations can entail the delineation of the pharmacokinetics and biodistribution of [^{111}In]-DPC11870-11 aided by utilizing serial scintigraphic imaging (0–24 hours after injection) and by *ex vivo* counting of dissected tissue samples (6 and 24 hours after injection). Receptor-mediated *in vivo* localization of the radiobiopharmaceutical agent can also be more precisely determined following the administration of an excess of non-radioactive indium-labeled agent 2 minutes before the administration of the [^{111}In]-labeled LTB_4 antagonist.

Animal Model: Rabbit (n = 3 New Zealand White rabbits with *Escherichia coli* infection induced in the left thigh muscle).

Interpretation and Analysis: In rabbits with intramuscular *E. coli* infection, abscesses can be visualized as early as 2 hours after injection. Accumulation in the abscess increases with time, resulting in the acquisition of excellent images at 6 hours postinjection. Blood clearance is rapid in the initial hours after injection (alpha-half-life = 30 ± 6 minutes, 85%; beta-half-life = 25.7 ± 0.8 hours, 15%). Abscess-to-background ratios, as derived from the region-of-interest (ROI) analysis, increase to 34 ± 7 at 24 hours after injection. Images of both groups show moderate uptake in the liver, spleen, kidneys, and bone marrow. No activity can be appreciated in the bladder, indicating almost complete retention in the kidneys. Accumulation within the abscess can be blocked completely by the injection of an excess of non-radioactive agent, indicating a specific receptor-ligand interaction of the radiolabeled agent in infected/septic tissues. Biodistribution data show that after saturation of the LTB_4 receptor, the abscess uptake, in percent injected dose per gram, is significantly reduced (0.03 ± 0.02 vs 0.24 ± 0.06, $p = 0.008$). The modified LTB_4 antagonist identifies/detects infectious foci rapidly after injection because of specific receptor-ligand interactions. Due to high abscess-to-background ratios, and the fact that no accumulation of radioactivity is observed in the gastrointestinal tract, this compound has excellent characteristics for revealing infectious and inflammatory foci.

A bivalent leukotriene B_4 antagonist for scintigraphic imaging of infectious foci. van Eerd JE, Oyen WJ, Harris TD, Rennen HJ, Edwards DS, Liu S, Ellars CE, Corstens FH, Boerman OC. J Nucl Med 2003 Jul; 44 (7), pp. 1087–91.

Abscess Formation: Sepsis/Leukotriene B_4 (LTB_4) Receptor Complexes
[^{99m}Technetium] ([^{99m}Tc])-RP517

Cellular/Molecular Mechanism of Action: The agent [^{99m}Tc]-RP517 recognizes and physically binds to LTB_4 receptor complexes.

Background Information: In a search for a rapid and accurate imaging agent for scintigraphic detection of infection and inflammation, an LTB_4 receptor antagonist, [^{99m}Tcm]-RP517, has been developed that contains a hydrazino nicotinamide moiety.

Application: Systemic detection and characterization of septic foci, including organized abscesses.

Route of Administration: [^{99m}Tc]-RP517 (intravenous).

Implementation: Determination of the *in vivo* behavior of [^{99m}Tc]-RP517 and its capacity to detect created abscesses utilizing gamma camera imaging in addition to establishing the *ex vivo* biodistribution of the agent several hours postinjection. Complementary studies entail cannulation of the choledochal duct for quantitative monitoring of hepatobiliary radiopharmaceutical clearance.

Animal Model: Rabbit septic abscesses induced by intravenous injection of *Escherichia coli* bacteria.

Animal Model: Rabbit (choledochal duct cannulation).

Interpretation and Analysis: The receptor binding fraction of the radiolabeled RP517 exceeds 70%. Accumulation of [^{99m}Tc]-RP517 within abscesses can be visualized as early as 1 hour postinjection. Due to rapid blood clearance ($t_{1/2}$ alpha = 18 ± 0.6 minutes, $t_{1/2}$ beta = 6.5 ± 0.4 hours) and high abscess uptake, the abscess-to-muscle ratios increase with time from 7.0 ± 2.3 at 1 hour postinjection to 44.3 ± 4.6 at 20 hours postinjection. The agent is mainly cleared via the hepatobiliary route: 50% of the radiolabel can be recovered in the small bowel at 1 hour postinjection, whereas 85% can be found in the cecum and sigmoid at 20 hours postinjection. In conclusion, [^{99m}Tc]-RP517 can facilitate rapid visualization of *E. coli* abscesses in rabbits. The agent is rapidly cleared from the blood, mainly via the hepatobiliary route. High abscess-to-background target ratios are feasible, but accumulation within the intestines may limit the applicability of the agent for detecting infectious processes within the abdominal cavity. The development of a more hydrophilic

analogue of [^{99m}Tc]-RP517 could improve the clinical applicability of the imaging agent.

A [^{99m}Tc]-labeled leukotriene B_4 receptor antagonist for scintigraphic detection of infection in rabbits. Brouwers AH, Laverman P, Boerman OC, Oyen WJ, Barrett JA, Harris TD, Edwards DS, Corstens FH. Nucl Med Commun 2000 Nov; 21 (11), pp. 1043–50.

Abscess Formation/Septic (*Staphylococcus Aureus*) *[^{99m}Technetium] ([^{99m}Tc])-Biotin-PEG-Distearoylphosphatidylethanolamine Avidin*

Cellular/Molecular Mechanism of Action: Avidin binds with high affinity to biotin to form a complex. In this investigation, Avidin is applied (injected) separately to accelerate the clearance of biotinlyated liposomes from the intravascular compartment after a portion of the dose has accumulated within the septic target lesion.
Application: Detection and characterization of localized regions of septic inflammation (e.g., abscess).
Route of Administration: Intravascular.
Semi-Synthetic Methods: Biotinylated liposomes can be radiolabeled with [^{99m}Tc]-hydrazinonicotinamide.
Implementation: Evaluation of biotin coupled to PEG-distearoylphosphatidylethanolamine (DSPE) that is incorporated into PEG liposomes and then radiolabeled with [^{99m}Tc]-hydrazinonicotinamide. *In vitro* binding studies can be performed to determine the optimal biotin concentration in liposomes. In rats the biodistribution of [^{99m}Tc]-biotin-PEG liposomes is then compared with the biodistribution of normal (non-biotinylated) [^{99m}Tc]-PEG liposomes. Complementary *in vivo* investigations can be employed to assess both the effect of the biotin content and the optimal avidin dose for efficient clearance of liposomes. Liposomes containing 0.5 or 1.0 mol% biotin-PEG-DSPE are then compared *in vivo* for their ability to accumulate within septic foci. Avidin is subsequently injected 4 hours after injection of liposomes.

Animal Model: Rats (septic foci created by injection of *S. aureus* into the left calf musculature). Avidin is then injected 4 hours after injection of liposome preparations.
Interpretation and Analysis: Biotinylation of the liposomes does not affect their *in vivo* behavior. All biotin-PEG liposome formulations show good *in vitro* avidin binding with 50% inhibitory concentrations ranging from 36 to 8 μmol/L. With avidin doses higher than 100 μg, both preparations are rapidly cleared from the circulation. As a result, abscess-to-blood ratios increase 5-fold. In an *in vivo* environment, avidin promotes clearance of radiolabeled PEG liposomes and improves the detection of subcutaneous *S. aureus* abscesses applying [^{99m}Tc]-biotin-PEG liposomes as an imaging agent. The infection can best be visualized only after injection of 100 μg avidin. Therefore, biotin-coated [^{99m}Tc]-PEG liposomes in combination with the injection of avidin can lead to improved imaging of infection or non-septic inflammation, especially when localized in regions with high blood-pool activity.

Improved imaging of infections by avidin-induced clearance of [^{99m}Tc]-biotin-PEG liposomes. Laverman P, Zalipsky S, Oyen WJ, Dams ET, Storm G, Mullah N, Corstens FH, Boerman OC. J Nucl Med 2000 May; 41 (5), pp. 912–8.

Abscess Formation: Septic/Targeting Method Optimization *Anti–Diethylenetriamine Pentaacetic Acid (DTPA) Antibody/[In]-DTPA-Bovine Serum Albumin/ [^{111}Indium] ([^{111}In])-DTPA (Bivalent)/ [3-Phase Injection Scheme]*

Application: A 3-step approach to imaging infectious and inflammatory foci has been developed and optimized. The approach relies on the non-specific localization of an anti-DTPA antibody in inflamed tissue.
Route of Administration: Anti-DTPA antibody, [In]-DTPA-bovine serum albumin, and [^{111}In]-diDTPA (intravenous).
Semi-Synthetic Methods: An N2S2 core is attached to the diDTPA compound, thereby allowing [^{99m}Tc] radiolabeling.
Implementation: Evaluation of a 3-step strategy involving the selection of the most suitable radiolabeled hapten in combination with tuning an optimal dosing schedule to achieve maximal targeting of focal septic inflammatory lesions. In this context, initial "priming" with the anti-DTPA antibody DTIn-1 (0.67, 2, or 6 nmol per rat) is performed. In the 2nd phase (1–24 hours later), the anti-DTPA activity within the intravascular department is blocked with unlabeled In-DTPA-bovine serum albumin (0.3, 1, or 3 nmol per rat). In the 3rd step (5–30 minutes later), the radiolabeled hapten (monovalent or bivalent [^{111}In]-DTPA) is administered. The *in vivo* distribution of the radiolabel is monitored by scintigraphic imaging and by *ex vivo* counting of dissected tissues.

Animal Model: Rat (Wistar strain) injected with *Staphylococcus aureus* in the left calf muscle.
Interpretation and Analysis: Scatchard analysis reveals that the affinity of DTIn-1 for bivalent [^{111}In]-diDTPA is 6 times higher than the affinity for monovalent [^{111}In]-DTPA ($K_a = 0.87 \times 10^{-9}$ mol/L vs 5.3×10^{-9} mol/L). The uptake of the bivalent chelate within abscesses is 2.5-fold higher than that for monovalent [^{111}In]-DTPA. Most important, the bivalent chelate is completely retained in the abscess over time. Using the bivalent chelate, the optimal dosing scheme can be determined with respect to the DTIn-1 dose (2 nmol per rat), the blocking agent dose (1 nmol per rat), and radiolabeled chelate dose (40 pmol per rat). The procedure is rapid; the infectious focus is clearly visualized at 1 hour postinjection of the [^{111}In]-labeled diDTPA (Note: 5 hours after administration of the anti-DTPA antibody). The non-targeted radiolabel is rapidly cleared into the urine and is only retained within abscess and the kidney (4–6% of injected dose). The 3-step diagnostic protocol enables rapid imaging of infectious foci with minimal uptake in non-inflamed tissues.

A 3-step pretargeting strategy to image infection. Boerman OC, van Eerd J, Oyen WJ, Corstens FH. J Nucl Med 2001 Sep; 42 (9), pp. 1405–11.

Colitis/Chemically Induced (Acute) *[^{99m}Technetium] ([^{99m}Tc])-HYNIC-Interleukin-8 (IL-8)/ [^{99m}Tc]-Granulocytes*

Cellular/Molecular Mechanism of Action: Interleukin-8 binds to its corresponding membrane-associated receptor complex expressed at relatively high densities by neutrophil and monocyte cell types. Granulocytes are chemotactically attracted to sites of septic and non-septic inflammation.
Physiological Mechanism of Action: Interleukin-8 can function as a chemotactic agent for granulocyte populations.
Implementation: Evaluation of acute colitis conditions facilitated by scintigraphic image analysis with [^{99m}Tc]-HYNIC-IL-8 and [^{99m}Tc]-granulocytes (purified cell populations). Gamma camera images are acquired at 2 minutes and at 1, 2, and 4 hours postinjection. At 4 hours after injection, the uptake of the radiolabel within specific tissues and organs can be determined (postmortem analysis). The dissected colon can be imaged and the inflammatory lesions scored macroscopically. For each affected colon segment, the colitis index (affected colon-to-normal colon uptake ratio, CI) is calculated and correlated with the macroscopically scored severity of inflammation.

Animal Model: Rabbit (chemically induced acute colitis).
Interpretation and Analysis: Both agents can facilitate visualization of colitis conditions within 1 hour after injection. [^{99m}Tc]-HYNIC-IL-8 images of colonic abnormalities are more accurate and

the intensity of uptake in regions of affected colon continuously increase up to 4 hours after injection, whereas no further increase 1 hour after injection is noticed scintigraphically with [^{99m}Tc]-HMPAO-granulocytes. Absolute uptake within affected colon is much higher for IL-8 than for radiolabeled granulocytes with the percentage of injected dose per gram (%ID/g) equal to 0.41 ± 0.04 %ID/g and 0.09 ± 0.054 %ID/g/hr after injection, respectively. With increasing severity, the CI at 4 hours after injection for [^{99m}Tc]-HYNIC-IL-8 is approximately 4.4 ± 0.6, 13.5 ± 0.5, and 25.8 ± 1.0; for granulocytes, the CI 4 hours after injection is 1.5 ± 0.1, 3.4 ± 0.2, and 6.4 ± 0.5, respectively. Values for CI correlate with the severity of tissue inflammation ($r = 0.95$, $p < 0.0001$ for IL-8; $r = 0.95$, $p < 0.0001$ for granulocytes). Therefore, within 1 hour after injection, visualization of the extent of colonic inflammation *in vivo* is possible with [^{99m}Tc]-HYNIC-IL-8 and [^{99m}Tc]-HMPAO-granulocytes. Within 2 hours after injection, [^{99m}Tc]-IL-8 allows a good evaluation, and within 4 hours after injection, a meticulous evaluation of the severity of inflammatory bowel disease (IBD) is possible. Although [^{99m}Tc]-HMPAO-granulocytes are able to delineate the extent of IBD within 2 hours after injection, an accurate estimation of the severity of inflammation is not possible. [^{99m}Tc]-HYNIC-IL-8 is an inflammation-imaging agent that has demonstrated promising results. [^{99m}Tc]-IL-8 can be prepared off-the-shelf and yields excellent imaging with high target-to-background ratios.

Instrumentation: Gamma camera imaging capabilities.

Rapid imaging of experimental colitis with [^{99m}Tc]-interleukin-8 in rabbits. Gratz S, Rennen HJ, Boerman OC, Oyen WJ, Corstens FH. J Nucl Med 2001 Jun; 42 (6), pp. 917–23.

Colitis/Inflammatory Bowel Disease (IBD) *[^{99m}Technetium] ([^{99m}Tc])-Liposome*

Cellular/Molecular Mechanism of Action: Liposomes have a marked tendency to accumulate in inflamed colon in an animal model of experimental colitis (rats).

Application: Selective accumulation of diagnostic agents or therapeutic pharmaceuticals within inflamed structures of the colon.

Considerations: Therapeutic intervention in IBDs is often associated with severe toxicity related to the non-specific and ubiquitous interaction of drugs with organs and tissues. In order to prevent side effects from aggressive and prolonged treatment with glucocorticoids and immunosuppressive agents, preferential accumulation of these potent drugs in diseased tissue is desired.

Implementation: Evaluation of the disposition of [^{99m}Tc]-liposomes monitored by gamma camera imaging and determination of the biodistribution of radioactivity in various organs.

Animal Model: Rat.

Interpretation and Analysis: Images reveal distinct accumulation of radioactivity in the colon within reagions of colitis, while the abdomen of normal rats is conspicuously free of any visible radioactivity. Although images acquired 4 hours after [^{99m}Tc]-liposome injection are clear enough for diagnostic purposes, the real potential of liposomes for drug delivery is evident in 24-hour images, where the major organs of liposome accumulation are dwarfed by intense colon activity in animals with colitis. On necropsy, 13.5% ± 5.48 of the activity is within the inflamed colon compared to only 0.1% in the normal colon, yeilding a 135:1 target-to-non-target ratio. Blood-borne radioactivity is 9% ± 2.12 (colitis) and 25.7% ± 4.27 (normal), indicating that the decrease in circulating liposomes is associated with an increase in liposome accumulation in the inflammatory site. The other 2 major organs that accumulate liposomes are the spleen (10.7% normal vs 11% colitis) and liver (8% normal vs 10.1% colitis). Liposomes therefore have an innate propensity to accumulate in sites of inflammation and they also have the potential of being loaded with therapeutic drugs or diagnostic agents for targeted delivery in colitis conditions.

Endothelial Inflammation (Acute)/ Bleomycin-Induced: Pulmonary Injury *[^{111}Indium] ([^{111}In])-Anti-ICAM-1 Monoclonal Antibody (MAb)*

Cellular/Molecular Mechanism of Action: The antibody component of [^{111}In]-anti-ICAM-1 monoclonal IgG antibody recognizes and physically binds to intracellular adhesion molecule-1 expressed on the exterior surface membrane of "activated" endothelial cells.

Application: Detection of endothelial injury and acute vascular inflammatory responses, including bleomycin-induced pulmonary injury.

Route of Administration: [^{111}In]-Anti-ICAM-1 monoclonal IgG antibody (intravenous).

Implementation: Evaluation of alterations in endothelial intracellular adhesion molecule expression following bleomycin-induced pulmonary injury employing [^{111}In]-anti-ICAM-1 monoclonal IgG antibody (^{111}In*αICAM-1) compared to [^{111}In]-labeled normal mouse IgG (^{111}In*nmIgG). Tissue samples are harvested 24 hours following image acquisition and their radioactivity measured. Lung tissue can be cryostat-sectioned to detect the presence of ICAM-1 by immunofluorescence, and the upregulation of LFA-1α identified in blood polymorphonuclear leukocytes (PMNs) using fluorescence-activated cell-sorter (FACS) analysis.

Animal Model: Rat (lung injury induced by intratracheal bleomycin injection 4 or 24 hours before humane sacrifice).

Interpretation and Analysis: The percent injected dose/organ of [^{111}In]-anti-ICAM-1 monoclonal IgG antibody in lungs both at 4 and 24 hours postbleomycin increases significantly with the application of [^{111}In]-anti-ICAM-1 monoclonal IgG antibody, in contrast to values in either uninjured control states or the administration of [^{111}In]*nmIgG. At 4 and 24 hours postinjury, the target-to-blood (T/B) ratio is 8/1 and 6/1, respectively. For [^{111}In]*nmIgG, the T/B ratio at 4 hours is 0.5/1 and 0.4/1 at 24 hours. [^{111}In]-anti-ICAM-1 monoclonal IgG antibody preparations administered postbleomycin-induced pulmonary injury produce 4- and 24-hour images that can easily be distinguished from uninjured reference controls. All images with [^{111}In]*nmIgG show only cardiac blood-pool and liver activity with little lung activity. Lung ICAM-1 immunofluorescence intensity increases in the bleomycin-treated pulmonary samples, compared to uninjured lungs. Expression of LFA-1α on PMNs increases 19% and 210% at 4 hours and 24 hours postinjury, respectively, compared to control values. Biodistribution and imaging data therefore are capable of demonstrating that [^{111}In]-anti-ICAM-1 monoclonal IgG antibody is capable of detecting early acute bleomycin-induced lung injury. Immunofluorescence and FACS data suggest that [^{111}In]-anti-ICAM-1 monoclonal IgG antibody uptake is a specific process. Monoclonal antibody preparations possessing such attributes has potential as an early radionuclide detector of acute inflammations.

Early detection of bleomycin-induced lung injury in rat using indium-111-labeled antibody directed against intercellular adhesion molecule-1. Weiner RE, Sasso DE, Gionfriddo MA, Syrbu SI, Smilowitz HM, Vento J, Thrall RS. J Nucl Med 1998 Apr; 39 (4), pp. 723–8.

Gastrointestinal Inflammation/Intestinal Lesions: Non-Steroidal Anti-Inflammatory Drug (NSAID)-Associated/Intestinal Lesions: Crohn's Ileitis *[^{111}Indium] ([^{111}In]) Leukocytes/Tauro 23 [^{75}Se]-Selena-25-Homocholic Acid/[^{58}Co]-Labeled Cyanocobalamine*

Cellular/Molecular Mechanism of Action: The agent tauro 23 [^{75}Se]-selena-25-homocholic acid is a bile acid analog, while

[^{58}Co]-labeled cyanocobalamine is an analogue of vitamin B_{12}. Radiolabeled [^{111}In] leukocytes behave *in vivo* in a manner highly analogous to normal endogenous leukocytes of the same cell type.

Application: The agents tauro 23 [^{75}Se]-selena-25-homocholic acid and [^{58}Co]-labeled cyanocobalamine can be applied to evaluate intestinal absorption. Conversely, [^{111}In] leukocytes can be used to detect foci of inflammation and sepsis.

Route of Administration: Intravenous.

Implementation: Evaluation of residual inflammatory lesions following discontinuation of NSAIDS using [^{111}In] leukocyte scintigraphy imaging methodologies.

Human Model #1: Cases of rheumatoid arthritis (n = 90) and osteoarthritis (n = 7).

Human Model #2: Cases treated with NSAIDs (n = 19); healthy controls (n = 20); and Crohn's ileitis (n = 13).

Interpretation and Analysis: [^{111}In] leukocyte facilitated scintigraphic imaging techniques reveal that cases of rheumatoid arthritis and osteoarthritis that have been treated with NSAIDs display gastrointestinal lesions. More specifically, approximately two-thirds of all cases evaluated on long-term treatment display inflammatory lesions that may persist for up to 16 months following discontinuation. The prevalence and magnitude of the intestinal inflammation is unrelated to the type and dose of non-steroidal drugs and previous or concomitant 2nd-line drug treatment. There is a significant inverse correlation ($r = -0.29$, $p < 0.05$) between fecal [^{111}In] excretion and hemoglobin levels in cases treated with NSAIDs. The kinetics of fecal [^{111}In] excretion in cases treated with NSAIDs is almost identical to that of cases with small bowel Crohn's disease. Small bowel radiological examination of cases on NSAIDs reveals that approximately one-sixth have asymptomatic ileal disease with ulceration and strictures (e.g., n = 3/18 cases). Evaluation of cases being treated with anti-inflammatory agents, normal healthy controls, and cases of Crohn's ileitis with tauro 23 [^{75}Se]-selena-25-homocholic acid and [^{58}Co]-labeled cyanocobalamine reveals several abnormalities. On day 4, more than half the cases with rheumatoid arthritis have evidence of bile acid malabsorption, but ileal dysfunction is much milder than seen in patients with Crohn's ileitis.

Non-steroidal anti-inflammatory drug–induced intestinal inflammation in humans. Bjarnason I, Zanelli G, Smith T, Prouse P, Williams P, Smethurst P, Delacey G, Gumpel MJ, Levi AJ. Gastroenterology 1987 Sep; 93 (3), pp. 480–9.

Gastrointestinal Septic Inflammation/Appendicitis *[^{99m}Technetium] ([^{99m}Tc])-Labeled Anti-CD15 IgM Immunoglobulin*

Cellular/Molecular Mechanism of Action: Anti-CD15 immunoglobulin M monoclonal antibody (MAb) recognizes and binds to CD15 complexes expressed on the exterior surface membrane of polymorphonuclear neutrophils. The difference between IgG and IgM immunoglobulin is that IgM is the principal immunoglobulin produced after the 1st exposure to a given antigen, while IgG is the principal immunoglobulin produced after a 2nd exposure to the same antigen. IgM is composed of a pentomer of IgG-like molecules and generally has less specific antigen-binding properties than does IgG, but IgM has a higher propensity to generate multimolecular antigen/antibody complexes.

Application: Detection and monitoring of septic foci and lesions.

Route of Administration: Anti-CD15 immunoglobulin M MAb (LeuTech: intravenous).

Dosage: Anti-CD15 immunoglobulin M MAb (LeuTech: 10–20 mCi of [^{99m}Tc]).

Semi-Synthetic Methods: Preparations of anti-CD15 immunoglobulin M MAb (e.g., LeuTech) avidly bind to circulating and sequestered human polymorphonuclear neutrophils *in vivo,* eliminating the need for *in vitro* cell labeling and the risks of blood handling.

Implementation: Evaluation of the safety and efficacy of anti-CD15 immunoglobulin M MAb (LeuTech) imaging in cases of suspected acute appendicitis. An effective procedure includes the acquisition of serial dynamic and static planar images for up to 3 hours after intravenous administration. Scans are read as positive or negative for acute appendicitis or the presence of other intra-abdominal infection (septic lesions). The institutional diagnosis is validated by surgery and histopathology of the appendix, results of other diagnostic studies, or 2-week clinical follow-up. Scans are positive for appendicitis in 39 of 40 patients with appendicitis at surgery (sensitivity 98%) and negative for appendicitis in 49 of 58 patients without appendicitis (specificity 84%). Accuracy, positive predictive value, and negative predictive value are 90, 81, and 98%, respectively. In patients with appendicitis and positive scans more than 50% of the images are positive at 4 minutes, and all are positive by 1 hour. Mean time of 1st positive image is 15 minutes. There are usually no serious adverse reactions. Imaging with LeuTech is a highly sensitive test for the detection of appendicitis in equivocal cases. There are advantages of this agent over the other currently used radiotracers in terms of convenience and time to diagnosis, particularly with respect to the rapidity with which acute appendicitis will be seen on the images.

Human Model: Cases presenting with equivocal clinical signs (e.g., n = 99).

Interpretation and Analysis: Scans are usually positive in a very high percentage of cases of suspected appendicitis with validation of the condition confirmed at surgery (e.g., n = 39/40; sensitivity 98%). Conversely, images are negative for appendicitis in a large percentage of suspect cases that do not have appendicitis (e.g., n = 49/58, specificity = 84%). Accuracy, positive predictive, and negative predictive values are approximately 90, 81, and 98%, respectively. In conditions with appendicitis and positive scans more than 50% of the images are positive at 4 minutes, and essentially all images are positive by 1 hour. Mean time of 1st positive image is about 15 minutes. Serious adverse reactions are generally rare. Imaging with anti-CD15 immunoglobulin M MAb (LeuTech) is considered a highly sensitive diagnostic agent for detecting appendicitis in equivocal cases. Advantages of this agent over the other currently used radiotracers include greater convenience and shorter time to diagnosis, particularly with regard to the expedient nature that acute appendicitis can be visualized on scintigraphic images.

Scintigraphic determination of equivocal appendicitis. Rypins EB, Kipper SL. Am Surg 2000 Sep; 66 (9), pp. 891–5.

Musculoskeletal/Osteomyelitis (Septic)/ Vascular Permeability *[^{99m}Techetium] ([^{99m}Tc])-Sulesomab/[^{99m}Tc]-Labeled Human Serum Albumin ([^{99m}Tc]-HSA)*

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-sulesomab and [^{99m}Tc]-HSA have similar molecular weights (molecular mass). Human serum albumin within the intravascular compartment is primarily responsible for maintaining plasma oncotic pressure. However, when it is labeled and applied for diagnostic purposes, it per se does not exert any true biological/molecular mechanism of action.

Background Information: [^{99m}Tc]-sulesomab (Fab fragment of anti-NCA-90) is used as an *in vivo* granulocyte labeling agent for imaging foci of inflammation. It is not clear to what extent it targets cells that have already migrated into the interstitial space of an

inflammatory lesion, as opposed to circulating cells. The contribution to image signals from radioprotein diffusion in settings of increased vascular permeability has not been thoroughly documented.

Application: Detection and characterization of septic osteomyelitis conditions.

Route of Administration: [^{99m}Tc]-sulesomab and [^{99m}Tc]-HSA (intravascular).

Implementation: Evaluation of orthopedic infections (e.g., septic osteomyelitis) utilizing [^{99m}Tc]-sulesomab and [^{99m}Tc]-HSA as a function of their local kinetic profiles. [^{99m}Tc]-sulesomab and [^{99m}Tc]-HSA are administered in sequence separated by an interval of 2 to 6 days. Images are obtained 1, 3, 4, and 6 hours after injection, and multiple venous blood samples obtained for the calculation of blood clearance parameters. Patlak-Rutland (P-R) analysis is performed to measure lesion and control tissue protein clearance. Target-to-background tissue (T/Bkg) ratios are calculated for each radioprotein and compared with the T/Bkg ratio for [^{111}In] leukocytes. [^{99m}Tc]-Sulesomab binding to granulocytes can be measured *in vivo*, in *ex vivo* preparations, and *in vitro* to primed and activated granulocytes.

Human Model: Cases of septic osteomyelitis (n = 7) already previously detected as being clearly positive on [^{111}In] leukocyte scintigraphy analysis.

Interpretation and Analysis: After intravenous injection, $< 5\%$ of the circulating radioactivity is cell-bound with both radioproteins so that the P-R curves can therefore be assumed to represent extravascular uptake of free protein. The blood clearance (mean $\pm$ standard deviation) of sulesomab is 23.4 $\pm$ 11.7 mL/min, which is approximately 5 times greater than that of HSA (4.8 $\pm$ 3.1 mL/min). Similarly, clearance into the lesion by sulesomab is consistently higher than for HSA (on average about 3 times as high). Nevertheless, the T/Bkg ratios for sulesomab and HSA are similar, except at 6 hours when that of HSA (2.14 $\pm$ 0.6) is higher than that for sulesomab (1.93 $\pm$ 0.5; p approximately 0.01). Both values are considerably less than the T/Bkg ratio on the [^{111}In] leukocyte images, which, at 22 hours, is 12.3 $\pm$ 5.3. Moderate clearance of sulesomab, but not HSA, occurs in control tissue regions. Granulocytes bind significantly more [^{99m}Tc]-sulesomab *in vitro* when primed or activated. In conclusion, (i) sulesomab does not localize in inflammation as a result of binding to circulating granulocytes; (ii) sulesomab "diffuses" into sites of inflammation non-specifically as a function of increased vascular permeability; nevertheless, it may be cleared after local binding to primed granulocytes or bind to activated, migrated extravascular granulocytes; and (iii) HSA produces a similar or higher T/Bkg ratio than sulesomab because sulesomab is cleared into normal tissues and because image positivity in inflammation is significantly dependent on local blood-pool expansion.

Mechanism of accumulation of [^{99m}Tc]-sulesomab in inflammation. Skehan SJ, White JF, Evans JW, Parry-Jones DR, Solanki CK, Ballinger JR, Chilvers ER, Peters AM. J Nucl Med 2003 Jan; 44 (1), pp. 11–8.

Musculoskeletal/Osteomyelitis: Septic Inflammatory Response/[Diabetes Mellitus] *[^{131}I]-Interleukin-8 (IL-8) (Recombinant)/ [^{99m}Technetium] ([^{99m}Tc])-Hydroxyethylene Diphosphonate*

Cellular/Molecular Mechanism of Action: Neutrophils perceive IL-8 as a chemotactic factor and express membrane-associated IL-8 receptor complexes.

Background Information: The chemotactic cytokine IL-8 plays an important role in the attraction (chemotactic migration) and activation of polymorphonuclear leukocytes in conditions of septic (infection) and non-septic inflammation.

Application: Detection and characterization of septic inflammatory disease states. In this context, [^{131}I]-rhIL-8 accumulates rapidly within infected foci in osteomyelitis and cellulites.

Route of Administration: [^{131}I]-rhIL-8 (intravenous).

Semi-Synthetic Methods: Radioiodination of human recombinant IL-8 (rhIL-8) with [^{131}I] (specific activity, 0.4–0.7 MBq at 11–18 μCi [^{131}I]/μg IL-8).

Implementation: Evaluation of septic disease states applying [^{131}I]-rhIL-8 as an imaging agent.

Human Model: Cases of diabetes mellitus (n = 8 active foot infections with evidence of osteomyelitis; n = 2 successfully-treated osteomyelitis; n = 1 cellulitis of the thumb).

Interpretation and Analysis: Focal accumulation of [^{131}I]-rhIL-8 can be seen in a high percentage of cases with active foot infection (e.g., n = 8/8). In other affected appendages, the image profile may appear diffuse (e.g., n = 1/1 thumb cellulitis). In cases successfully treated for osteomyelitis, multiphase [^{99m}Tc]-hydroxyethylene diphosphonate bone scans may be negative early, but late-phase (> 3 hours) uptake can detect degenerative lesions that are not initially detected by imaging with [^{131}I]-rhIL-8 (e.g., n = 2/2 cases). [^{131}I]-rhIL-8 accumulates rapidly within infected foci of osteomyelitis conditions and cellulitis but not in successfully treated infections or degenerative joint disease.

Imaging of human infection with [^{131}I]-labeled recombinant human interleukin-8. Gross MD, Shapiro B, Fig LM, Steventon R, Skinner RW, Hay RV. J Nucl Med 2001 Nov; 42 (11), pp. 1656–9.

Bone/Osteomyelitis Septic Inflammatory Responses/*Staphylococcus Aureus*–Induced *[^{99m}Technetium] ([^{99m}Tc])-PEG Liposomes/ [^{99m}Tc]-HYNIC-IgG/[^{99m}Tc]-MDP/[^{111}Indium] ([^{111}In])-Granulocytes/Gallium-67 ([^{67}Ga])-Citrate*

Background Information: Assessment of disease activity and disease extent in chronic osteomyelitis remains a difficult diagnostic problem. Radiography is not particularly sensitive. Scintigraphic techniques can be more helpful, but routinely available agents lack specificity (e.g., [^{99m}Tc]-methylene diphosphonate (MDP), [^{67}Ga]-citrate) or are laborious to prepare (e.g., [^{111}In] leukocytes).

Route of Administration: [^{99m}Tc]-PEG liposomes (intravenous); [^{99m}Tc]-HYNIC-IgG (intravenous); [^{99m}Tc]-MDP (intravenous); [^{111}In]-granulocytes (intravenous); or [^{67}Ga]-citrate (intravenous).

Dosage: [^{99m}Tc]-PEG liposomes (37 MBq rabbit); [^{99m}Tc]-HYNIC-IgG (37 MBq rabbit); [^{99m}Tc]-MDP (37 MBq rabbit); [^{111}In]-granulocytes (18 MBq rabbit); or [^{67}Ga]-citrate (18 MBq rabbit).

Implementation: Scintigraphic image analysis of osteomyelitis lesions of the femur is performed 4 weeks after bacterial infusion applying [^{99m}Tc]-PEG liposomes, [^{99m}Tc]-HYNIC-IgG, and [^{99m}Tc]-MDP on 3 consecutive days and imaged up to 4 hours (MDP) or 22 hours (liposomes and IgG) postinjection. On day 4, preparations of [^{111}In]-granulocytes or [^{67}Ga]-citrate are injected and imaged up to 44 hours after injection. Uptake in the septic femur is determined by drawing regions-of-interest (ROIs). Ratios of infected-to-sham-operated femur are calculated. After the last image, rabbits are killed, and the left and right femur scored for microbiologic and histopathologic evidence of osteomyelitis.

Animal Model: Chronic osteomyelitis is induced by infusing *S. aureus* organisms into the right reamed and washed femoral canal (rabbits). The canal is then closed with cement and a sham operation performed on the left femur. Routine radiographs are obtained immediately after surgery and before scintigraphy.

Interpretation and Analysis: [^{99m}Tc]-PEG liposomes and [^{99m}Tc]-HYNIC-IgG can correctly identify lesions of osteomyelitis in a high percentage of cases (e.g., n = 6/6). In a small percentage of cases, [^{111}In]-granulocytes and [^{67}Ga]-citrate can produce equivocal results (e.g., 15% or n = 1/6), and [^{99m}Tc]-MDP may not detect osteomyelitis lesions (e.g., 15% or n = 1/6). The uptake in the affected region does not differ significantly between the imaging agents, although [^{99m}Tc]-MDP tends to have higher values (MDP, 4.75 ± 1.23 percent injected dose per gram [%ID/g]; [^{67}Ga], 2.05 ± 0.54 %ID/g; granulocytes, 1.56 ± 0.83 %ID/g; liposomes, 1.75 ± 0.76 %ID/g; and IgG, 1.96 ± 0.27 %ID/g). The ratios of infected-to-normal femur are also not significantly different for the respective radiopharmaceuticals. Radiography visualizes only severe osteomyelitis conditions. [^{99m}Tc]-PEG liposomes and [^{99m}Tc]-HYNIC-IgG perform at least as well as [^{111}In]-granulocytes and [^{67}Ga]-citrate in the localization of chronic osteomyelitis (e.g., rabbits). The ease of preparation, the better image quality, and the lower radiation dose suggest that [^{99m}Tc]-PEG liposomes and [^{99m}Tc]-HYNIC-IgG might be suitable alternatives for [^{67}Ga]-citrate and [^{111}In]-granulocytes in the scintigraphic evaluation of osteomyelitis.

Scintigraphic evaluation of experimental chronic osteomyelitis. Dams ET, Nijhof MW, Boerman OC, Laverman P, Storm G, Buma P, Lemmens JA, van der Meer JW, Corstens FH, Oyen WJ. J Nucl Med 2000 May; 41 (5), pp. 896–902.

Bone/Osteomyelitis Septic Inflammatory Responses/*Staphylococcus Aureus*–Induced *Gallium-67 ([^{67}Ga])-Citrate/ [^{99m}Technetium] ([^{99m}Tc])-Methylene Diphosphonate (MDP)/[^{99m}Tc]-Interleukin-8 (IL-8)*

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-MDP is believed to be assimilated by active osteoblasts/osteoclasts at sites of dynamic bone growth and destruction.

Background Information: Early and accurate diagnosis of osteomyelitis remains a clinical problem. Acute osteomyelitis often occurs in infants and most often is located in the long bones. Radiologic images show changes only in the advanced stages of disease. Scintigraphic imaging with [^{99m}Tc]-MDP or bone scanning is much more sensitive in detecting acute osteomyelitis but lacks specificity.

Application: Detection and characterization of septic osteomyelitis lesions.

Route of Administration: [^{99m}Tc]-MDP (intravenous), [^{99m}Tc]-IL-8 (intravenous).

Dosage: [^{99m}Tc]-MDP (18.5 MBq), [^{99m}Tc]-IL-8 (18.5 MBq).

Implementation: Scintigraphic characterization of osteomyelitis lesions 10 days following lesion induction (e.g., n = 10 rabbits; n = 5/group) utilizing injections of either 18.5 MBq [^{111}In]-granulocytes or 18.5 MBq [^{67}Ga]-citrate. Images are performed both 24 hours and 48 hours postinjection. On day 12, serial images are acquired at 0, 1, 2, 4, 8, 12, and 24 hours after injection of either 18.5 MBq [^{99m}Tc]-MDP or 18.5 MBq [^{99m}Tc]-IL-8 preparations. Uptake in the infected femur is determined by establishing regions-of-interest (ROIs). Ratios of infected femur (target) to sham-operated femur (background) (T/Bs) are then calculated. After the final images are obtained, the right femur is harvested at postmortem to facilitate dissection and analysis of tissue samples for microbiological and histopathological evidence of osteomyelitis.

Animal Model: Acute pyogenic osteomyelitis can be induced in rabbits (e.g., n = 10) by inserting sodium morrhuate and *S. aureus* into the medullary cavity of the right femur. The cavity is then closed with liquid cement. A sham operation is performed on the left femur. Routine radiographs are obtained just before scintigraphy imaging. Acute osteomyelitis develops in a high percentage of rabbits (e.g., n = 8/10).

Interpretation and Analysis: All imaging agents can correctly detect acute osteomyelitis in a very high percentage of cases (e.g., n = 8/8). The extent of infection can be optimally visualized with [^{67}Ga]-citrate and by delayed bone scanning, whereas diaphyseal photopenia is noted with both [^{99m}Tc]-IL-8 and [^{111}In]-granulocytes. Some false-negative (e.g., n = 1/8) and false-positive (e.g., n = 1/8) image results can be observed with [^{111}In]-granulocytes and [^{99m}Tc]-MDP, respectively. Quantitative analysis of the images reveals that the uptake in the infected region is highest with [^{67}Ga]-citrate (4.9 ± 0.8 percent injected dose [%ID]) and [^{99m}Tc]-MDP (4.7 ± 0.7 %ID), whereas the uptake in the infected area is significantly lower with [^{99m}Tc]-IL-8 (2.2 ± 0.2 %ID) and [^{111}In]-granulocytes (0.8 ± 0.2 %ID) ($p < 0.0042$). In contrast, the T/Bs are significantly higher for [^{99m}Tc]-IL-8 (T/B, 6.2 ± 0.3 at 4 hours after injection) than for [^{67}Ga]-citrate, [^{99m}Tc]-MDP, and [^{111}In]-granulocytes, which have ratios of 1.5 ± 0.4, 1.9 ± 0.2, and 1.4 ± 0.1, respectively ($p < 0.0001$). Radiography correctly reveals acute osteomyelitis in only a relatively small percentage of cases (e.g., n = 2/8). In experimentally-induced osteomyelitis, [^{99m}Tc]-IL-8 clearly detects osteomyelitic lesions. Although the absolute uptake in the osteomyelitic area is significantly lower than that obtained with [^{99m}Tc]-MDP and [^{67}Ga]-citrate, the T/Bs are significantly higher for [^{99m}Tc]-IL-8 because of fast background clearance. The ease of preparation, good image quality, and lower radiation burden suggest that [^{99m}Tc]-IL-8 may be a suitable imaging agent for the scintigraphic evaluation of acute osteomyelitis.

[^{99m}Tc]-interleukin-8 for imaging acute osteomyelitis. Gratz S, Rennen HJ, Boerman OC, Oyen WJ, Burma P, Corstens FH. J Nucl Med 2001 Aug; 42 (8), pp. 1257–64.

Bone/Osteomyelitis/Leukocyte Detection *[^{99m}Technetium] ([^{99m}Tc])-Labeled Antigranulocyte Antibody (IgG) Fragments*

Cellular/Molecular Mechanism of Action: Antigranulocyte antibody possesses binding avidity for membrane-associated antigens that are unique to neutrophil populations. Neutrophils are chemotactically attracted to areas of (septic) inflammation. The Fab′ fragment of IgG has the advantage of being devoid of the Fc fragment so that it minimally binds to membrane-associated Fc receptor complexes expressed by the mononuclear phagocytic system residing within the liver and spleen, thereby experiencing low levels of non-specific binding.

Application: Detection and characterization of septic inflammatory lesions.

Route of Administration: [^{99m}Tc]-labeled antigranulocyte antibody fragment (intravenous).

Dosage: [^{99m}Tc]-labeled antigranulocyte antibody fragment (740 MBq [20 mCi]).

Implementation: Immunoscintigrams performed with [^{99m}Tc]-labeled antigranulocyte antibody fragments for the detection of focal lesions of septic inflammation. Validation of findings can be established by histopathology, magnetic resonance imaging, computed tomography, and monitoring of clinical signs.

Interpretation and Analysis: The percentage of true-positive images obtained with [^{99m}Tc]-labeled antigranulocyte antibody fragments for the detection of septic inflammation is approximately 67% (e.g., n = 20/30). The number of false-positive results is relatively low (e.g., n = 3/30). Approximately 20% of images will be true-negatives (e.g., n = 6/30). Consequently, [^{99m}Tc]-labeled antigranulocyte antibody fragments can achieve a high level of sensitivity (e.g.,

95%), moderately high specificity (e.g., 67%), and high diagnostic accuracy (e.g., 86%). Therefore, [^{99m}Tc]-labeled monoclonal antibody-Fab′ fragments are suitable for the detection of osteomyelitis. Its clinical application is simple, and its use guarantees a reliable and accurate diagnostic result just 1–2 hours after injection, making a late scan unnecessary. False-positive results have been reported with conditions of coxarthrosis or hyperostosis, while false-negative results can occur with perforating ulcers of the foot.

Imaging osteomyelitis with [^{99m}Tc]-labeled antigranulocyte antibody Fab′ fragments. Von Rothenburg T, Schaffstein J, Ludwig J, Vehling D, Koster O, Schmid G. Clin Nucl Med 2003 Aug; 28 (8), pp. 643–7.

Osteomyelitis/Interleukin-8 (IL-8) Receptors *[^{99m}Technetium] ([^{99m}Tc])-Labeled IL-8*

Cellular/Molecular Mechanism of Action: The cytokine IL-8 binds with high affinity to the CXCR1 and CXCR2 receptor complexes on neutrophil populations.

Application: Previous investigations have shown that [^{99m}Tc]-IL-8 can rapidly and effectively delineate foci of infection and inflammation in rabbit models of intramuscular infection, colitis, and osteomyelitis.

Semi-Synthetic Methods: A derivative of hydrazinonicotinamide (HYNIC) can be used as a bifunctional coupling agent to label protein fractions with the [^{99m}Tc] radioisotope.

Implementation: Delineate [^{99m}Tc]-IL-8 pharmacokinetic profiles in conditions of neutropenia compared to states of normal health, and determine the ability of [^{99m}Tc]-IL-8 to detect septic abscesses. Complementary investigations include characterization of the interaction of [^{99m}Tc]-IL-8 with blood cells in circulation in normal rabbits, and the distribution of the radiolabel relative to circulating white and red blood cells and plasma. The *in vivo* kinetics of [^{99m}Tc]-IL-8 is established by quantitative analysis of whole-body images acquired between 0 and 6 hours after injection. The results from such analyses (*in vivo* biodistribution) can be validated by *ex vivo* counting of radioactivity in dissected tissue samples.

Animal Model: Turpentine-induced abscesses in both neutropenic and normal rabbits.

Interpretation and Analysis: The abscess uptake (percentage of injected dose per gram of tissue [%ID/g] ± standard error of measurement [SEM]) in immunocompetent rabbits (0.41 ± 0.05) is 10 times higher than that in neutropenic rabbits (0.038 ± 0.014), demonstrating specificity of the target uptake of [^{99m}Tc]-IL-8. Abscess-to-muscle ratios ± SEM are also 10 times higher (110 ± 10 vs 10 ± 5). Lung and spleen uptake in normal rabbits is 3 times higher than that in neutropenic rabbits. The blood clearance of the radiolabel in neutropenic rabbits is similar to that in normal rabbits. In circulation, most of [^{99m}Tc]-IL-8 (70%) is found within the plasma fraction. Less than one-third is associated with red blood cells, and only a very low percentage (< 2.5%) is associated with white blood cells. Image analysis reveals a gradual increase in abscess uptake over time of up to > 15 %ID, which can be confirmed by *ex vivo* gamma counting of infected muscle tissue. The highest increase in uptake in abscesses is observed after 2 hours following injection, when most of the [^{99m}Tc]-IL-8 is cleared from the blood, suggesting specific neutrophil-mediated accumulation of [^{99m}Tc]-IL-8 within abscesses. Furthermore, region-of-interest (ROI) analysis demonstrates gradual accumulation of [^{99m}Tc]-IL-8 within abscesses accompanied by a simultaneous clearance of activity from the lungs, suggesting that neutrophil-associated [^{99m}Tc]-IL-8 initially trapped in the lungs migrates to abscess at later time points, favoring neutrophil-bound transportation from the lungs to abscesses. Substantial support is given for the hypothesis that [^{99m}Tc]-IL-8 localizes in abscesses, mainly bound to peripheral neutrophils. Accumulation within abscesses is a highly specific, neutrophil-driven process. As assessed by *in vivo* and *ex vivo* analysis, the total fraction that accumulates in inflamed tissue is extremely high (up to > 15 %ID) compared with that of other agents used for imaging infection and inflammation.

Kinetics of [^{99m}Tc]-labeled interleukin-8 in experimental inflammation and infection. Rennen HJ, Boerman OC, Oyen WJ, Corstens FH. J Nucl Med 2003 Sep; 44 (9), pp. 1502–9.

Neutrophil Leukotriene B_4 (LTB_4) Receptors/ Induced Myocardial Ischemia Insult *[^{99m}Technetium] ([^{99m}Tc])-RP517/ [F]-RP517 (Fluorescent Analogue)*

Cellular/Molecular Mechanism of Action: In whole blood, [F]-RP517 (500 nmol/L) preferentially labels neutrophil LTB_4 receptor complexes.

Background Information: [^{99m}Tc]-RP517 is a new LTB_4 receptor antagonist developed for imaging acute septic and non-septic inflammatory reactions. A unique property of [^{99m}Tc]-RP517 is its ability to label white blood cells *in vivo* after intravenous injection.

Route of Administration: [^{99m}Tc]-RP517 (intravenous).

Implementation: Evaluate the relative [^{99m}Tc]-RP517 binding to human leukocyte subtypes and the [^{99m}Tc]-RP517 uptake pattern in canine myocardium within inflammatory lesions induced either by coronary occlusion and reperfusion or tumor necrosis factor-alpha (TNF-α) injection. Initial analysis involves delineation of the potential for RP517 analogues to bind to neutrophil populations *in vitro* within whole blood and *in vivo* within the intravascular compartment. Acquisition of such data can be achieved through the use of fluorescence-activated cell sorter analysis performed on whole human blood samples (n = 2) and isolated neutrophils (n= 4) with a fluorescent analogue of [^{99m}Tc]-RP517 referred to as [F]-RP517. Evaluation *in vivo* can entail administration of [^{99m}Tc]-RP517 to determine its biodistribution within the myocardium before and after coronary occlusion. To facilitate *ex vivo* images of the myocardium, the left ventricle can be divided into 72 segments for flow and [^{99m}Tc]-RP517 uptake analyses.

Animal Model: Canine (n = 9 dogs; anesthetized, open-chest surgical exposure before coronary occlusion [90 minutes] and reperfusion [120 minutes]; or n = 3 dogs before intramyocardial TNF-α administration).

Interpretation and Analysis: In whole blood, [F]-RP517 (500 nmol/L) preferentially labels neutrophils. In isolated neutrophil populations, [F]-RP517 (10 nmol/L) binding is inhibited by 44% in the presence of LTB_4 (400 nmol/L). In the analysis of *ex vivo* cardiac samples, an inverse exponential relationship can be detected between [^{99m}Tc]-RP517 uptake and occlusion flow (r = 0.73). In the same 15 segments, [^{99m}Tc]-RP517 uptake is highly correlated with the neutrophil enzyme myeloperoxidase (r = 0.91). *Ex vivo* images reveal tracer uptake in the reperfused area (ischemic to normal count ratio = 2.7 ± 0.2). Therefore, it can be concluded that RP517 binds to the neutrophil LTB_4 receptor complex after intravenous injection. After reperfusion, [^{99m}Tc]-RP517 uptake correlates with myeloperoxidase content and can be observed on *ex vivo* images, indicating that this tracer may have potential as an inflammation-imaging agent.

Assessment of myocardial inflammation produced by experimental coronary occlusion and reperfusion with [^{99m}Tc]-RP517, a new leukotriene B_4 receptor antagonist that preferentially labels neutrophils *in vivo.* Riou LM, Ruiz M, Sullivan GW, Linden J, Leong-Poi H, Lindner JR, Harris TD, Beller GA, Glover DK. Circulation 2002 Jul 30; 106 (5), pp. 592–8.

Sepsis/Bacterial Infection: Selective Organism Detection

[^{99m}Technetium] ([^{99m}Tc])-Labeled Ciprofloxacin/ [^{99m}Tc]-Hexamethylpropyleneamine Oxime (HMPAO)-Labeled White Blood Cell (WBC)

Cellular/Molecular Mechanism of Action: Ciprofloxacin when applied as an imaging agent localizes in high concentrations within living bacteria.

Application: [^{99m}Tc]-labeled ciprofloxacin (infecton) has been developed for detecting infectious foci.

Route of Administration: [^{99m}Tc]-labeled ciprofloxacin and [^{99m}Tc]-HMPAO-labeled WBC preparations are injected intravenously.

Dosage: [^{99m}Tc]-HMPAO-labeled WBC (185–200 MBq): [^{99m}Tc]-labeled ciprofloxacin (370–400 MBq).

Considerations: Previous reports have described greater specificity of radiolabeled ciprofloxacin for bacterial infections compared to radiolabeled WBC scans.

Implementation: Performance of scintigraphic image analysis in suspected cases of bone or joint sepsis using [^{99m}Tc]-labeled ciprofloxacin and [^{99m}Tc]-WBC preparations (e.g., n = 51; n = 21/51 prosthetic implant materials). Procedures that can complement or validate findings observed in scintigraphic image analyses include biochemical, radiologic, and microbiologic data in addition to assessment of clinical history (e.g., outcomes). Scintigraphic images are performed at 1 and 4 hours postinjection of [^{99m}Tc]-labeled ciprofloxacin or [^{99m}Tc]-HMPAO-WBCs. At least a 2-day and at most a 7-day lag-time period occurs between imaging agent administration and the acquisition of scintigraphic images.

Interpretation and Analysis: Scintigraphic imaging with [^{99m}Tc]-labeled ciprofloxacin and [^{99m}Tc]-HMPAO-labeled WBC populations in cases of bone or joint sepsis produces a variety of image assessments including true-positives (e.g., n = 30/32 vs n = 20/32); false-positives (e.g., n = 4/24 vs n = 1/24); true-negatives (e.g., n = 20/24 vs 23/24); and false-negatives (e.g., n = 2/22 vs 12/22), respectively. Corresponding values for sensitivity, specificity, and accuracy are approximately 94%, 83%, and 89% ([^{99m}Tc]-labeled ciprofloxacin), and 63%, 96%, and 77% ([^{99m}Tc]-HMPAO-labeled WBC), respectively. Differences between the 2 agents are statistically significant ($p < 0.001$). [^{99m}Tc]-labeled ciprofloxacin and WBC scan results are in general concordance for approximately 77% of sites (e.g., n = 43/56). [^{99m}Tc]-HMPAO-labeled WBC results for vertebral infections are the most notable. [^{99m}Tc]-ciprofloxacin scans are positive in a large percentage of cases with vertebral osteomyelitis (e.g., n = 83% or 5/6). WBC scans can show photon-deficient areas in some of these cases (e.g., n = 4/6), but normal distribution in the remaining scenarios (e.g., n = 2/6). [^{99m}Tc]-ciprofloxacin is a useful agent for detecting infectious foci in bones and joints. Moreover, the [^{99m}Tc]-labeled ciprofloxacin scans seem to be a more powerful tool in diagnosing vertebral infections than WBC scintigraphy.

Usefulness of [^{99m}Tc]-ciprofloxacin (infecton) scan in diagnosis of chronic orthopedic infections: Comparative study with [^{99m}Tc]-HMPAO leukocyte scintigraphy. Sonmezoglu K, Sonmezoglu M, Halac M, Akgun I, Turkmen C, Onsel C, Kanmaz B, Solanki K, Britton KE, Uslu I. J Nucl Med. 2001 Apr; 42 (4), pp. 567–74.

Septic Inflammatory Disease/Radiolabeling Efficiency/Radiolabel Stability: *In Vivo*/Kinetic Profile: *In Vivo*/Dosimetry: *In Vivo*

Stabilized [^{99m}Technetium] ([^{99m}Tc])-Exametazime-Labeled Leukocytes

Background Information: Labeling leukocytes with [^{99m}Tc]-exametazime is a validated technique for imaging infection and inflammation. A new radiolabeling technique has recently been described that enables leukocyte labeling with a more stable form of [^{99m}Tc]-exametazime.

Application: Detection and characterization of inflammatory and septic disease states.

Route of Administration: Stabilized [^{99m}Tc]-exametazime-labeled leukocytes (intravascular).

Semi-Synthetic Methods: Labeling of autologous leukocyte populations with [^{99m}Tc] using stabilized exametazime.

Implementation: Evaluation of the *in vivo* radioactive biodistribution, kinetic profile, and dosimetry estimates of stabilized [^{99m}Tc]-exametazime-labeled autologous leukocytes. Serial whole-body imaging and blood sampling are acquired up to 24 hours after injection. Cell-labeling efficiency and *in vivo* viability, organ dosimetry, and clearance calculations are calculated from the blood samples and imaging data as well as samples of urine and stool collected up to 36 hours after initial injection.

Human Model: Normal healthy volunteers (n = 10).

Interpretation and Analysis: Cell-labeling efficiencies of 87.5% ± 5.1% can be achieved, which is similar to or better than that reported with the standard preparation of [^{99m}Tc]-exametazime. *In vivo* stability of the radiolabeled leukocytes is also similar to *in vitro* results with stabilized [^{99m}Tc]-exametazime and better than previously reported for the *in vivo* stability of non-stabilized [^{99m}Tc]-exametazime-labeled leukocytes. Organ dosimetry and radiation absorbed doses are similar with a whole-body absorbed dose of 1.3×10^{-3} mGy/MBq. Urinary and fecal excretion of activity is minimal, and visual assessment of images shows little renal parenchymal activity and no bowel activity for up to 2 hours following injection.

Cell labeling and *in vivo* stability appears to improve compared to leukocytes labeled with non-stabilized [^{99m}Tc]-exametazime preparations. Advantages of the methodology include increased preparation cost-effectiveness of stabilized [^{99m}Tc]-exametazime and an extended window for clinical usage, with good visualization of abdominal structures on early images. No significant increase in specific organ and whole-body dosimetry estimates has been noted compared with previous estimates using non-stabilized [^{99m}Tc]-exametazime-labeled leukocytes.

Biodistribution and radiation dosimetry of stabilized [^{99m}Tc]-exametazine-labeled leukocytes in normal subjects. Robins PD, Salazar I, Forstrom LA, Mullan BP, Hung JC. J Nucl Med 2000 May; 41 (5), pp. 934–40.

Septic or Non-Septic Inflammation/Differentiation

[^{99m}Technetium] ([^{99m}Tc])-UBI 29-41/[^{99m}Tc]-Sc-UBI 29-41

Cellular/Molecular Mechanism of Action: A [^{99m}Tc]-labeled peptide derived from ubiquicidine referred to as [^{99m}Tc]-UBI 29-41 targets bacterial and fungal infections but not sterile inflammatory processes.

Application: Differentiation between septic inflammation due to bacterial pathogens from conditions of non-septic inflammatory responses.

Route of Administration: [^{99m}Tc]-UBI 29-41 and [^{99m}Tc]-Sc-UBI 29-41 (intravenous).

Semi-Synthetic Methods: Radiochemical analyses of [^{99m}Tc]-UBI 29-41 and a radiolabeled scrambled version of this peptide, referred to as [^{99m}Tc]-Sc-UBI 29-41, reveal that both peptides can be (i) labeled rapidly (within 10 minutes); (ii) labeled effectively with little colloid formation (less than 5% of the total radioactivity); and (iii) labeled with very little free pertechnetate (or radioactive intermediates) in the preparations containing radiolabeled peptide.

Implementation: Delineation of the relative capacity of [^{99m}Tc]-UBI 29-41 and [^{99m}Tc]-Sc-UBI 29-41 to differentiate between septic

inflammatory responses and conditions of non-septic inflammation. In this manner, greater insight can be achieved in delineating regions of the amino acid sequence of UBI 29-41 that are the most critical for selective imaging of bacterial infections.

Animal Model: Murine (*Staphylococcus aureus*–infected mice).

Interpretation and Analysis: Association of peptides with bacteria has been shown to be temperature dependent. Based on *in vitro* observations, labeling of peptides with [^{99m}Tc] by a direct method is rapid, efficient, and safe. Scintigraphy demonstrates that radioactivity is rapidly removed from the circulation (the half-life of UBI 29-41 and Sc-UBI 29-41 is 16 and 21 minutes, respectively) and occurs primarily as a function of renal clearance mechanisms. Analysis of murine blood reveals that only a small proportion of the intravenously injected [^{99m}Tc]-peptides is associated with blood cells. Although both types of radiolabeled peptides accumulate rapidly at sites of infection, the values for [^{99m}Tc]-UBI 29-41 are higher ($p < 0.05$) than for [^{99m}Tc]-Sc-UBI 29-41. Moreover, injection of excess unlabeled UBI 29-41, but not Sc-UBI 29-41, in scenarios of *S. aureus* infections prior to injection of [^{99m}Tc]-UBI 29-41 significantly ($p < 0.05$) reduces the accumulation of this radiopharmaceutical at sites of sepsis. In addition, significantly ($p < 0.01$) higher amounts of [^{99m}Tc]-UBI 29-41 can be appreciated at the site of infection (e.g., mice) using a carrier-free formulation of radiolabeled UBI 29-41 peptide in contrast to unpurified preparations containing radiolabeled UBI 29-41 peptide. Such *in vivo* data implies that the radiolabeled amino acid sequence [^{99m}Tc]-UBI 29-41 contributes to its accumulation at sites of infection.

Radiochemical and biological characteristics of [^{99m}Tc]-UBI 29-41 for imaging of bacterial infections. Welling MM, Mongera S, Lupetti A, Balter HS, Bonetto V, Mazzi U, Pauwels EK, Nibbering PH. Nucl Med Biol 2002 May; 29 (4), pp. 413–22.

Septic or Non-Septic Inflammation/Differentiation *[^{99m}Technetium] ([^{99m}Tc])-Labeled Ubiquicidin (UBI)*

Cellular/Molecular Mechanism of Action: Human UBI is an antimicrobial peptide that physically binds to bacterial organisms.

Application: Discrimination between bacterial infections and sterile inflammatory reactions.

Route of Administration: [^{99m}Tc]-labeled UBI (intravascular).

Implementation: Evaluate the *in vitro* binding of various [^{99m}Tc]-labeled natural or synthetic peptides (based on the sequence of the human antimicrobial peptide UBI or human lactoferrin [hLF]) to bacteria and leukocytes populations. Complementary investigations can entail the *in vivo* evaluation of these same radiolabeled peptides injected in animal models (e.g., mice) intraperitoneally infected with *Klebsiella pneumoniae* in order to identify sequences that preferentially bind to bacteria compared to host cell populations. The amount of radioactivity associated with bacteria and leukocytes is then quantitated. Discovery of the ability for candidate [^{99m}Tc]-labeled peptides to discriminate between bacterial infections and sterile inflammatory processes can be established *in vivo* (e.g., mice) utilizing intramuscular injections of various bacterial organisms (e.g., multidrug-resistant *Staphylococcus aureus*) and lipopolysaccharides (LPS) of bacterial origin (sterile inflammatory process). Complementary validation studies can entail determination of [^{99m}Tc]-labeled UBI 29-41 and UBI 18-35 distribution in rabbits having an experimental thigh muscle infection with *K. pneumoniae* and in rabbits injected with LPS fractions.

Animal Model: Murine (mouse sepsis: intraperitoneal injection of *K. pneumoniae*.

Animal Model: Rabbit (intramuscular injection of *K. pneumoniae* (septic foci model) and lipopolysaccharide fractions (sterile LPS inflammatory model).

Interpretation and Analysis: In both *in vitro* and *in vivo* binding assays, the 2 peptides, UBI 29-41 and UBI 18-35, represent 2 candidate platforms that appear to be appropriate for image analysis of infection. The radiolabeled peptides can detect infections due to both gram-positive and gram-negative bacteria in mice as early as 5–30 minutes after injection, with a target-to-non-target (T/NT) ratio between 2 and 3; maximum T/NT ratios can be seen within 1 hour after injection. In rabbits, high T/NT ratios (> 5) for [^{99m}Tc]-labeled UBI 29-41 are observed from 1 hour after injection. No accumulation of candidate [^{99m}Tc]-labeled UBI-derived peptides can be observed in thighs of mice and rabbits previously injected with LPS fractions. Scintigraphic investigations into the biodistribution of [^{99m}Tc]-labeled UBI peptides reveal that they are rapidly removed from circulation by renal excretion. Similar data can be observed for [^{99m}Tc]-labeled defensin 1-3. Data for [^{99m}Tc]-labeled hLF and related peptides indicate that these compounds are less favorable for the detection of infection. Taken together, [^{99m}Tc]-labeled UBI 18-35 and UBI 29-41 enable discrimination between bacterial infections and sterile inflammatory processes in both mice and rabbits. Based on their characteristics and properties, these peptides are candidate platforms for the preferential detection of septic bacterial infections.

[^{99m}Technetium]-labeled antimicrobial peptides discriminate between bacterial infections and sterile inflammations. Welling MM, Paulusma-Annema A, Balter HS, Pauwels EK, Nibbering PH. Eur J Nucl Med 2000 Mar; 27 (3), pp. 292–301.

Septic and Non-Septic Inflammation/ Central Nervous System (CNS) Allergic Encephalitis/ Glucocorticoid Response/Leukocytes: *In Vivo* Labeling of Neutrophil and Macrophage Tuftsin Receptors/Multiple Sclerosis: Potential Applications *[^{99m}Technetium] ([^{99m}Tc])-RP128*

Cellular/Molecular Mechanism of Action: The agent RP128 functions as a chelation agent for [^{99m}Tc], in addition to recognizing and binding to tuftsin receptors expressed by neutrophils and monocyte-macrophages.

Application: The agent [^{99m}Tc]-RP128 can be applied for the *in vivo* labeling of neutrophil and macrophage tuftsin receptor complexes for the purpose of detecting and characterizing focal sites of septic inflammation. Potential applications include the detection and characterization of human multiple sclerosis (MS).

Route of Administration: Intravascular.

Implementation: Administration of [^{99m}Tc]-RP128 for the *in vivo* detection of CNS foci of inflammation (e.g., experimental allergic encephalomyelitis [EAE]).

Animal Model: Mouse (e.g., murine EAE developed as a model for human MS).

Interpretation and Analysis: The radiopharmaceutical [^{99m}Tc]-RP128 can be detected at significantly increased levels within EAE-diseased CNS tissues at 0.5, 1.0, and 3.0 hours postinjection using a dual radioisotope technique to correct for non-extravasated tracer ($p < 0.05$). Moreover, extravascular accumulation of [^{99m}Tc]-RP128 can be clearly demonstrated in inflammatory tissues with minimal loss of sensitivity when the secondary isotopic correction for blood volume is omitted. In addition, [^{99m}Tc]-RP128 can successfully monitor glucocorticoid suppression of inflammation ($p < 0.05$), which is detected as a typical dose response to increasing steroid concentration. Clearly, [^{99m}Tc]-RP128 can quantitatively detect CNS inflammation and assess responses to therapy, indicating potential value as an imaging agent both clinically and as a molecule

probe for investigative research purposes. Furthermore, the rapid *in vivo* labeling by [^{99m}Tc]-RP128 of specific inflammatory cells combined with the ability to monitor the progress of anti-inflammatory therapeutics may validate the use of this cellular radiolabeling agent for detecting a variety of inflammatory conditions.

The detection and quantitation of inflammation in the central nervous system during experimental allergic encephalomyelitis using the radiopharmaceutical [^{99m}Tc]-RP128. Paul C, Peers SH, Woodhouse LE, Thornback JR, Goodbody AE, Bolton C. J Neurosci Methods 2000 May 15; 98 (1), pp. 83–90.

INTEGUMENTARY SYSTEM

See neoplastic disease section within this chapter for topics related to melanoma and metastatic melanoma.

METABOLIC AND METABOLISM

Lipoprotein Metabolism and Distribution *[^{99m}Technetium] [^{99m}Tc])-Very Low-Density Lipoproteins (VLDLs)/[^{99m}Tc]-Peroxynitrite-Modified Beta-VLDL/[^{99m}Tc]-Native Beta-VLDL*

Background Information: Free radicals superoxide (O_2^-) and nitric oxide (*NO) are generated by blood vessels and can rapidly react to produce a peroxynitrite anion ($ONOO^-$), a powerful oxidant that modifies lipoproteins, making them more atherogenic.
Cellular/Molecular Mechanism of Action: Radiolabeled lipoproteins behave and are perceived by the body in essentially the same manner that endogenous lipoprotein fractions are when pertaining to interactions with corresponding receptor complexes, transport, and metabolization.
Application: Trace the binding of VLDLs to cell membrane–associated receptor complexes and processes and characterization of VLDL metabolism.
Route of Administration: Intravenous.
Implementation: Evaluate the effect of peroxynitrite-induced modifications on beta-VLDL as to its biodistribution and plasma clearance rate, as well as uptake (assimilation) by THP-1 cells. Examples of preparations that can be applied for diagnostic purposes includes [^{99m}Tc]-labeled VLDLs either in their natural form or as peroxynitrite-modified beta-VLDL [^{99m}Tc]-per-beta-VLDL.

Animal Model: Rabbit (New Zealand White strain with hypercholesterolemia).
Interpretation and Analysis: Very low-density lipoproteins can be successfully radiolabeled with [^{99m}Tc] in a manner that produces a diagnostic agent capable of assessing selective transport mechanisms and targeted organ-specific biodistribution patterns. Following injection the peroxynitrite-modified beta-VLDL ([^{99m}Tc]-per-beta-VLDL) is cleared from circulation faster than the native beta-VLDL ([^{99m}Tc-nat-beta-VLDL) in both normocholesterolemic rabbits (NC) and in hypercholesterolemic rabbits (HC). In HC rabbits, the fractional clearance of [^{99m}Tc]-labeled beta-VLDL is significantly lower than in NC rabbits. Tissue/organ accumulation, *in vivo* of [^{99m}Tc]-labeled beta-VLDL, expressed per gram of tissue has been observed to be kidney > liver > spleen > adrenal gland ≥ lung > aortic arch > heart > or = abdominal aorta > thoracic aorta > psoas muscle. High accumulations in the kidneys suggest processing of [^{99m}Tc]-labeled apolipoproteins by receptors present in kidney cells. The tissue/organ accumulation of [^{99m}Tc]-nat-beta-VLDL in the whole organ occurs in the following order: liver > kidney > heart > spleen > adrenal gland > aorta in HC and NC rabbits. The uptake of [^{99m}Tc]-per-beta-VLDL by the spleen is greater than the uptake by the heart in both groups. Uptake of [^{99m}Tc]-per-beta-VLDL by THP-1 cells *in vitro* is higher than that of [^{99m}Tc]-nat-beta-VLDL. These results show that peroxynitrite-modified beta-VLDL is rapidly removed from plasma and accumulates in several tissues, mainly in the liver and kidney. This may be particularly important in hypercholesterolemic situations that could favor the accumulation of native and peroxynitrite-modified beta-VLDL in several tissues.

Peroxynitrite-modified [^{99m}Tc]-beta-VLDL: tissue distribution and plasma clearance rate. Silva EL, Meneghetti JC, Coelho IJ, Abdalla DS. Free Radic Biol Med 2001 Aug 15; 31 (4), pp. 440–9.

MUSCULOSKELETAL SYSTEM

Bone Activity: Elevated in Skeletal Disorders *[^{99m}Technetium] ([^{99m}Tc])-Labeled Methylene Diphosphonate (MDP)*

Cellular/Molecular Mechanism of Action: The agent MDP is incorporated into hydroxyapetite crystal by biochemically viable osteoblast cell populations residing within skeletal tissues.
Application: Diagnosis of skeletal fractures, osteomyelitis, primary osteosarcoma, secondary metastasis, and other abnormalities affecting the physical and biochemical integrity of bone.
Route of Administration: [^{99m}Tc]-labeled MDP (intravenous).
Implementation: Patients on long-term hemodialysis suffer from dialysis-associated arthropathy due to the deposition of dialysis amyloid. In most clinically affected joints, with the exception of shoulders and hands, an increased uptake of radioisotope can consistently be appreciated in structures surrounding periarticular bone structures. In addition, intense soft-tissue radioisotope uptake around clinically affected large joints can be detected. Similar scintigraphic patterns are not appreciated in negative reference control subjects. A semi-quantitative scale of uptake analysis reveals a degree of correlation with (i) perception of pain and ankle/feet radioisotope uptake; and (ii) number of radiological lesions and degree of isotope uptake in the wrists and knees. In cases of renal failure that are receiving renal dialysis management, it has been proposed that the uptake of radioisotope can be attributed to its binding in affected joint structures to amyloid deposits, soft-tissue calcification, or areas of increased bone turnover.
Interpretation and Analysis: Represents a scintigraphic scanning technique that cannot make a definite diagnosis of amyloid and, therefore, cannot be expected to supersede histological diagnosis. However, it is a useful adjuvant investigative tool that is of particular importance in those cases that are unable or unwilling to undergo biopsy procedures.
Instrumentation: Scintigraphic gamma camera.

Technetium-99-labelled methylene diphosphonate uptake scans in patients with dialysis arthropathy. Sethi D, Naunton Morgan TC, Brown EA, Jewkes RF, Gower PE. Nephron 1990; 54 (3), pp. 202–7.

Bone Skeletal Structures *[^{99m}Technetium] ([^{99m}Tc])-Labeled Methylene Diphosphate (MDP)*

Cellular/Molecular Mechanism of Action: Incorporated into the hydroxyapetite matrix of bone tissues by functional osteoblast populations.
Background Information: Scintigraphy with technetium MDP gives a positive result in bone tissue with a state of elevated metabolic activity. This can be used in the diagnosis of mechanical stress lesions in bone.

Diagnostic Application: Detect sites of active osteoblastic activity such as that appreciated near septic inflammation and fractures.
Route of Administration and Dosage: Intravenous injection.
Implementation: Soft tissue phase imaging is performed at approximately 1 hour postadministration. Following completion of the soft tissue phase, cases are imaged a 2nd time at approximately 3 hours following intravenous injection.
Interpretation and Analysis: Radiolabeled MDP accumulates in soft tissue structures during the initial 30- to 60-minute period following intravenous administration. Detection is achieved with the aid of a computer-integrated scintigraphy (nuclear medicine) camera. Soft tissue lesions affecting joint, tendon, and ligament structures are detected by an increase in the local accumulation of radiolabeled MDP.

Following completion of soft tissue phase imaging, bone phase images are acquired approximately 3 hours following intravenous injection. Orthopedic lesions can be detected as an increase in the local accumulation of radiolabeled MDP within skeletal tissues.

Scintigraphy with [^{99m}Tc]-MDP in the diagnosis of stress lesions in bone. Motta F, Pietrogrande V. Ital J Orthop Traumatol 1985 Jun; 11 (2), pp. 215–22.

Bone/Osteomyelitis Bone Scanning *[^{99m}Technetium] ([^{99m}Tc])-Labeled Antigranulocyte Antibody Fragments*

Cellular/Molecular Mechanism of Action: Antigranulocyte antibody possesses binding avidity for membrane-associated antigens that are unique to neutrophil populations. Neutrophils are chemotactically attracted to areas of (septic) inflammation. The Fab′ fragment of IgG has the advantage of being devoid of the Fc fragment so that it minimally binds to membrane-associated Fc receptor complexes expressed by the mononuclear phagocytic system residing within the liver and spleen (low level of non-specific binding).
Application: Detection and characterization of septic inflammatory lesions.
Route of Administration: [^{99m}Tc]-labeled antigranulocyte antibody fragment (intravenous).
Dosage: [^{99m}Tc]-labeled antigranulocyte antibody fragment [740 MBq (20 mCi)].
Implementation: Immunoscintigrams performed with [^{99m}Tc]-labeled antigranulocyte antibody fragments for the detection of focal lesions of septic inflammation. Validation of findings can be established by histopathology, magnetic resonance imaging, computed tomography, and monitoring of clinical signs.
Interpretation and Analysis: The percentage of true-positive images obtained with [^{99m}Tc]-labeled antigranulocyte antibody fragments for the detection of septic inflammation is approximately 67% (e.g., n = 20/30). The number of false-positive results is relatively low (e.g., n = 3/30). Approximately 20% of images will be true-negatives (e.g., n = 6/30). Consequently, [^{99m}Tc]-labeled antigranulocyte antibody fragments can achieve a high level of sensitivity (e.g., 95%), moderately high specificity (e.g., 67%), and high diagnostic accuracy (e.g., 86%). Therefore, [^{99m}Tc]-labeled monoclonal antibody-Fab′ fragments are suitable for the detection of osteomyelitis. In a clinical environment, preparations and their applications are simple, while providing a reliable and accurate diagnostic result just 1–2 hours after injection, making a late scan unnecessary. Coxarthrosis or hyperostosis can lead to false-positive results. A perforating ulcer of the foot may result in a false-negative conclusion.

Imaging osteomyelitis with [^{99m}Tc]-labeled antigranulocyte antibody Fab′ fragments. Von Rothenburg T, Schaffstein J, Ludwig J, Vehling D, Koster O, Schmid G. Clin Nucl Med 2003 Aug; 28 (8), pp. 643–7.

Bone/Osteomyelitis Bone Scanning *[^{99m}Technetium] ([^{99m}Tc])-PEG Liposomes/[^{99m}Tc]-HYNIC-IgG/[^{99m}Tc]-Methylene Diphosphonate (MDP)/[^{111}Indium] ([^{111}In])-Granulocytes/Gallium-67 ([^{67}Ga])-Citrate*

Background Information: Assessment of disease activity and disease extent in chronic osteomyelitis remains a difficult diagnostic problem. Radiography is not particularly sensitive. Scintigraphic techniques can be more helpful, but routinely available agents lack specificity (e.g., [^{99m}Tc]-MDP, [^{67}Ga]-citrate) or are relatively laborious to prepare (e.g., [^{111}In] leukocytes).
Route of Administration: [^{99m}Tc]-PEG liposomes (intravenous); [^{99m}Tc]-HYNIC-IgG (intravenous); [^{99m}Tc]-MDP (intravenous); [^{111}In]-granulocytes (intravenous); or [^{67}Ga]-citrate (intravenous).
Dosage: [^{99m}Tc]-PEG liposomes (37 MBq rabbit); [^{99m}Tc]-HYNIC-IgG (37 MBq rabbit); [^{99m}Tc]-MDP (37 MBq rabbit); [^{111}In]-granulocytes (18 MBq rabbit); or [^{67}Ga]-citrate (18 MBq rabbit).
Implementation: Scintigrahic image analysis of osteomyelitis lesions of the femur 4 weeks after bacterial infusion applying [^{99m}Tc]-PEG liposomes, [^{99m}Tc]-HYNIC-IgG, and [^{99m}Tc]-MDP on 3 consecutive days and imaged up to 4 hours (MDP) or 22 hours (liposomes and IgG) postinjection. On day 4, preparations of [^{111}In]-granulocytes or [^{67}Ga]-citrate are administered and then imaged up to 44 hours after injection. Uptake in the septic femur is determined by drawing regions-of-interest (ROIs). Ratios of infected-to-sham-operated femur are calculated. After the last image, the left and right femur are scored for microbiologic and histopathologic evidence of osteomyelitis (research models).

Animal Model: Chronic osteomyelitis is induced by infusing *Staphylococcus aureus* into the right reamed and washed femoral canal (e.g., rabbits). The canal is sealed with cement and a sham operation is performed on the left femur. Routine radiographs are obtained immediately after surgery and before scintigraphic imaging.
Interpretation and Analysis: [^{99m}Tc]-PEG liposomes and [^{99m}Tc]-HYNIC-IgG can correctly identify lesions of osteomyelitis in a high percentage of cases (e.g., n = 6/6). In a small percentage of cases, [^{111}In]-granulocytes and [^{67}Ga]-citrate can produce equivocal results (e.g., 15% or n = 1/6), and [^{99m}Tc]-MDP may miss lesions of osteomyelitis (e.g., 15% or n = 1/6). The uptake in the affected region does not differ significantly between the imaging agents, although [^{99m}Tc]-MDP tends to have higher values (MDP, 4.75 ± 1.23 percent injected dose per gram [%ID/g]; [^{67}Ga], 2.05 ± 0.54 %ID/g; granulocytes, 1.56 ± 0.83 %ID/g; liposomes, 1.75 ± 0.76 %ID/g; and IgG, 1.96 ± 0.27 %ID/g). The ratios of infected-to-normal femur are also not significantly different for the respective radiopharmaceuticals. Radiography can visualize only severe osteomyelitis. [^{99m}Tc]-PEG liposomes and [^{99m}Tc]-HYNIC-IgG perform at least as well as [^{111}In]-granulocytes and [^{67}Ga]-citrate in localizing chronic osteomyelitis (e.g., rabbits). The ease of preparation, the better image quality, and the lower radiation dose suggest that [^{99m}Tc]-PEG liposomes and [^{99m}Tc]-HYNIC-IgG might be suitable alternatives to [^{67}Ga]-citrate and [^{111}In]-granulocytes for the scintigraphic evaluation of osteomyelitis conditions.

Scintigraphic evaluation of experimental chronic osteomyelitis. Dams ET, Nijhof MW, Boerman OC, Laverman P, Storm G, Buma P, Lemmens JA, van der Meer JW, Corstens FH, Oyen WJ. J Nucl Med 2000 May; 41 (5), pp. 896–902.

Bone/Osteomyelitis Bone Scanning
Gallium-67 ([^{67}Ga])-Citrate/[^{99m}Technetium] ([^{99m}Tc])-Methylene Diphosphonate (MDP)/[^{99m}Tc]-Interleukin-8 (IL-8)

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-MDP is believed to be assimilated by active osteoblasts/osteoclasts at sites of dynamic bone growth and destruction.

Background Information: Early and accurate diagnosis of osteomyelitis remains a clinical problem. Acute osteomyelitis often occurs in infants and most often is located in long-bone skeletal structures. Radiologic images show changes only in the advanced stages of disease. Scintigraphic imaging with [^{99m}Tc]-MDP, or bone scanning, is much more sensitive in detecting acute osteomyelitis but lacks specificity.

Application: Detection and characterization of septic osteomyelitis lesions.

Route of Administration: [^{99m}Tc]-MDP (intravenous), [^{99m}Tc]-IL-8 (intravenous).

Dosage: [^{99m}Tc]-MDP (18.5 MBq), [^{99m}Tc]-IL-8 (18.5 MBq).

Implementation: Scintigraphic characterization of osteomyelitis lesions 10 days following induction (e.g., n = 10 rabbits; n = 5/group) utilizing injections of either 18.5 MBq [^{111}In]-granulocytes or 18.5 MBq [^{67}Ga]-citrate. Images are performed both 24 hours and 48 hours postinjection. On day 12, serial images are acquired at 0, 1, 2, 4, 8, 12, and 24 hours after injection of either 18.5 MBq [^{99m}Tc]-MDP or 18.5 MBq [^{99m}Tc]-IL-8 preparations. Uptake in the infected femur is determined by establishing regions-of-interest (ROIs). Ratios of infected femur (target) to sham-operated femur (background) (T/Bs) are then calculated. After the final images are acquired, the right femur is harvested postmortem to facilitate dissection and analysis of tissue samples for microbiological and histopathological evidence of osteomyelitis.

Animal Model: Rabbit. Acute pyogenic osteomyelitis can be induced in rabbits (e.g., n = 10) by inserting sodium morrhuate and *Staphylococcus aureus* into the medullary cavity of the right femur. The cavity is then closed with liquid cement. A sham operation is performed on the left femur. Routine radiographs are obtained just before scintigraphy imaging. Acute osteomyelitis develops in a high percentage of rabbits (e.g., n = 8/10).

Interpretation and Analysis: All imaging agents can correctly detect acute osteomyelitis in a very high percentage of cases (e.g., n = 8/8). The extent of infection can be optimally visualized with [^{67}Ga]-citrate and by delayed bone scanning, whereas diaphyseal photopenia is noted with both [^{99m}Tc]-IL-8 and [^{111}In]-granulocytes. Some false-negative (e.g., n = 1/8) and false-positive (e.g., n = 1/8) image results can be observed with [^{111}In]-granulocytes and [^{99m}Tc]-MDP, respectively. Quantitative analysis of the images reveals that the uptake in the infected region is highest with [^{67}Ga]-citrate (4.9 ± 0.8 percent injected dose [%ID]) and [^{99m}Tc]-MDP (4.7 ± 0.7 %ID), whereas the uptake in the infected area is significantly lower with [^{99m}Tc]-IL-8 (2.2 ± 0.2 %ID) and [^{111}In]-granulocytes (0.8 ± 0.2 %ID) ($p < 0.0042$). Conversely, the T/Bs are significantly higher for [^{99m}Tc]-IL-8 (T/B, 6.2 ± 0.3 at 4 hours after injection) than for [^{67}Ga]-citrate, [^{99m}Tc]-MDP, and [^{111}In]-granulocytes, which have ratios of 1.5 ± 0.4, 1.9 ± 0.2, and 1.4 ± 0.1, respectively ($p < 0.0001$). Radiography correctly reveals acute osteomyelitis in only a relatively small percentage of cases (e.g., n = 2/8). In experimentally-induced osteomyelitis, [^{99m}Tc]-IL-8 clearly detects the osteomyelitic lesion. Although the absolute uptake in the osteomyelitic area is significantly lower than that obtained with [^{99m}Tc]-MDP and [^{67}Ga]-citrate, the T/Bs are significantly higher for [^{99m}Tc]-IL-8 because of fast background clearance. The ease of preparation, good image quality, and lower radiation burden suggest that [^{99m}Tc]-IL-8 may be a suitable imaging agent for the scintigraphic evaluation of acute osteomyelitis.

[^{99m}Tc]-interleukin-8 for imaging acute osteomyelitis. Gratz S, Rennen HJ, Boerman OC, Oyen WJ, Burma P, Corstens FH. J Nucl Med 2001 Aug; 42 (8), pp. 1257–64.

Bone/Osteomyelitis Bone Scanning
[^{99m}Technetium] ([^{99m}Tc])-Labeled Interleukin-8 (IL-8)

Cellular/Molecular Mechanism of Action: The cytokine IL-8 binds with high affinity to the CXCR1 and CXCR2 receptors on neutrophils.

Application: Previous investigations have shown that [^{99m}Tc]-IL-8 can rapidly and effectively delineate foci of infection and inflammation in rabbit models of intramuscular infection, colitis, and osteomyelitis.

Semi-Synthetic Methods: A derivative of hydrazinonicotinamide (HYNIC) can be used as a bifunctional coupling agent to label protein fractions with [^{99m}Tc] preparations.

Implementation: Delineation of [^{99m}Tc]-IL-8 pharmacokinetic profiles in conditions of neutropenia compared to normal states and determination of the ability of [^{99m}Tc]-IL-8 to detect septic abscesses. Delineation of the interaction of [^{99m}Tc]-IL-8 with blood cells circulating in normal rabbits can be established through assessment of the distribution of the radiolabel relative to circulating white and red blood cells and plasma. The *in vivo* kinetics of [^{99m}Tc]-IL-8 is established by quantitative analysis of whole-body images acquired between 0 and 6 hours after injection. Results from such analyses (*in vivo* biodistribution) can be validated by *ex vivo* counting of radioactivity in dissected tissue samples.

Animal Model: Turpentine-induced abscesses in both neutropenic and normal rabbits.

Interpretation and Analysis: The abscess uptake (percentage of injected dose per gram of tissue [%ID/g] ± standard error of measurement [SEM]) in immunocompetent rabbits (0.41 ± 0.05) is 10 times higher than that in neutropenic rabbits (0.038 ± 0.014), demonstrating specificity for target uptake of [^{99m}Tc]-IL-8. Abscess-to-muscle ratios ± SEM are also 10 times higher (110 ± 10 vs 10 ± 5). Lung and spleen uptake in normal rabbits is 3 times higher than that in neutropenic rabbits. The blood clearance of the radiolabel in neutropenic rabbits is similar to that in normal rabbits. In circulation, most of [^{99m}Tc]-IL-8 (70%) is found within the plasma fraction. Less than one-third is associated with red blood cells, and only a very low percentage (< 2.5%) is associated with white blood cells. Image analysis reveals a gradual increase in abscess uptake over time up to > 15 %ID, which can be confirmed by *ex vivo* gamma counting of infected muscle tissue. The highest increase in uptake within abscesses is observed after 2 hours following injection, when most of the [^{99m}Tc]-IL-8 clears from the blood, suggesting specific neutrophil-mediated accumulation of [^{99m}Tc]-IL-8 in abscesses. Furthermore, region-of-interest (ROI) analysis demonstrates gradual accumulation of [^{99m}Tc]-IL-8 in abscesses accompanied by a simultaneous clearance of activity from the lungs, suggesting that neutrophil-associated [^{99m}Tc]-IL-8 initially trapped in the lungs ultimately migrates to abscesses at a later point in time, favoring neutrophil-bound transportation from the lungs to the abscess. Substantial support exists for the hypothesis that [^{99m}Tc]-IL-8 localizes in the abscess and is mainly bound to peripheral neutrophils. Accumulation within abscesses is a highly specific, neutrophil-driven process. As determined by *in vivo* and *ex vivo* analysis, the total fraction that accumulates within inflamed tissues is extremely high (up

to > 15 %ID) compared with that of other agents used for imaging infection and inflammation.

Kinetics of [^{99m}Tc]-labeled interleukin-8 in experimental inflammation and infection. Rennen HJ, Boerman OC, Oyen WJ, Corstens FH. J Nucl Med 2003 Sep; 44 (9), pp. 1502–9.

Collagen Synthesis (Abnormal)/Osteosarcoma/ Pancreatic Accumulation/Renal Uptake/ Hepatic Clearance/Collagen Synthesis: Abnormal *(2S,4R)-4-[^{18}F]Fluoro-L-Proline/ (2S,4S)-4-[^{18}F]Fluoro-L-Proline*

Cellular/Molecular Mechanism of Action: (2S,4R)-4-[^{18}F]fluoro-L-proline and (2S,4S)-4-[^{18}F]fluoro-L-proline are radiofluorinated analogues of proline that are systemically perceived as the amino acid proline by endogenous protein synthesis pathways (e.g., collagen production).

Implementation: Evaluate the diastereomeric effect on uptake and metabolic behavior of (2S,4R)-4-[^{18}F]fluoro-L-proline (*trans*-[^{18}F]FPro) and (2S,4S)-4-[^{18}F]fluoro-L-proline (*cis*-[^{18}F]FPro) in view of their potential suitability as tracers for abnormal collagen synthesis. No-carrier-added 4-[^{18}F]fluoro-L prolines can be prepared in about 150 minutes utilizing previously described methodologies (50–60% radiochemical yield).

Animal Model: Murine (mouse osteosarcoma).

Interpretation and Analysis: The compounds *cis*-[^{18}F]F-Pro and *trans*-[^{18}F]F-Pro both exhibit high *in vivo* stability. The tumor uptake of *cis*-[^{18}F]F-Pro in osteosarcomas is high and at 240 minutes postinjection reaches 11.8 ± 2.2 percent injected dose per gram (%ID)/g compared to 7.07 ± 1.68 %ID/g for *trans*-[^{18}F]F-Pro. In contrast to *trans*-[^{18}F]F-Pro, which shows fast and complete renal clearance, the *cis* isomer is accumulated within the pancreas and shows hepatic clearance and renal reuptake. Speciation studies on tissue homogenates reveal protein incorporation only for *cis*-[^{18}F]F-Pro. However, due to the relatively slow protein incorporation rate of *cis*-[^{18}F]F-Pro, the tumor uptake of both compounds in colon carcinomas, mammary carcinomas, and osteosarcomas 1 hour postinjection predominantly reflects amino acid transport.

Preclinical evaluation of 4-[^{18}F]fluoroprolines: Diastereomeric effect on metabolism and uptake in mice. Wester HJ, Herz M, Senekowitsch-Schmidtke R, Schwaiger M, Stöcklin G, Hamacher K. Nucl Med Biol 1999 Apr; 26 (3), pp. 259–65.

Joints/Rheumatoid Arthritis Inflammation/ Intestinal Lesions: Non-Steroidal Anti-Inflammatory Drug (NSAID)-Associated/Intestinal Lesions: Crohn's Ileitis *[^{111}Indium] ([^{111}In]) Leukocytes/Tauro 23 [^{75}Se]-Selena-25-Homocholic Acid/[^{58}Co]-Labeled Cyanocobalamine*

Cellular/Molecular Mechanism of Action: The agent tauro 23 [^{75}Se]-selena-25-homocholic acid is a bile acid analog, while [^{58}Co]-labeled cyanocobalamine is an analogue of vitamin B_{12}. Radiolabeled [^{111}In] leukocytes behave *in vivo* like endogenous leukocytes of the same cell type.

Application: The agents tauro 23 [^{75}Se]-selena-25-homocholic acid and [^{58}Co]-labeled cyanocobalamine can be applied to evaluate intestinal absorption. Conversely, [^{111}In] leukocytes can be used to detect foci of septic and non-septic inflammation.

Route of Administration: Intravenous.

Implementation: Evaluation of residual inflammatory lesions following discontinuation of NSAIDs using [^{111}In] leukocyte scintigraphy imaging methodologies.

Human Model #1: Cases of rheumatoid arthritis (n = 90) and osteoarthritis (n = 7).

Human Model #2: Cases treated with NSAIDs (n = 19); healthy controls (n = 20); and Crohn's ileitis (n = 13).

Interpretation and Analysis: Using [^{111}In] leukocyte imaging techniques reveals that cases of rheumatoid arthritis and osteoarthritis that are administered NSAIDs frequently develop gastrointestinal lesions. More specifically, approximately two-thirds of all cases evaluated on long-term treatment display inflammatory lesions that may persist for up to 16 months following discontinuation. The prevalence and magnitude of intestinal inflammation is unrelated to the type and dose of non-steroidal drugs and previous or concomitant 2nd-line drug treatment. There is a significant inverse correlation ($r = -0.29$, $p < 0.05$) between fecal [^{111}In] excretion and hemoglobin levels in cases treated with NSAIDs. The kinetics of fecal [^{111}In] excretion in cases treated with NSAIDs is almost identical to that of cases with small bowel Crohn's disease. Small bowel radiological examination of cases on NSAIDs reveals that approximately one-sixth have asymptomatic ileal disease with ulceration and strictures (e.g., n = 3/18 cases). Evaluation of cases on anti-inflammatory agents, normal healthy controls, and cases of Crohn's ileitis with tauro 23 [^{75}Se]-selena-25-homocholic acid, and [^{58}Co]-labeled cyanocobalamine reveals several abnormalities. On day 4, more than half the cases with rheumatoid arthritis have evidence of bile acid malabsorption, but the ileal dysfunction is much milder than seen in patients with Crohn's ileitis.

Non-steroidal anti-inflammatory drug–induced intestinal inflammation in humans. Bjarnason I, Zanelli G, Smith T, Prouse P, Williams P, Smethurst P, Delacey G, Gumpel MJ, Levi AJ. Gastroenterology 1987 Sep; 93 (3), pp. 480–9.

Muscle/Exercise Intolerance (Anaerobic)/Left Ventricular (LV) Dysfunction *^{201}Thallium ([^{201}Tl])*

Cellular/Molecular Mechanism of Action: [^{201}Tl] is a radioactive isotope that emits photons.

Background Information: Decreased blood flow to working muscles makes an important contribution to exercise intolerance in patients with chronic heart failure.

Application: Radioactive marker system that can be utilized to detect alterations in muscle metabolism.

Route of Administration: [^{201}Tl] (intravenous).

Implementation: Evaluate whether maldistribution of skeletal muscle blood flow is closely related to exercise intolerance in conditions of cardiac dysfunction.

One investigative strategy for assessing this consideration involves the acquisition of images from whole-body thallium scintigraphy during 1-leg exercise challenge in cases of LV dysfunction. Blood flow distribution to exercising and resting legs is quantified by expressing regional thallium counts as a percentage of whole-body counts at rest, at the level of anaerobic threshold, and at peak exercise.

Human Model: Left ventricular dysfunction (LV ejection fraction < 45%; n = 11 cases).

Interpretation and Analysis: At anaerobic threshold, thallium activity of exercising muscle increases from 4.2 ± 0.7% to 14.0 ± 2.5% ($p < 0.05$) in the thigh and from 1.7 ± 0.3% to 4.1 ± 0.9% ($p < 0.05$) in the calf, compared to resting values. Consequently, the ratio of thallium activity between exercising and resting legs increases to 2.7 ± 0.7 ($p < 0.05$) in the thigh and to 2.3 ± 0.7 ($p < 0.05$) in the calf. When plotted as a function of anaerobic threshold, thallium activity of the exercising thigh ($r = 0.78$, $p < 0.05$) and the thallium

ratio between exercising and resting thigh ($r = 0.69$, $p < 0.05$) declines with a reduction in exercise tolerance. These correlations are not observed in calves. Whole-body thallium scintigraphy demonstrates a maldistribution of leg blood flow in conditions with reduced aerobic exercise capacity, suggesting that this abnormality could play an important role in exercise intolerance in cases with such forms of cardiac dysfunction.

Quantitative evaluation of blood flow distribution to exercising and resting skeletal muscles in patients with cardiac dysfunction using whole-body thallium-201 scintigraphy. Wada O, Asanoi H, Miyagi K, Ishizaka S, Kameyama T, Ishise H, Seto H, Inoue H. Clin Cardiol 1997 Sep; 20 (9), pp. 785–90.

Muscle/Myopathy: Statin-Induced
Methoxy-Isobutyl-Isonitrile (MIBI)

Background Information: Statin-induced myopathy is the most commonly seen side effect in users of this family of drugs. Different forms present either with or without creatine phosphokinase (CK) elevations, signs of *in vivo* oxidation injury or not, or a combination of both. The pathogenetic background, however, still remains obscure. Besides its effectiveness for myocardial and tumor scintigraphy, MIBI is also useful in detecting muscle metabolic abnormalities, which is recognized as an increase in the level of MIBI uptake in diseased muscular segments.

Implementation: Evaluation of statin-induced myopathy utilizing MIBI whole-body imaging techniques performed immediately during statin exposure.

Human Model: Cases of statin-induced myopathy displaying either elevated CK, isoprostanes, or muscle pains at varying combinations ($n = 7$; $n = 5$ males; $n = 2$ females; age = 36–56 years). Reference controls can include cases suffering from lung or breast cancer that are also exposed to statins preparations ($n = 16$; $n = 6/16$ males, $n = 10$ females).

Interpretation and Analysis: No uptake abnormalities can be appreciated in any muscular segment in either affected cases or reference controls. Therefore, MIBI scintigraphy is not useful, apparently, in diagnosing and eventually localizing statin-induced myopathy. Scintigraphy with MIBI is of no diagnostic benefit for gaining further insight into statin-induced myopathy.

Statin-induced myopathy does not show up in MIBI scintigraphy. Lupattelli G, Palumbo B, Sinzinger H. NuclMed Commun 2001 May; 22 (5), pp. 575–8.

NEOPLASTIC DISEASE

Adrenal Gland: Function and Structure
[^{131}I]-6-β-Iodomethyl-Norcholesterol/[^{75}Se]-Selenomethyl-Norcholesterol/[^{131}I]-, [^{123}I]-, and [^{124}I]-Metaiodobenzylguanidine (MIBG)/[^{18}F]-Metafluoro-Benzyl-Guanidine/[^{123}I]-Tyr-Octreotide/[^{111}In]-Diethylenetriamine Pentaacetic Acid (DTPA)-Octreotide/[^{111}In]-DOTA-Octreotide/[^{18}F]-2-Fluoro-d-Deoxyglucose/Metyrapone/Tyrosine Analogues/Low-Density Lipoproteins (LDL Receptors)/Bombesin/Vasoactive Intestinal Polypeptide/Cholecystokinin/Opiate Peptides/[^{11}C]-Acetate

Background Information: Over the last 30 years nuclear medicine imaging of the adrenal gland and its lesions has been achieved by exploitation of a number of physiological characteristics associated with this organ. By seeking and utilizing features that are quantitatively or qualitatively different from those of the adjacent tissues, it is possible to functionally characterize the adrenal gland and associated disease states. In most instances lesions affecting both the cortex and the medulla retain the basic physiology related to their original tissue type. Diagnostic strategies widely used in clinical practice include assessment of (i) radiolabeled cholesterol analog uptake and storage facilitated by LDL receptors and cholesterol ester storage pools in the adrenal cortex utilizing the agents [^{131}I]-6-β-iodomethyl-norcholesterol or [^{75}Se]-selenomethyl-norcholesterol; (ii) catecholamine Type I presynaptic, uptake mechanisms, and intracellular granule uptake and storage processes in the adrenal medulla and extra-adrenal paraganglia utilizing [^{131}I]-, [^{123}I]-, and [^{124}I]-MIBG and [^{18}F]-metafluoro-benzyl-guanidine; (iii) cell surface receptor binding of peptides/neurotransmitters/modulators such as those for the family of 5 subtypes of somatostatin receptors ([^{123}I]-tyr-octreotide, [^{111}In]-DTPA-octreotide, [^{111}In]-DOTA-octreotide, and many others); (iv) increases in tumor glycolysis, particularly in the most malignant adrenal-associated cell types, utilizing [^{18}F]-2-fluoro-d-deoxyglucose, that can be expected to detect certain neoplastic lesions such as malignant pheochromocytomas (particularly the minority of cases that are not detected with MIBG) and adrenal incidentalomas (particularly with known extra-adrenal malignancies).

There are a variety of adrenal tissue characteristics that have the potential for being exploited for various purposes that are not currently employed for clinical uses but nevertheless, have potential as imaging agents. These include (i) inhibitors of adrenal cortical steroid hormone synthesis enzymes (e.g., radiolabeled analogues of metyrapone); (ii) radiolabeled lipoproteins that bind to adrenocortical LDL receptors; (iii) inhibitors of catecholamine biosynthesis enzymes (e.g., radiolabeled analogues of tyrosine and related amino acids); and (iv) cell surface receptors for various peptides and hormones that may be overexpressed by adrenal cortical or adrenal medullary tumors (e.g., radiolabeled analogues of adenocorticotropic hormone [ACTH] for adrenocortical cells of zona fasciculata or zona glomerulosa origin; neurotransmitter/hormone message peptides binding to cell surface receptors such as bombesin, vasoactive intestinal polypeptide, cholecystokinin, and opiate peptides). The adrenal cortex can also synthesize cholesterol from acetate, and preliminary studies with [^{11}C]-acetate positron emission tomography have shown interesting results.

Application: Detection and characterization of the adrenal gland function and dysfunction.

Functional scintigraphy of the adrenal gland. Rubello D, Bui C, Casara D, Gross MD, Fig LM, Shapiro B. Eur J Endocrinol 2002 Jul; 147 (1), pp. 13–28.

Adrenal Gland/Pheochromocytoma
[^{131}I]-Metaiodobenzylguanidine (MIBG)/Metaclopramide

Background Information: Pheochromocytoma accounts for approximately 25% of incidentally discovered adrenal masses. Certain diagnostic procedures (e.g., adrenal arteriography, needle biopsy of an adrenal mass), anesthesia, and abdominal surgery may cause a sudden release of catecholamines from a pheochromocytoma and induce paroxysmal attacks of hypertension. In addition, pheochromocytoma is well known to cause unsuspected operating room deaths. Therefore, it is important to carefully separate this functioning neoplasm from other types of adrenal gland masses.

Pheochromocytoma cases frequently have elevated plasma concentrations of adrenaline (epinephrine)and noradrenaline (norepinephrine), in addition to increases in urinary vanillylmandelic acid, metanephrine, and/or normetanephrine.

Application: Detection of pheochromocytoma.

Route of Administration: Metaclopramide (IV); [^{131}I]-MIBG (IV).

Considerations: In situations where results from these assays are negative, both the metaclopramide (provocative test) and [^{131}I] or [^{123}I]-MIBG (MIBG scintigraphy) can be performed as an alternative diagnostic strategy.
Implementation: Compare results of non-invasive tests including (i) assay of urinary catecholamines and their metabolites; (ii) a provocative pharmacologic test using metoclopramide (MCP test); and (iii) [^{131}I]-MIBG scintigraphy to screen for pheochromocytoma.

Human Model: Cases with an incidentally discovered adrenal mass (n = 10; n = 6 pheochromocytomas, n = 4 non-functioning adrenocortical adenomas).
Interpretation and Analysis: Urinary excretion of catecholamines, metanephrines, and vanillylmandelic acid may be positive in only 50% of cases (e.g., n = 5/10; n = 4 negative; n = 1 false-negative; sensitivity = 83%, specificity = 100%). The [^{131}I/^{123}I]-MIBG reagent uptake within adrenal lesions is increased to the extent that they can be visualized in diagnostic nuclear medicine studies. The MCP test may be positive in less that 50% of cases (e.g., n = 3/7; n = 3/7 negative; n = 1 false-negative; sensitivity = 75%, specificity = 100%). MIBG scintigraphy may similarly be positive in over 50% of cases (e.g., n = 4/7; n = 1/7 negative; n = 2/7 false-negative; sensitivity = 67%, specificity = 100%). Such observations suggest that all patients with an incidentally discovered adrenal mass should undergo a determination of the 24-hour urinary excretion of catecholamines and their metabolites, including metanephrines. If this urine assay is negative, other non-invasive tests, including the MCP test and MIBG scintigraphy, should be considered in selected patients with radiographic characteristics indicative of pheochromocytoma.

Non-invasive screening for pheochromocytoma in patients with an incidentally discovered adrenal mass: Usefulness of provocative test with metoclopramide and [^{131}I]-metaiodobenzylguanidine scintigraphy. Yokoyama H, Tsuji Y. Hinyokika Kiyo 1999 Oct; 45 (10), pp. 677–80.

Adrenal Gland/Pheochromocytoma (Silent/ Subclinical) *[^{131}I]-Metaiodobenzylguanidine (MIBG)/Glucagon*

Application: Diagnosis of subclinical/silent pheochromocytoma.
Route of Administration: Glucagons (IV) and [^{131}I]-MIBG (IV).
Considerations: Adrenal masses can sometimes be incidentally discovered during abdominal ultrasonography and computed tomography in cases that are normotensive and asymptomatic in addition to having nearly normal plasma catecholamine levels. However, urinary levels of catecholamine metabolites are often slightly elevated in such cases.
Implementation: Evaluation of suspected conditions of pheochromocytoma. Note: A cutoff at 4.0 cm for tumor mass size can be set in order to differentiate between benign and malignant tumors.
Interpretation and Analysis: A glucagon provocative test and [^{131}I]-MIBG scintigram can be useful for diagnosing atypical pheochromocytoma. In cases classified as incidentaloma, 82.5% are benign masses, of which 35.0% (n = 36/85) are non-functional adrenal cortical adenomas, and 11.7% (n = 12/85) are pheochromocytoma. Cases with malignant masses are significantly younger than cases with adenomas ($p < 0.05$), and adenomas are significantly smaller than malignant masses ($p < 0.001$). During the diagnosis of atypical incidentaloma, it's essential to evaluate hormonal activity and assess the risk for malignancy. In instances where the mass size is 4 cm or larger, the risk of malignancy increases. More attention should be paid to subclinical hyperfunctional state.

A clinical analysis of 103 cases of adrenal incidentaloma. Tao H, Lu ZL, Li HZ. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2003 Apr; 25 (2), pp. 172–5.

Hypertension following minor trauma: A rare presentation of pheochromocytoma. Dueck A, Poenaru D, Kamal I. Pediatr Surg Int 1999; 15 (7), pp. 508–9.

Apoptosis/Neoplastic Disease *[^{99m}Technetium] ([^{99m}Tc])-Annexin V/ [Cyclophosphamide]/TUNEL Tissue Analysis/ [^{14}C]-Iodoanitpyrine*

Cellular/Molecular Mechanism of Action: Annexin V is a human protein that possesses a high affinity for phosphatidylserine.
Application: Annexin V can be labeled with [^{99m}Tc] to detect *in vivo* apoptosis.
Route of Administration: [^{99m}Tc]-annexin V (intravenous).
Semi-Synthetic Methods: Radiolabeling of annexin V with [^{99m}Tc] (specific activity, 3.0 MBq/μg protein).
Implementation: Evaluate the effectiveness of [^{99m}Tc]-annexin V to characterize *in vivo* the degree of apoptosis in engrafted hepatoma after the 1st dose of chemotherapy. Investigation can involve determining the biodistribution of [^{99m}Tc]-annexin V within innoculated allogenic hepatoma lesion 20 hours after administration of a cyclophosphamide dose. Radioactivity within specific tissues is then determined 6 hours after [^{99m}Tc]-annexin V administration. Tumor uptake of [^{14}C]-iodoanitpyrine is employed as a marker of tumor blood flow. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) of tissue harvested at necropsy is then performed to detect evidence of apoptosis within tumor tissue specimens.

Animal Model: Rat (inoculated with allogenic hepatoma cells [KDH-8] in the left calf muscle, followed 11 days later by randomized administration of a single dose of cyclophosphamide [150 mg/kg intraperitoneally], while those not receiving the chemotherapeutic serve as a negative reference control).
Interpretation and Analysis: Cyclophosphamide treatment significantly increases tumor uptake (percent activity of injected dose per gram of tissue after normalization to the animal's weight [%ID/g/kg]) of [^{99m}Tc]-annexin V (0.070 ± 0.007 %ID/g/kg for treated rats and 0.046 ± 0.009 %ID/g/kg for controls, $p < 0.001$). [^{14}C]-iodoantipyrine uptake is similar in the treated and untreated groups. The number of TUNEL-positive cells in the tumor is significantly larger in the treated rats (297.70 ± 50.34 cells/mm^2) than in the control rats (168.45 ± 23.60 cells/mm^2, $p < 0.001$). Tumor uptake of [^{99m}Tc]-annexin V correlates with the number of TUNEL-positive cells within tumor specimens ($r = 0.712$; $p < 0.001$). Tumor uptake of [^{99m}Tc]-annexin V is significantly increased by a single dose of cyclophosphamide treatment, and the increase is concordant with the number of TUNEL-positive cells in the tumor. Such observations suggest the utility of [^{99m}Tc]-annexin V as a non-invasive means to assess tumor response, although further testing, including clinical evaluation, is required.

Detection of apoptotic tumor response *in vivo* after a single dose of chemotherapy with [^{99m}Tc]-annexin V. Mochizuki T, Kuge Y, Zhao S, Tsukamoto E, Hosokawa M, Strauss HW, Blankenberg FG, Tait JF, Tamaki N. J Nucl Med 2003 Jan; 44 (1), pp. 92–7.

Carcinomas/Gastrin-Releasing Peptide (GRP) Receptors/[Mammary Carcinoma]/ Prostatic Carcinoma/[Small Cell Lung Cancer]/ [Pancreatic Cancer] *[^{111}Indium] ([^{111}In])-Bombesin (BBN)/[^{111}In]-DOTA-X-BBN[7-14]NH$_2$*/Beta-Ala, 5-Ava Spacer/8-Aoc Spacer

Cellular/Molecular Mechanism of Action: Bombesin has been known to bind to GRP receptors with high affinity and specificity.

Background Information: Gastrin-releasing peptide receptors have been shown to be expressed with high densities on several types of cancer cells, including prostate, breast, small cell lung, and pancreas cancers.

Application: Hydrocarbon spacer chains can effectively improve the specificity of detecting and characterizing human prostatic carcinoma cell types when employing [^{111}In]-DOTA-bombesin as a template molecule for the purpose of recognizing GRP receptor complexes.

Implementation: Evaluation of the relative tumor "uptake" and pharmacokinetic parameters for various [^{111}In]-labeled bombesin analogues as potential candidates for specific targeting of human prostate cancers.

***Ex Vivo* Model:** Human (PC-3 androgen-independent human prostate cancer cells).

Animal Model: Murine (CF-1 mice and PC-3 human prostate cancer-derived xenograft in severe combined immunodeficiency [SCID] mice).

Interpretation and Analysis: *In vitro* competitive binding assays, using PC-3 androgen-independent human prostate cancer cells, reveal values < 2.5 nmol/L for inhibitory concentration of 50% for analogues with β-Ala, 5-Ava, and 8-Aoc spacers. *In vivo* biodistribution studies of the [^{111}In]-DOTA-X-BBN[7-14]NH_2 conjugate performed in CF-1 mice at 1 hour after injection reveal that radioactivity uptake within the pancreas (Note: A GRP receptor expressing tissue) increases as a function of hydrocarbon spacer length (Data: 0.20 ± 0.04 percent injected dose [%ID] per gram for the analogue with no spacer to a maximum of 26.97 ± 3.97 %ID/g for the analogue with 8-Aoc spacer). Radioactivity is cleared efficiently from the blood pool by excretion mainly through the renal/urinary pathway (e.g., 71.6 ± 1.8 %ID at 1 hour postinjection for 8-Aoc spacer analog). *In vivo* pharmacokinetic studies of the [^{111}In]-DOTA-8-Aoc-BBN[7-14]NH_2 conjugate conducted on PC-3 human prostate cancer-derived xenografts in SCID mice show a specific uptake of radioactivity within tumors, with 3.63 ± 1.11 %ID/g observed at 1 hour postinjection. High tumor-to-blood and tumor-to-muscle ratios of approximately 6:1 and 45:1, respectively, can be appreciated at 1 hour after injection. Relative to the radioactivity observed in tumors at 1 hour after injection, 43%, 19%, and 9% of the radioactivity is retained at, respectively, 24, 48, and 72 hours postinjection. Radiometallated DOTA-X-BBN[7-14]NH_2 constructs with hydrocarbon spacers ranging from 5 to 8 carbon atoms therefore appear to be viable candidates for further development as diagnostic and therapeutic radiopharmaceuticals for GRP-positive neoplastic disease states.

Novel series of [^{111}In]-labeled bombesin analogues as potential radiopharmaceuticals for specific targeting of gastrin-releasing peptide receptors expressed on human prostate cancer cells. Hoffman TJ, Gali H, Smith CJ, Sieckman GL, Hayes DL, Owen NK, Volkert WA. J Nucl Med 2003 May; 44 (5), pp. 823–31.

Carcinomas/Mammary Carcinoma/Prostatic Carcinoma/Vascular Endothelium Carcinoma/ Human Carcinoid Bon Cells/Rat Pancreatic Tumor Cells (CA20948)
[^{111}Indium] ([^{111}In])-[c(Arg-Gly-Asp-D-Tyr-Lys)]/ [^{125}I]-[c(Arg-Gly-Asp-D-Tyr-Lys)]

Cellular/Molecular Mechanism of Action: Purified fractions of [c(Arg-Gly-Asp-D-Tyr-Lys)] appear to bind specifically and with high affinity to $\alpha_v\beta_3$ receptors on neovascular blood vessel sections of different major human cancers, like prostate and breast cancer, that express this receptor type.

Background Information: Tumors depend on sufficient blood supply for their growth. They are able to promote new blood vessel formation (neoangiogenesis) via angiogenic factors. Inhibition of this process results in tumor involution or necrosis. RGD (Arg-Gly-Asp) peptides have been described to antagonize neoangiogenesis, for example, by binding to $\alpha_v\beta_3$ receptors on blood vessels.

Implementation: Evaluate the ability to visualize neoangiogenesis in tumors *in vitro* and *in vivo*, with an RGD analogue [c(Arg-Gly-Asp-D-Tyr-Lys)], coupled to the chelator diethyleletriame pentaacetic acid (DTPA) to facilitate [^{111}In] and [^{125}I] radiolabeling.

Interpretation and Analysis: In autoradiography and immunohistochemistry studies, [^{125}I]-labeled [c(Arg-Gly-Asp-D-Tyr-Lys)] appears to bind specifically and with high affinity to $\alpha_v\beta_3$ receptors on neovascular blood vessel sections of different major human cancers, like prostate and breast cancer, that express this receptor type. The radioiodinated pharmaceutical also binds to and becomes internalized within human carcinoid Bon cells and rat pancreatic CA20948 tumor cells. Internalization is receptor specific and appears as well to be time and temperature dependent. Administration of different peptide amounts (0.1, 0.5, and 100 μg) *in vivo* in rats reveals that the optimum amount of radiolabeled analogue appears to be 0.1 μg/rat, because uptake decreases with increasing peptide doses. Receptor-specific accumulation of [^{111}In]-labeled analogue can be appreciated in transplantable CA20948 pancreatic tumors. The introduction of the DTPA group in this peptide results in increased renal clearance of the radiopharmaceutical, in contrast to the non-DTPA-conjugated compound that is cleared predominantly via the liver. [^{111}In] emits auger and conversion electrons besides gamma radiation; therefore, this radiopharmaceutical is suitable not only for tumor scintigraphy but also has potential applicability for radionuclide therapy of major human cancers as well. Moreover, after coupling to the chelator DOTA, the analogue can be radiolabeled in a stable manner with beta-emitters, for example [^{90}Y] and [^{177}Lu], expanding its theoretical potential.

Evaluation of a radiolabeled cyclic DTPA-RGD analogue for tumor imaging and radionuclide therapy. van Hagen PM, Breeman WA, Bernard HF, Schaar M, Mooij CM, Srinivasan A, Schmidt MA, Krenning EP, de Jong M. Int J Cancer (Radiat. Oncol. Invest.) 2000 Aug 20; 90 (4), pp. 186–98.

Carcinomas/Lung Carcinoma: Small Cell Type/ Mammary Carcinoma/Prostatic Cancer (Carcinoma)/Pancreatic Cancer (Carcinoma)
[^{99m}Technetium] ([^{99m}Tc])-MAG3-Coupled Bombesin (BN) Peptide

Background Information: Several human cancers, including small cell lung, prostate, breast, gastric, colon, and pancreatic cancers, express receptors for BN-like peptides. Bombesin peptides that bind specifically to these receptors are useful for the *in vivo* detection of BN receptor expressing cancers.

Application: Detection and characterization of neoplastic disease states, including small cell lung, prostate, breast, gastric, colon, and pancreatic cancers that express receptors for BN-like peptides.

Semi-Synthetic Methods: MAG3-coupled BN peptide (MAG3-BN) can be prepared by solid-phase synthesis and radiolabeled with [^{99m}Tc] by an exchange method.

Implementation: Evaluation of the *in vitro* binding avidity (targeting capacity) of [^{99m}Tc]-MAG3-BN for BN receptor complexes expressed by MDA-MB-231 and MCF-7 human breast cancer cell lines. Complementary investigations can entail determination of *in vivo* radioactivity biodistribution profiles.

Animal Model: Murine (normal and nude mice bearing BN receptor-positive tumors).
Interpretation and Analysis: Radiolabeling of MAG3-BN with [^{99m}Tc] produces a single radioactive species (> 95%). *In vitro* cell-binding analyses validate the affinity and specificity of [^{99m}Tc]-MAG3-BN toward BN receptors. *In vivo* biodistribution in mice reveals that [^{99m}Tc]-MAG3-BN clears rapidly from the blood and most non-targeted tissues, while being excreted mainly via the kidneys. Uptake in BN receptor-positive tissues and in the tumor is low to moderate. [^{99m}Tc]-MAG3-BN displays good radiolabeling together with certain favorable biological characteristics and may be a useful peptide radiopharmaceutical in the detection of BN receptor expressing cancers *in vivo*.

Synthesis, radiolabeling, and biological characteristics of a bombesin peptide analogue as a tumor-imaging agent. Okarvi SM, al-Jammaz I. Anticancer Res 2003 May–Jun; 23 (3B), pp. 2745–50.

Central Nervous System (CNS)/Astrocytoma (Low Grade): CNS/Intra-Axial Brain Tumors *[^{201}Thallium] ([^{201}Tl])-Pentetreotide/ [^{111}Indium] ([^{111}In])-Pentetreotide/[Gd]-Enhanced Magnetic Resonance Imaging (MRI)*

Background Information: Highly undifferentiated glial tumors do not express somatostatin receptors (SSR) in contrast to low-grade astrocytomas that contain SSR. To differentiate a malignant glioma from a low-grade astrocytoma and to distinguish an SSR-positive intra-axial brain tumor from an SSR negative 1, [^{111}In]-pentetreotide brain single photon emission computed tomography (SPECT) can be prospectively undertaken.
Application: Detection and characterization of intra-axial brain tumors.
Implementation: Evaluation of intra-axial brain tumors utilizing [^{201}Tl] and [^{111}In]-pentetreotide brain SPECT performed using a 3-day to 4-day interval before surgery. Results form SPECT analyses are compared with those of [Gd]-enhanced MRI.

Human Model: Cases with intra-axial brain tumors (n = 8; n = 3/8 glioblastoma multiforme, n = 1/8 low-grade astrocytoma, n = 1/8 lymphoma, n = 1/8 medulloblastoma, n = 1/8 neurocytoma, n = 1/8 metastatic tumor).
Interpretation and Analysis: Increased uptake of [^{111}In]-pentetreotide can be appreciated in a high percentage of cases with glioblastoma multiforme (n = 2 grade (−); n = 1 grade (+); positive uptake n = 3/3 or 100% of cases) despite lack of SSR.

Low-grade astrocytomas exhibit minimal uptake of [^{111}In]-pentetreotide (grade +). Remaining tumors have intense uptake of [^{111}In]-pentetreotide. [^{201}Tl] SPECT reveals similar findings to those observed with [^{111}In]-pentetreotide scintigraphy except in some conditions of glioblastoma multiforme (e.g., n = 2). Rare cases that have a negative [^{201}Tl] scan will usually show increases in [^{111}In]-pentetreotide uptake. Similarly, a small percentage of cases that have minimal uptake of [^{111}In]-pentetreotide will usually display increased thallium uptake. The uptake pattern of both [^{201}Tl] and [^{111}In]-pentetreotide appears to correlate with [Gd]-enhanced MRI. [^{111}In]-pentetreotide scintigraphy is sensitive for the detection of intra-axial brain tumors; however, it has no role in assessing the tumor grading and in the definition of receptor profiles.

Indium-111-pentetreotide imaging in intra-axial brain tumors: Comparison with thallium-201 SPECT and MRI. Lee JD, Kim DI, Lee JT, Chang JW, Park CY. J Nucl Med 1995 Apr; 36 (4), pp. 537–41.

Central Nervous System (CNS)/Glioma *[^{123}I]-Iodo-α-Methyl Tyrosine (IMT)*

Application: Scintigraphy using [^{123}I]-IMT is useful in the preoperative characterization of gliomas, in detecting recurrent glioma, and in the biological reevaluation of residual or recurrent tumors. Certain non-parenchymal brain metastatic tumors also appear to be detected utilizing the same imaging agents.
Route of Administration: [^{123}I]-IMT (intravenous).
Implementation: Evaluation of [^{123}I]-IMT SPECT as a modality for the management of intracerebral metastases and lymphomas. [^{123}I]-IMT or [^{123}I]-IMT uptake is quantified as a ratio between maximal tumor accumulation and average uptake in the contralateral hemisphere.

Human Model: Cases with CNS neoplasia (n = 31 patients; n = 28 metastases of extracerebral solid tumors; n = 7 cerebral lymphomas). Note: Histopathological detection of high-grade lymphomas, melanomas, and carcinomas originating from the lung, unknown primary, breast, colon, renal cell, ovary, vagina, frontal sinus.
Interpretation and Analysis: All tumors except renal cell (e.g., n = 2) and small cell lung carcinoma (e.g., n = 1) metastases accumulate [^{123}I]-IMT (91%). The highest IMT uptake can be found in some lung carcinoma metastases. [^{123}I]-IMT uptake is highly variable and is similar in primary and recurrent tumors. Significant accumulation of IMT is seen in the majority of tumors, so that this technique might be helpful for the management of cerebral metastases and lymphomas.

[^{123}I]-iodo-α-methyl tyrosine SPECT in non-parenchymal brain tumors. Matheja P, Weckesser M, Rickert Ch, Franzius Ch, Palkovic S, Riemann B, Schober O. Nuklearmedizin 2002; 41 (4), pp. 191–6.

Central Nervous System (CNS)/Blood-Brain Barrier (BBB): Osmotic Disruption/Glioma *[^{99m}Technetium] ([^{99m}Tc])-Epidermal Growth Factor (EGF)/Intravenous vs Intracarotid Administration/ [Mannitol]*

Cellular/Molecular Mechanism of Action: Mannitol is an osmotic agent that can be applied to disrupt the BBB.
Background Information: Previous reports have described methods for labeling EGF with [^{99m}Tc] and have shown that the product, [^{99m}Tc]-EGF, is retained within EGF receptor-positive intracerebral C6EGFR rat gliomas following intratumoral (i.t.) injection of the radioligand.
Application: Mannitol when administered to the intravascular compartment can reduce the severity of cerebral edema presumably through osmotic draw of free water across the BBB. In addition, agents including [^{99m}Tc]-EGF can potentially be applied to characterize optimum delivery modalities and identify neoplastic lesions that may be susceptible to EGF-bioconjugate based platforms for therapeutic intervention.
Route of Administration: [^{99m}Tc]-EGF (intravenous or intracarotid).
Implementation: Evaluation of the potential use of [^{99m}Tc]-EGF as a tumor targeting agent for gliomas. Radiolocalization is determined following intravenous or intracarotid injection with or without hyperosmotic mannitol-induced disruption of the BBB (BBB-D).

Animal Model: Rat (Fisher strain bearing intracerebral implants of C6EGFR gliomas).

Interpretation and Analysis: As determined by gamma-scintillation counting, 4 hours after intracarotid injection of [^{99m}Tc]-EGF, 0.34% of the injected dose per gram (%ID/g) is localized in C6EGFR tumors that express 10^5–10^6 EGFR sites per cell, compared to 0.07 %ID/g for *in vivo* models bearing C6 wild-type gliomas that do not express EGFR. Corresponding tumor-to-brain ratios are approximately 5.6 and 1.6, respectively. Tumors can be visualized by external gamma scintigraphy with *in vivo* models bearing C6EGFR but not C6 wild-type gliomas, thereby establishing that radiolocalization is dependent upon receptor expression. Intracarotid administration of [^{99m}Tc]-EGF significantly increases tumor uptake compared to intravenous injection (0.34 vs 0.14 %ID/g, $p < 0.04$). Blood-brain barrier hyperosmotic disruption induced with mannitol, followed by intracarotid injection of [^{99m}Tc]-EGF, however, does not significantly enhance tumor uptake compared to intracarotid injection without BBB-D (0.45% vs 0.34 %ID/g, $p > 0.1$). The uptake of [^{99m}Tc]-EGF is approximately 4–9 %ID/g in the liver and 12–20 %ID/g in the kidneys after intracarotid or intravenous administration. External gamma scintigraphy of regions-of-interest (ROIs) over the liver and kidneys reveals that approximately 70–80% of the whole-body radioactivity accumulates in these organs, and only 0.47–0.83% in the tumor following intravenous or intracarotic administration of [^{99m}Tc]-EGF. Therefore, EGF can be used as a specific targeting agent for EGFR (+) brain tumors. However, it is unlikely that systemic injection of EGF-based bioconjugates can deliver sufficient amounts of the ligand to brain tumors for therapeutic purposes, and direct delivery by means of either intratumoral injection or a variant of it such as convection-enhanced delivery will be required.

Evaluation of systemically administered radiolabeled epidermal growth factor as a brain tumor targeting agent. Yang W, Barth RF, Leveille R, Adams DM, Ciesielski M, Fenstermaker RA, Capala J. J Neurooncol 2001 Oct; 55 (1), pp. 19–28.

Central Nervous System (CNS)/Glioma: Luciferase-Transfected *Antisense Nucleotide Radiopharmaceuticals/ [^{125}I]Peptide Nucleic Acid (PNA)/Antitransferrin Receptor/[^{125}I]PNA Avid/Biotin Monoclonal Antibody (MAb)*

Application: Model system for the development of antisense radiobiopharmaceutical imaging agents capable of facilitating the *in vivo* detection and characterization of neoplastic diseases affecting the CNS.

Route of Administration: [^{125}I]PNA antisense conjugates are administered intravenously.

Semi-Synthetic Methods: Antisense radiopharmaceuticals can be used *in vivo* to image gene expression in the brain if these polar molecules could be made transportable through (across) the blood-brain barrier. The present studies describe an antisense imaging agent comprised of an iodinated PNA conjugated to a MAb with affinity for the rat transferrin receptor. The molecular targeting strategy utilizes avidin-biotin technology. The PNA has a 16-mer antisense strand corresponding to the sequence around the methionine initiation codon of the luciferase mRNA.

Implementation: Evaluation of the capacity for [^{125}I]PNA conjugates to recognize and physically interact with glioma neoplastic cell types in a selective manner.

Animal Model: Rat glioma cells (C6 type) are permanently transfected with a luciferase expression plasmid for development of rat experimental brain tumor model. Frozen sections of the brain for film autoradiography are then acquired 2 hours following intravenous injection of [^{125}I]PNA conjugates.

Interpretation and Analysis: The expression of the luciferase transgene in the tumors *in vivo* can be confirmed by measurement of luciferase enzyme activity in tumor cell extracts. No image of luciferase gene expression can be obtained after the administration of either the unconjugated antiluciferase PNA or a PNA conjugate that is an antisense strand to the messenger RNA (mRNA) of a viral transcript. In contrast, tumors can be successfully imaged with [^{125}I]PNA that is an antisense strand to the luciferase sequence and conjugated to the targeting antibody. In conclusion, these studies demonstrate that gene expression in the brain *in vivo* can be imaged with antisense radiobiopharmaceuticals that are conjugated to a brain drug-targeting system.

Antisense imaging of gene expression in the brain *in vivo.* Shi N, Boado RJ, Pardridge WM. Proc Natl Acad Sci U S A 2000 Dec 19; 97 (26), pp. 14709–14.

Central Nervous System (CNS)/Glioma Proliferative Activity/Early-Delayed Retention Ratios *[^{99m}Technetium] ([^{99m}Tc])-Methoxy-Isobutyl-Isonitrile (MIBI)/[^{201}Thallium] ([^{201}Tl])/MI/MIB Index/ [Antiglioma Ki-67 Antigen IgG Validation]*

Background Information: [^{99m}Tc]-MIBI, like [^{201}Tl], is a highly efficient agent for the diagnosis and monitoring of glioma tumors. Although [^{201}Tl] uptake is known to be partly associated with proliferative activity, little is known about the correlation between MIBI uptake and proliferation activity in gliomas.

Application: Detection and characterization of CNS glioma lesions, including estimation of proliferative activity.

Implementation: Evaluate the degree of correlation between MIBI uptake and proliferative activities in gliomas. Validation of results can be estimated utilizing monoclonal antibody with affinity for Ki-67 antigen (MIB-1 staining method). By comparing the results with those of [^{201}Tl], it is possible to determine which tracer is suitable for estimating proliferative activities. In this context, cases are subjected to MIBI and [^{201}Tl] SPECT. Early (10 minutes after injection) and delayed images (3 hours after injection) are then obtained for both MIBI and [^{201}Tl] scintigraphy. Single photon emission computed tomography (SPECT) parameters, early ratio (ER), delayed ratio (DR), and retention index (RI) are obtained with both radiopharmaceuticals. Sample lesions obtained by surgical excision are then immunostained with monoclonal antibody directed against Ki-67 antigen (MIB-1 staining method) in order to facilitate calculation of the MI/MIB index. To evaluate the relationship between proliferative activity and SPECT parameters, correlation analysis can be performed to further interpret experimental findings.

Human Model: Cases of presurgical glioma (n = 24; n = 6 low-grade gliomas, n = 5 anaplastic astrocytomas, n = 13 glioblastomas). Surgical excision performed in all cases following initial evaluation with both MIBI and [^{201}Tl] scintigraphy.

Interpretation and Analysis: Values for MI correlate with calculated MIBI uptake ratios (r = 0.75 for ER and r = 0.7 for DR). Both DR and RI for [^{201}Tl] also correlate with MI, but to a lesser degree (r = 0.6 for DR and r = 0.59 for RI). There appears to be no significant correlation between the MIB-1 index and other parameters. Methoxy-isobutyl-isonitrile-uptake parameters demonstrate a stronger positive correlation with the MIB-1 index than that of [^{201}Tl]. With the use of MIBI SPECT, it is possible to estimate the proliferative activity of CNS gliomas non-invasively.

The correlation between [^{99m}Tc]-MIBI uptake and MIB-1 as a nuclear proliferation marker in glioma: A comparative study with [^{201}Tl]. Nagamachi S,

Jinnouchi S, Nabeshima K, Nishii R, Flores L 2nd, Kodama T, Kawai K, Tamura S, Yokogami K, Samejima T, Wakisaka S. Neuroradiology 2001 Dec; 43 (12), pp. 1023–30.

Central Nervous System (CNS)/Glioma/ Somatostatin Receptor Type 2 Postive Neoplasias *[^{90}Y]-Labeled DOTA0-D-Phe$_1$-Tyr$_3$-Octreotide (DOTATOC)*

Cellular/Molecular Mechanism of Action: The agent DOTATOC competes specifically with somatostatin binding to somatostatin receptor (Type 2). In this context, it functions as a metabolically stable small regulatory peptide.

Background Information: Human gliomas, especially of low-grade type, have been shown to express high-affinity somatostatin Type 2 receptors.

Application: Detection and characterization of glioma neoplastic conditions affecting the CNS. The activity:dose ratio (MBq:Gy) can potentially be utilized as a measure for the stability of peptide retention in receptor-positive tissue and may possibly function as a parameter for predicting clinical course.

Implementation: Evaluation of anaplastic glioma patients in a pilot study using the diffusible peptidic vector [^{90}Y]-labeled DOTATOC for receptor targeting. The radiopharmakon can be locoregionally injected into a stereotactically inserted Port-a-cath.

Human Model: Cases of glioma (n = 7 low-grade; n = 4 anaplastic type).

Interpretation and Analysis: The agent DOTATOC competes specifically with somatostatin binding to somatostatin Type 2 receptors in the low nanomolar range as shown by displacement curves of [^{125}I]-[Tyr$_3$]-octreotide in tumor tissue sections. Diagnostic [^{111}In]-labeled DOTATOC scintigraphy following local injection displays homogeneous to nodular intratumoral vector distribution. The cumulative activity of regionally injected peptide-bound [^{90}Y] amounts to 370–3,300 MBq, which is equivalent to an effective dose range between 60 ± 15 and 550 ± 110 Gy. Activity can be injected in 1 to 4 fractions according to tumor volumes; 1,110 MBq of [^{90}Y]-labeled DOTATOC is the maximum activity per single injection applied in some investigations. Disease stabilization and shrinking of a cystic low-grade astrocytoma can be expected in a certain percentage of cases (e.g., n = 6/7). Signs of toxicity if they are observed include secondary perifocal edema. The activity:dose ratio (MBq:Gy) represents a measure for the stability of peptide retention in receptor-positive tissues and might predict the clinical course. Therefore, somatostatin receptor positive human gliomas, especially of low-grade type, can be successfully targeted by intratumoral injection of DOTATOC peptide.

Locoregional regulatory peptide receptor targeting with the diffusible somatostatin analogue [^{90}Y]-labeled DOTA0-D-Phe$_1$-Tyr$_3$-octreotide (DOTATOC): A pilot study in human gliomas. Merlo A, Hausmann O, Wasner M, Steiner P, Otte A, Jermann E, Freitag P, Reubi JC, Müller-Brand J, Gratzl O, Mäcke HR. Clin Cancer Res 1999 May; 5 (5), pp. 1025–33.

Central Nervous System (CNS)/Neural Crest Tumors/ Neuroblastoma (NB)/Ganglioneuroma (GN)/ Ganglioneuroblastoma *[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)*

Application: Detection of neural crest tumors applying nuclear medicine imaging techniques.

Dosage: [^{123}I]-MIBG.

Considerations: Scintigraphy with [^{123}I]-MIBG should be the primary imaging procedure followed by computed tomography (CT) in the investigation of cases suffering from neural crest tumors. Results are compared with bone scans and CT images, as well as surgical pathologic findings.

Implementation: Evaluation of neural crest tumor conditions with serial MIBG scans to determine the impact of imaging results on the clinical treatment of pediatric cases with neural crest tumors.

Human Model: Cases with neural crest tumors (n = 27; n = 25/27 with initial diagnoses of NB; n = 1/27 ganglioneuroblastoma; n = 1/27 GN).

Interpretation and Analysis: At initial diagnosis, when compared with bone and CT scans, MIBG imaging does not identify any unsuspected lesions resulting in changes in staging. In NB conditions, approximately 50% of cases that initially have positive MIBG scans will have serial studies that normalize during therapy (e.g., n = 13/25). However, after completion of therapy, over 50% of cases will have a disease relapse (e.g., n = 8/13). Although areas of active disease may be well delineated by other standard imaging modalities, only 50% (e.g., n = 4/8) have MIBG study results that are positive in sites of relapse. Some cases of GN (e.g., n = 1 at diagnosis and 3 after NB therapy) demonstrate an uptake of MIBG that is similar in appearance to that in NB. Therefore, MIBG imaging does not change the staging or alter treatment during therapy for most cases. Normalization of positive study results is an unreliable indicator of outcome for children with NB. Furthermore, when relapses occur, MIBG scans identify only 50% of those with active NB. The uptake of MIBG in GN is indistinguishable from that in NB. In some clinical scenarios, results of serial MIBG studies do not have a significant impact on patient treatment.

Both [^{123}I]-MIBG scintigraphy and x-ray CT are imaging techniques that are capable of accurately detecting neural crest tumors. Slightly more accurate detection of metastatic disease can be observed with [^{123}I]-MIBG scintigraphy. Computed tomography of tumors is useful in planning appropriate metastatic intervention.

The role of [^{131}iodine]-metaiodobenzylguanidine scanning in the correlative imaging of patients with neuroblastoma. Andrich MP, Shalaby-Rana E, Movassaghi N, Majd M. Pediatrics 1996 Feb; 97 (2), pp. 246–50.

Central Nervous System (CNS)/Neuroblastoma (NB) *[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)*

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is a radioiodinated analogue of guanidine nucleotide.

Application: Improved specificity and sensitivity in the detection of pediatric NB can be achieved applying [^{123}I]-MIBG as a diagnostic probe for scintigraphy procedures applied in concert with magnetic resonance imaging (MRI) diagnostic modalities.

Route of Administration: [^{123}I]-MIBG (intravascular).

Implementation: Evaluation of the comparative effectiveness of MRI and [^{123}I]-MIBG scintigraphic imaging for detecting and characterizing NB lesions (n = 50 examinations for each diagnostic modality). Separate and combined analyses of MRI and MIBG scintigraphic images are then compared with clinical and histologic findings.

Human Model: Cases of NB (n = 28 with n = 193 neuroblastoma lesions).

Interpretation and Analysis: In the diagnosis of NB lesions, MIBG scintigraphy, MRI, and combined analysis show a sensitivity of 69%, 86%, and 99% and a specificity of 85%, 77%, and 95%, respectively. On MRI, some false-positive images may be recorded (e.g., n = 15/50) that can be associated with posttherapeutic reactive changes (e.g., n = 10/15); benign adrenal tumors (e.g., n = 3/15); or enlarged lymph nodes (e.g., n = 2/15). Scintigraphy with MIBG may also produce false-positive findings (e.g., n = 10) that can be

associated with ganglioneuromas (e.g., n = 2/10); benign liver tumors (e.g., n = 2/10); or physiologic uptake (e.g., n = 6/10). The occurrence of false-negative MRI scans for NB can also occur (e.g., n = 13 NB metastases; n = 2 residual masses managed with chemotherapy). Occasionally, primary or residual NBs and metastasis (orbital) can be misinterpreted on MRI as Wilms' tumor, reactive changes after surgery, or rhabdomyosarcoma. A relatively large number of bone metastases (e.g., n = 32) or other sites of NB metastases (e.g., n = 6) may show MIBG uptake in addition to some adrenal NB lesions.

Combined image interpretation can noticeably improve sensitivity and specificity (e.g., n = 1 false-negative [bone metastasis]; n = 3 false-positive [n = 2/3 ganglioneuromas, n = 1/3 pheochromocytoma]). Therefore, in the assessment of NB lesions in pediatric cases, MRI shows a higher sensitivity and MIBG scintigraphy a higher specificity. However, integrated imaging shows an increase in both sensitivity and specificity.

Integrated imaging using MRI and [^{123}I] metaiodobenzylguanidine scintigraphy to improve sensitivity and specificity in the diagnosis of pediatric neuroblastoma. Pfluger T, Schmied C, Porn U, Leinsinger G, Vollmar C, Dresel S, Schmid I, Hahn K. AJR Am J Roentgenol 2003 Oct; 181 (4), pp. 1115–24.

Central Nervous System (CNS)/Neuroblastoma (NB)/Xenotransplants/Spontaneous Arising Neuroblastoma
[^{131}I]-Metaiodobenzylguanidine ([^{131}I]-MIBG)

Cellular/Molecular Mechanism of Action: [^{131}I]-MIBG is a radioiodinated analogue of guanidine nucleotide.
Application: Detection and characterization of NB lesions within the CNS.
Route of Administration: [^{131}I]-MIBG (intraperitoneal).
Dosage: [^{131}I]-MIBG (18 μCi/g: mouse).
Implementation: Evaluate [^{131}I]-MIBG scintigraphy for image localization of high-risk NB xenotransplanted and spontaneously arising NBs (e.g., murine model). In instances when measured tumor volumes exceed 200 mm^3, each mouse receives an intraperitoneal injection of 18 μCi/g [^{131}I]-MIBG. Pinhole scintigraphy can be applied to evaluate the MIBG biodistribution and visualize individual tumors.

Animal Model: Murine (mouse model of high-risk NB employing xenotransplanted and spontaneously arising NBs). Neuroblastoma xenografts are created by subcutaneous inoculation of human NB cell suspensions into the flanks of athymic nude mice. In addition, spontaneous paraspinal NBs can be detected by direct palpation in MYCN trans-genic mice.
Interpretation and Analysis: Image analysis reveals the absorption of radiolabeled MIBG and visualization of tumors. Analysis of the images allows quantification of relative MIBG uptake and determination of linear and area measurements of tumors. In this context, high-energy pinhole imaging effectively demonstrates uptake of radiolabeled MIBG by human NB tumors in murine experimental models. The technique allows for the *in vivo* assessment of tumor burden. In the future, this method will be used to evaluate its sensitivity for detecting metastatic spread as well as for investigating the therapeutic efficacy of high-dose [^{131}I]-MIBG in combination with radiosensitizing agents.
Instrumentation: Gamma camera equipped with a 3-mm pinhole on 1 head and an HEGP collimator on the other.

Pinhole imaging of [^{131}I]-metaiodobenzylguanidine ([^{131}I]-MIBG) in an animal model of neuroblastoma. Accorsi R, Morowitz MJ, Charron M, Maris JM. Pediatr Radiol 2003 Oct; 33 (10), pp. 688–92.

Central Nervous System (CNS)/Neuroendocrine Neoplasias: Catecholamine-Secreting/ Neuroblastoma/Pheochromocytoma
[^{131}I]-Metaiodobenzylguanidine ([^{131}I]-MIBG)/ [^{123}I]-MIBG

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is classified as a norepinephrine analog.
Application: Metaiodobenzylguanidine has been in clinical use for 15 years and has been shown to have high sensitivity (about 85%) and specificity (> 95%) for the location of all types of pheochromocytomas. Similar levels of success have been observed with the diagnostic detection of neuroblastoma. Other neuroendocrine lesions have been imaged at lower levels of sensitivity, including carcinoids, medullary thyroid cancer, and non-secretory paragangliomas.
Interpretation and Analysis: Alternative analogues of radiolabeled somatostatin may have greater utility for improved detection of neuroendocrine lesions, including carcinoids, medullary thyroid cancer, and non-secretory paragangliomas. Metaiodobenzylguanidine scintigraphy may also provide a different *in vivo* diagnostic approach for evaluating the sympathetic autonomic nervous system, particularly with respect to cardiac function.
Instrumentation: Various radiolabels for MIBG and its analogues permit planar scintigraphy, single photon emission computed tomography (SPECT), positron emission tomography (PET), intraoperative probe localization, and radiopharmaceutical therapy.

The current status of metaiodobenzylguanidine and related agents for the diagnosis of neuro-endocrine tumors. Shapiro B, Sisson JC, Shulkin BL, Gross MD, Zempel S. Quart J Nucl Med 1995 Dec; 39 (4 Suppl 1), pp. 3–8.

Central Nervous System (CNS)/Neuroendocrine Neoplasia: Catecholamine-Secreting
[^{131}I]-Metaiodobenzylguanidine ([^{131}I]-MIBG)/ [^{123}I]-MIBG

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is classified as a norepinephrine analog.
Application: Radioiodinated MIBG ([^{131}I] or [^{123}I]) is used as a radiopharmaceutical that preferentially accumulates within certain neuroendocrine tumors, especially pheochromocytomas and neuroblastomas.
Route of Administration: [^{131}I]-MIBG and [^{123}I]-MIBG (intravenous).
Dosage: [^{131}I]-MIBG and [^{123}I]-MIBG.
Interpretation and Analysis: [^{131}I]-MIBG and [^{123}I]-MIBG permit non-invasive whole-body scintigraphic screening for benign and malignant, familial and sporadic, intra-adrenal and extra-adrenal pheochromocytomas and primary and metastatic neuroblastomas, with high sensitivity (85–90%) and specificity (> 95%). Metaiodobenzylguanidine also concentrates in presynaptic terminals of adrenergic, autonomically innervated organs such as the heart and may be used as a non-invasive *in vivo* probe for characterizing this segment of the autonomic nervous system. As an additional benefit, large doses of [^{131}I]-MIBG and [^{123}I]-MIBG are also capable of selectively delivering radiotherapeutic doses to malignant pheochromocytomas and advanced stages or refractory forms of neuroblastoma.
Instrumentation: Nuclear medicine imaging with a scintillation camera.

Imaging of catecholamine-secreting tumors: Uses of MIBG in diagnosis and treatment. Shapiro B. Baillieres Clin Endocrinol Metab 1993 Apr; 7 (2), pp. 491–507.

Neuroendocrine: Pheochromocytoma/[Elevated Glucagon-Receptor Expression]
Des-His$_1$[Glu$_9$]Glucagon Amide/[^{125}I-Thyr$_{10}$]Glucagon

Cellular/Molecular Mechanism of Action: [^{125}I-Thyr$_{10}$] glucagon is a radiolabeled analogue of glucagon. Des-His$_1$[Glu$_9$] glucagon amide is a glucagon receptor antagonist.
Background Information: Pheochromocytoma lesion express glucagon receptor complexes at elevated densities on their exterior membranes.
Application: Potential diagnostic probe for detecting conditions of pheochromocytoma. Des-His$_1$[Glu$_9$]glucagon amide (GR-A) abolishes the secretory response to glucagon by pheochromocytomas.
Route of Administration: Experimental (*in vitro*).
Implementation: Evaluation of glucagon messenger RNA (mRNA) expression and glucagon receptor expression in pheochromocytoma lesions.

Human *Ex Vivo* Model: Pheochromocytoma lesions (n = 3 and n = 4 normal adrenal medullas).
Interpretation and Analysis: Glucagon-receptor mRNA can be detected by reverse transcription-polymerase chain reaction in human pheochromocytomas but not in normal adrenal medullas. Quantitative autoradiography demonstrates the presence of abundant [^{125}I-Thyr$_{10}$]glucagon binding sites in pheochromocytomas, which can be displaced by both cold glucagon and the glucagon receptor antagonist Des-His$_1$[Glu$_9$]glucagon amide (GR-A). The adrenal medulla is weakly labeled, and binding is not displaced by GR-A. Glucagon enhances epinephrine and norepinephrine release by pheochromocytoma slices with minimal and maximal effective concentrations being 10^{-8} M and 10^{-6} M, respectively. Adrenomedullary slices display a weak catecholamine response only in the presence of 10^{-5} M glucagon.

GR-A abolishes the secretory response to glucagon by pheochromocytomas but not of adrenal medullas. Collectively, these findings indicate that human pheochromocytomas, but not the adrenal medulla, express glucagon receptors and possess a marked secretory response to glucagon, thereby providing the rationale to explain the specificity of the glucagon provocative test in the diagnosis of pheochromocytoma.

Human pheochromocytomas, but not adrenal medulla, express glucagon-receptor gene and possess an *in vitro* secretory response to glucagon. Albertin G, Aragona F, Gottardo L, Malendowicz LK, Nussdorfer GG. Peptides 2001 Apr; 22 (4), pp. 597–600.

Central Nervous System (CNS)/Neuroendocrine Tumors/Somatostatin Receptor Expression
Octreotide/Somatostatin Analogues

Somatostatin and other neuropeptides are expressed in tumors originating from neuronal precursors and paraganglia, namely medulloblastoma, central primitive neuro-ectodermal tumors (cPNETs), neurocytoma, gangliocytoma, olfactory neuroblastoma, and paraganglioma. In medulloblastoma, the most common malignant tumor in childhood, there is extensive expression of somatostatin in addition to somatostatin receptors (SSTR) Type 2 complexes. Although density of SSTR-2 and intensity of the expression of somatostatin genes have no prognostic significance in medulloblastoma, their presence may bring along important information on oncogenesis and relate medulloblastoma to cPNETs. Radiolabeled octreotide scintigraphy may be useful in the follow-up of such conditions, allowing differentiation between scar and tumoral tissue. Moreover, on the basis of octreotide-induced inhibition of cell proliferation in medulloblastoma, a trial with octreotide in cases with recurrent or high-risk tumor is warranted. Meningiomas and low-grade astrocytic gliomas, even if not displaying a clear neuroendocrine phenotype, have high levels of SSTR-2. In meningiomas, SSTR scintigraphy is not part of the routine preoperative assessment; moreover, a therapeutic trial with somatostatin analogues in cases with recurrent or inoperable meningiomas should be carried out with great caution, because somatostatin and octreotide slightly increase cell proliferation in cultured meningiomatous cells. Low-grade gliomas (World Health Organization grade 2), and a smaller fraction of anaplastic astrocytomas, express SSTR-2, while glioblastomas usually do not. Unfortunately, radiolabeled-octreotide scintigraphy is not useful in the differential diagnosis of gliomas, because the results are altered by the disruption of the blood-brain barrier (BBB). In addition, radionuclide-labeled somatostatin analogues are not useful in the therapy of low-grade gliomas, because an intact BBB prevents them from reaching target SSTR-2 complexes. Recently, a pilot study in gliomas has proposed the use of a radiolabeled somatostatin analogue with a locoregional approach in order to overcome the intact BBB.

Neuroendocrine tumors in the brain. Cavalla P, Schiffer D. Ann Oncol 2001; 12 Suppl 2, pp. S131–4.

Cholecystokinin (CCK-B) Receptor Expression/ Meningiomas/Gastroenteropancreatic Tumors/ Medullary Thyroid Carcinoma/Small Cell Lung Cancer/Astrocytomas/Gastrointestinal Tumors/ Stromal Ovarian Cancer
[^{111}Indium] ([^{111}In])-Labeled MP2288/[^{111}In]-Labeled MP2286

Cellular/Molecular Mechanism of Action: [^{111}In]-labeled MP2288 and [^{111}In]-labeled MP2286 recognize and physically bind to CCK-B receptor complexes.
Background Information: Receptors for regulatory peptides such as somatostatin or vasoactive intestinal polypeptide are expressed by a number of human neoplasms and can be visualized *in vivo* with peptide receptor scintigraphy. Recently, the CCK-B receptor, which binds both gastrin and cholecystokinin with high affinity, has been shown using *in vitro* methods to be overexpressed in a number of human tumor tissues, including medullary thyroid carcinomas, small cell lung cancers, astrocytomas, gastrointestinal tumors, and stromal ovarian cancers.
Semi-Synthetic Methods: Design of a novel, unsulfated CCK octapeptide analogue linked to the diethylenetriamine pentaacetic acid (DTPA) and DOTA metal chelating agents.
Implementation: Evaluation of the binding avidity of unsulfated CCK octapeptide analogues linked to the metal chelating agents DTPA and DOTA for CCK-B receptor-positive tissues from human tumors.

Animal Model: Rat (biodistrubution studies).
Interpretation and Analysis: Assays reveal that the most potent compounds are DTPA-[Nle28, 31]-CCK(26-33) (MP2286) and DTPA-[d-Asp26,Nle28,31]-CCK(26-33) (MP2288) with an IC_{50} of 1.5 nM. For comparison, analogues with C-terminal DTPA, such as [Nle28,31,Aphe33(*p*-NH-DTPA)]-CCK(26-33) and CCK-(26-33)-NH$(CH_2)_2$ NH-DTPA, have an IC_{50} of $>$ 100 nM. DOTA-[D-Asp26, Nle28,31]-CCK(26-33) has an IC_{50} of 3.9 nM. The compounds are selective for CCK-B receptors, as they do not bind with high affinity to CCK-A receptors expressed in human tumors (meningiomas or gastroenteropancreatic tumors). Biodistribution

studies with [^{111}In]-labeled MP2286 and MP2288 show that the primary mode of clearance is renal, and the primary sites of uptake (percent injected dose per gram [%ID/g] 24 hours postinjection) are the kidney (0.270 and 0.262, respectively) and gastrointestinal tract (rat). The CCK-B receptor expressing gastric mucosa shows specific *in vivo* accumulation of [^{111}In]-labeled MP2288 can be blocked in the presence of excess unlabeled MP2288. [^{111}In]-labeled MP2286 and MP2288 are also found to be stable in human plasma, whereas both compounds are degraded in urine (> 40% after 3 hours at 37°C). The affinity, specificity, biodistribution, and stability of these 2 DTPA-CCK analogues indicate that these compounds have substantial promise for use in the *in vivo* visualization of CCK-B receptor expressing tumors.

Unsulfated DTPA- and DOTA-CCK analogues as specific high-affinity ligands for CCK-B receptor expressing human and rat tissues *in vitro* and *in vivo*. Reubi JC, Waser B, Schaer JC, Laederach U, Erion J, Srinivasan A, Schmidt MA, Bugaj JE. Eur J Nucl Med 1998 May; 25 (5), pp. 481–90.

Ehrlich Solid Tumor: Murine Transplant/Sarcoma-180 Solid Tumor: Murine Transplant *[^{75}Se]-Labeled L-Selenomethionine/[^{75}Se]-Labeled D-Selenomethionine*

Cellular/Molecular Mechanism of Action: Semi-synthetic *D*-selenomethionine and *L*-selenomethionine are perceived by cells as analogues of the amino acid methionine.
Route of Administration: Intravascular.
Semi-Synthetic Methods: *D*-SeMet-[^{75}Se] can be enzymatically prepared from commercially available *L*-selenomethionine-[^{75}Se] (*L*-SeMet-[^{75}Se]) by using amino acid racemase and immobilized *L*-amino acid oxidase.
Implementation: Evaluation of the radioactive biodistribution of [^{75}Se]-labeled *D*-selenomethionine (*D*-SeMet-[^{75}Se]) and capacity to recognize solid tumors and sarcoid neoplasias.
Animal Model: Murine (tumor-bearing mice with Ehrlich solid tumors and sarcoma-180 solid tumors).
Interpretation and Analysis: No difference can be appreciated in the excretion rate for *D*-SeMet-[^{75}Se] and *L*-SeMet-[^{75}Se] into the urine and feces in normal mice within 48 hours after administration. The *in vivo* uptake of *D*-SeMet-[^{75}Se] in both Ehrlich solid tumors and sarcoma-180 solid tumors is several times higher than that for *L*-SeMet-[^{75}Se] but the uptake of *D*-SeMet-[^{75}Se] and *L*-SeMet-[^{75}Se] is similar within the pancreas. Such findings indicate that *D*-SeMet-[^{75}Se] might be useful as a tumor-imaging agent. *In vitro* experiments on Ehrlich ascites tumor cells show that *D*-SeMet-[^{75}Se] is incorporated into the tumor cells by an Na^+-dependent active transport system similar to *L*-SeMet-[^{75}Se] and that a transport mechanism specific for *D*-SeMet-[^{75}Se] might be present in tumor cells in addition to a transport system common to both *D*- and *L*-forms.

Tumor accumulation of D-selenomethionine-[^{75}Se] in tumor-bearing mice. Goto R, Unno K, Takeda A, Okada S, Tamemasa O. J Pharmacobiodyn 1987 Sep; 10 (9), pp. 456–61.

Endothelium: Vascular (Carcinoma)/Mammary Carcinoma/Prostatic Carcinoma/Human Carcinoid Bon Cells/Rat Pancreatic Tumor Cells (CA20948)/$\alpha_v\beta_3$ Receptor Complexes *[^{111}Indium] ([^{111}In])-[c(Arg-Gly-Asp-D-Tyr-Lys)]/ [^{125}I]-[c(Arg-Gly-Asp-D-Tyr-Lys)]*

Cellular/Molecular Mechanism of Action: Purified fractions of [c(Arg-Gly-Asp-D-Tyr-Lys)] appear to bind specifically and with high affinity to $\alpha_v\beta_3$ receptors on neovascular blood vessel sections of different major human cancers, like prostate and breast cancer, that express this receptor type.
Background Information: Tumors depend on sufficient blood supply for their growth. They are able to promote new blood vessel formation (neoangiogenesis) via angiogenic factors. Inhibition of this process results in tumor involution or necrosis. RGD (Arg-Gly-Asp) peptides have been described to antagonize neoangiogenesis, for example, by binding to $\alpha_v\beta_3$ receptors on blood vessels.
Implementation: Evaluate the ability to visualise neoangiogenesis in tumors *in vitro* and *in vivo*, with an RGD analogue [c(Arg-Gly-Asp-D-Tyr-Lys)], coupled to the chelator diethyleletriame pentaacetic acid (DTPA) to facilitate [^{111}In] and [^{125}I] radiolabeling.
Interpretation and Analysis: In autoradiography and immunohistochemistry studies, [^{125}I]-labeled [c(Arg-Gly-Asp-D-Tyr-Lys)] appears to bind specifically and with high affinity to $\alpha_v\beta_3$ receptors on neovascular blood vessel sections of different major human cancers, like prostate and breast cancer, that express this receptor type. The radioiodinated pharmaceutical also binds to and becomes internalized within human carcinoid Bon cells and rat pancreatic CA20948 tumor cells. Internalization is receptor-specific and appears as well to be time and temperature dependent. Administration of different peptide amounts (0.1, 0.5, and 100 μg) *in vivo* in rats reveals that the optimum amount of radiolabeled analogue appears to be 0.1 μg/rat, because uptake decreases with increasing peptide doses. Receptor-specific accumulation of [^{111}In]-labeled analogue can be appreciated in transplantable CA20948 pancreatic tumors. The introduction of the DTPA group in this peptide results in increased renal clearance of the radiopharmaceutical, in contrast to the non-DTPA-conjugated compound that is cleared predominantly via the liver. [^{111}In] emits auger and conversion electrons besides gamma radiation; therefore, this radiopharmaceutical is suitable not only for tumor scintigraphy but also has potential applicability for radionuclide therapy of major human cancers as well. Moreover, after coupling to the chelator DOTA, the analogue can be radiolabeled in a stable manner with beta-emitters, for example [^{90}Y] and [^{177}Lu], expanding its theoretical potential.

Evaluation of a radiolabeled cyclic DTPA-RGD analogue for tumor imaging and radionuclide therapy. van Hagen PM, Breeman WA, Bernard HF, Schaar M, Mooij CM, Srinivasan A, Schmidt MA, Krenning EP, de Jong M. Int J Cancer (Radiat. Oncol. Invest.) 2000 Aug 20; 90 (4), pp. 186–98.

Folate Receptors/Neoplastic Disease *Gallium-67 ([^{67}Ga])Folate-DF-Folate-(α)/[^{67}Ga] Folate-DF-Folate-(γ)*

Cellular/Molecular Mechanism of Action: Folate is used as a carrier targeting molecule for the radioisotopes due to its binding avidity for folate receptors expressed at relatively high concentrations by certain tumor cell types compared to normal cell types. Deferoxamine functions as a chelating agent for certain radioisotopes (e.g., [$^{67}Ga^{3+}$]).
Application: Identification of folate receptor positive neoplastic lesions applying nuclear medicine imaging techniques.
Route of Administration: Intravenous.
Semi-Synthetic Methods: The vitamin folic acid can be covalently linked to the chelating agent deferoxamine (DF) via an amide bond using a simple carbodiimide coupling reaction. A mixture of 2 isomers, DF-folate-(α) and DF-folate-(γ), can be generated by producing chemical interactions with a carboxyl group at either the α- or γ- position of folic acid, respectively. Isomer separation is feasible applying anion-exchange chromatography and NH_4HCO_3 gradients.

Experimental Interpretation: Competitive binding studies reveal that only the DF-folate-(γ) is recognized by the folate receptors on KB cells and interacts with an affinity comparable to unconjugated folic acid. The DF-folate conjugates can be radiolabeled with the γ-emitting radionuclide [$^{67}Ga^{3+}$] to test for uptake by cultured KB cells overexpressing the folate receptor.
Interpretation and Analysis: The cellular accumulation of [^{67}Ga]-DF-folate-(γ) tracer exhibits rapid uptake kinetics in cell culture with a $T_{1/2}$ of approximately 3 minutes. The KB cell–associated [^{67}Ga]-DF-folate-(γ) radioactivity is competitively blocked by free folic acid, indicating that uptake of [^{67}Ga]-DF-folate-(γ) is specifically mediated by the folate receptor. Because folate receptors are overexpressed on the surface of many neoplastic cells, these results suggest that [^{67}Ga]-DF-folate-(γ) complex might be useful as a diagnostic agent for the non-invasive imaging of folate receptor-positive tumors.
Instrumentation: Computer-integrated nuclear medicine scintigraphy imaging camera.

Preparation of [^{66}Ga]- and [^{68}Ga]-labeled Ga(III)-deferoxamine-folate as potential folate-receptor-targeted PET radiopharmaceuticals. Mathias CJ, Lewis MR, Reichert DE, Laforest R, Sharp TL, Lewis JS, Yang ZF, Waters DJ, Snyder PW, Low PS, Welch MJ, Green MA. Nucl Med Biol 2003 Oct; 30 (7), pp. 725–31.

Folic Acid Receptor Expression/Neoplastic Disease *[^{99m}Technetium] ([^{99m}Tc])-Labeled Folic Acid*

Cellular/Molecular Mechanism of Action: In the context of diagnostic pharmacology, folic acid binds to folic acid receptor complexes variably expressed on the surface membrane of different cell types.
Background Information: It is known that membrane folic acid receptors are responsible for the cellular accumulation of folate and folate analogues such as methotrexate and overexpressed by various tumor cells. However, these receptors are highly restricted in normal differentiated tissues. Results of limited *in vitro* and *in vivo* animal studies suggest that folate receptors can potentially function as a targeting platform for tumor imaging.
Application: Detection and characterization of neoplastic lesions.
Route of Administration: [^{99m}Tc]-labeled folic acid (intravenous).
Dosage: [^{99m}Tc]-labeled folic acid (370 KBq/rat).
Semi-Synthetic Methods: Use of ethylenedicysteine (EC) as a chelating agent for the production of [^{99m}Tc]-EC-folate.
Implementation: Evaluate the labeling efficiency of [^{99m}Tc]-labeled folic acid and its potential use as a tumor-seeking agent. Tissue distribution of [^{99m}Tc]-EC-folate can be determined by evaluations made at 20 minutes and 1, 2, and 4 hours (n = 3 observations/time interval). Validation of receptor-mediated processes can be established employing blocking studies utilizing the co-administration of "cold folic acid" (50 and 150 μmol/kg) in concert with [^{99m}Tc]-EC-folate in tumor-bearing rats. Planar imaging and whole-body autoradiograms are then performed and findings compared to [^{99m}Tc]-EC (control).
Animal Model: Rats (tumor-bearing).
Interpretation and Analysis: In animal studies, tumor/blood count density ratios at 20 minutes to 4 hours increase from 0.81 ± 0.09 to 1.23 ± 0.13 with [^{99m}Tc]-EC-folate. Conversely, these values show a time-dependent decrease from 0.77 ± 0.32 to 0.65 ± 0.01 with [^{99m}Tc]-EC within the same time period. Tumor/muscle and tumor/blood count density ratios significantly decrease with folic acid co-administration. Planar images and autoradiograms confirm that tumors can be visualized clearly with [^{99m}Tc]-EC-folate.

[^{99m}Tc]-ethylenedicysteine-folate: A new tumor-imaging agent. Synthesis, labeling and evaluation in animals. Ilgan S, Yang DJ, Higuchi T, Zareneyrizi F, Bayhan H, Yu D, Kim EE, Podoloff DA. Cancer Biother Radiopharm 1998 Dec; 13 (6), pp. 427–35.

Folate Receptors (Ovarian Carcinoma) *[^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)-Folate*

Cellular/Molecular Mechanism of Action: [^{111}In]-DTPA-folate is a radioactive chelation analogue of folic acid that recognizes and physically binds to folate receptors expressed at relatively high membrane densities by certain neoplastic cell types.
Application: [^{111}In]-DTPA-folate can be applied as a tumor-selective imaging agent in diagnostic nuclear medicine for the purpose of detecting and monitoring neoplastic disease states (e.g., ovarian malignancies). Based on the biological properties of folate, it could also function as a tumor-specific drug delivery platform.
Background Information: The cell-membrane folate receptor is a potential molecular target for tumor-selective drug delivery, including radiolabeled folate-chelate conjugates for diagnostic imaging.
Route of Administration: [^{111}In]-DTPA-folate (intravenous bolus).
Implementation: Evaluation of the diagnostic potential for [^{111}In]-DTPA-folate to function as a diagnostic agent for malignant ovarian disease or endometrial cancer utilizing [^{111}In]-DTPA-folate as an imaging agent (phase I/II clinical trials). Prior to the administration of [^{111}In]-DTPA-folate, some nuclear medicine facilities prefer to administer unlabeled "free" folic acid followed by whole-body imaging at 30 minutes, 4 hours, and 24 hours postinjection of the folate radiopharmaceutical agent. Imaging with single photon emission computed tomography (SPECT) instrumentation can also be performed at the appropriate time-delayed intervals.
Human Model: Female cases of ovarian cancer (n = 35) enrolled in a phase I/II clinical study (n = 33 completing the surgical follow-up required by the study protocol that serves to define disease status). Individual cases either have a pathologically proven malignancy or are scheduled for surgery to resolve suspected new ovarian cancer growth (n = 26 cases), recurrent ovarian cancer (n = 5 cases), or endometrial cancer (n = 2 cases). In some cases, evaluated (e.g., n = 19/26) unlabeled "free" folic acid is administered followed by whole-body imaging at 30 minutes, 4 hours, and 24 hours postinjection of the folate radiopharmaceutical agent.
Interpretation and Analysis: In cases subjected to surgical intervention (e.g., n = 33), slightly less than half have new or recurrent malignant tumors that can be detected (e.g., n = 14/33). A large percentage of newly diagnosed ovarian carcinomas can be identified blindly by multiple professional readers (e.g., n = 7/7 or 100% sensitivity). Conversely, sensitivity for detecting recurrent ovarian-related malignancies can be significantly less (e.g., n = 7/33) in cases where the interpretation is performed without prior knowledge, compared to the evaluation of images with prior knowledge of disease (e.g., n = 28/33 or 84.5% for unmasked readings). Such findings indicate that a correlation exists between anatomic imaging studies (computed tomography) and the diagnostic detection of such lesions. A number of cases may be determined to have benign masses (e.g., n = 18). In the context of a limited population size, the specificity of [^{111}In]-DTPA-folate scintigraphy is approximately 76% and 82% for masked and unmasked image interpretation, respectively. [^{111}In]-DTPA-folate is safe, and possibly effective, for differentiating between malignant and benign ovarian masses based on interpretation of scintigraphic images.

Evaluation of [^{111}In]-DTPA-folate as a receptor-targeted diagnostic agent for ovarian cancer: Initial clinical results. Siegel BA, Dehdashti F, Mutch DG, Podoloff DA, Wendt R, Sutton GP, Burt RW, Ellis PR, Mathias CJ, Green MA, Gershenson DM. J Nucl Med 2003 May; 44 (5), pp. 700–7.

Gastrin-Releasing Peptide (GRP) Receptors/ Prostatic Carcinoma/[Mammary Carcinoma]/ [Small Cell Lung Cancer]/[Pancreatic Cancer] *[^{111}Indium] ([^{111}In])-Bombesin (BBN)/[^{111}In]-DOTA-X-BBN[7-14]NH_2 / Beta-Ala, 5-Ava Spacer/ 8-Aoc Spacer*

Cellular/Molecular Mechanism of Action: Bombesin has been known to bind to GRP receptors with high affinity and specificity.

Background Information: Gastrin-releasing peptide receptors have been shown to be expressed at relatively high densities on several types of cancer cells, including cancers of the prostate, breast, small cell lung, and pancreas.

Application: Hydrocarbon spacer chains can effectively improve the specificity of detecting and characterizing human prostatic carcinoma cell types when employing [^{111}In]-DOTA-bombesin as a template molecular for the purpose of recognizing GRP receptor complexes.

Implementation: Evaluation of the relative tumor "uptake" and pharmacokinetic parameters for various [^{111}In]-labeled BBN analogues as potential candidates for the specific targeting of human prostate cancers.

***Ex Vivo* Model:** Human (PC-3 androgen-independent human prostate cancer cells).

Animal Model: Murine (CF-1 mice and PC-3 human prostate cancer-derived xenografts in severe combined immunodeficient [SCID] mice).

Interpretation and Analysis: *In vitro* competitive binding assays, using PC-3 androgen-independent human prostate cancer cells, reveal values $<$ 2.5 nmol/L for inhibitory concentrations of 50% for analogues with β-Ala, 5-Ava, and 8-Aoc spacers. *In vivo* biodistribution studies of the [^{111}In]-DOTA-X-BBN[7-14]NH_2 conjugates performed on CF-1 mice 1 hour after injection reveal that uptake of radioactivity within the pancreas (Note: A GRP receptor expressing tissue) increases as a function of hydrocarbon spacer length (Data: 0.20 $\pm$ 0.04 percent injected dose per gram [%ID/g] for the analogue with no spacer to a maximum of 26.97 $\pm$ 3.97 %ID/g for the analogue with 8-Aoc spacer). Radioactivity is cleared efficiently from the blood pool by excretion mainly through the renal/urinary pathway (e.g., 71.6 $\pm$ 1.8 %ID at 1 hour postinjection for the 8-Aoc spacer analog). *In vivo* pharmacokinetic studies of the [^{111}In]-DOTA-8-Aoc-BBN[7-14]NH_2 conjugate conducted on PC-3 human prostate cancer-derived xenografts in SCID mice show a specific uptake of radioactivity within tumors, with 3.63 $\pm$ 1.11 %ID/g observed at 1 hour postinjection. High tumor-to-blood and tumor-to-muscle ratios of approximately 6:1 and 45:1, respectively, can be appreciated at 1 hour after injection. Relative to the radioactivity observed in the tumor at 1 hour after injection, 43%, 19%, and 9% of the radioactivity is retained at, respectively, 24, 48, and 72 hours postinjection.

These studies show that radiometallated DOTA-X-BBN[7-14]NH_2 constructs with hydrocarbon spacers ranging from 5 to 8 carbon atoms are suitable candidates for further development as diagnostic and therapeutic radiopharmaceuticals for patients with GRP-positive cancers.

Novel series of [^{111}In]-labeled bombesin analogues as potential radiopharmaceuticals for specific targeting of gastrin-releasing peptide receptors expressed on human prostate cancer cells. Hoffman TJ, Gali H, Smith CJ, Sieckman GL, Hayes DL, Owen NK, Volkert WA. J Nucl Med 2003 May; 44 (5), pp. 823–31.

Gastrointestinal/Colon Cancer/Guanyl Cyclase-C (GC-C) Receptor (LS-180 Cell Type) *[^{125}I]-Tyr$_5$-6-Ahx-Phe$_{19}$-ST[1-19]/[^{111}Indium] ([^{111}In])-DOTA-NCS-6-Ahx-Phe$_{19}$-ST[1-19]*

Cellular/Molecular Mechanism of Action: [^{111}In]-DOTA-NCS-6-Ahx-Phe$_{19}$-ST[1-19] and [^{125}I]-Tyr$_5$-6-Ahx-Phe$_{19}$-ST [1-19] both recognize and bind to GC-C receptor complexes.

Application: Detection and characterization of human colon cancer (e.g., LS-180 cell strains).

Route of Administration: [^{111}In]-DOTA-NCS-6-Ahx-Phe$_{19}$-ST[1-19] and [^{125}I]-Tyr$_5$-6-Ahx-Phe$_{19}$-ST[1-19] (intravenous).

Implementation: Evaluation of the relative *in vitro* competitive binding of [^{111}In]-DOTA-NCS-6-Ahx-Phe$_{19}$-ST[1-19] for GC-C receptor complexes expressed by human colon cancer LS-180 cells compared to [^{125}I]-Tyr$_5$-6-Ahx-Phe$_{19}$-ST[1-19].

Animal Model: Murine (tumor-bearing severe combined immunodeficient [SCID] mice supporting LS-180 colon cancer lesions).

Interpretation and Analysis: Competitive *in vitro* binding studies of [^{111}In]-DOTA-NCS-6-Ahx-Phe$_{19}$-ST[1-19] and [^{125}I]-Tyr$_5$-6-Ahx-Phe$_{19}$-ST[1-19] for GC-C receptors on human colon cancer cells (type LS-180) reveal an IC_{50} value of 7.7 $\pm$ 0.1.6 nM. *In vitro* cellular residualization studies with [^{111}In]-DOTA-NCS-ST peptide and GC-C receptor mediated stimulated cGMP production with LS-180 cells demonstrate that this peptide selectively binds to LS-180 cells in an agonistic fashion. *In vivo* biodistribution studies in mice bearing LS-180 tumors reveal that the [^{111}In]-DOTA-NCS-ST peptide targets the tumor with a specific uptake of 0.94 $\pm$ 0.31 percent injected dose per gram (%ID/g) at 1 hour postinjection and approximately 23% is retained by the tumor at 4 hours postinjection. Radioactivity clears rapidly from the bloodstream with 84.5 $\pm$ 3.4 %ID found in the urine at 1 hour postinjection. High activity in urine and kidney and minimal activity in liver and intestines demonstrates preferential clearance of the radioactivity through the renal/urinary pathway. The specific *in vitro* and *in vivo* accumulation of the radioactivity within LS-180 human colonic cancer cells highlights the potential of radiometallated-DOTA-ST analogues as diagnostic/therapeutic radiopharmaceuticals.

In vivo evaluation of an [^{111}In]-labeled ST-peptide analogue for specific-targeting of human colon cancers. Gali H, Sieckman GL, Hoffman TJ, Owen NK, Chin DT, Forte LR, Volkert WA. Nucl Med Biol 2001 Nov; 28 (8), pp. 903–9.

Gastrointestinal/Colorectal Cancer *[^{111}Indium] ([^{111}In])-Labeled Carcinoembryonic Antigen (CEA) Monoclonal Antibody (MAb)*

Application: Detection of colorectal cancer applying nuclear medicine imaging techniques following curative resection with the simultaneous expression of elevated carcinoembryonic antigen levels and negative clinical diagnostic parameters.

Dosage: Anti-carcinoembryonic antigen MAb Type ZCE-025 (1 mg) labeled with 5.5 mCi of [^{111}In] co-administered with 9 to 39 mg of unlabeled ZCE-025 antibody.

Implementation: Diagnostic scanning is performed 3–7 days after infusion by planar and emission computed tomography.

Interpretation and Analysis: In clinical trials, ZCE-025 MAb imaging detects tumor recurrence or metastasis in 11 of 13 cases

with tumor sites identified within the pelvis (2 cases), abdominal wall (2), retroperitoneum (1), lymph nodes (3), liver (2), bone (2), and lung (1). The accurate localization of colorectal carcinoma recurrences by means of [^{111}In]-ZCE-025 MAb demonstrates the usefulness of this diagnostic agent in scenarios associated with elevated carcinoembryonic antigen levels and negative results from clinical and radiologic workup evaluations.

Improved tumor localization with increasing dose of indium-111-labeled anti-carcinoembryonic antigen monoclonal antibody ZCE-025 in metastatic colorectal cancer. Patt YZ, Lamki LM, Haynie TP, Unger MW, Rosenblum MG, Shirkhoda A, Murray JL. J Clin Oncol 1988 Aug; 6 (8), pp. 1220–30.

Indium 111 ZCE-025 immunoscintigraphy in occult recurrent colorectal cancer with elevated carcinoembryonic antigen level. Doerr RJ, Abdel-Nabi H, Merchant B. Arch Surg 1990 Feb; 125 (2), pp. 226–9.

Gastrointestinal/Colorectal Carcinoma/ Intraoperative Lesion Localization
[^{125}I]-B72.3 Anticolorectal Carcinoma Monoclonal Antibody (MAb)

Cellular/Molecular Mechanism of Action: [^{125}I]-B72.3 MAb selectively binds to antigenic complexes expressed by colorectal carcinoma.
Application: Intraoperative detection and localization of neoplastic lesions using a handheld gamma probe.
Route of Administration: [^{125}I]-B72.3 MAb (intravenous).
Implementation: Evaluation of an intraoperative approach for tumor localization using radiolabeled MAb B72.3 involving the use of a handheld gamma-detecting probe (GDP). Surgical procedures can be performed between 5 and 35 days postadministration of [^{125}I]-labeled MAb B72.3 IgG preparations. Analysis of resected tumor and normal tissues involves measurement of relative [^{125}I]-labeled MAb B72.3 IgG (e.g., injected dose/gram tissue).
Interpretation and Analysis: Applying a handheld GDP, surgeons intraoperatively can successfully localize lesions 68% of the time (e.g., n = 21/31). Resected tumor radiolocalization indices range from 0.5 to 543 with radiolocalization indices of 3.0 or higher in 71% (e.g., n = 22/31) of tumor specimens. Approximately 68% of carcinoma lesions that can be positively detected intraoperatively with a handheld GDP will also have levels of tumor antigen expression that can be detected with an immunoperoxidase-based assay. Alternatively, approximately 8% of lesions that cannot be detected intraoperatively are also antigen negative. About 24% of carcinoma lesions cannot be localized intraoperatively based on the use of the GDP, but immunohistopathology analysis reveals positive expression of detectable levels of tumor antigen. The [^{125}I]-labeled MAb B72.3 IgG reagent recognizes all histologic grades of colorectal cancer. In most situations, higher *in vivo* probe ratios and radiolocalization indices can be observed when intraoperative handheld gamma scans are performed 20–35 days following injection of MAb and preparations are successful in detecting colorectal carcinoma lesions that have metastasized to mesenteric and periaortic lymph nodes, liver, lung, and perirectal soft tissue. A strong degree of correlation ($p = 0.001$) can be appreciated between the intraoperative detection of tumorous lesions with increased MAb B72.3 localization and the *in vitro* measurement of cpm/gram of tumor tissue compared to normal/healthy tissue samples.

Intraoperative radioimmunolocalization of colorectal carcinoma with a handheld gamma probe and MAb B72.3: Comparison of *in vivo* gamma probe counts with *in vitro* MAb radiolocalization. Tuttle SE, Jewell SD, Mojzisik CM, Hinkle GH, Colcher D, Schlom J, Martin EW. Int J Cancer 1988 Sep 15; 42 (3), pp. 352–8.

Gastrointestinal/Colorectal Carcinoma/Prognosis Prediction of Therapeutic Sensitivity
[^{18}F]-5-Fluorouracil (5-FU)

Application: Radiopharmaceutical biodynamic evaluation of 5-FU chemotherapeutic efficacy.
Considerations: The colon carcinoma cell Type 38 is 5-FU sensitive in contrast to R1-rhabdomyosarcoma, which is 5-FU resistant.
Implementation: Delineation of the relationship between [^{18}F]-5-fluorouracil cellular pharmacodynamics and chemotherapeutic sensitivity to 5-FU chemotherapy in colorectal carcinoma cell types.
Semi-Synthetic Methods: Optimized methods for the synthesis of [^{18}F]-labeled 5-FU have been described.
Interpretation and Analysis: Instead of initial tumor uptake, chemotherapeutic sensitivity to 5-FU correlates best with carcinoma [^{18}F] efflux out of lesion sites.

An optimized synthesis of [^{18}F]-labeled 5-fluorouracil and a reevaluation of its use as a prognostic agent. Visser GW, Gorree GC, Braakhuis BJ, Herscheid JD. Eur J Nucl Med 1989; 15 (5), pp. 225–9.

Gastrointestinal/Colorectal Carcinoma: Recurrent Postresection/Surgical Intervention Decision Making/Nuclear Scan vs Computed Tomography (CT) Scan
[^{111}Indium] ([^{111}In])-CYT-103 Monoclonal Antibody (MAb)

Background Information: Recurrent colorectal cancer is seen in as many as 40% of cases after curative resection. In view of the limitations of endoscopic and cross-sectional imaging, external immunoscintigraphy has been added to follow-up regimens in conditions that are at high risk of recurrent disease.
Application: Detection and characterization of colorectal carcinoma as an aid to deciding the merits of surgical intervention.
Route of Administration: Intravascular.
Considerations: Provocative agents (histamine, glucagon, tyramine) are rarely needed. Preoperative localization of tumor lesions can be done with nephrotomography, intravenous pyelography (IVP), computerized axial tomography, ultrasound, [^{131}I]-19-iodocholesterol scan, arteriography, and venography.
Semi-Synthetic Methods: Labeling of CYT-103 MAb (1.0 mg) with [^{111}In] radioisotope (approximately 4.3 mCi).
Implementation: Evaluation of the utility of immunoscintigraphy in conditions of suspected colorectal carcinoma utilizing MAb (e.g., B72.3 or CYT-103) site-specifically labeled with [^{111}In] conjugate. Planar and single photon emission computed tomography (SPECT) imaging is then performed 2–5 days after infusion of, for example, [^{111}In]-CYT-103).
Human Model: Cases of suspected recurrences of previous "curative" colorectal carcinoma resections.
Interpretation and Analysis: Local or regional recurrences (4 cases) and liver metastasis (6 cases) may be detected by physical examination and computed tomography (CT). Alternatively, some cases (e.g., n = 9) may have occult disease with increasing serum carcinoembryonic antigen (CEA) levels and negative conventional workups. Serum CEA levels are elevated (mean, 22 ng/ml) in the vast majority of cases (e.g., 100%). A final diagnosis of recurrence or metastasis can be established in a number of cases by 2nd-look surgery or biopsy (e.g., n = 18). Tumors may potentially be identified at the following locations: pelvis (e.g., n = 12 cases), abdominal wall (e.g., n = 2 cases), retroperitoneum (e.g., n = 1 case), liver (e.g., n = 5 cases), and omentum (e.g., n = 2 cases). Superiority of MAb scans

is supported by its capacity to detect pelvic and intra-abdominal recurrences (100%) at a higher success rate than CT scan (43%). Liver metastases are identified equally well by both modalities. [^{111}In]-CYT-103 scan findings may influence the management of approximately 55% of affected cases (e.g., n = 10/18). Surgery may be avoided in conditions with scan-detected disseminated metastases. Correct identification of occult local recurrences can be made in some cases (e.g., n = 6). Isolated liver metastasis may be confirmed in instances of equivocal CT scan results. Finally, additional intra-abdominal lesions may also be detected. Such observations suggest an important and beneficial role for [^{111}In]-CYT-103 MAb imaging in conditions of suspected recurrent colorectal carcinomas, particularly in cases with negative cross-sectional imaging results; such imaging may prevent cases from undergoing unnecessary surgical exploration.

In-111 CYT-103 monoclonal antibody imaging in patients with suspected recurrent colorectal cancer. Doerr RJ, Herrera L, Abdel-Nabi H. Cancer]1993 Jun 15; 71 (12 Suppl), pp. 4241–7.

Gastrointestinal/Colorectal Carcinoma/Targeting and Pretargeting with Monoclonal Antibody (MAb) Mediated/Human Colorectal Carcinoma Xenographs (SW1222) in Mice *[^{64}Cu]-DOTA-Biotin/[^{64}Cu]-DOTA-NR-LU-10*

Background Information: Pretargeting involves administration of a tumor-targeting MAb covalently linked to a molecule having a high-affinity binding site for a rapidly distributed radiolabeled effector molecule.

Application: Pretargeted [^{64}Cu]-DOTA-biotin should afford markedly superior positron emission tomography (PET) images and therapeutic efficacy due to its extremely rapid tumor uptake and blood clearance.

Semi-Synthetic Methods: DOTA-biotin (biotinylated 1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid) and the intact immunoconjugate DOTA-NR-LU-10 are labeled to high specific activities with [^{64}Cu], and the serum stabilities and target binding capabilities of each agent are then assayed (*in vitro*).

Implementation: Compare different pretargeting strategies employing conventionally labeled antibody for targeting tumors with the intermediate-lived radionuclide [^{64}Cu], which has shown promise for PET imaging and radioimmunotherapy of cancer. One approach to characterizing "pretargeting" strategies can involve the evaluation of preparations for their ability to recognize and preferentially accumulate within colorectal carcinoma xenografts. Semi-synthetic preparations of [^{64}Cu]-DOTA-biotin are administered, with and without MAb-streptavidin conjugate NR-LU-10/SA pretreatement and the synthetic clearing agent biotin-GalNAc(16); or injected with [^{64}Cu]-DOTA-NR-LU-10. Biodistribution of both agents is then established from 5 minutes to 48 hours after injection.

Animal Model: Murine (nude mice bearing SW1222 human colorectal carcinoma xenografts).

Interpretation and Analysis: Both [^{64}Cu]-DOTA-biotin and [^{64}Cu]-DOTA-NR-LU-10 are 100% stable *in vitro* in serum. [^{64}Cu]-DOTA-biotin exhibits > 98% specific binding to immobilized streptavidin, whereas the immunoreactivity of [^{64}Cu]-DOTA-NR-LU-10 averages nearly 80%. Biodistributions in SW1222-bearing mice show that NR-LU-10/SA-pretargeted [^{64}Cu]-DOTA-biotin attains a peak tumor uptake of 18.9% of the injected dose per gram (%ID/g) at 1 hour, with a concomitant rapid disappearance from blood and renal excretion. In the absence of pretargeting, [^{64}Cu]-DOTA-biotin has very similar biodistribution and clearance properties, except with extremely low non-specific tumor uptake. Conversely, [^{64}Cu]-DOTA-NR-LU-10 reaches 80.3 %ID/g in tumor tissue, after 48 hours, whereas blood clearance is considerably slower than pretargeted [^{64}Cu]-DOTA-biotin. Comparison of the time-activity curves for tumor uptake and blood clearance of pretargeted [^{64}Cu] and [^{64}Cu]-labeled antibody reveals that the maximum tumor accumulations of radioactivity are similar for each agent (e.g., 17.9% injected activity per gram [%IA/g] and 20.7 %IA/g, respectively). However, the tumor-to-blood ratio of areas under the curves is 14 times higher for pretargeted [^{64}Cu]-DOTA-biotin because of the substantial increase in blood clearance of the small effector molecule. The extremely rapid tumor uptake and blood clearance of pretargeted [^{64}Cu]-DOTA-biotin should afford markedly superior PET images and therapeutic efficacy, compared with conventionally labeled [^{64}Cu]-DOTA-NR-LU-10. Further comparison of the therapeutic efficacy, toxicity, and dosimetry of these 2 agents is warranted.

In vivo evaluation of pretargeted [^{64}Cu] for tumor imaging and therapy. Lewis MR, Wang M, Axworthy DB, Theodore LJ, Mallet RW, Fritzberg AR, Welch MJ, Anderson CJ. J Nucl Med 2003 Aug; 44 (8), pp. 1284–92.

Head and Neck Neoplastic Disease/Benign vs Malignant *[^{201}Thallium] [(^{201}Tl]) Chloride/Gallium-67 ([^{67}Ga])-Citrate*

Application: Differentiation between malignant tumors and benign tumors of the head and neck by means of [^{201}Tl] chloride scintigraphy.

Route of Administration: Intravenous.

Considerations: [^{201}Tl] chloride scintigraphy and [^{67}Ga] scintigraphy performed in conditions of benign or malignant neoplasia initially entail establishing the blood flow index and the tumor retention index by means of [^{201}Tl] scintigraphy. The blood flow index = the count of tumor/the count of control in the early dynamic scan from 30 to 120 seconds after injection; and the tumor retention index = (the count of tumor/the count of control in the delayed dynamic scan 2.5 hours after injection)/(tumor count/control count in the early dynamic scan from 4 to 5 minutes after injection).

Interpretation and Analysis: Early and delayed scans with [^{201}Tl] demonstrate a very high correlation with the blood flow index. The tumor retention index has a tendency to increase in malignant tumors, especially in poorly differentiated tumors and highly invasive tumors. Likewise, the tumor retention index decreases in benign tumors. Results from such obseverations are statistically significant and reveal that [^{201}Tl] scintigraphy is superior to [^{67}Ga] scintigraphy. Scintigraphy with [^{201}Tl] is also useful in detecting neoplastic lesions of relatively small size/mass. The tumor retention index of [^{201}Tl] is useful for the differentiation of malignant tumors of the head and neck from benign tumors of the same areas.

Hepatic Neoplasia: Metastatic Tumor Imaging *[^{75}Se]-Methionine*

Application: Detection of metastatic hepatic tumors applying nuclear medicine imaging methodologies.

Considerations: Preparations of [^{75}Se]-methionine can function as a valuable diagnostic agent for the detection of malignant lesions affecting hepatic tissues.

Various types of biological activity have been shown to be provided by [^{75}Se]-methionine for the detection of metastatic liver tumors. Concerning the spectrum of imaging agents applied in diagnostic nuclear medicine, hepatic neoplasias manifest themselves

with a great degree of variability (e.g., enhanced to absent incorporation into "cold zones" detected with [^{113m}In]-colloid).

Interpretation and Analysis: Analysis of data from human cancer patients with neoplastic lesions affecting various sites within the liver has revealed that the diagnostic value of [^{75}Se]-methionine when applied in combination with [^{113m}In] colloid approaches 90% approximately.

Insulin-Like Growth Factor (IGF) Binding Proteins/ Neoplastic Disease
[^{125}I]-Labeled-IGF

Cellular/Molecular Mechanism of Action: Insulin-like growth factors recognize and bind to membrane-associated receptor complexes [insulin-like growth factor binding proteins].

Background Information: Insulin-like growth factor binding proteins (IGFBPs) play a critical role in modulating IGF activity.

Application: Detection and characterization of certain neoplastic cell types.

Considerations: [^{125}I]-labeled IGF-I is rapidly degraded within the cytosol of some types of neoplastic cell.

Semi-Synthetic Methods: Production of [^{125}I]-labeled-IGF-I by radioiodination of IGF-I using chloramines-T reagent.

Implementation: Evaluate the biodistribution and tumor targeting ability of radiolabeled IGF-I. Complementary investigations can entail *in vitro* and *in vivo* assessment of binding properties for [^{125}I]-labeled IGF-I for IGFBPs. Validation of the relative specificity of uptake can in part be established by co-administration of unlabeled peptide. Because a large amount of the IGF-I is catabolized *in vivo*, valuable information can also be acquired by delineating the *in vitro* catabolism of IGF-I by tumor cells. *In vitro* and *in vivo* chromatography studies can be performed to evaluate the binding profile to IGFBPs and measure serum/urine catabolite concentrations.

Animal Model: Rat and mouse (tumor-bearing nude mice).

Interpretation and Analysis: Binding of radiolabeled IGF-I to IGFBPs *in vitro* occurs in a dose-dependent manner. However, there is a difference in complex formation between serum and heparinized plasma. In heparinized plasma, radioactivity shifts from a 30–50-kDa complex to a 150-kDa complex and to a free ligand, because the formation of IGFBPs/heparin complexes decreases its binding affinity for IGF-I. In plasma prepared with acid citrate dextrose a binding pattern identical to that of serum is observed. Moreover, there is a binding difference between mouse and rat. The [^{125}I]-labeled IGF-I is catabolized very quickly when incubated at 37°C but not at all at 4°C. In tumor-bearing nude mice, the uptake of radioactivity in normal tissues decreases quickly, particularly in the kidney. In mice co-injected with unlabeled carrier, the radioactivity in most normal tissues is lower and the tumor uptake higher than in mice without carrier. [^{125}I]-labeled IGF-I is avidly bound to IGFBPs, both *in vitro* and *in vivo*. By partially saturating such molecular interactions with unlabeled peptides, a favorable biodistribution can be achieved, including faster clearance from normal tissue and higher tumor uptake, which thereby results in better tumor-to-non-tumor ratios. Nevertheless, the rapid catabolism and release of the radiolabel from tumor tissue results in a suboptimal targeting agent.

Effects of insulin-like growth factor binding proteins on insulin-like growth factor-I biodistribution in tumor-bearing nude mice. Sun BF, Kobayashi H, Le N, Yoo TM, Drumm D, Paik CH, McAfee JG, Carrasquillo JA. J Nucl Med 2000 Feb; 41 (2), pp. 318–26.

Leukemia: Human Jurkat Cell Line
[^{99m}Technetium] ([^{99m}Tc])Tat-Peptide 4/ [Fluorescein-5-Maleimide]-Tat-Peptide

Cellular/Molecular Mechanism of Action: [Tc/Re$_V$]complexes can rapidly translocate across cell membranes into intracellular compartments.

Background Information: Rapid and efficient delivery of radioactive metal complexes to the cell interior would enable novel applications in medical imaging and radiotherapy.

Application: [^{99m}Tc]Tat-peptide 4 can potentially be applied for the diagnostic imaging of certain neoplastic disease types.

Route of Administration: [^{99m}Tc]Tat-peptide 4 (intravenous).

Semi-Synthetic Methods: Membrane permeant peptide conjugates incorporating HIV-1 Tat transactivation protein sequences (GRKKRRQRRR) and an appropriate peptide-based motif (epsilon-KGC) that provides an N_3S donor core for chelating technetium and rhenium can be synthesized. Oxotechnetium$_V$ and oxorhenium$_V$ Tat-peptide complexes can be prepared by facile transchelation reactions with permetalates, [Sn^{+2}]chloride, and sodium glucoheptonate. RP-HPLC reveals 2 major [^{99m}Tc]Tat-peptide species (4) that differ in retention time by approximately 2 minutes corresponding to 2 [Re]Tat-peptide species (7) shown to have identical mass, consistent with the formation of 2 isomers, likely the oxo-metal diastereomers. [^{99m}Tc]Tat-peptides are stable to transchelation *in vitro*. To facilitate analysis using fluorescent microscopy, the C-terminus thiol of the prototypic Tat-peptide can be labeled with fluorescein-5-maleimide (yielding conjugate 8).

Implementation: Evaluation of binding avidity and stability of [^{99m}Tc]Tat-peptide 4 in the presence of human Jurkat cell populations.

Tissue Culture Model: Human Jurkat KB 3-1 and KB 8-5 leukemia cell types.

Animal Model: Murine (*in vivo* investigations).

Interpretation and Analysis: In human Jurkat cells, [^{99m}Tc]Tat-peptide 4 show concentrative cell accumulation (30-fold greater than extracellular concentration) and rapid uptake kinetics ($t_{1/2} < 2$ minutes) in a diastereomeric-comparable manner. Paradoxically, uptake is enhanced in 4°C buffer compared to findings noted at 37°C, while depolarization of membrane potential as well as inhibition of microtubule function and vesicular trafficking show no inhibitory effect. Cells preloaded with "4" show rapid washout kinetics into peptide-free solution. Modification of [^{99m}Tc]Tat-peptide by the deletion of the N-terminus Gly with or without biotinylation minimally impacts net-cell uptake.

Fluorescence microscopy reveals that [fluorescein-5-maleimide]-Tat-peptide (conjugate 8) can directly localize the preparation within the cytosol and nuclei (possibly nucleolus) of human Jurkat, KB 3-1, and KB 8-5 tumor cells. Preliminary *in vivo* imaging studies in mice following intravenous administration of prototypic [^{99m}Tc]Tat-peptide "4" reveals an initial whole-body distribution and rapid clearance by both renal and hepatobiliary excretion. Analysis of murine blood *in vivo* and human serum *ex vivo* reveals $> 95\%$ intact complex, while murine urine *in vivo* contains 65% parent complex. Thus, these novel Tat-peptide chelate conjugates, capable of forming stable [Tc/Re$_V$]complexes, rapidly translocate across cell membranes into intracellular compartments and can be readily derivatized for further targeted applications in molecular imaging and radiotherapy.

Novel Tat-peptide chelates for direct transduction of technetium-^{99m} and rhenium into human cells for imaging and radiotherapy. Polyakov V, Sharma V, Dahlheimer JL, Pica CM, Luker GD, Piwnica-Worms D. Bioconjug Chem 2000 Nov–Dec; 11 (6), pp. 762–71.

Lymph Nodes: Sentinel Lymphadenectomy Guide/ Melanoma
[^{99m}Technetium] ([^{99m}Tc])-Sulfur Colloid (SC)/ [^{99m}Tc]-Human Serum Albumin (HAS)/ Isosulfan Blue Dye

Cellular/Molecular Mechanism of Action: Isosulfan blue dye serves as a pigment-based marker system for biological fluid compartments. [^{99m}Tc]-HSA and [^{99m}Tc]-SC can be phagocytized by reticuloendothelial cell populations residing within peripheral lymph nodes.

Background Information: Radiopharmaceutical agents appear to improve the accuracy of sentinel node (SN) identification in patients with early-stage melanoma, but the optimal radiopharmaceutical agent and its timing from injection to surgery remains controversial.

Application: [^{99m}Tc]-SC and [^{99m}Tc]-HSA can be used intraoperatively as probes for lymphatic mapping in radiopharmaceutical-directed sentinel lymphadenectomy (LM/SL) procedures.

Route of Administration: Isosulfan blue dye, [^{99m}Tc]-SC, and [^{99m}Tc]-HSA can be administered intraoperatively into lymphatic vessels.

Implementation: Cutaneous lymphoscintigraphy can be performed before LM/S utilizing 1 of 3 techniques, including (i) HSA ([^{99m}Tc]-HSA) injected at least 24 hours before LM/SL (n = 124 cases); (ii) filtered sulfur colloid ([^{99m}Tc]-SC) injected no more than 4 hours before LM/SL (same-day SC, n = 95 cases); or (iii) [^{99m}Tc]-SC injected at least 18 hours before LM/SL (prior-day SC, n = 28 cases). At the time of LM/SL, isosulfan blue dye is injected alone (SC groups) or with a 2nd dose of HSA (HSA group). A handheld gamma probe is used to determine the radioactive (hot) counts of blue-stained and non-stained nodes, and the *in vivo* and *ex vivo* node-to-background count ratios of the nodes compared.

Human Model: Melanoma (n = 247; American Joint Committee on Cancer stage I or II).

Interpretation and Analysis: Preoperative LS can identify a large percentage of drainage basins (e.g., n = 299 in 247 cases). Lymphatic mapping with radiopharmaceutical-directed sentinel lymphadenectomy (LM/SL) is capable of identifying at least 1 sentinel lymph node in a large percentage of cases. Specific examples include: (i) lymph node basins using same-day SC (98% or n = 119/121); (ii) lymph node basins using HSA (97% or n = 142/146); and (iii) lymph node basins using prior-day SC (100% or n = 32/32). There is no difference ($p = 0.62$) in the accuracy rate between the 3 techniques in the scenario described for a total of 463 SNs in 247 cases. Same-day SC yields higher intraoperative node-to-background count ratios than do either of the other techniques ($p < 0.0001$). Same-day SC also has the greatest relative change in radioactivity between the blue sentinel node and the postexcision basin ($p < 0.0001$), and the highest rate of SNs that are both blue and hot (*in vivo* or *ex vivo* ratio ≥ 2, $p = 0.05$). Therefore, LS and LM/SL performed on the same day with a single injection of filtered [^{99m}Tc]-SC serves as the most useful method for probe-directed LM/SL. The technique demonstrates the highest *in vivo* and *ex vivo* count ratios, falloff of radioactivity between the excised nodes and postexcision basin, and concordance between blue dye and hot nodes. It should be recommended as the method of choice for probe-directed LM/SL.

Standardized probe-directed sentinel node dissection in melanoma. Essner R, Bostick PJ, Glass EC, Foshag LJ, Haigh PI, Wang HJ, Morton DL. Surgery 2000 Jan; 127 (1), pp. 26–31.

Lymph Nodes: Sentinel Lymph Node Detection
[^{99m}Technetium] ([^{99m}Tc])-Diethylenetriamine Pentaacetic Acid (DTPA)-Mannosyl-Dextran

Application: Sentinel node characterization/detection.

Route of Administration: Foot pads (rabbits: n = 4).

Dosage: [^{99m}Tc]-DTPA-mannosyl-dextran (0.22 nmol) or filtered [^{99m}Tc]-sulfur colloid (3.7 MBq in 0.050 mL).

Considerations: Receptor affinity can be measured by Scatchard assay of rabbit liver. Axillary, popliteal, and iliac lymph nodes and each injection site can be assayed for radioactivity at 1 and 3 hours after injection.

Synthetic Chemistry and Analysis: Synthesis begins with a 2-step process that attaches a high density of amino-terminated leashes to a dextran backbone. Allyl-bromide is reacted with pharmaceutical-grade dextran to yield allyl-dextran. After diafiltration with water, filtration, and lyophilization, the product is reacted with aminoethanethiol and ammonium persulfate. The resulting amino-conjugated dextran is then dialyzed, filtered, and lyophilized. The mixed anhydride method can then be used to covalently link together DTPA, 2-imino-2-methoxyethyl-1-D-mannose and amino-conjugated dextran. The molecular diameter of the product can be measured by dynamic light scattering. Amino, mannose, and DTPA densities are measured by trinitrobenzene sulfonate assay, sulfuric acid/phenol assay, and inductively coupled plasma spectroscopy of [gadolinium]-DTPA-mannosyl-dextran, respectively. DTPA-mannosyl-dextran had a molecular weight of 35,800 g/mol and a molecular diameter of 7.1 nm. The final amine, mannose, and DTPA densities are 23, 55, and 8 mol per dextran. Labeling yields are in excess of 98% and stable for 6 hours. Specific activities of 74 3 106 GBq/mol can be achieved. The equilibrium dissociation constant for binding to the mannose-terminated glycoprotein receptor is 0.12 6 0.07 nmol/L.

Interpretation and Analysis: The popliteal extraction at both 1 and 3 hours is significantly ($p < 0.05$) higher for [^{99m}Tc]-DTPA-mannosyl-dextran (90.1% ± 10.7% and 97.7% ± 2.0%, respectively) than for filtered [^{99m}Tc]-sulfur colloid (78.8 ± 6.5 and 67.4% ± 26.8%, respectively). [^{99m}Tc]-DTPA-mannosyl-dextran exhibits significantly faster injection site clearance than does filtered [^{99m}Tc]-sulfur colloid. The [^{99m}Tc]-DTPA-mannosyl-dextran percent injected dose (%ID) for the front and rear paws is 52.6 ± 10.5 and 52.3 ± 8.0 at 1 hour and 45.7 ± 8.5 and 43.6 ± 8.2 at 3 hours after administration. Filtered [^{99m}Tc]-sulfur colloid %ID for the front and rear paws is 70.4 ± 11.0 and 66.3 ± 15.1 at 1 hour and 55.5 ± 7.8 and 66.9 ± 8.5 at 3 hours. Lymph node accumulation of each agent at either 1 or 3 hours is not significantly different. [^{99m}Tc]-DTPA-mannosyl-dextran is a receptor-based sentinel node radiotracer that exhibits the desired properties of rapid injection site clearance and low distal node accumulation. This molecule is the 1st member of a new class of diagnostic agents based on a macromolecular backbone with a high density of sites for the attachment of substrates and imaging reporters. This molecule is 1 of the 1st members of a relatively new class of diagnostic agents based on a macromolecular backbone with a high density of sites for the attachment of substrates and imaging reporters.

Detection of gastric and colonic sentinel nodes through endoscopic administration of [^{99m}Tc]-DTPA-mannosyl-dextran in pigs. Méndez J, Wallace AM, Hoh CK, Vera DR. J Nucl Med 2003 Oct; 44 (10), pp. 1677–81.

In vivo evaluation of 3 different [^{99m}Tc]-labeled radiopharmaceuticals for sentinel lymph node identification. Edreira MM, Colombo LL, Perez JH, Sajaroff EO, de Castiglia SG. Nucl Med Commun 2001 May; 22 (5), pp. 499–504.

Sentinel lymph node identification and sampling in women with early breast cancer using [^{99m}Tc]-labeled dextran 500 and patent blue V dye. Xavier NL, Amaral BB, Cerski CT, Fuchs SC, Spiro BL, Oliveira OL, Menke CH, Biazús JV, Cavalheiro JA, Schwartsmann G. Nucl Med Commun 2001 Oct; 22 (10), pp. 1109–17.

Lymphoma: Malignant
[^{125}I]-Tyr$_3$-Octreotide/ [^{111}Indium] ([^{111}In])-[Diethylenetriamine Pentaacetic Acid-D-Phe$_1$]-Octreotide

Cellular/Molecular Mechanism of Action: Octreotide is a somatostatin analogue that functions as a ligand that recognizes and physically binds to somatostatin receptor complexes.
Application: Characterization of biopsy specimens utilizing autoradiography techniques, and the detection and monitoring of primary and metastatic (human) malignant lymphoma lesions when applied as a non-invasive *in vivo* imaging agent.
Route of Administration: [^{125}I]-[Tyr$_3$]-octreotide (intravenous) [^{111}In]-[DTPA-D-Phe$_1$]-octreotide (intravenous).
Considerations: Somatostatin receptor complexes are expressed by a wide variety of (human) neoplastic disease states and can serve as valuable pathobiochemical tissue markers (indicators) that can be capitalized upon because of their potential for *in vivo* use as diagnostic tools for (human) malignant lymphomas.
Implementation: Evaluation of immune-based autoradiographs of surgically excised malignant tissue samples applying [^{125}I]-[Tyr$_3$]-octreotide as radioligand (binding affinity KD = 1.2 nM). In cases of B-cell non-Hodgkin's lymphoma classified as low-grade, intermediate-grade, and high-grade malignancy, a very high percentage will positively express somatostatin receptor complexes (e.g., 91%, 100%, 70%, respectively).
Interpretation and Analysis: High-density somatostatin receptor complex expression in low-grade malignancy B-cell non-Hodgkin's lymphoma is largely confined to neoplastic follicles, and most of the somatostatin receptors expressed in cases of high-grade malignancy are present at a very high density. Some T-cell lymphomas and Hodgkin's lymphomas are also positive for the expression of somatostatin receptor complexes.

Lymphoma lesions can also be non-invasively localized *in vivo* with the diagnostic imaging agent [^{111}In]-[DTPA-D-Phe$_1$]-octreotide. Positive accumulation of [^{111}In]-[DTPA-D-Phe$_1$]-octreotide on scintigraphic images closely correlates with surgically excised neoplastic tissues [^{125}I]-[Tyr$_3$]-octreotide positive confirmed by receptor ligand autoradiography analysis.
Instrumentation: Scintigraphic gamma camera.

In vitro and *in vivo* detection of somatostatin receptors in human malignant lymphomas. Reubi JC, Waser B, van Hagen M, Lamberts SW, Krenning EP, Gebbers JO, Laissue JA. Int J Cancer 1992 Apr 1; 50 (6), pp. 895–900.

Lymphoma/Non-Hodgkin's Lymphoma (NHL)/ Hodgkin's Disease (HD)
[Gallium-67] ([^{67}Ga])/[^{18}Flourine]-Fluorodeoxyglucose ([^{18}F]-FDG)

Background Information: Until recently, [^{67}Ga] scintigraphy (GS) has been the best available functional imaging modality for evaluating NHL and HD conditions.
Application: [^{18}F]-FDG and [^{67}Ga] scintigraphy.
Implementation: As [^{67}Ga] is a tumor viability agent, the role of [^{67}Ga] scintigraphy is primarily utilized as a complementary follow-up diagnostic procedure.
Interpretation and Analysis: The diagnostic accuracy of [^{67}Ga] scintigraphy in detecting lymphoma is based on transferring the imaging protocol, knowledge of potential physiological and benign sites of [^{67}Ga] uptake, and the [Ga] avidity characteristics of individual lymphoma cell types. A residual mass that persists on computed tomography (CT) scans after treatment poses a common clinical dilemma that may indicate the presence of viable lymphoma requiring further treatment, or it can be benign, consisting of only fibrotic and necrotic tissues. Scintigraphy with [^{67}Ga] can successfully differentiate between these conditions. Routine follow-up with [^{67}Ga] scintigraphy may allow early diagnosis of recurrence and early initiation of treatment. Reversion of a positive [^{67}Ga] scintigraphy to a negative test, and the rapidity with which this occurs, has a high predictive value for prognosis (morbidity/mortality). Lymphoma lesions that appear normal on [^{67}Ga] scintigraphy early during treatment protocols have a better prognosis than lymphoma with a persistence of pathological findings. Other tumor-seeking single photon emitting agents, such as [^{201}thallium], [^{99m}technetium]-methoxyisobutylisonitrile, and [^{111}indium]- octreotide, have been investigated in lymphoma, primarily as an alternative to [^{67}Ga] scintigraphy in specific clinical settings, but are of limited value. The role of radioimmunoscintigraphy is gaining importance in conjunction with radioimmunotherapy. [^{18}F]-FDG imaging of lymphoma using either dedicated or camera-based positron emission tomography (PET) systems is gradually replacing [^{67}Ga] scintigraphy for assessment of lymphoma. [^{18}F]-FDG overcomes some of the limitations of [^{67}Ga] scintigraphy while sharing its tumor viability characteristics. The extensive clinical knowledge and experience accumulated over 3 decades with [^{67}Ga] scintigraphy in lymphoma provides a solid background as well as a model for the assessment of new functional imaging techniques.

Gallium-67 scintigraphy: A cornerstone in functional imaging of lymphoma. Even-Sapir E, Israel O. Eur J Nucl Med Mol Imaging 2003 Jun; 30 Suppl 1, pp. S65–81. Epub 2003 Mar 18.

Lymphoma: Non-Hodgkin's Type (NHL)/ (Staging of Recurrent or Newly Diagnosed NHL)
[^{99m}Technetium] ([^{99m}Tc])-Labeled Anti-lymphoma IgG Fragment

Application: [^{99m}Tc]-labeled anti-lymphoma IgG fragment can be used to stage recurrent or newly diagnosed NHL and to assess targeting prior to the initiation of radioimmunotherapy (RIT).
Route of Administration: [^{99m}Tc]-labeled anti-lymphoma IgG fragment.
Dosage: [^{99m}Tc]-labeled anti-lymphoma IgG fragment [bectumomab (LymphoScan)].
Interpretation and Analysis: In the evaluation of graded tumors (e.g., low-grade; intermediate-grade; high-grade histiological subtypes), the combined application of computed x-ray tomography, radiogallium imaging, fluorodeoxyglucose (FDG) positron emission tomography (PET), and bone marrow biopsy can detect a relatively large number of lesions. Radiolabeled [^{99m}Tc]-labeled antilymphoma IgG fragment can detect approximately 56% of these total lesions and in some instances detects lesions that cannot be identified by other conventional diagnostic modalities. The anatomical location where radiolabeled [^{99m}Tc]-labeled anti-lymphoma IgG fragment is least valuable as a diagnostic imaging agent is in the central abdomen and thoracic cavity. Conversely, radiolabeled [^{99m}Tc]-labeled antilymphoma IgG fragment demonstrates a superior capability to detect lesions within the head and neck region of the body. Relative to [^{67}Ga]-citrate imaging, the performance of [^{99m}Tc]-

labeled anti-lymphoma IgG fragment would have to be considered variable with no clear relationship to anatomic location. However, it does provide better targeting of low and intermediate grade NHL lesions. By comparison, radiogallium out-performs [^{99m}Tc]-labeled antilymphoma IgG fragment imaging (e.g., at n = 23/117 lesion sites, n = 19/23 = high or intermediate-grade disease). Alternatively, [^{99m}Tc]-labeled antilymphoma IgG fragment is superior to radiogallium at other anatomical sites (e.g., n = 6/117 lesions sites, n = 5/6 = low-grade tumor). In summary, nuclear medicine imaging with [^{99m}Tc]-labeled anti-lymphoma IgG fragment has demonstrated potential value as a pre-RIT probe for targeting of B-cell NHL, and it excels at defining small volume, low-grade disease. Unfortunately, as a purely diagnostic agent, its performance is variable.

Radioimmunoscintigraphy (RIS) with bectumomab (Tc^{99m}-labeled IMMU-LL2, Lymphoscan) in the assessment of recurrent non-Hodgkin's lymphoma (NHL). Lamonica D, Czuczman M, Nabi H, Klippenstein D, Grossman Z. Cancer Biother Radiopharm 2002 Dec; 17 (6), pp. 689–97.

Mammary Carcinoma/Detection and Disease Monitoring
[^{125}I]-Tyr(3)]-Octreotide/[^{125}I]-Vasoactive Intestinal Peptide (VIP)/[^{125}I]-Tyr(4)-Bombesin/[^{125}I]-[Leu(31),Pro(34)]-PYY

Cellular/Molecular Mechanism of Action: Recognizes and physically binds to the exterior surface of human mammary carcinoma cell types.
Application: Detection, characterization, and therapeutic "targeting" of human breast cancer cell types (*in vivo*). Breast cancers can express different types of peptide receptors such as somatostatin, VIP, gastrin-releasing peptide (GRP), and hypothalamic neuropeptide Y Type 1 (NPY-Y1) receptors. Investigations performed *in vitro* strongly suggest that the combination of radiolabeled GRP and Y1 analogues could be used as vehicles in the form of selective *in vivo* peptide receptor scintigraphic imaging and radiotherapeutic delivery relevant to both primary and metastatic mammary carcinoma cell populations.
Route of Administration: Intravenous.
Implementation: Detection and characterization of mammary carcinoma neoplastic disease states.
Interpretation and Analysis: In the *in vitro* analysis of primary and metastatic mammary carcinoma tissue samples, the membrane-associated receptor complexes for somatostatin, VIP, GRP, and NPY-Y1 can be detected by ligand-affinity autoradiography applying [^{125}I]-Tyr(3)-octreotide, [^{125}I]-VIP, [^{125}I]-[Tyr(4)]-bombesin and [^{125}I]-[Leu(31), Pro(34)]-PYY, respectively. Majority of neoplastic tissue samples analyzed in this fashion (88%) express at least 1 of the membrane-associated receptor complexes at a relatively high density (> 2,000 dpm/mg tissue). Based on these findings, the receptor probe reagents could function in a valuable capacity as *in vivo* targeting agents for detecting the existence of mammary carcinoma, characterization and monitoring of disease progression, evaluation of response to therapy, and selective delivery of antineoplastic modalities. A 2nd smaller group of neoplastic tissue samples (12%) can be recognized that express none of the 4 receptor complexes evaluated at relatively high densities (< 2,000 dpm/mg tissue).

Relative receptor density expression analyses of neoplastic tissue samples displaying a high level of expression for at least 1 of the complexes reveals that GRP, NPY-Y1, vasoactive intestinal polypeptide Type 1 (VPAC), and somatostatin receptors are present, respectively, in 74%, 66%, 37%, and 21% of the tissue samples evaluated. Estimated mean density values are 9,819 ± 530 dpm/mg tissue for GRP receptors, 9,135 ± 579 dpm/mg for NPY-Y1 receptors, 4,337 ± 528 dpm/mg for somatostatin receptors, and 3,437 ± 306 dpm/mg for VPAC-1 receptors. Important to these observations is the finding that a very high percentage of cells of this tumor type (93%) express NPY-Y1, GRP receptors, or both NPY-Y1 and GRP complexes (93%). Lymph node metastases have receptor expression profiles that closely correlate with those noted for primary tumor tissues.
Instrumentation: Nuclear medicine scintillation imaging camera with computer-integrated instrumentation.

Co-expressed peptide receptors in breast cancer as a molecular basis for *in vivo* multireceptor tumor targeting. Reubi C, Gugger M, Waser B. Eur J Nucl Med Mol Imaging 2002 Jul; 29 (7), pp. 855–62.

Mammary Carcinoma/Detection and Disease Monitoring
[^{123}I]-Anti–Human Milk Fat Globulin (HMFG1)/[^{99m}Technetium] ([^{99m}Tc])-Anti-Carcinoembryonic Antigen (CEA)

Cellular/Molecular Mechanism of Action: Humoral immune-mediated recognition of neoplastic cell-specific membrane-associated antigens expressed by mammary carcinoma facilitated by simple antigen/antibody complex formation. Examples of unique neoplastic cell antigens associated with the exterior membrane include anti-HMFG1 and anti-CEA.
Application: Detection of primary, residual, multicentric, and recurrent mammary carcinoma.
Route of Administration: Intravenous.
Dosage: Murine-origin anti-CEA monoclonal antibody (MAb) Type CYT 380 (0.5–2.0 mg) labeled with [^{99m}Tc] (13–22 millicurie [mCI]) and anti-HMFG1 MAb (0.25–1.0 mg: Unipath, U.K.) labeled with [^{123}I] (4-17 mCI).
Considerations: Radiolabeled MAbs used in this study are potentially useful diagnostic agents for the evaluation of primary or recurrent breast carcinoma conditions, particularly in the scenarios where conventional methodologies are limited.
Implementation: Investigations have assessed the potential proficiency of radioimmunodetection (RAID) of primary, residual, multicentric, and recurrent breast carcinoma using 2 radiolabeled murine MAbs with binding avidity for (i) anti-HMFG1; and (ii) anti-CEA.

Human Model: Cases with suspicious clinical and/or mammographic evidence of primary or recurrent breast carcinoma (n = 13) in a phase I-II prospective, consecutive, non-randomized, non-controlled study; case subjects receive intravenous infusions of [^{99m}Tc]-anti-CEA monoclonal Type CYT 380 (n = 5 cases) or [^{123}I]-anti-HMFG1 monoclonal antibody (n = 8 cases).
Interpretation and Analysis: Both types of radiolabeled monoclonal antibodies bind specifically to breast cancer lesions, resulting in successful RAID profiles (e.g., n = 10/12 cases [n = 5/5 cases with [^{99m}Tc]-anti-CEA; and n = 5/7 receiving [^{123}I]-anti-HMFG group resulting in 83.3% level of accuracy]). Sensitivity is relatively high (87.5%) based on the correlation of true-positive scans with observations noted at the time of surgical intervention. Monoclonal antibody facilitated scans can accurately diagnose lesions of primary invasive mammary carcinoma confirmed histiologically (e.g., n = 3/4). Detection of the presence of residual carcinoma may be slightly less effective following wide surgical excision (e.g., 1 of 2 cases) or in conditions with soft tissue metastases (e.g., n = 3). Some conditions may display patterns of a true-negative scan (specificity 75%) that may be observed in cases presented with suspicious mammographic recurrence post-lumpectomy (n = 2) or those with questionable soft tissue recurrence (n = 1). Somewhat infrequently, cases with primary breast carcinoma may have a false-negative scan or others may display false-positive scans in the presence of fibrosis

that develops following lumpectomy and/or radiation therapy. Adverse reactions to radiolabeled immunoglobulin injection are not common, but the possibility of a hypersensitivity reaction should be a consideration if serial scintigraphic images are performed over an extended period of time. Radioummunodetection (scintigraphic imaging) can be confirmed by immunohistochemistry in a high percentage of cases (e.g., n = 6/9).

Diagnosis of breast carcinoma with radiolabeled monoclonal antibodies (MAbs) to carcinoembryonic antigen (CEA) and human milk fat globulin (HMFG). Rosner D, Nabi H, Wild L, Ortman-Nabi J, Hreshchyshyn MM. Cancer Invest 1995; 13 (6), pp. 573–82.

Mammary Carcinoma/Detection and Disease Monitoring
N-(N-Benzylpiperidin-4-yl)-2-[^{18}F]-Fluorobenzamide

Cellular/Molecular Mechanism of Action: Selectively assimilated by human mammary carcinoma.
Application: Detection and characterization of human mammary carcinoma populations that express sigma receptor complexes. In this context, the agent can potentially function as a positron emission tomography (PET) imaging agent for breast cancer.
Route of Administration: N-(N-benzylpiperidin-4-yl)-2-[^{18}F]-fluorobenzamide.
Implementation: Detection of mammary carcinoma neoplastic lesions *in vivo* applying PET imaging modalities.
Animal Model: Mice (severe combined immunodeficient [SCID] mice bearing MDA-MB231 tumor and native explants of human mammary carcinoma).
Interpretation and Analysis: Investigations performed *in vivo* in SCID mice bearing MDA-MB231 tumors reveal (i) a high uptake of compound #2 in neoplastic lesions (3.8%/g); and (ii) ratios of tumor/muscle and tumor/blood of approximately 6.2 and 7.0, respectively, at 1 hour postinjection. Pretreatment of SCID mice with haldol increases the uptake of compound "2" in blood, muscle, and other well-perfused organs, while decreasing its uptake by tumors. The ratios of tumor/muscle and tumor/blood decrease from 6.2 and 7.0 to 1.3 and 1.1, respectively, at 1 hour postinjection. At 2 hours postinjection, the ratios of tumor/muscle and tumor/blood decrease from 4.9 and 7.8 to 1.4 and 1.4, respectively. The tumor uptake of compound #2 in SCID mice bearing primary tumor explants from human cases of breast cancer can be lower than observed with MDA-MB231 tumors (1.66%/g vs 3.78%/g), and the ratios of tumor/muscle and tumor/blood are 3.5 and 3.7, respectively, at 1 hour postinjection. Such findings suggest that compound "2" may be a potential ligand for (PET) imaging of breast cancer.

N-(*N*-Benzylpiperidin-4-yl)-2-[^{18}F]fluorobenzamide: A potential ligand for PET imaging of breast cancer. Shiue C, Shiue GG, Benard F, Visonneau S, Santoli D, Alavi AA. Nucl Med Biol 2000 Nov; 27 (8), pp. 763–7.

Mammary Carcinoma/Estrogen Receptor Alpha (ERα)
4-Para-Phenylvinyl Estradiol Analogues

Cellular/Molecular Mechanism of Action: Synthetic 17α-E-(4-substituted phenyl)vinyl estradiol represents a class of ligands that retain significant binding avidity for the ERα-HBD.
Application: Detection and characterization of estradiol receptor alpha (ERα-HBD) or imaging the distribution and expression density of estradiol receptor alpha complexes.
Semi-Synthetic Methods: An array of 4-*para*-substituted phenylvinyl estradiol derivatives can be fabricated using a combination of reagent formulations in combination with solid-phase Pd(0)-catalyzed methods.
Interpretation and Analysis: The compounds (e.g., 5a through 5j) are evaluated for binding affinity using the estrogen receptor alpha hormone binding domain (HDB) isolated from transfected BL21 cell lines. Although these compounds are somewhat lower in relative binding affinity (RBA at 25°C is 1–60%) than estradiol (100%), most have higher affinity than the unsubstituted parent phenylvinyl estradiol (RBA = 9%).

Because the substituents did not generate a structure-activity relationship directly based on physicochemical properties, evaluations are performed using molecular modeling and molecular dynamics to establish key interactions between the ligand (e.g., *para* substituent especially) and the protein molecule. The observed relative binding affinities are directly related to the calculated binding energies. Amino acids juxtaposed to the *para* position play a significant but not dominant role in binding. In particular, 4-substitution tends to increase receptor binding affinity compared to the unsubstituted analogue, as exemplified by product 5e (4-$COCH_3$), which has the highest RBA value (60%) of the series. Palladium(0)-catalyzes coupling reactions on solid support or in solution using suitably substituted iodo arenes and 17α-E-tributylstannylvinyl estradiols offer a flexible approach to their preparation.

Molecular modeling studies of the receptor suggest that there exists additional ligand accessible regions within the ERα-HBD to generate interactions that may enhance receptor affinity or modify efficacy in developing new therapeutic agents. Studies to undertake modification in the properties and/or position of the aryl substituents in subsequent series to further define that role are in progress.

Synthesis and evaluation of 17alpha-20E-21-(4-substituted phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17beta-diols as probes for the estrogen receptor alpha hormone binding domain. Hanson RN, Lee CY, Friel CJ, Dilis R, Hughes A, DeSombre ER. J Med Chem 2003 Jul 3; 46 (14), pp. 2865–76.

Mammary Carcinoma: Estrogen Receptor Expression
[^{99m}Technetium] ([^{99m}Tc)-N_2S_2]-Z-Aminotamoxifen/ [^{99m}Tc-N_2S_2]-E-Aminotamoxifen/[Rhenium-N_2S_2]-Z-Aminotamoxifen/[Rhenium- N_2S_2]-E-Aminotamoxifen

Cellular/Molecular Mechanism of Action: Aminotamoxifen recognizes and binds to membrane-associated estrogen receptor complexes.
Application: Imaging agent for the detection and characterization of breast cancer.
Route of Administration: Intravascular.
Semi-Synthetic Methods: The bifunctional chelator agent N_2S_2 is conjugated to Z- and E-aminotamoxifens through an amide linkage. Both [^{99m}Tc] and rhenium can be complexed with N_2S_2 employing mechanisms of simple chelation.
Implementation: Detection and characterization of mammary carcinoma lesions (*in vivo*).
Interpretation and Analysis: For the Z-isomer, chelation with rhenium in the presence of sodium acetate yields a mixture of 2 isomers, anti and syn, in a 1:1 ratio and in the presence of hydroxide results in only the anti isomer. Both the Z- and E-tamoxifen conjugates can be chelated with the [^{99m}Tc] radioisotope at the tracer level, yielding a single isomer product. Such preparations can be designated as antibase on chromatographic comparison to rhenium complexes. Radiochemical yields are consistently greater than 80%, with Sep-Pak column purification yielding a final product with > 99% radiochemical purity and no residual starting material. Both *in*

vitro and *in vivo* biological evaluation of the tamoxifen chelates indicate very limited estrogen receptor binding.

Single isomer technetium-^{99m} tamoxifen conjugates. Hunter DH, Luyt LG. Bioconjug Chem 2000 Mar–Apr; 11 (2), pp. 175–81.

Mammary Carcinoma/Estrogen Receptor Expression
[^{123}I]-Labeled Estradiol Derivatives

Background Information: Radiolabeled estrogen receptor ligands are tracers that can be used for functional receptor diagnosis. Their specificity toward receptors, together with the fact that only 50–70% of mammary carcinomas are receptor positive, renders them unsuitable for detection of primary tumors or metastases, and this means that estrogen receptor scintigraphy can be used neither for tumor screening nor for staging. However, both [^{18}F]-labeled and [^{123}I]-labeled estradiol derivatives are suitable for *in vivo* imaging of estrogen receptors.
Application: Detection and characterization of mammary carcinoma lesions that express estrogen receptor complexes.
Route of Administration: [^{123}I]-labeled estradiol derivatives (intravascular).
Interpretation and Analysis: The high specificity of both [^{18}F]-labeled and [^{123}I]-labeled estradiol derivatives *in vivo* in animal experiments and related *in vitro* studies have been reproduced *in vivo* in humans.

Tracers with positron radiation emitters are, however, hardly suitable for broad application, owing to the short half-life of [^{18}F], which means that users need to be situated close to a cyclotron and a correspondingly equipped radiochemical laboratory. The number of available positron emission tomography (PET) scanners, on the other hand, has increased over the last few years, especially in Germany, so that this, at least, does not present a limiting factor. All the same, [^{123}I]-labeled estradiol derivatives will find more widespread application, because the number of gamma cameras incorporating modern multihead systems is several times greater. The results of studies with [^{123}I]-E2 scintigraphy published to date are very promising, even given the initial technical problems just mentioned. As a method of examination, it could be enhanced by using improved tracers with a higher tumor contrast and less disturbance from overlapping in diagnostically relevant locations, for instance, by selecting tracers with higher activities whose excretion is more renal than hepatobiliary. The use of modern multihead camera systems can also be expected to improve the photon yield.

Estrogen receptor scintigraphy. Scheidhauer K, Scharl A, Schicha H. Q J Nucl Med 1998 Mar; 42 (1), pp. 26–32.

Mammary Carcinoma/*N*-Nitrosomethylurea (NMU)-Induced/Glioma: Central Nervous System
[^{99m}Technetium] ([^{99m}Tc])-T3,4BCPC/ Potential Delivery Platform

Cellular/Molecular Mechanism of Action: The exact mechanism that facilitates the selective uptake of T3,4BCPC by certain neoplastic cell types has not been precisely established.
Application: Potential application as a detectable delivery modality that selectively recognizes certain neoplastic cell types (e.g., glioma, mammary carcinoma).
Route of Administration: T3,4BCPC (intravenous).
Dosage: T3,4BCPC (5.0 mg/kg body weight; murine fibrosarcoma model).
Semi-Synthetic Methods: The new water-soluble photosensitizer 5,10,15,20-tetrakis[3,4-*bis*(carboxymethyleneoxy)-phenyl] chlorin (T3,4BCPC) has been prepared, characterized, and methods developed for radiolabeling with [^{99m}Tc] preparations.
Implementation: Determination of biodistribution profiles in tumor models in order to establish the comparative properties of the tumor-seeking radiopharmaceuticals [^{99m}Tc^{V}]-dimercaptosuccinic acid (DMSA), [^{201}Thallous]-chloride ([^{201}Tl]Cl), and [^{99m}Tc]-citrate using a gamma camera imaging systems. In the 2nd phase of evaluation, *in vivo* efficacy of the compound is determined by establishing its potential as a photodynamic therapeutic agent achieved by the conduction of a preclinical photodynamic therapy (PDT) study performed in fibrosarcoma-bearing mice (postinjection of T3,4BCPC).
Animal Model: Murine (C_3H/J virus-induced spontaneous mammary tumors).
Animal Model: Murine (fibrosarcoma-bearing).
Animal Model: Rats (Wistar strain).
Animal Model: Rat (NMU-induced mammary tumors).
Animal Model: Rat (transplanted C_6-glioma).
Interpretation and Analysis: Accumulation of activities within the liver, kidney, bladder, and large intestine at 4 hours postinjection indicates that the labeled ligand is largely eliminated through renal excretion and partly through the hepatobiliary system. In NMU-induced rat mammary tumors, the labeled ligand shows a 5-fold tumor-to-muscle (T/M) ratio compared to [^{99m}Tc^{V}]-DMSA (3-fold) and [^{201}Tl]Cl (3-fold). In the case of C_3H/J virus-induced spontaneous mammary tumors, differences are not marked. However, in transplanted rat C_6-glioma, the T/M ratio of the labeled compound is appreciably higher (4-fold) than that noted with [^{99m}Tc^{V}]-DMSA (2-fold), [^{201}Tl]Cl (3-fold), and [^{99m}Tc]-citrate (more than 3-fold). Therefore, radiolabeled T3,4BCPC may have potential utility for detecting neoplastic lesions. Photodynamic therapy applications of T3,4BCPC in fibrosarcoma-bearing mice evaluated following delivery of a laser dose of 20 mW for 60 seconds reveals an 80% destruction of tumor mass. The molecule may therefore be useful for PDT of cancer. The labeled agent could also be useful in monitoring the progression/regression of tumors before, during, and after chemotherapy, radiation therapy, or PDT.

Preparation and biological evaluation of the new chlorin photosensitizer T3,4BCPC for detection and treatment of tumors. Murugesan S, Shetty SJ, Srivastava TS, Samuel AM, Noronha OP. J Photochem Photobiol B 2002 Aug; 68 (1), pp. 33–8.

Mammary Carcinoma/Vasoactive Intestinal Peptide Receptor (VIP-R) Complexes
[^{99m}Technetium] ([^{99m}Tc])-Hexamethylpropyleneamine Oxime (HMPAO)-[SSL-VIP] Liposomes

Cellular/Molecular Mechanism of Action: Vasoactive intestinal peptide recognizes and physically binds to its respective receptor complex.
Background Information: Targeted delivery of radionuclides and therapeutic agents to specific biomarkers of breast cancer has important implications for the diagnosis and therapy of breast cancer. Vasoactive intestinal peptide receptors have approximately a greater expression density in human breast cancer compared to normal breast tissue.
Semi-Synthetic Methods: Incorporation of VIP (28 amino acid mammalian neuropeptide), as a breast cancer targeting moiety for selectively imaging of breast cancer. Vasoactive intestinal peptide is covalently attached to the surface of sterically stabilized liposomes (SSL) containing encapsulated [^{99m}Tc]-HMPAO radionuclide.

Implementation: Evaluation of liposome preparations as a potential delivery platform/imaging agent for mammary carcinoma. Complementary investigations can delineate the pharmacokinetics and biodistribution of [^{99m}Tc]-HMPAO encapsulating SSL with and without VIP.

Animal Model: Rats with *n*-methyl nitrosourea (MNU)-induced *in situ* breast cancers.

Interpretation and Analysis: The presence of VIP does not alter the size, and [^{99m}Tc]-HMPAO encapsulation ability of SSL. It also does not alter the pharmacokinetic profile of SSL. Long-circulating liposomes with and without VIP on their surface accumulate at significantly higher quantities in breast cancer compared to normal breast, indicating passive targeting of these constructs to cancer tissues. Importantly, in breast cancer, [^{99m}Tc]-HMPAO encapsulating SSL with VIP shows significantly more accumulation than SSL without VIP. The tumor-to-non-tumor ratio is also significantly higher for [^{99m}Tc]-HMPAO encapsulating VIP-SSL than [^{99m}Tc]-HMPAO encapsulating SSL without VIP, suggesting active targeting of VIP-SSL to breast cancer. Collectively, these data showed that [^{99m}Tc]-HMPAO encapsulating VIP-SSL can be successfully used for targeted imaging of breast cancer lesions.

VIP grafted sterically stabilized liposomes for targeted imaging of breast cancer: *In vivo* studies. Dagar S, Krishnadas A, Rubinstein I, Blend MJ, Onyuksel H. J Control Release 2003 Aug 28; 91 (1–2), pp. 123–33.

Mammary Carcinoma/Ineffective Diagnostic Imaging Agent for Neoplasia
[^{99m}Technetium] ([^{99m}Tc])-Medronate/[Methylene Diphosphate]

Background Information: Breast scintigraphy (BS) with the bone-seeking agent [^{99m}Tc]-medronate (MDP) can be usefully combined with mammography to diagnose and characterize questionable breast lumps. However, this radiotracer does not seem to provide any further prognostic information about breast cancer.

Application: Detection and characterization of mammary carcinoma lesions.

Implementation: Delineate the prognostic yield of MDP-BS in an effort to search for correlations that exist between scintigraphic findings and major biological and histologic parameters. Cases of mammary carcinoma are subjected to scintigraphic imaging with [^{99m}Tc]-MDP bone scan for the purpose of preoperative staging, as well as conventional breast imaging. Cancer/background ratio (c/b index) with lesion histotype, diameter, grading, and the tissue concentrations of steroid receptors, cathepsin D, Type 1 tymidine kinase, pS2, and p53 proteins are then statistically compared.

Interpretation and Analysis: The agent MDP-BS fails to detect a rather large percentage of mammary neoplastic lesions in the small to medium size range (n = 11/44 false-negative with a size range = 0.5–2.0 cm). Similary, the agents detected a relatively small percentage of mammographically questional lesions (e.g., n = 7/16), but can correctly visualize some multifocal cases (e.g., n = 2). Unfortunately, MDP-BS depicts no metastatic axillary lymph nodes. There is no statistically significant correlation between the c/b index and prognostic markers.

Different from evaluating BS with [^{99m}Tc]-methoxy-isobutyl-isonitrile, [^{201}T1], [^{18}F]-FDG, [^{111}In]-OCT, and radiolabeled estrogens, MDP-BS (despite its good overall accuracy) appears to have no prognostic role. In fact, despite the well-known capability of soft tissue lesions to take up the tracer, MDP tumor trapping seems to depend mainly on the increased permeability of neovessels and on interstitial space enlargement. Few reports are available in the literature about correlations between *in vivo* MDP uptake by the breast cancer and prognostic parameters. The lack of any statistical significance is in agreement with the theory and confirms the little prognostic value of MDP-BS. Nevertheless, further trials are warranted on larger series of cases to validate the referenced study's findings.

Human Model: Cases of primary breast cancer (n = 44).

The prognostic significance of [^{99m}Tc]-MDP breast scintigraphy. A comparison with histological and molecular parameters. Dimonte M, Leo G, Marsigliante S, Manca C. Radiol Med (Torino) 1999 Nov; 98 (5), pp. 352–5.

Melanoma
[^{99m}Technetium] ([^{99m}Tc])Oxotechnetium^{V}-Complexes of Amine-Amide-Dithiol (AADT)

Cellular/Molecular Mechanism of Action: [^{99m}Tc]Oxotechnetium(V) complexes of AADT chelates containing tertiary amine substituents possess binding affinity for melanoma cell types.

Application: Diagnostic imaging agent for the non-invasive *in vivo* detection of melanoma and associated metastases.

Route of Administration: Intravenous.

Semi-Synthetic Methods: Complexation is achieved by mixing the AADT-$CH_2[CH_2]$nNR2 (n = 1, 2; R = Et, n-Bu) ligand with a [^{99m}Tc]oxotechnetium^{V}-glucoheptonate precursor to make AADT-[^{99m}Tc]oxotechnetium^{V} complexes in nearly quantitative yield.

Implementation: Evaluation of the binding affinity of [^{99m}Tc] oxotechnetium^{V}-complexes of amine-amide-dithiol for sigma receptor complexes.

Interpretation and Analysis: Evaluation of the binding affinity for sigma receptor complexes reveals that preparations possess values in the low μM range with K_i values in the 7.8–26.1 and 0.18–2.3 μM range for $sigma_1$ and $sigma_2$ receptors, respectively. Cellular *in vitro* uptake of [^{99m}Tc] complexes by intact B16 murine melanoma cells at 37°C after a 60-minute incubation varies between 12% for complex 2 (n = 1, R = n-Bu) to 68% for complex 4 (n = 2, R = n-Bu). Complexes 1-Tc–4-Tc *in vitro* in the C57Bl/B16 mouse melanoma model significantly localize within tumor lesions. Complex 1-Tc (n = 1, R = Et) provide *in vivo* tumor uptake of 7.6 percent injected dose per gram (%ID/g) at 1 hour postadministration with values of > 4 that can be observed for initial melanoma/blood (M/B), melanoma/spleen (M/S), and melanoma/lung (M/L) ratios. At a time period of 6 hours postadministration, the observed measurements for these same ratios substantially increase (e.g., 10.8, 10.1, 7.3, respectively). While complex 3-Tc (n = 3, R = Et) can have an initial tumor uptake level of 3.7 %ID/g at 1 hour postadministration (M/B, M/S, M/L ratios > 2), a greater tumor retention and slightly faster clearance from non-tumor-containing organs results in 6-hour M/B, M/S, and M/L ratios of 19.1, 19.1, and 12.7, respectively.

[^{99m}Tc]oxotechnetium(V) complexes amine-amide-dithiol chelates with dialkylaminoalkyl substituents as potential diagnostic probes for malignant melanoma. Friebe M, Mahmood A, Bolzati C, Drews A, Johannsen B, Eisenhut M, Kraemer D, Davison A, Jones AG. J Med Chem 2001 Sep 13; 44 (19), pp. 3132–40.

Melanoma/Melanocyte-Stimulating Hormone (MSH) Receptor
[^{111}Indium] ([^{111}In])-DOTA-ReCCMSH/[^{111}In]-DOTA-CCMSH/[^{111}In]-DOTA-CMSH/[^{111}In]-DOTA-NDP

Cellular/Molecular Mechanism of Action: Possesses binding avidity for MSH receptor complexes.

Application: Detection and characterization of neoplastic cell types (melanoma) that express MSH receptor complexes.

Semi-Synthetic Methods: Production of (i) DOTA-ReC-CMSH; (ii) its reduced non-metalated linear homolog, DOTA-CCMSH; and (iii) an analogue in which rhenium cyclization is replaced by disulfide bond cyclization → DOTA-[$Cys_{4,10}$,D-Phe_7]α-MSH_{4-13} (CMSH). DOTA can also be conjugated to the amino terminus of 1 of the highest-affinity α-MSH receptor-binding peptides, [Nle_4,D-Phe_7]α-MSH (NDP), as a linear peptide standard. The DOTA-conjugated α-MSH analogues can subsequently be [^{111}In] radiolabeled.

Implementation: Evaluation of the effect of rhenium-mediated peptide cyclization on melanoma targeting, biodistribution, and clearance kinetics of the α-MSH analogue 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) coupled ReO-cyclized [$Cys_{3,4,10}$,D-Phe_7]α-MSH_{3-13} (DOTA-ReCCMSH). One approach to achieving such an objective can entail assessment of the *in vitro* receptor-binding affinity of (i) [^{111}In]-DOTA-ReCCMSH; (ii) [^{111}In]-DOTA-CCMSH; (iii) [^{111}In]-DOTA-[$Cys_{4,10}$,D-Phe_7]α-MSH_{4-13} (CMSH); and (iv) [^{111}In]-DOTA [Nle_4,D-Phe_7]α-MSH (NDP) for B16/F1 murine melanoma cell types in addition to determing their relative *in vivo* biodistribution properties in tumor-bearing and non-tumor-bearing mice.

Animal Model: Murine (C57 and melanoma tumor-bearing C57 mice).

Interpretation and Analysis: The tumor uptake values of [^{111}In]-DOTA-ReCCMSH are significantly higher than those of the other closely related [^{111}In]-DOTA-α-MSH conjugates. Even at 24 hours after injection, a comparison of the tumor uptake values for [^{111}In]-DOTA-coupled ReCCMSH (4.86 ± 1.52 percent injected dose [%ID]/g), CCMSH (1.91 ± 0.56 %ID/g), CMSH (3.09 ± 0.32 %ID/g), and NDP (2.47 ± 0.79 %ID/g) highlight the high tumor retention property of ReCCMSH. Rhenium-coordinated cyclization results in less renal radioactivity accumulation of [^{111}In]-DOTA-ReCCMSH (8.98 ± 0.82 %ID/g) than of [^{111}In]-DOTA-CCMSH (63.2 ± 15.6 %ID/g), [^{111}In]-DOTA-CMSH (38.4 ± 3.6 %ID/g), and [^{111}In]-DOTA-NDP (12.0 ± 1.96 %ID/g) at 2 hours after injection and its urinary clearance significantly increases (92 %ID at 2 hours postinjection). A high radioactivity uptake ratio of tumor-to-normal tissue can be observed for [^{111}In]-DOTA-ReCCMSH (e.g., 489, 159, 100, and 49 for blood, muscle, lung, and liver, respectively, at 4 hours after injection). The novel ReO-coordinated cyclic structure of DOTA-ReCCMSH contributes significantly to its enhanced tumor-targeting and renal clearance properties. Such characteristics make DOTA-ReCCMSH an excellent candidate for melanoma radiodetection and radiotherapy.

Evaluation of a [^{111}In]-DOTA-rhenium cyclized α-MSH analog: A novel cyclic-peptide analogue with improved tumor-targeting properties. Chen J, Cheng Z, Owen NK, Hoffman TJ, Miao Y, Jurisson SS, Quinn TP. J Nucl Med 2001 Dec; 42 (12), pp. 1847–55.

Melanoma/Melanocyte-Stimulating Hormone (MSH) Receptor Complexes
[^{111}Indium] ([^{111}In])-DOTA-MSH(OCT)/[^{111}In]-DOTA-NDP-MSH

Cellular/Molecular Mechanism of Action: The octapeptide [βAla$_3$, Nle_4, Asp_5, D-Phe_7, Lys_{10}]-α-MSH_{3-10} referred to as MSH (OCT) is an analogue of α-MSH.

Background Information: Scintigraphic imaging of metastatic melanoma lesions requires highly tumor-specific radiopharmaceuticals. Because both melanotic and amelanotic melanomas overexpress melanocortin-1 receptors (MC1R), radiolabeled analogues of α-MSH are potential candidates for development as melanoma diagnostic imaging agents.

Application: Detection and characterization of malignant melanoma lesions.

Route of Administration: [^{111}In]-DOTA-MSH(OCT) and [^{111}In]-DOTA-NDP-MSH (intravenous).

Semi-Synthetic Methods: Molecular design of the octapeptide analogue MSH(OCT) utilizes conjugation reactions involving its *N*-terminal amino group to the metal chelator in the form of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) to enable incorporation of radiometals (e.g., [^{111}In]) into the peptide.

Implementation: Evaluation of the *in vitro* ligand binding avidity of non-radioactive DOTA-MSH(OCT) for MC1R. Complementary investigations can involve determining the *in vivo* biodistribution of [^{111}In]-DOTA-MSH(OCT) in normal reference control and tumor-bearing models.

Animal Model: Murine (B16-F1 melanoma tumor-bearing mice).

Interpretation and Analysis: The non-radioactive ligand DOTA-MSH(OCT) possesses a relatively high level of *in vitro* binding avidity for MC1R complexes (IC_{50} 9.21 nM). *In vivo* [^{111}In]-DOTA-MSH(OCT) exhibits a favorable biodistribution profile after injection into tumor-bearing *in vivo* models. The radiopeptide is rapidly cleared from blood through the kidneys and, most importantly, accumulates preferentially within melanoma lesions. Lung and liver melanoma metastases can be clearly imaged on tissue section autoradiographs 4 hours after injection of [^{111}In]-DOTA-MSH(OCT). A comparative study of [^{111}In]-DOTA-MSH(OCT) with [^{111}In]-DOTA-[Nle_4, D-Phe_7]-α-MSH ([^{111}In]-DOTA-NDP-MSH) demonstrates that DOTA-MSH(OCT) peptide is a superior ligand reagent, particularly with regard to the amount of radioactivity taken up by non-malignant organs, including bone, the most radiosensitive tissue. Therefore, [^{111}In]-DOTA-MSH(OCT) is a promising melanoma imaging agent.

DOTA α-melanocyte-stimulating hormone analogues for imaging metastatic melanoma lesions. Froidevaux S, Calame-Christe M, Sumanovski L, Tanner H, Eberle AN. Ann N Y Acad Sci 2003 Jun; 994, pp. 378–83.

Melanoma/Melanocyte Stimulating Hormone (MSH) Receptor Expression/Non-Specific Renal Uptake Inhibition Strategies
Melanocyte-Stimulating Hormone (MSH) Analogues/[^{99m}Technetium] ([^{99m}Tc])-CCMSH/Lys_{11} Replacement/Lysine Co-Administration

Cellular/Molecular Mechanism of Action: Melanocyte-stimulating hormone and related analogues physically bind to receptor complexes associated with the exterior surface membrane of melanoma cell types.

Physiological Mechanism of Action: Preliminary reports have demonstrated that [^{99m}Tc]-labeled cyclic [$Cys_{3,4,10}$, D-Phe_7]α-MSH_{3-13} (CCMSH) exhibits high tumor uptake and retention values in a murine melanoma tumor model.

Application: Detection and characterization of primary or metastatic melanoma lesions.

Route of Administration: Intravascular.

Implementation: Evaluation of the tumor targeting capacity of [^{99m}Tc]-CCMSH for comparision with radiolabeled α-MSH peptide analogues including [^{125}I]-(Tyr_2)-[Nle_4, D-Phe_7]α-MSH [^{125}I]-(Tyr_2)-NDP]; [^{99m}Tc]-CGCG-NDP; [^{99m}Tc]-Gly_{11}-CCMSH; and [^{99m}Tc]-Nle_{11}-CCMSH. Initial delineation of *in vitro* receptor binding, internalization, and cellular retention of radiolabeled α-MSH analogues can be performed using B16/F1 murine cell lines. Further characterization of the *in vivo* properties of these radiobiopharmaceutical agents can be determined in mouse melanoma models.

Complementary investigations can be performed to establish molecular strategies for reducing non-specific renal uptake of imaging agents. Examples of 2 strategies that can be employed to decrease non-specific kidney uptake of [^{99m}Tc]-CCMSH include (i) substitution of Lys_{11} in CCMSH with Gly_{11} or Nle_{11}; and (ii) lysine co-injection.

Animal Model: Murine (C57 strain mice and severe combined immunodeficient [SCID] mice, inoculated subcutaneously with B16/F1 murine and TXM-13 human melanoma cell populations).

Interpretation and Analysis: The *in vitro* receptor binding, internalization, and cellular retention of radiolabeled α-MSH analogues in B16/F1 murine cell line reveals that > 70% of the receptor-bound radiolabeled analogues are internalized together with the receptor. Ninety percent of the internalized [^{125}I]-(Tyr_2)-NDP, whereas only 36% of internalized [^{99m}Tc]-CCMSH, is released from the cells into the medium during a 4-hour incubation period (37°C).

In an *in vivo* environment, tumor uptake values of 11.32 and 2.39 [percent injected dose (%ID)/g] can be observed for [^{99m}Tc]-CCMSH at 4 hours post-injection, resulting in tumor:blood uptake ratios of approximately 39.0 and 11.5 in murine melanoma-C57 and human melanoma-SCID mouse models, respectively. Biodistribution data for the modified peptides reveals that Lys_{11} replacement dramatically decreases kidney uptake, whereas the tumor uptakes of [^{99m}Tc]-Nle_{11}- and [^{99m}Tc]-Gly_{11}-CCMSH are significantly lower than that of [^{99m}Tc]-CCMSH. Lysine coinjection significantly decreases kidney uptake (e.g., from 14.6 %ID/g to 4.5 %ID/g at 4 hours after injection in murine melanoma-C57 mice) without creating significant changes in value for tumor uptake of [^{99m}Tc]-CCMSH. Therefore, the compact cyclic structure of [^{99m}Tc]-CCMSH, its resistance to degradation, and its enhanced intracellular retention are the major contributing factors to the superior *in vivo* tumor targeting properties of [^{99m}Tc]-CCMSH. The Lys_{11} residue in [^{99m}Tc]-CCMSH is critical to *in vivo* tumor targeting, and lysine co-injection rather than lysine replacement can significantly decrease non-specific renal radioactivity accumulation without impeding the high melanoma-targeting properties of [^{99m}Tc]-CCMSH. The metal-cyclized CCMSH molecule displays excellent potential for development as a melanoma-specific diagnostic and therapeutic agent.

Melanoma-targeting properties of [^{99m}technetium]-labeled cyclic α-melanocyte-stimulating hormone peptide analogues. Chen J, Cheng Z, Hoffman TJ, Jurisson SS, Quinn TP. Cancer Res 2000 Oct 15; 60 (20), pp. 5649–58.

Melanoma (Metastatic)/Melanocyte-Stimulating Hormone (MSH) Receptor Expression *[^{111}Indium] ([^{111}In])-DOTA-NDP-MSH/ [^{111}In]-DOTA-MSH(OCT)*

Cellular/Molecular Mechanism of Action: The peptide [Nle_4, D-Phe_7]-α-MSH is a very potent α-MSH analogue that recognizes and physically binds to MSH receptor complexes. Similarly, [βAla_3, Nle_4, Asp_5, D-Phe_7, Lys_{10}]-α-MSH_{3-10} is an α-MSH octapeptide analog.

Application: Scintigraphic imaging of metastatic melanoma lesions requires highly tumor-specific radiolabeled compounds. Because both melanotic and amelanotic melanomas overexpress receptors for α-MSH (receptor name: melanocortin Type 1 receptor, or MC1R), radiolabeled α-MSH analogues are potential candidates for development as diagnostic imaging agents for melanoma conditions.

Route of Administration: [^{111}In]-DOTA-NDP-MSH and [^{111}In]-DOTA-MSH(OCT) are presumably administered intravenously.

Semi-Synthetic Methods: Covalent conjugation of [Nle_4, D-Phe_7]-α-MSH (NDP-MSH) and a newly designed α-MSH octapeptide analog, [βAla_3, Nle_4, Asp_5, D-Phe_7, Lys_{10}]-α-MSH_{3-10} (MSH(OCT)) to the metal chelator 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), thereby enabling radiometal incorporation into the complex.

Implementation: Evaluation of the (i) potential *in vitro* binding avidity of [^{111}In]-DOTA-NDP-MSH and [^{111}In]-DOTA-MSH(OCT) for MC1R; (ii) melanogenic activity of [^{111}In]-DOTA-NDP-MSH and [^{111}In]-DOTA-MSH(OCT) in isolated mouse B16F1 cells; and (iii) *in vivo* biodistribution of the hormone receptor ligands in mouse models of primary and metastatic melanoma.

Animal Model: Murine (melanoma B16F1-bearing mice).

Interpretation and Analysis: DOTA-MSH(OCT) binds with high affinity (inhibitory concentration of 50% IC_{50} = 9.21 nmol/L) to the MC1R, although with a lower potency than does DOTA-NDP-MSH (IC_{50} = 0.25 nmol/L). In B16F1 melanoma-bearing mice, both [^{111}In]-DOTA-NDP-MSH and [^{111}In]-DOTA-MSH(OCT) exhibit high MC1R-mediated uptake by melanoma, which differs by a factor of only 1.5 at 4 hours postadministration. The main route of excretion for both radioconjugates is the kidneys, whereby [^{111}In]-DOTA-MSH(OCT) produces somewhat higher kidney values than does [^{111}In]-DOTA-NDP-MSH. Conversely, the latter conjugate is much more poorly cleared from other non-malignant tissues, including bone, the most radiosensitive organ. Therefore, [^{111}In]-DOTA-MSH(OCT) displays higher uptake ratios of tumor-to-non-target tissue (e.g., tumor-to-bone ratio at 4 hours post-administration is approximately 4.9 for [^{111}In]-DOTA-NDP-MSH and 53.9 for [^{111}In]-DOTA-MSH(OCT)). Lung and liver melanoma metastases can easily be visualized on tissue section autoradiographs after injection of [^{111}In]-DOTA-MSH(OCT). Radio-reversed-phase high-performance liquid chromatography analysis of urine samples reveals that most [^{111}In]-DOTA-MSH(OCT) is excreted intact 4 hours after injection, indicating good *in vivo* stability. Therefore, [^{111}In]-DOTA-MSH(OCT) appears to exhibit more favorable overall performance than does [^{111}In]-DOTA-NDP-MSH in murine models of primary and metastatic melanoma, making it a promising melanoma imaging agent.

A novel DOTA-α-melanocyte-stimulating hormone analogue for metastatic melanoma diagnosis. Froidevaux S, Calame-Christe M, Tanner H, Sumanovski L, Eberle AN. J Nucl Med. 2002 Dec; 43 (12), pp. 1699–706.

Metastatic Neoplastic Disease/Carcinoma Metastasis to Bone/Recurrent Tumor and Postradiotherapy Change/Paget's Disease *[^{111}Indium] ([^{111}In])-Labeled Bleomycin*

Cellular/Molecular Mechanism of Action: Preferential uptake by cancer cells, including neoplastic conditions located within the thorax, abdomen, or pelvis.

Route of Administration: Intravenous.

Dosage: [^{111}In] (2 mCi) chelated to 2 mg bleomycin.

Considerations: The optimum time to perform scintigraphy examinations is 72 hours after intravenous injection.

Implementation: Establish the optimum time to visualize tumor [^{111}In]-labeled bleomycin uptake.

Interpretation and Analysis: A relatively high percentage of the injected dose of [^{111}In]-labeled bleomycin accumulates within tumors to the extent that it is sufficient for acquiring positive visual images (e.g., n = 53/62 cancer cases). Extent of tumor involvement can occasionally be underestimated (n = 7/62) or overestimated (n = 5/62; usually with 2° infection). Related investigations have suggested that indium provides the most clinically relevant information

when distinguishing between recurrent tumor and postradiotherapy changes in the thorax and pelvis, the diagnosis of recurrent carcinoma within the pelvis, and the distinction between bony metastases from carcinoma of the prostate and Paget's disease. Further clinical trials are necessary to assess these situations.

Indium 111-A-bleomycin—A new tracer for imaging orofacial neoplasms. Kment G, Matejka M, Porteder H, Sinzinger H. J Oral Maxillofac Surg 1988 Sep; 46 (9), pp. 742–5.

Kinetics of [^{111}In]-labeled bleomycin in patients with brain tumors: Compartmental vs non-compartmental models. Ryynänen PM, Savolainen SE, Aronen HJ, Korppi-Tommola ET, Huhmar HM, Kallio ME, Hiltunen JV. Ann Nucl Med 1998 Dec; 12 (6), pp. 313–21.

Multidrug-Resistance-1 (MDR1) Phenotype *[^{99m}Technetium] ([^{99m}Tc])-SestaMIBI/XR9576/ Vinorelbine (Navelbine)*

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-sestaMIBI is a substrate of the multidrug transporter complex P-glycoprotein (Pgp).
Background Information: *In vitro*, retention of [^{99m}Tc]-sestaMIBI by cells that overexpress Pgp can be enhanced by the addition of Pgp inhibitors. XR9576 (Tariquidar) is a potent and selective non-competitive inhibitor of Pgp that is active at 25–80 nM.
Application: Functional imaging agent for the MDR1 neoplastic cell phenotype.
Implementation: A phase I trial employing XR9576 in combination with vinorelbine (Navelbine) to assess conditions of metastatic cancer using a [^{99m}Tc]-sestaMIBI scan (baseline and at 48–96 hours later by a 2nd scan 1–3 hours after the administration of XR9576). Time activity curves and areas-under-the-curves (AUCs) are obtained for tumor, liver, lung, and heart, and tissue:heart AUC ratios are calculated.

Human Model: Phase I trial (n = 26 metastatic neoplasia cases).
Interpretation and Analysis: XR9576 enhances [^{99m}Tc]-sestaMIBI accumulation and retention in the liver of all but 2 cases with a mean change of +128%. Furthermore, in cases with tumor masses visible with [^{99m}Tc]-sestaMIBI (e.g., n = 13/17), the tumor:heart [^{99m}Tc]-sestaMIBI AUC (0–3 hours) increases after XR9576 administration, with increases of 36 to 263% detected in certain instances (e.g., n = 8 cases). Therefore, the *in vivo* administration of XR9576 inhibits [^{99m}Tc]-sestaMIBI efflux in both the normal liver and in drug-resistant tumors. Such findings provide convincing evidence of the existence of XR9576-inhibitable [^{99m}Tc]-sestaMIBI efflux in a large fraction of drug-resistant tumors. One can predict that efflux of Pgp substrates also occurs in these tumors. XR9576 provides an efficient way to inhibit this efflux and offers the potential to increase drug exposure in human cancer.

Increased [^{99m}Tc]-sestaMIBI accumulation in normal liver and drug-resistant tumors after the administration of the glycoprotein inhibitor XR9576. Agrawal M, Abraham J, Balis FM, Edgerly M, Stein WD, Bates S, Fojo T, Chen CC. Clin Cancer Res 2003 Feb; 9 (2), pp. 650–6.

Multidrug-Resistance-Associated Protein (MRP)/ [Leukemia] *[^{99m}Technetium] ([^{99m}Tc])-Tetrofosmin ([^{99m}Tc]-TF)/ Verapamil*

Cellular/Molecular Mechanism of Action: Tetrofosmin is a substrate molecule for MRP expressed by certain neoplastic cell types.
Application: Detection and characterization of neoplastic cell types (e.g., leukemia) that possess resistance to chemotherapeutic intervention due to the expression of MRP. Such knowledge can provide prognostic information and the basis for the design of alternative strategies for therapeutic intervention.
Route of Administration: [^{99m}Tc]-TF (intravenous).
Implementation: Evaluation of the kinetics of cellular accumulation and retention of [^{99m}Tc]-TF in leukemia wild-type cell lines and its corresponding chemotherapeutic-resistant cell line to determine if [^{99m}Tc]-TF is a substrate for MRP with and without the presence of verapamil.

Tissue Culture Model: Leukemia wild-type HL60/WT cell line and a corresponding doxorubicin-resistant HL60/DOX cell line that expresses the MRP but without P-glycoprotein (Pgp) overexpression (37°C).
Interpretation and Analysis: [^{99m}Tc]-TF net accumulation is significantly lower in HL60/DOX (1.35 ± 0.23%) than in HL60/WT (12.79 ± 0.47%) at 60 minutes ($p < 0.001$). Three minutes after exchanging the incubation solution with the tracer-free medium, only 18.20 ± 0.34% of [^{99m}Tc]-TF remains in HL60/DOX, whereas 84.74 ± 0.65% is retained in HL60/WT ($p < 0.001$). In the presence of 10 μM verapamil, [^{99m}Tc]-TF net accumulation in HL60/DOX is 302% of the control and washout is significantly delayed. [^{99m}Tc]-TF would be a substrate for MRP and [^{99m}Tc]-TF may be used as a functional *in vivo* imaging agent of MRP.

Technetium-^{99m}-tetrofosmin would be a substrate for multidrug-resistance-associated protein (MRP): Comparison between a leukemia cell line with high MRP gene expression and its parental cell line. Li XF, Kinuya S, Yokoyama K, Konishi S, Ma YY, Watanabe N, Shuke N, Bunko H, Michigishi T, Tonami N. Cancer Biother Radiopharm 2001 Feb; 16 (1), pp. 17–23.

Multidrug-Resistance (MDR) Protein/Predicted Chemotherapeutic Response Rate/Osteosarcoma *[^{99m}Technetium] ([^{99m}Tc])-Methoxy-Isobutyl-Isonitrile (MIBI)/[^{99m}Tc]-MIBI Washout Rate*

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-MIBI is a pharmaceutical substrate transported by the MDR-related proteins.
Background Information: *In vitro* studies have demonstrated that [^{99m}Tc]-MIBI is a transport substrate of MDR-related proteins. Minimal knowledge is available about the potential of [^{99m}Tc]-MIBI scintigraphy as a functional imaging tool for the *in vivo* detection of MDR-associated protein (MRP) expression in osteosarcoma. Little research has been devoted to characterizing the potential role of MRP and [^{99m}Tc]-MIBI imaging in schemes for the prediction of clinical outcome.
Application: Detection and characterization of MDR protein expression in neoplastic tissues (e.g., osteosarcoma) in a manner that can assist in predicting response to therapeutic intervention.
Implementation: Evaluation of osteosarcoma conditions before and after initiation of chemotherapy. Tumor-to-background ratios of both early (10 minutes) and delayed (1 hour) images and the percentage washout rate (WR%) of [^{99m}Tc]-MIBI are calculated. Immunohistochemical analysis of MRP and P-glycoprotein (Pgp) can be performed on biopsy specimens, and the response to preoperative chemotherapy assessed by histopathologic examination. In this manner, it will be possible to examine if scintigraphic parameters are of any benefit in distinguishing the functional capacity of Pgp and MRP.

Human Model: Cases of osteosarcoma (n = 24).
Interpretation and Analysis: Approximately 62.5% of osteosarcomas display significant MRP expression levels (e.g., n = 15/24).

The level of MRP expression significantly correlates with WR% of [^{99m}Tc]-MIBI ($r = 0.58$, $p = 0.003$), and the WR% of [^{99m}Tc]-MIBI is significantly faster in cases with higher MRP expression than in those with a low MRP score ($p = 0.007$). The clearance rate of [^{99m}Tc]-MIBI is significantly slower in tumor samples with negative or low expression of both Pgp and multidrug-resistance protein ($16\% \pm 6.2\%$) when compared with osteosarcomas that express high levels of both proteins ($31.7\% \pm 8.7\%$) ($p = 0.001$). There appears not to be a significant difference between the WR% of [^{99m}Tc]-MIBI in tumors with co-expression of both proteins and in tumors with high expression of either Pgp or MRP. Both the rate of MRP expression and the WR% of [^{99m}Tc]-MIBI significantly correlate with response rate. Therefore, the WR% of [^{99m}Tc]-MIBI correlates with MRP expression levels. Both the WR% of [^{99m}Tc]-MIBI and MRP expression correlate with response to therapeutic intervention. [^{99m}Tc]-MIBI can therefore be used as a general probe for functional imaging of both Pgp and MRP; however, it is not capable of differentiating the functional status of either MDR-related glycoprotein.

[^{99m}Tc]-MIBI imaging as a predictor of therapy response in osteosarcoma compared with multidrug-resistance-associated protein and P-glycoprotein expression. Burak Z, Moretti JL, Ersoy O, Sanli U, Kantar M, Tamgac F, Basdemir G. J Nucl Med 2003 Sep; 44 (9), pp. 1394–401.

Multidrug-Resistance (MDR) Protein/ P-Glycoprotein (Pgp) Transport Activity/ [Mammary Carcinoma]/[Lung Tumor]
[^{99m}Technetium] ([^{99m}Tc])$(CO)_3$(Methoxy-Isobutyl-Isonitrile [MIBI])$_3$] + (Tc-CO-MIBI)

Cellular/Molecular Mechanism of Action: [^{99m}Tc$(CO)_3$ $(MIBI)_3$] + (Tc-CO-MIBI) can function as a transport substrate for MDR protein 1 (Pgp).

Background Information: Multidrug resistance mediated by the overexpression of MDR1 Pgp is 1 of the best characterized obstacles to successful chemotherapy in conditions of neoplastic disease. Furthermore, the protective function of Pgp-mediated efflux of xenobiotics in various organs has a profound effect on the bioavailability of drugs in general. Thus, there is an expanding requirement to non-invasively interrogate Pgp transport activity *in vivo*.

Application: Detection and characterization of MDR protein/Pgp transport function and genetic expression. Secondary applicability includes the capacity to facilitate evaluation of the efficacy of inhibitors of MDR protein/Pgp transport function and genetic expression.

Implementation: Evaluation of the recognition properties (binding avidity) of [^{99m}Tc](I)-tricarbonyl complexes in the form of [^{99m}Tc$(CO)_3(MIBI)_3$]+ (Tc-CO-MIBI) for Pgp.

***Ex Vivo* Model:** Drug-sensitive KB 3-1 cell lines; colchicine-selected drug-resistant KB 8-5 cell types; chemotherapeutic-sensitive MCF-7 mammary carcinoma cell lines; H69 lung tumor cell lines.

Animal Model: Murine (MDR1a/1b(−/−)-gene-deleted mice and wild-type mice.

Interpretation and Analysis: Tc-CO-MIBI has a 60-fold higher accumulation in drug-sensitive KB 3-1 cells compared to colchicine-selected drug-resistant KB 8-5 cells. In KB 8-5 cells, tracer enhancement can be achieved with the potent MDR modulator LY335979 (EC50 = 62 nM). Similar behavior can be observed using drug-sensitive MCF-7 breast adenocarcinoma cells and MCF-7/MDR1 stable transfectants, thereby confirming that Tc-CO-MIBI is specifically excluded by MDR1 Pgp overexpression. By comparison, net accumulation in control H69 lung tumor cells is 9-fold higher than in MDR-associated protein (MRP1)-expressing H69AR cells, indicating only modest transport by MRP1. Biodistribution analysis following tail vein injection of Tc-CO-MIBI shows delayed liver clearance as well as enhanced brain uptake and retention in MDR-gene-deleted mice vs wild-type mice, directly demonstrating that Tc-CO-MIBI is a functional *in vivo* probe for detecting Pgp transport activity.

Characterization of a novel [^{99m}Tc]-carbonyl complex as a functional probe of MDR1 P-glycoprotein transport activity. Dyszlewski M, Blake HM, Dahlheimer JL, Pica CM, Piwnica-Worms D. Mol Imaging 2002 Jan–Mar; 1 (1), pp. 24–35.

Neoplastic Disease
Cobalt-57-Bleomycin ([^{57}Co]-BLM)

Cellular/Molecular Mechanism of Action: Isomerism of bleomycin affects its affinity for neoplastic cell types. Bleomycin Type I isomer is superior with respect to this particular property.

Background Information: [^{57}Co]-BLM has proven to be the most stable and useful tumor-diagnostic agent among several radiolabeled BLMs. However, the considerably long half-life of [^{57}Co] causes logistical difficulties in handling and precludes its extensive use. In previous studies, it has been demonstrated that BLM produces 2 geometrical isomers when forming a cobalt chelation complex.

Application: Detection of neoplastic lesions by nuclear medicine imaging methodologies.

Interpretation and Analysis: Both tumor accumulation in tumor-bearing mice and stability of DNA binding of Type I isomer are higher than those for Type II.

Instrumentation: Computer-integrated nuclear medicine imaging methodologies.

Planar cobalt-57 bleomycin scintigraphy compared with CT-scan in the diagnosis and staging of lung cancer. Verhoeven GT, Kho GS, Ausema L, Krenning EP, Hilvering C. Neth J Med 1994 Apr; 44 (4), pp. 116–21.

Tumor affinity and DNA interactions of [^{57}Co]-bleomycin (author's transl). Kakinuma J, Honjo Y, Orii H. Radioisotopes 1982 Apr; 31 (4), pp. 175–8.

Neoplastic Disease
3′-([^{82}Br], [^{36}Cl], or [^{123}I]) "Arabino" Pyrimidine Nucleosides

Application: Detection of neoplastic lesions.

Dosage: 3′-radiohalogenated ([^{36}Cl], [^{82}Br], or [^{123}I]) "arabino" pyrimidine nucleosides.

Interpretation and Analysis: No preferential tissue uptake can be observed. The compounds are excreted mainly unchanged in the urine. The 3′-[$^{3\ 6}$Cl]- and 3′-[^{82}Br]-3′-deoxyarabino nucleosides exhibit biliary uptake. The low uptake of injected radioactivity by the tumor is probably due to the combined effects of the lack of a C-3′ hydroxyl group in the "ribo" configuration, the presence of a halogen group related to chemical properties, the structural rigidity imposed by the physical presence of a halogen group, and the short biological half-lives of the compounds.

Tumor uptake of radiolabeled pyrimidine bases and pyrimidine nucleosides in animal models: VI. 1-(3′-[^{36}Cl]-chloro-, 1-(3′-[^{82}Br]-bromo-, and 1-(3′-[^{123}I]-iodo-3′-deoxy-beta-D-arabinofuranosyl)uracil. Lee YW, Mercer JR, Wiebe LI, Knaus EE. Int J Appl Radiat Isot 1984 Nov; 35 (11), pp. 1057–61.

Neoplastic Disease
[^{131}I]-1-(2-Phenoxy-Ethyl)-2-Nitroimidazole

Cellular/Molecular Mechanism of Action: The highly lipophilic and protein-bound derivatives of 1-(2-phenoxy-ethyl)-2-nitroimidazole (PENI; RGW-609) are known radiosensitizers.

Background Information: IPENI and DIPENI are radiosensitizers by inference only; that is, they have not been tested for radiosensitizer properties. However, PENI, the parent compound, has been shown to be active as a radiosensitizer, and the peak potentials (polarographic) for both IPENI and DIPENI fall near those of MISO, PENI, and most other 2-nitroimidazole sensitizers.
Application: The low concentration levels in target tissues achieved by [^{131}I]-PENI mitigate against its use as a diagnostic imaging agent in nuclear medicine scintigraphy.
Semi-Synthetic Methods: The agents can be prepared by introducing 1 (IPENI) and 2 (DIPENI) iodine atoms into the phenyl ring via electrophilic substitution. The electron affinity of all 3 compounds, as determined by differential pulse polarography, is similar to that for MISO, a radiosensitizer that has undergone clinical trials, but protective (P) values are 2–3 orders of magnitude greater than for MISO, and percent protein binding (%PB) values are as high as 30% *in vitro* compared to less than 1% for MISO. [^{131}I]-PENI is prepared by catalyzed halogen exchange with Na[^{131}I] facilitating a greater than 90% yield, and a product that is chemically and radiochemically stable in solution for at least 2 weeks.
Implementation: Evaluation of the biodistribution profiles for [^{131}I]-PENI in conditions of neoplastic disease.

Animal Model: Murine (BDF/1 strain mice bearing EMT-6 tumors).
Interpretation and Analysis: Whole-body studies show rapid hepatic extraction and biliary elimination with little or no accumulation in any other tissue, (including tumor and fat), indicating that P and %PB values have little impact on *in vivo* distribution and disposition. [^{131}I]-PENI is not measurably deiodinated *in vivo*.

Electron-affinic compounds for labeling hypoxic cells: The synthesis and characterization of 1-[2-(2-iodophenoxy)-ethyl]-2-nitroimidazole. Wiebe LI, Jette DC, Chapman JD. Nuklearmedizin 1984 Apr; 23 (2), pp. 63–7.

Neoplastic Disease and Inflammation
Gallium-67 ([^{67}Ga])

Cellular/Molecular Mechanism of Action: Although many hypotheses concerning the mechanism of uptake of [^{67}Ga] into tumors and inflammation have been proposed, a unified consensus has not been established.
Application: [^{67}Ga] has been used in nuclear medicine as an imaging agent for neoplastic disease and inflammation.
Implementation: The uptake of [^{67}Ga] by hepatic tissues in carbon tetrachloride (CCl_4)-treated rats is slightly enhanced by the administration of $FeCl_3$ 5 minutes before the injection of the radioimaging agent.

Animal Model: Rat (CCl_4-induced hepatic injury).
Route of Administration: Intravascular.
Considerations: Previously, it was unknown if transferrin (Tf) was involved in [^{67}Ga] uptake by hepatic tissues. However, it is well known that [Fe^{+3}] can inhibit the binding of [^{67}Ga] to transferrin. If the mechanism of [^{67}Ga] uptake is clarified, the sensitivity of diagnostic imaging with [^{67}Ga] could be improved.
Interpretation and Analysis: The entering of [^{67}Ga] into hepatocytes in livers exposed to CCl_4 is similar to the uptake by liver tissue. In addition, the administration of [$FeCl_3$] slightly increases the entry of [^{67}Ga] in to hepatocytes. These results suggest that free [^{67}Ga] ion (not [^{67}Ga]-transferrin complexes) enters hepatocytes in the livers of CCl_4-treated rats.

Free [^{67}Ga] enters into the hepatocytes of carbon tetrachloride-treated rats. Abe S, Hasegawa S, Nirasawa M, Sato N, Ohkubo Y. Hepatol Res 2003 Mar; 25 (3), pp. 306–11.

Neoplastic Disease/Interleukin-2 (IL-2) Receptor Expression
Gallium-67 ([^{67}Ga]) Ga-2-(p-SCN-Bz)-NOTA Anti-IL-2 IgG

Cellular/Molecular Mechanism of Action: Immunoglobulin-based recognition and binding to IL-2 receptor.
Application: The low molecular weight protein (LMWP) has been proposed as a diagnostic agent for the detection of neoplastic disease states.
Semi-Synthetic Methods: The disulfide-linked fragment (dsFv) of the antibody to the alpha subunit of the IL-2 receptor can be radiolabeled with a [^{67}Ga] Ga-2-(*p*-SCN-Bz)-NOTA derivative linked through an isothiocyanato group to either the epsilon-amino group of lysine or the alpha-amino group of the N-terminal amino acids.
Considerations: Because different amino acids in the dsFv are radiolabeled applying the method described, it is possible to study the relative residence times of the various catabolites.
Implementation: Evaluation of the residence time for methionine and lysine conjugates within the same kidney.
Interpretation and Analysis: Approximately $>$ 60% of the injected dose localizes in the mouse kidney. The major catabolites ($>$ 95%) in the kidney can be identified as the Ga-2-(p-SCN-Bz)-NOTA conjugated with either lysine or methionine, with no evidence of [^{67}Ga] transchelation. The methionine conjugate has a significantly shorter residence time than the lysine conjugate in the same kidney. Labeling the appropriate amino acid in a LMWP may lead to reduced residence times and increased diagnostic or therapeutic ratios. Appropriately synthesized radiobiological agents of this design can preferentially accumulate within neoplastic tissues, producing a high "target-to-background" ratio on nuclear medicine scintigraphic images.
Instrumentation: Computer-integrated nuclear medicine scintigraphy instrumentation.

Biodistribution and catabolism of Ga-67-labeled anti-Tac dsFv fragment. Wu C, Jagoda E, Brechbiel M, Webber KO, Pastan I, Gansow O, Eckelman WC. Bioconjug Chem 1997 May–Jun; 8 (3), pp. 365–9.

Neoplastic Disease/Oxytocin Receptor Complex Expression/Endothelial Carcinoma/Mammary Carcinoma/Glioblastoma/Neuroblastoma/Vascular Expression
[^{111}Indium] ([^{111}In])-DOTA-Lys$_8$-Vasotocin

Cellular/Molecular Mechanism of Action: Lys_8-vasotocin (LVT) is an analogue of oxytocin that possesses high binding affinity for oxytoxin receptor complexes.
Semi-Synthetic Methods: Covalent cross-linking the chelating agent 1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid (DOTA) to LVT.
Implementation: Evaluation of the ability for the radioactive ligand [^{111}In]-DOTA-Lys_8-vasotocin to recognize and physically bind to oxytocin receptor complexes expressed by mammary carcinoma and glioblastoma cell types.

***Ex Vivo* Model:** MCF-7 (mammary carcinoma); MOG-U-V-W (glioblastoma) and transiently transfected COS7 cell lines.

Animal Model: Murine (TS/A mouse mammary tumor).
Interpretation and Analysis: The agent DOTA-LVT retains high *in vitro* binding affinity for human oxytoxin receptor complexes endogenously expressed by cell lines such as MCF-7 breast carcinoma and MOG-U-V-W glioblastoma cells lines, as well as to tran-

siently transfected COS7 cells. In investigations performed *in vivo*, [^{111}In]-DOTA-LVT specifically binds to oxytoxin receptor-positive TS/A mouse mammary tumors. Based on the nature of these laboratory findings, DOTA-LVT could potentially function as a platform for imaging and possibly the therapeutic management of oxytoxin receptor-positive human tumors such as breast and endometrial carcinomas, neuroblastomas, and glioblastomas.

[^{111}In]-labeled 1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid-lys$_8$-vasotocin: A new powerful radioligand for oxytocin receptor expressing tumors. Bussolati G, Chinol M, Chini B, Nacca A, Cassoni P, Paganelli G. Cancer Res 2001 Jun 1; 61 (11), pp. 4393–7.

Neoplastic Disease/Membrane-Associated Cancer Antigens/[^{99m}Technetium] ([^{99m}Tc]) Anti-Carcinoembryonic Antigen (CEA) Monoclonal Antibody (MAb)/Colorectal Cancer/Bladder Cancer/Thyroid Cancer
[^{99m}Tc]- CEA MAb

Cellular/Molecular Mechanism of Action: Monoclonal antibody is used as a targeting modality for unique antigens expressed on the external surface membrane of neoplastic cell types.

Background Information: Monoclonal antibody can be produced with binding avidity for almost any protein, glycoprotein, or lipoprotein antigen greater than 10 kDA in mass.

Examples of preparations other than radiolabled anti-CEA include (i) [^{111}In]-labeled anti-MUC1 mucin for invasive bladder cancer; and (ii) [^{131}I]-MN-14 F(ab)$_2$ anti-CEA MAb for medullary thyroid cancer.

Although this allows flexibility in the design of new agents, binding avidities and specificity of binding can vary between different monoclonal antibody preparations.

Diagnostic Application: Detection of neoplastic disease states that express membrane-associated CEA at relatively high densities.

Route of Administration: Intravascular.

Dosage: [^{99m}Tc]-anti-CEA monoclonal antibody (925 MBq)

Considerations: Intact radiolabeled IgG immunoglobulin will accumulate within the spleen and liver due to binding to Fc-receptor sites expressed on the exterior surface membrane of cell populations comprising the reticuloendothelial (RE) or mononuclear phagocytic system. Enzymatic cleavage of the Fc fragment with either pepsin or papain results in the generation of Fab′ or Fab′$_2$. Such IgG molecular fragments display a significantly higher target-to-background ratio than intact IgG. Examples of radiolabeled MAbs applied for either diagnostic or therapeutic purposes include those targeted against p57 protein fractions expressed by malignant melanoma cell types.

Implementation: Evaluate the usefulness of immunoscintigraphy with an anti-CEA MAb fragment labeled with [^{99m}Tc] for early detection of colorectal cancer recurrence in patients with rising serum CEA levels. Cases are then subjected to immunoscintigraphy imaging studies (e.g., n = 8 cases; n = 59 acquired images). Immunoscintigraphy is performed after injection of anti-CEA MAb. Planar images of the thorax, abdomen, and pelvis, as well as single photon emission computed tomography (SPECT) of the abdomen and pelvis are obtained 4 and 24 hours after injection. Abdominal computed tomography (CT) scans can additionally be performed prior to scintigraphic imaging. Findings are subsequently validated by histopathological analysis (e.g., n = 28 cases) or by imaging and clinical follow-up of at least 6 months following the immunoscintigraphy (e.g., n = 31 cases).

Human Model: Cases of colorectal cancer (e.g., n = 51 consecutive patients; n = 27 women, n = 24 men; population mean age 68.9 ± 10.2 years) with rising CEA levels (e.g., 16.2 ± 18.2 ng/ml).

Interpretation and Analysis: A considerable number of cases will not show recurrence during the follow-up period (e.g., n = 41). Conversely, slightly less than two-fifths of all cases will have a confirmed diagnosis of extrahepatic abdominal or pelvic disease (e.g., n = 18/51); approximately one-fifth of cases will have liver metastases (e.g., n = 11/51); slightly less than one-fifth of cases will have thoracic involvement (e.g., n = 9/51); while a relatively small number will have bone metastasis (e.g., n = 2/51). In cases with pelvic and extrahepatic abdominal disease, immunoscintigraphy will be true-positive in most instances (e.g., n = 14/18 true-positive and n = 4/18 false-positive). Of the cases classified as true-positive, only about half are detectable by CT imaging (e.g., n = 7/14).

In the remaining cases with no detectable lesions on immunoscintigraphy images, most of these results can be classified as true-negatives (e.g., n = 37/41 true-negative, n = 4/41 false-negative). Therefore, the sensitivity and specificity for immunoscintigraphy in extrahepatic abdominal and pelvic disease is approximately 78% and 90%, respectively. CT results show a lower sensitivity of 61% ($p < 0.05$) and specificity of 83%. Liver metastases may not be detected by CT (e.g., n = 9 cases), but such lesions may alternatively only be detected in a small number of cases utilizing immunoscintigraphy (e.g., n = 2/9). Therefore, scintigraphy with anti-CEA MAb fragment labeled with [^{99m}Tc] is superior to CT for the detection of pelvic and extrahepatic abdominal recurrence of colorectal cancer, while CT is more sensitive in the detection of liver and lung metastases. Immunoscintigraphy has a limited usefulness in the detection of distant metastases, but it may be helpful in the diagnosis of suspected colorectal recurrence in patients with non-conclusive CT findings, when FDG-PET is not available.

Is there a role for [^{99m}Tc]-anti-CEA monoclonal antibody imaging in the diagnosis of recurrent colorectal carcinoma? Fuster D, Maurel J, Muxí A, Setoain X, Ayuso C, Martín F, Ortega ML, Fuertes S, Pons F. Q J Nucl Med 2003 Jun; 47 (2), pp. 109–15.

Neoplastic Disease Imaging/[Cardiac Pool and Bone Distribution]/[*In Vivo* Stability]
[^{57}Co]-Bleomycin/[^{111}Indium] ([^{111}In])-Bleomycin/[^{99m}Technetium] ([^{99m}Tc])-Bleomycin/[^{197}Hg]-Bleomycin

Investigations with bleomycin labeled with radionuclides other than [^{57}Co] in human cases suffering from cancer and in tumor-bearing animals have been described. In human cases, [^{57}Co]-bleo appears to be a better tumor-seeking radiopharmaceutical than [^{111}In]-bleo, [^{99m}Tc]-bleo, or [^{197}Hg]-bleo. This can be explained by a higher stability *in vivo* and better tumor-seeking property of [^{57}Co]-bleo, in addition to less disturbing activity in the cardiac pool and in bone or other normal tissues when assessing scintigraphic images. Results with [^{111}In]-bleo labeled in acidic solution are essentially no different from those with [^{111}In]-bleo labeled in neutral solution. Results of [^{197}Hg]-bleo are almost identical with those of [^{197}Hg]Cl$_2$ regarding the tumor-seeking properties as well as distribution profiles in normal tissues and organs. Probably the complex of [^{197}Hg] to bleomycin is not stable *in vivo*. The superiority of [^{57}Co]-bleo over [^{99m}Tc]-bleo and [^{197}Hg]-bleo, and also over [^{67}Cu]-bleo, has been confirmed in tumor-bearing animals. Such findings indicate that the use of bleomycin as a tumor-seeking pharmaceutical labeled with [^{111}In], [^{99m}Tc], [^{197}Hg], or [^{67}Cu] seems to be very limited.

Clinical evaluation of radiolabeled bleomycin for tumor detection. Rasker JJ, Beekhuis H, van de Poll MA, Versluis A, Jurjens H, Woldring MG. Nuklearmedizin 1978 Dec; 17 (6), pp. 238–48.

Neoplastic Disease States/Glioma/ Mammary Tumors (Carcinogen-Induced) *[^{99m}Technetium] ([^{99m}Tc])-Field Bean Protease Inhibitor (FBPI)/[$^{99m}Tc^{V}$]-Dimercaptosuccinic Acid (DMSA)/[^{201}Thallous] Chloride (TlCl)/ [$^{99m}TcO_4^-$]-Citrate*

Application: Detection and characterization of gliomas of the central nervous system and possibly other types of neoplastic disease.

Route of Administration: [$^{99m}TcO_4^-$]-FBPI (intravenous)

Semi-Synthetic Methods: Purified FBPI can be labeled with [$^{99m}TcO_4^-$] functioning as a tracer molecule. Labeling is done in the presence of Sn^{2+} serving as a reducing agent and the resulting yield is approximately 95%. At room temperature, the product is stable for at least a 2-hour incubation period.

Implementation: Evaluation of the biodistribution for [^{99m}Tc]-labeled FBPI to ascertain its ability to locate within tumor lesions. Biodistribution analysis is determined at various time intervals following administration.

Animal Model: Rat (Wistar strain bearing carcinogen-induced mammary tumors or transplanted C6 glioma).

Interpretation and Analysis: Biodistribution analysis of [^{99m}Tc]-FBPI reveals that it undergoes rapid blood clearance from the systemic circulation (approximately 5 hours). The complex is predominantly eliminated through renal and hepatobiliary excretory mechanisms. The tumor-to-muscle (T/M) ratios obtained with [^{99m}Tc]-FBPI in C6 glioma explants are nearly 2- to 5-fold higher than observed with several conventional tumor-seeking radiopharmaceuticals such as [^{99m}Tc]-DMSA, [^{201}Thallous] chloride ([^{201}Tl]Cl]), and [^{99m}Tc]-citrate. Similarly, the T/M ratio obtained with [^{99m}Tc]-FBPI in a mammary tumor model, on the other hand, appears to be 2–3-fold higher than observed with [^{99m}Tc]-DMSA and [^{201}Tl]Cl]. The ratio is, however, comparable with that obtained with [^{99m}Tc]-citrate. Therefore, [^{99m}Tc]-FBPI has the specific potentials for imaging gliomas and possibly other tumors as well.

[^{99m}Tc]-labeled field bean protease inhibitor can function as an efficient tumor detecting agent. Murugesan S, Banerji AP, Noronha OP, Samuel AM, Fernandes AO. Indian J Exp Biol 2001 Aug; 39 (8), pp. 742–7.

Neoplastic Disease/Insulin-Like Growth Factor (IGF) Binding Proteins *[^{125}I]-Labeled-IGF*

Cellular/Molecular Mechanism of Action: Insulin-like growth factor recognizes and binds to membrane-associated receptor complexes (IGF binding proteins [IGFBPs]).

Background Information: IGFBPs play a critical role in modulating IGF activity.

Application: Detection and characterization of certain neoplastic disease types.

Considerations: [^{125}I]-labeled IGF-I is degraded quite rapidly within the cytosol of some neoplastic cell types.

Semi-Synthetic Methods: Production of [^{125}I]-labeled-IGF-I by the radioiodination of IGF-I using chloramines-T reagent.

Implementation: Evaluate the biodistribution and tumor targeting ability of radiolabeled IGF (e.g., [^{125}I]-IGF-I). Because IGFBPs play a critical role in modulating IGF activity, the binding properties of [^{125}I]-labeled IGF-I to IGFBPs can be investigated under both *in vitro* and *in vivo* conditions. Complementary investigations can be initiated to study the *in vitro* catabolism of IGF-I by tumor cells. Validation of the relative specificity of uptake can in part be established by co-administration of unlabeled peptide. *In vitro* and *in vivo* chromatography studies are performed to evaluate the binding profile to IGFBPs and measure serum/urine catabolite concentrations.

Animal Model: Rat and mouse (tumor-bearing nude mice).

Interpretation and Analysis: Binding of radiolabeled IGF-I to IGFBPs *in vitro* occurs in a dose-dependent manner. However, there is a difference in complex formation between serum and heparinized plasma. In heparinized plasma, radioactivity shifts from a 30- to 50-kDa complex to a 150-kDa complex and to a free ligand, because the binding of heparin with IGFBPs decreases its affinity for IGF-I. In plasma prepared with acid citrate dextrose a binding pattern identical to that of serum is observed. Moreover, there is a binding difference between mouse and rat. The [^{125}I]-labeled IGF-I is catabolized very quickly when incubated at 37°C but not at all at 4°C. In tumor-bearing nude mice, the uptake of radioactivity in normal tissues decreases quickly, particularly in the kidneys. In mice co-injected with unlabeled carrier, the radioactivity in most normal tissues is lower and the tumor uptake higher than in the mice without carrier. [^{125}I]-labeled IGF-I is avidly bound to IGFBPs, both *in vitro* and *in vivo*. By partially saturating this binding with unlabeled peptides, a favorable biodistribution can be achieved, including faster clearance from normal tissue and higher tumor uptake, which results in better tumor-to-non-tumor ratios. Nevertheless, the rapid *in vivo* catabolism and release of the radiolabel from tumor tissue result in a suboptimal targeting agent.

Effects of insulin-like growth factor binding proteins on insulin-like growth factor-I biodistribution in tumor-bearing nude mice. Sun BF, Kobayashi H, Le N, Yoo TM, Drumm D, Paik CH, McAfee JG, Carrasquillo JA. J Nucl Med 2000 Feb; 41 (2), pp. 318–26.

Neoplastic Disease: Topoisomerase I and II/ Adenocarcinoma (HT29)/Gliobastoma (U87MG)/ Melanoma (A375M Antigen Positive) *[^{11}C]-9-Methoxyphenazine-1-Carboxamide/ [^{11}C]2-(4-Pyridyl)Quinoline-8-Carboxamide/ [^{11}C]DACA/[Topoisomerases I and II]*

Cellular/Molecular Mechanism of Action: The tricyclic carboxamide N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA) is a DNA-intercalating agent capable of inhibiting both topoisomerases I and II enzyme fractions.

Background Information: The tricyclic carboxamide DACA is currently in phase II clinical trial. Many related analogues have been developed, but despite their potent *in vitro* cytotoxicities, they exhibit poor extravascular distribution.

Application: Delineation of rational drug selection.

Semi-Synthetic Methods: Each candidate compound shares a common structural feature, *N*-dimethyl side chain, which is radiolabeled with carbon-11 (positron-emitting radioisotope).

Implementation: Evaluate the biodistribution and metabolite profiles of the prototype compound, DACA, with 3 analogues to aid rational drug selection in order to identify agents that are "minimal intercalators" with lower DNA association constants. In this fashion, promising candidates can be identified in emerging preclinical studies in animals that can ultimately be evaluated in humans using positron emission tomography (PET) or nuclear medicine.

Animal Model: Murine (mice bearing human tumor xenografts).

Interpretation and Analysis: Acridine DACA, phenazine SN 23490, pyridoquinoline SN 23719, and dibenzodioxin SN 23935 are cytotoxic when evaluated with *in vitro* assays utilizing HT29, U87MG, and A375M cell lines (IC_{50} of 1.4 to 1.8 μM, 0.4 to 0.6 μM, 1.3 to 1.6 μM, and 24 to 36 μM, respectively). *Ex vivo* biodistribution studies with carbon-11 radiolabeled compounds in mice

bearing human tumor xenografts show rapid clearance of [^{11}C]-radioactivity (parent drug and metabolites) from blood and major organs. Rapid hepatobiliary clearance and renal excretion can also be observed. There is low (< 5% of injected dose/gram [%ID/g]) and variable uptake of [^{11}C]-radioactivity in 3 tumor types for all of the compounds. Tumor (U87MG) to blood [^{11}C]-radioactivity for [^{11}C]DACA, [^{11}C]-9-methoxyphenazine-1-carboxamide (SN 23490), [^{11}C]2-(4-pyridyl)quinoline-8-carboxamide (SN 23719), and [^{11}C]dibenzo[1,4]dioxin-1-carboxamide (SN 23935) at 30 minutes are approximately 2.9 ± 1.1, 2.3 ± 0.6, 2.6 ± 0.6, and 0.7 ± 0.2, respectively. For SN 23719, the distribution of [^{11}C]-radioactivity in normal tissues and tumors determined *ex vivo* is in broad agreement with that determined *in vivo* by whole-body PET scanning. [^{11}C]DACA is rapidly and extensively metabolized to several plasma metabolites and a major tumor metabolite. Conversely, [^{11}C]SN 23935, [^{11}C]SN 23490, and [^{11}C]SN 23719 shows less extensive metabolism. In tumor samples, parent [^{11}C]DACA and [^{11}C]SN 23935 is present between 0.3 and 1.5 %ID/g, whereas [^{11}C]SN 23490 and [^{11}C]SN 23719 is present between 1.5 and 2.8 %ID/g. Therefore, by using a strategy employing [^{11}C]-labeling, it is possible to determine the tissue distribution and metabolic stability of novel tricyclic carboxamides.

Given the objective directed at identifying analogues with potentially improved *in vivo* activity against solid tumors, the agents SN 23490 and SN 23719 have more favorable distribution and metabolic stability compared with DACA and SN 23935. Such observations suggest that further development and evaluation are warranted. The radiolabeling strategy used allows *ex vivo* and *in vivo* evaluation of promising anticancer agents in animals and offers the potential of rapid translation to studies in humans using PET. Because of their mechanism of action, this class of agents also has the potential of being developed into diagnostic biopharmaceutical probes.

Comparative biodistribution and metabolism of carbon-11-labeled N-[2-(dimethylamino)ethyl]acridine-4-carboxamide and DNA-intercalating analogues. Osman S, Rowlinson-Busza G, Luthra SK, Aboagye EO, Brown GD, Brady F, Myers R, Gamage SA, Denny WA, Baguley BC, Price PM. Cancer Res 2001 Apr 1; 61 (7), pp. 2935–44.

Neurotensin-Positive Tumors/Adenocarcinomas/ Pancreatic Cancer
[^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)-Neurotensin [Arg$_8$, Arg$_9$, and Ile$_{12}$]/ Fluorescein-Neurotensin [Arg$_8$, Arg$_9$, and Ile$_{12}$]

Cellular/Molecular Mechanism of Action: Native neurotensin (NT) is a tridecapeptide that binds to neurotensin receptor (NTR) and induces tumor growth.

Background Information: The prevalence of NTR in several human tumors makes it an attractive target for the delivery of cytotoxic drugs and design of cancer imaging agents. Unfortunately, NT has a short plasma half-life, which hinders its use for *in vivo* biomedical applications. Numerous reports suggest that Arg$_8$-Arg$_9$ and Tyr$_{11}$-Ile$_{12}$ amide bonds are particularly susceptible to degradation by proteolytic enzymes.

Semi-Synthetic Methods: Substitution of Arg$_8$, Arg$_9$, and Ile$_{12}$ amino acids with the corresponding commercially available mimics. Preparations can be conjugated to DTPA for subsequent radiolabeling to produce [^{111}In]-DTPA or conjugated with fluorescein.

Animal Model: Mouse (severe combined immunodeficient [SCID] bearing NTR-positive human adenocarcinoma [HT29] xenografts).

Interpretation and Analysis: The surrogate amino acids are amenable to standard Fmoc peptide synthesis strategies, and the resulting compounds are stable in biological media for > 4 hours and bind to NTR with high affinity. Conjugation of DTPA to the synthetic peptides and subsequent labeling with [^{111}In]-DTPA for nuclear imaging or fluorescein for optical imaging does not diminish NTR binding avidity. Evaluation of the *in vivo* biodistribution of a representative [^{111}In]-DTPA-NT peptide analogue in HT29-bearing SCID mice reveals that the compound is primarily retained in tumor tissue (2.2 %ID/g) and the kidneys (4.8 %ID/g) at 4 hours post-injection. Co-injection of cold NT and the radiolabeled NT peptide analogue inhibits tumor but not kidney uptake, and demonstrates that retention of the radiolabeled compound in tumor tissue is mediated by NTR specific uptake while it accumulates in the kidneys by a non-specific mechanism. These findings show that NT peptide analogues are robust and can deliver imaging agents to NTR-positive tumors such as pancreatic cancer.

Novel bioactive and stable neurotensin peptide analogues capable of delivering radiopharmaceuticals and molecular beacons to tumors. Achilefu S, Srinivasan A, Schmidt MA, Jimenez HN, Bugaj JE, Erion JL. J Med Chem 2003 Jul 17; 46 (15), pp. 3403–11.

Otolaryngological Conditions/Thyrotoxicosis and Solitary Thyroid Nodules/Thyrotoxicosis/ Solitary Thyroid Nodules/Parathyroid Adenoma/ Salivary and Lacrimal Gland/Bone
[^{99m}TcO^{-4}]/[^{123}I]/[^{99m}TcO^{-4}]/[^{201}Tl]/[^{99m}TcO^{-4}]/ [^{99m}TcO^{-4}]-MDP

Background Information: Nuclear medicine has a distinct role to play in otolaryngological practice. Accurate diagnosis of endocrine conditions is now possible using precise *in vitro* hormone measurements. Specific clinical questions can be answered using *in vivo* investigations. [^{99m}TcO^{-4}]/[^{123}I] scintigraphy is used to evaluate thyrotoxicosis and solitary thyroid nodules. [^{99m}TcO^{-4}]/[^{201}T1] subtraction scanning is of value in the preoperative localization of parathyroid adenomas and [^{99m}TcO^{-4}] is particularly useful in assessing salivary and lacrimal gland function and drainage. [^{99m}TcO^{-4}]-MDP bone scanning is useful in the evaluation of osteomyelitis, temporomandibular joint dysfunction, bone graft viability, and some facial fractures. The role of radioisotopes in the management of differentiated and medullary carcinoma of the thyroid is now well established.

Application: Detection of thyrotoxicosis and solitary thyroid nodules by scintigraphic imaging.

Interpretation and Analysis: Although there are many other agents available to image head and neck cancer, few can actually achieve the required diagnostic sensitivity and specificity. The introduction of monoclonal antibodies into routine imaging has been hampered by distinct practical problems and a search is now on for more sensitive non-specific diagnostic agents. It is now possible to evaluate new [^{99m}Tc]-labeled tumor-imaging agents using animal tumor model systems, and the use of radioactivity in all aspects of otolaryngological research adds an extra quantitive dimension. Together with single photon emission computed tomography (SPECT), and the introduction of positron emission tomography (PET) to image the physiology of normal tissues and tumors, the use of radionuclide investigations can lead only to an increase in ear, nose, and throat (ENT) diagnostic sensitivity and specificity and, subsequently, to an overall improvement in the diagnosis, staging, and treatment of cancer affecting the head and neck.

Nuclear medicine in otolaryngology. Watkinson JC. Clin Otolaryngol 1990 Oct; 15 (5), pp. 457–69.

Ovarian Carcinoma: Imaging *[^{111}Indium] ([^{111}In])-(hCTMO1) Monoclonal Antibody*

Cellular/Molecular Mechanism of Action: Immunoglobulins designed to recognize and physically bind to ovarian carcinoma antigens.
Application: Detection and characterization of ovarian carcinoma cell types.
Route of Administration: Intravenous.
Dosage: (D1) 0.1 mg per kg body weight of radiolabeled antibody; (D2) 1 mg per kg body weight; and (D3) 1 mg per kg body weight of unlabeled antibody followed 1 hour later by 0.1 mg per kg body weight of radiolabeled antibody. Images are acquired using a gamma camera at 1 hour and up to 96 hours after injection.
Implementation: Evaluation of suspected cases of primary ovarian carcinoma. Images are acquired using a gamma camera at 1 hour and up to 96 hours after injection.

Human Model: Cases with a high suspicion of primary ovarian carcinoma on the basis of ultrasound or computed tomography (CT) imaging and serum CA125 concentrations, and those in whom there is a suspicion of recurrent ovarian carcinoma after being treated for histologically confirmed carcinoma.
Interpretation and Analysis: At the low dose of antibody the sensitivity for detection of ovarian carcinoma is 70%. After increasing the dose of antibody, and also after predosing with unlabeled antibody, the sensitivity increases to 100%, but there is a large number of false-positive results observed at the higher dose, and therefore the specificity is lower. The liver and bone marrow are the organs with the highest activities due to the binding of labeled antibody to the Fc receptor expressed by cells of the mononuclear phagocytic system. An increased accumulation of radiolabeled immunoglobulin within a specific anatomical location or organ system implies the presence of ovarian carcinoma.

An immunoscintigraphic evaluation of the engineered human monoclonal antibody (hCTMO1) for use in the treatment of ovarian carcinoma. Davies Q, Perkins AC, Roos JC, Molthoff CF, Verheijen RH, Frier M, Kenemans P, Broadhead T, Sopwith M, Symonds EM. Br J Obstet Gynaecol 1999 Jan; 106 (1), pp. 31–7.

Ovarian Cancer: Carcinoma/Sensitivity and Specificity/Folate Receptor Expression *[^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)-Folate*

Cellular/Molecular Mechanism of Action: [^{111}In]-DTPA-folate is a radioactive chelation analogue of folic acid that recognizes and physically binds to folate receptors expressed at relatively high membrane densities by certain neoplastic cell types.
Application: [^{111}In]-DTPA-folate can be applied as a tumor-selective imaging agent in diagnostic nuclear medicine for the purpose of detecting and monitoring neoplastic disease states (e.g., ovarian malignancies). Based on the biological properties of folate, it can also function as a tumor-specific drug delivery platform.
Background Information: The cell-membrane folate receptor is a potential molecular target for tumor-selective drug delivery, including radiolabeled folate-chelate conjugates for diagnostic imaging.
Route of Administration: [^{111}In]-DTPA-folate (intravenous bolus).
Implementation: Evaluation of the diagnostic potential for [^{111}In]-DTPA-folate to function as a diagnostic agent for malignant ovarian disease or endometrial cancer utilizing [^{111}In]-DTPA-folate as an imaging agent (phase I/II clinical trials). Prior to the administration of [^{111}In]-DTPA-folate, some nuclear medicine facilities prefer to administer unlabeled "free" folic acid followed by whole-body imaging at 30 minutes, 4 hours, and 24 hours postinjection of the folate radiopharmaceutical agent. Imaging with single photon emission computed tomography (SPECT) instrumentation can also be performed at the appropriate time-delayed intervals.

Human Model: Female cases of ovarian cancer (n = 35) enrolled in a phase I/II clinical study (n = 33 completing the surgical follow-up required by the study protocol that serves to define disease status). Individual cases either have a pathologically proven malignancy or are scheduled for surgery to resolve suspected new ovarian cancer growth (n = 26 cases), recurrent ovarian cancer (n = 5 cases), or endometrial cancer (n = 2 cases). In the first 19 of 26 cases, evaluated unlabeled "free" folic acid has been administered followed by whole-body imaging at 30 minutes, 4 hours, and 24 hours postinjection of the folate radiopharmaceutical agent.
Interpretation and Analysis: In cases subjected to surgical intervention (e.g., n = 33), slightly less than half have new or recurrent malignant tumors that can be detected (e.g., n = 14/33). A large percentage of newly diagnosed ovarian carcinomas can be identified blindly by multiple professional readers (e.g., n = 7/7 or 100% sensitivity). Conversely, sensitivity for detecting recurrent ovarian-related malignancies can be significantly less (e.g., n = 7/33) in cases where the interpretation is performed without prior knowledge, compared to the evaluation of images with prior knowledge of disease (e.g., n = 28/33 or 84.5% for unmasked readings). Such findings indicate that a correlation exists between anatomic imaging studies (computed tomography [CT]) and the diagnostic detection of such lesions. A number of cases may be determined to have benign masses (e.g., n = 18). In the context of a limited population size, the specificity of [^{111}In]-DTPA-folate scintigraphy is approximately 76% and 82% for masked and unmasked image interpretation, respectively. [^{111}In]-DTPA-folate is safe, and possibly effective, for differentiating between malignant and benign ovarian masses based on interpretation of scintigraphic images.

Evaluation of [^{111}In]-DTPA-folate as a receptor-targeted diagnostic agent for ovarian cancer: Initial clinical results. Siegel BA, Dehdashti F, Mutch DG, Podoloff DA, Wendt R, Sutton GP, Burt RW, Ellis PR, Mathias CJ, Green MA, Gershenson DM. J Nucl Med 2003 May; 44 (5), pp. 700–7.

Pancreatic/Pituitary/Adrenal/Somatostatin Receptors: Subtype 2 (sst2) *[^{111}Indium] ([^{111}In])-(DTPA0,Tyr$_3$)-Octreotide/[^{111}In]-(DOTA0,Tyr$_3$)-Octreotide/[^{111}In]-(DTPA0)-Octreotide*

Cellular/Molecular Mechanism of Action: Possesses physical binding avidity for subtype 2 somatostatin receptor (sst2) complexes.
Application: Detection and characterization of pituitary, pancreatic, and adrenal neoplastic lesions that express an overabundance of sst2 complexes.
Implementation: Evaluate the potential usefulness of radiolabeled [DTPA0,Tyr$_3$]octreotide and [DOTA0,Tyr$_3$]octreotide as radiopharmaceuticals for somatostatin receptor-targeted scintigraphy and radiotherapy. Analysis can involve the characterization of *in vitro* somatostatin receptor binding complemented by the determination of the *in vivo* pattern of metabolism of agents compared to [^{111}In]-DTPA0-octreotide.

Animal Model: Rats (CA20948 tumor-bearing Lewis rats).
Interpretation and Analysis: Comparing different peptide-chelator constructs, [DTPA0,Tyr$_3$]octreotide and [DOTA0,Tyr$_3$] octreotide have been found to have a higher affinity than [DTPA0] octreotide for subtype 2 somatostatin receptors (sst2) in mouse

AtT20 pituitary tumor cell membranes (all IC_{50} values obtained are in the low nanomolar range). Studies *in vivo* in CA20948 tumor-bearing Lewis rats reveal a significantly higher uptake of both [^{111}In]-labeled [DOTA0,Tyr_3]octreotide and [DTPA0,Tyr_3]octreotide in sst2-expressing tissues than after injection of [^{111}In]-(DTPA0)octreotide, showing that substitution of Tyr for Phe at position 3 in octreotide results in increased affinity for its receptor and in a higher target tissue uptake. Uptake of [^{111}In]-labeled [DTPA0] octreotide, [DTPA0,Tyr_3]octreotide, and [DOTA0,Tyr_3]octreotide in pituitary, pancreas, adrenals, and tumors is decreased to $<$ 7% of control by pretreatment with unlabeled octreotide (e.g., 0.5 mg/rat), indicating specific binding to sst2 complexes. Comparing different radionuclides, [^{90}Y]-(DOTA0,Tyr_3)-octreotide has the highest uptake in sst2-positive organs, followed by the [^{111}In]-(DOTA0,Tyr_3)-octreotide, whereas (DOTA0,[^{125}I]-Try_3)-octreotide uptake is low compared to that of other radiopharmaceuticals, when measured 24 hours after injection. Renal uptake of [^{111}In]-labeled (DTPA0)-octreotide, (DTPA0,Tyr_3)-octreotide, and (DOTA0,Tyr_3)-octreotide is reduced over 50% by intravenous injection of D-lysine (400 mg/kg), whereas radioactivity in blood, pancreas, and adrenals is not affected.

Preclinical comparison of [DTPA0] octreotide, [DTPA0,Tyr_3] octreotide, and [DOTA0,Tyr_3] octreotide as carriers for somatostatin receptor-targeted scintigraphy and radionuclide therapy. De Jong M, Bakker WH, Breeman WA, Bernard BF, Hofland LJ, Visser TJ, Srinivasan A, Schmidt M, Béhé M, Mäcke HR, Krenning EP. Int J Cancer 1998 Jan 30; 75 (3), pp. 406–11.

Pancreatic/Pituitary/Adrenal/Somatostatin Receptor-Positive Organs/Octreotide Receptor-Positive Organ Systems
[^{111}Indium] ([^{111}In])-(DOTA0,D-Phe$_1$,Tyr$_3$)-Octreotide/ D-Lysine

Cellular/Molecular Mechanism of Action: [^{111}In]-(DOTA0, D-Phe_1,Tyr_3)-octreotide recognizes and physically binds to somatostatin receptor-positive lesions.

Background Information: Indium-111-diethylenetriamine pentaacetic acid (DTPA)-octreotide or [^{111}In]-DTPAOC is used successfully for imaging somatostatin receptor-positive lesions. A new and promising application is its use in peptide-receptor radionuclide therapy (PRRT). For the latter purpose, [DOTA0,D-Phe_1,Tyr_3] octreotide (DOTATOC), which is suitable for stable radiolabeling with [^{90}Y], is probably even more promising. Significant renal uptake of these octreotide analogues exists, however, reducing the scintigraphic sensitivity for detection of small tumors in the perirenal region and limiting the possibilities for PRRT. Renal uptake of [^{111}In]-DTPAOC can be reduced to about 50% of control by L-lysine administration *in vivo* in rats.

Application: Different forms of lysine could potentially be applied to diminish non-specific renal tissue binding of [^{111}In]-(DOTA0,D-Phe_1,Tyr_3)-octreotide. The agent [^{111}In]-(DOTA0,D-Phe_1,Tyr_3)-octreotide can be applied for the detection and characterization of certain disease states associated with the expression of somatostatin receptor-positive lesions. The delivery platform DTPA-octreotide can be non-specifically associated with renal tissues during scintigraphic imaging studies. Different forms of lysine could potentially be applied to diminish non-specific renal tissue binding.

Route of Administration: [^{111}In]-DTPAOC (intravenously, intraperitoneally, or orally); [^{90}Y]-DOTATOC (intravenous).

Dosage: [^{111}In]-DTPAOC (0.2 MBq, 0.5 μg–0.5 mg); [^{90}Y]-DOTATOC (1 MBq, 0.5 μg).

Implementation: Evaluation of the renal uptake of [^{111}In]-DTPAOC in the presence or absence of D-lysine or L-lysine. At 1, 4, or 24 hours, organs are harvested and their corresponding radioactivity counted for comparision. Complementary investigations involve determination of similar differential organ uptake profiles applying [^{90}Y]-DOTATOC in the presence or absence of D-lysine.

Animal Model: Rat (male Wistar rats [200–250 g]).

Interpretation and Analysis: Administration of D-lysine or L-lysine in a single intravenous dose of 400 mg/kg results in more than 50% inhibition of kidney uptake of [^{111}In]-DTPAOC at all time points tested, independent of the mass of [^{111}In]-DTPAOC administered. Higher or repeated doses of lysine do not give a significantly higher percentage inhibition. D-lysine, given orally at a dose of 400 mg/kg at 30 or 15 minutes before [^{111}In]-DTPAOC injection, results in 30% and 20% inhibition of kidney uptake, respectively. L-lysine, given orally 30 minutes before [^{111}In]-DTPAOC administration, results in a 30% inhibition as well. Inhibition of kidney [^{111}In]-DTPAOC uptake by L-lysine after intraperitoneal administration is 40%. After L-lysine administration, [^{111}In]-DTPAOC is decreased in the kidneys and in somatostatin receptor-positive organs such as the pancreas and adrenal glands. Conversely, D-lysine does not have a significant effect on uptake in octreotide receptor-positive organs. Renal uptake of [^{90}Y]-DOTATOC is reduced by 65% with intravenous D-lysine, whereas radioactivity in blood, pancreas, and adrenal glands is not affected. D-lysine may be preferred to L-lysine for reduction of renal uptake of radioactivity during scintigraphy and PRRT because of its lower toxicity and because it should not interfere with the natural amino acid metabolic balance.

D-lysine reduction of indium-111 octreotide and yttrium-90 octreotide renal uptake. Bernard BF, Krenning EP, Breeman WA, Rolleman EJ, Bakker WH, Visser TJ, Mäcke H, de Jong M. J Nucl Med 1997 Dec; 38 (12), pp. 1929–33.

Parathyroid Adenomas
[^{99m}Technetium] ([^{99m}Tc])/ [^{201}Thallium] ([^{201}T1]) Subtraction Scanning

Application: Preoperative localization of parathyroid adenomas by scintigraphic imaging.

Considerations: Although there are many other agents available to image head and neck cancer, few can actually achieve the required diagnostic sensitivity and specificity. The introduction of monoclonal antibodies into routine imaging has been hampered by distinct practical problems and the search is now on for more sensitive non-specific diagnostic agents. It is now possible to evaluate new [^{99m}Tc]-labeled tumor-imaging agents using animal tumor models and the use of radioactivity in all aspects of otolaryngological research adds an extra quantitative dimension.

Together with single photon emission computed tomography (SPECT) and the introduction of positron emission tomography (PET) to image the physiology of normal tissues and tumors, the use of radionuclide investigations can lead only to an increase in ear, nose, and throat (ENT) diagnostic sensitivity and specificity and, subsequently, to an overall improvement in the diagnosis, staging, and treatment of cancer involving the head and neck.

Parathyroid Adenomas: Ectopic
[^{99m}Technetium] ([^{99m}Tc])-SestaMIBI/[^{201}Thallium] ([^{201}Tl])

Background Information: [^{99m}Tc]-sestaMIBI has been introduced for the preoperative localization of abnormal parathyroid glands in conditions of primary hyperparathyroidism with promising results. However, the sensitivity of sestaMIBI and thallium to detect abnormal parathyroid glands is partly dependent on gland size.

Implementation: Evaluate and compare the sensitivity of sestaMIBI subtraction scintigraphy with thallium subtraction scintigraphy in conditions of primary hyperparathyroidism with predominantly mild increases in serum calcium level utilizing [^{201}Tl] and [^{99m}Tc]-sestaMIBI scintigraphy.

Human Model: Cases with primary hyperparathyroidism (n = 39). The mean (± standard deviation) serum level of calcium is approximately 2.75 ± 0.17 mmol/L. In about 72% of cases (e.g., n = 28/39) serum levels of calcium are less than 2.85 mmol/L. These cases are classified as having mild abnormalities in serum calcium. The other cases are evaluated before surgery with both sestaMIBI and thallium subtraction scintigraphy. Note: Autonomous thyroid adenomas may preclude performing subtraction scintigraphy.

Interpretation and Analysis: SestaMIBI subtraction scintigraphy correctly localizes about 86% (n = 31/36) of parathyroid adenomas compared with only 47% (n = 17/36) by thallium subtraction scintigraphy ($p < 0.001$). Occurrence of false-positive results with sestaMIBI is low (may be seen with thyroid adenoma), while other scans may appear negative. Both the sestaMIBI and the thallium subtraction scintigraphy may localize 1 single enlarged gland in cases with multiple gland involvement (n = 3 cases). Prediction accuracy of multiglandular disease is very low. SestaMIBI subtraction scintigraphy is superior to thallium subtraction scintigraphy and has a high sensitivity for localizing a solitary parathyroid adenoma in cases with mild increase in serum calcium level. The sensitivity decreases in cases with multiglandular parathyroid disease and concomitant thyroid nodular abnormalities.

SestaMIBI vs thallium subtraction scintigraphy in parathyroid localization: A prospective comparative study in patients with predominantly mild primary hyperparathyroidism. Bergenfelz A, Tennvall J, Valdermarsson S, Lindblom P, Tibblin S. Surgery 1997 Jun; 121 (6), pp. 601–5.

Parathyroid Adenoma/Radio-Guided Resection *[^{99m}Technetium] ([^{99m}Tc])-SestaMIBI*

Background Information: Radio-guided resection of hyperfunctioning parathyroid glands has been shown to be technically feasible in cases of parathyroid adenomas. Radio-guided excision may obviate the need for intraoperative frozen sections because excised parathyroid adenomas uniformly have radionuclide *ex vivo* counts > 20% of background. The feasibility and applicability of radio-guided techniques for patients with parathyroid hyperplasia are unclear.

Application: Image analysis of the parathyroid gland.

Route of Administration: [^{99m}Tc]-sestaMIBI (intravascular).

Dosage: [^{99m}Tc]-sestaMIBI (10 mCi).

Implementation: Evaluate the ability of [^{99m}Tc]-sestaMIBI to image the parathyroid gland in a manner suitable to guide "same-day" exploratory (radio-guided) parathyroidectomy in conditions of hyperparathyroidism. Using a gamma probe, intraoperative scanning is performed, looking for *in vivo* radionuclide counts > background to localize abnormal parathyroid glands. After excision, radionuclide counts of each *ex vivo* parathyroid gland are determined and expressed as a percentage of background counts.

Human Model: Cases of hyperparathyoridism subjected to neck exploration (n = 77 primary; n = 25 secondary/tertiary).

***Ex Vivo* Model:** Parathyroid glands (n = 180 resected hyperfunctioning parathyroid glands).

Interpretation and Analysis: In conditions of hyperparathyroidism, radio-guided surgery is a sensitive adjunct for the intraoperative localization of both adenomatous and hyperplastic glands. Some clinical investigations have reported essentially a 100% success rate in localizing enlarged parathyroid glands with a gamma probe (e.g., n = 180/180). It has also been shown that the "> 20% rule" for *ex vivo* counts not only applies to parathyroid adenomas but also to hyperplastic glands. Therefore, radio-guided resection is equally effective and informative for both adenomatous and hyperplastic glands.

Radio-guided parathyroidectomy is equally effective for both adenomatous and hyperplastic glands. Chen H, Mack E, Starling JR. Ann Surg 2003 Sep; 238 (3), pp. 332–7; discussion 337–8.

Pituitary Adenoma/Radio-Guided Resection *[^{99m}Technetium] ([^{99m}Tc])-SestaMIBI/ [Ultrasonography]*

Background Information: Clinical or subclinical hyperparathyroidism is 1 of the most common endocrine disorders. Excessive secretion of parathyroid hormone is most frequently caused by an adenoma of the parathyroid gland. Unsuccessful surgery with persistent hyperparathyroidism, due to inadequate preoperative or intraoperative localization, may be observed in about 10% of patients. The conventional surgical approach for resolution of parathyroid adenoma is bilateral neck exploration. Imaging-based-localization techniques include ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and scintigraphy.

Application: [^{99m}Tc]-sestaMIBI scintigraphy can be used to preoperatively localize parathyroid adenomas in a manner that greatly facilitates performing minimally-invasive parathyroidectomy (MIP) procedures. In MIP, the incision is small, dissection is minimal, postoperative pain is less, and hospital stays are shorter.

Route of Administration: [^{99m}Tc]-sestaMIBI (intravascular).

Implementation: Detection and characterization of parathyroid adenoma conditions.

Interpretation and Analysis: Parathyroid scintigraphy with [^{99m}Tc]-sestaMIBI is based on the principle that this radioactive tracer molecule has a relatively long radioactive retention time within the parathyroid compared to thyroid tissue. Because of the frequent association of parathyroid adenomas with nodular goiter, the optimal imaging combination is [^{99m}Tc]-sestaMIBI scintigraphy and ultrasonography. Different protocols have been used for [^{99m}Tc]-sestaMIBI parathyroid scintigraphy, depending on the institutional logistics and experience (classical dual-phase scintigraphy, various subtraction techniques in combination with radioiodine, or [^{99m}Tc]-pertechnetate).

Performing a minimally-invasive parathyroidectomy procedure is greatly aided by the use of a gamma probe to facilitate intraoperative guidance based on the *in vivo* detection of regional radioactivity counts detected after the injection of [^{99m}Tc]-sestaMIBI. Different protocols used for gamma-probe-guided MIP are designed as a function of different timing intervals for administration and doses of injected tracer. Gamma-probe-guided MIP is a very effective surgical approach for treating conditions of primary hyperparathyroidism due to a solitary parathyroid adenoma. The procedure is technically easy, safe with a low morbidity rate, and has better cosmetic results and lower overall cost than conventional bilateral neck exploration. Specific guidelines should be followed when selecting cases for gamma-probe-guided MIP.

Preoperative localization and radio-guided parathyroid surgery. Mariani G, Gulec SA, Rubello D, Boni G, Puccini M, Pelizzo MR, Manca G, Casara D, Sotti G, Erba P, Volterrani D, Giuliano AE. J Nucl Med 2003 Sep; 44 (9), pp. 1443–58.

Proliferation of Neoplastic Cells/DNA Synthesis/ Thymidine Kinase Expression
5-Iodo-4′-Thio-2′-Deoxyuridine (ITdU)

Cellular/Molecular Mechanism of Action: 5-iodo-4′-thio-2′-deoxyuridine is a metabolically stable nucleoside that can function as a substrate for cellular thymidine kinase biochemical activity.
Application: Radioiodinated forms of ITdU and 5-iodo-2′-deoxyuridine (IUdR) can be applied to detect elevations in DNA synthesis in cell populations that positively express thymidine kinase activity. In this capacity, both ITdU and IUdR can serve as indicator molecules for measuring alterations in cellular proliferation especially relevant to the characterization of neoplastic disease states.
Route of Administration: Usually ITdU or IUdR would most frequently be administered intravenously/intravascularly. Theoretically, it may be possible to effectively image lesions if the agents are administered intrathecally for central nervous system (CNS) tumors, by direct injection into tumors, or into the arterial network supplying a suspected neoplastic lesion.
Semi-Synthetic Methods: Radiolabeling of ITdU and 5-iodo-1-(4-thio-beta-D-arabinofuranosyl)uracil (ITAU) with [^{125}I] utilizing a destannylation reaction of the trimethylstannyl precursor for each nucleoside. The products can be isolated in high yields and with a $>$ 99% level of radiochemical purity.
Implementation: Evaluation of ITdU and ITAU for their ability to be synthetically incorporated into nuclear DNA and serve as a marker for cellular mitosis/proliferation.

***Ex Vivo* Model:** Thymidine kinase-expressing L-M cells and thymidine kinase-deficient mutant L-M (TK$^-$) cells.
Interpretation and Analysis: [^{125}I]-ITdU is effectively phosphorylated by cytosolic nucleoside kinases and specifically incorporated into a thymidine kinase-expressing L-M cell rather than a thymidine kinase-deficient mutant L-M (TK$^-$) cell. In addition, *in vitro* cell metabolism studies employing [^{125}I]-ITdU reveal that [^{125}I]-ITdU is effectively and specifically incorporated into a DNA fraction ($>$ 90% at 60 minutes). Therefore, [^{125}I]-ITdU appears to be an effective DNA synthesis marker. In contrast, [^{125}I]-ITAU is neither remarkably phosphorylated by cytosolic nucleoside kinases nor notably incorporated into an L-M cell compared to L-M (TK$^-$) cell. [^{125}I]-ITdU and [^{125}I]-ITAU show a higher resistance to phosphorolytic cleavage by recombinant thymidine phosphorylase than does [^{125}I]-IUdR. Furthermore, biodistribution of [^{125}I]-ITdU and [^{125}I]-ITAU display better *in vivo* stability of radioiodination than do [^{125}I]-IudR preparations. [^{125}I]-ITdU also displays a significantly higher uptake in proliferating organs (thymus, spleen, small intestine, and bone) than in non-proliferating organs (brain, muscle, liver, and lung), as does [^{125}I]-IUdR, at 18 hours postinjection. As indicated by *in vitro* studies, [^{125}I]-ITAU does not show any significant uptake in proliferating organs. Therefore, radioiodinated ITdU is potentially useful as a proliferation-imaging agent, and further studies should clarify the usefulness of this compound as a SPECT tumor-imaging agent.

Rationale of 5-[^{125}I]-iodo-4′-thio-2′-deoxyuridine as a potential iodinated proliferation marker. Toyohara J, Hayashi A, Sato M, Tanaka H, Haraguchi K, Yoshimura Y, Yonekura Y, Fujibayashi Y. J Nucl Med 2002 Sep; 43 (9), pp. 1218–26.

Prostatic Carcinoma/"Enhanced Permeability and Retention" (EPR) Effect/Human Prostatic Carcinoma Transplants (Rat): Tumor-Specific Delivery Modality
[^{131}I]-[(N-2-(Hydroxypropyl)Methacrylamide] (HPMA)

Route of Administration: Intravenous.
Dosage: Equimolar concentrations of radioiodinated copolymers of HPMA with small amounts of methacryloyltyrosinamide (pHPMA) differing in molecular weight (23.4-kD, 27.3-kD, 30.5-kD, 44-kD, 58.4-kD, 60.1-kD).
Implementation: Investigations have described the optimal design of polymers intended for tumor detection and drug delivery based on the initial delineation of the fate and total body distribution of soluble synthetic macromolecules derived from copolymers of HPMA. Evaluations can be performed by monitoring scintigraphic images acquired following radiolabeling of the product with [^{131}I] during a 7-day time window. Scintigraphic data can be validated by determining absolute amounts of [^{131}I]-pHPMA in both tumor tissue and normal organs after sacrificing animals.

Animal Model: Detection of Dunning prostate carcinomas (subline R3327-AT1) transplanted into Copenhagen rats.
Interpretation and Analysis: Copolymers are cleared from the intravascular compartment in a molecular-weight dependent manner, either via excretion or by extravasation into normal and neoplastic tissues. While distribution patterns for pHPMAs in normal organs are quite similar, absolute amounts of copolymer uptake differ. The higher the molecular weight, the more radioactivity is taken up by the organs. Highest amounts of radioactivity are seen in the lung, liver, and spleen. In solid tumors, the kinetics of pHPMA accumulation is clearly dependent on molecular weight. pHPMAs below the renal threshold peaks at 24 hours postinjection and then remains constant. In contrast, copolymers above the renal clearance threshold display a continuous accumulation, reaching a significantly higher tumor uptake, presumably due to the very small or non-existent polymer release from tumor tissue. Absolute amounts of tumor uptake determined by dissection analysis are 0.5 $\pm$ 0.1% of injected dose/g tissue for the 27.3-kD pHPMA and 1.2 $\pm$ 0.1% for the 60.1-kD pHPMA, respectively.

In conclusion, laboratory results have demonstrated the influence of the molecular weight of the synthetic polymer pHPMA on plasma circulation time, excretion, and organ clearance. While pHPMAs are cleared from all normal tissues except the spleen quite effectively, these polymers accumulate in solid tumors in a size-dependent manner, due to the well-known EPR effect. Such information is of fundamental interest for ongoing studies on the pharmacokinetics of synthetic polymers, especially when these molecules are conjugated with targeting moieties and therapeutic or diagnostic agents.

Synthetic macromolecular drug carriers: Biodistribution of poly[(N-2-hydroxypropyl)methacrylamide] copolymers and their accumulation in solid rat tumors. Kissel M, Peschke P, Subr V, Ulbrich K, Schuhmacher J, Debus J, Friedrich E. PDA J Pharm Sci Technol 2001 May–Jun; 55 (3), pp. 191–201.

Prostatic Carcinoma/Gene Expression Imaging
[Gd]-Anti-Messenger RNA (mRNA) Oligonucleotide Strand/Trans-Membrane Carrier Peptide

Cellular/Molecular Mechanism of Action: The spatial orientation of gadolinium changes when placed within a magnetic field. Trans-membrane carrier proteins are transported across cell membrane structures by specific membrane-associated complexes. Oligonucleotides can be designed as complementary sequences to specific mRNA strands and the resulting cDNA/mRNA double strand complexes thereby effectively preventing translation of the mRNA strand. Diagnostically, complementary cDNA strands would theoretically accumulate intracellularly within cells that are actively transcribing the corresponding mRNA sequences that cDNA strands have been designed to recognize and physically bind to.

Background Information: The term "molecular imaging" can be broadly defined as the *in vivo* characterization and measurement of biological processes at the cellular and molecular level.
Application: Extrapolation of the concept and methodologies for the design of strategies for the diagnostic detection of *in vivo* gene expression. Example: Specific oncogene expression within neoplastic cell types.
Route of Administration: Theoretically intravenous would be the preferred method of delivery.
Semi-Synthetic Methods: Development of a novel intravital and intracellular contrast agent that is composed of a detection molecule, an oligonucleotide sequence (peptide nucleic acid [PNA]), and a trans-membrane carrier peptide that is composed of a peptide sequence similar to that of the homeodomain of the Antennapedia protein.
Implementation: Determine if a (i) labeled oligonucleotide complex can accumulate in tumor cells *in vitro* and *in vivo*; and (ii) whether the specificity of a PNA for the upregulated c-myc mRNA in the cell's cytoplasm has an effect on contrast agent retention within tumor cells.
Tissue Culture Model: HeLa cell lines.
Animal Model: Rat (Dunning R3327 AT1 rat prostate adenocarcinoma).
Interpretation and Analysis: Using the c-myc-specific and a c-myc-non-specific control PNA, an increase in signal intensity in the tumor cells can be observed after 10 minutes *in vitro* and *in vivo* (maximum reached *in vitro* in HeLa within 60 minutes compared to Dunning R3327 AT1 rat prostate adenocarcinoma cells *in vivo* within 30 minutes). Such increases of signal intensity can be maintained *in vitro* in HeLa cells for only 4 hours and in Dunning R3327 AT1 rat prostate adenocarcinoma cells *in vivo* at least for 5 hours by using the c-myc mRNA-specific PNA as a "retention" agent.

Intracellular visualization of prostate cancer using magnetic resonance imaging. Heckl S, Pipkorn R, Waldeck W, Spring H, Jenne J, von der Lieth CW, Corban-Wilhelm H, Debus J, Braun K. Cancer Res 2003 Aug 15; 63 (16), pp. 4766–72.

Pulmonary Carcinoma
N-[2-(Hydroxyethoxy)Methyl]-5-[^{3}H]Methyluracil (^{3}H-1)

Application: Unsuitable for diagnostic imaging of neoplastic disease states.
Semi-Synthetic Methods: The production of tritium-labeled acyclonucleoside N-[2-(hydroxyethoxy)methyl]-5-[^{3}H]methyluracil ([^{3}H]-3) can be synthesized from the precursor, 5-[^{3}H]-methyluracil, [^{3}H]-1, which is subsequently converted to a 2,4-bis-trimethylsilyl intermediate, which is then coupled with 2-acetoxyethoxymethyl bromide to yield 1-[(2-acetoxyethoxy)methyl-5-[^{3}H]methyluracil ([^{3}H]-2). Treatment of [^{3}H]-2 with sodium methoxide in methanol produces [^{3}H]-3 (specific activity 188 MBq mmol^{-1}).
Implementation: Evaluation of the tissue distribution of ^{3}H-3.
Animal Model: Murine (male BDF1 mice bearing Lewis lung carcinomas).
Interpretation and Analysis: The tissue distribution of [^{3}H]-3 reveals that long bones exhibit the highest tumor:tissue ratios. The kidney contains the highest radioactivity level relative to the tumor, suggesting a major urinary route of excretion. The primary radioactive blood component (89.21%) has a biological half-life of approximately 0.19 minutes. The candidate compound is unsuitable for use as a diagnostic agent for Lewis lung carcinoma because of low tumor uptake and rapid urinary elimination of injected radioactivity from the body.

Tumor uptake of radiolabelled pyrimidine bases and pyrimidine nucleosides in animal models-VIII. Synthesis and tissue distribution of N-[2-(hydroxyethoxy) methyl]-5-[3H]methyluracil. Lee YW, Iwashina T, Gati WP, Knaus EE, Wiebe LI. Int J Appl Radiat Isot 1985 May; 36 (5), pp. 395–8.

Renal Carcinoma/Associated Unique Antigen
[^{99m}Technetium] ([^{99m}Tc])-G250/Antigen-Specific Immunoglobulin

Cellular/Molecular Mechanism of Action: Immunoglobulins specifically designed to selectively interact with and bind to unique membrane-associated antigens expressed by renal carcinoma cell types.
Route of Administration: Most frequently given by intravenous infusion.
Considerations: Not all monoclonal antibody preparations are effective in their ability to selectively detect neoplastic lesions, and therefore their efficacy has to be predetermined (prescreened) prior to their application.
Interpretation and Analysis: Perfusion of tumor-bearing kidneys with [^{99m}Tc]-labeled G250 and RC38 antibody can be used to effectively visualize lesions of renal cell carcinoma following formation of Ag/Ab complexes with renal cell carcinoma-associated antigens. Superior preparations display minimal uptake in normal renal tissues (e.g., tumor-to-kidney ratios after perfusion = 2.2 to 2.7). Following rinsing for 3 hours with unlabeled perfusion fluid the tumor-to-kidney tissue ratios can increase from 2.7 to 8.6. Based on observations from preliminary investigations, it has been concluded that perfusion of tumor-bearing human kidneys with radiolabeled monoclonal antibodies is a relatively simple way to evaluate renal cell carcinoma-associated monoclonal antibodies as *in vivo* diagnostic agents.

Perfusion of tumor-bearing kidneys as a model for scintigraphic screening of monoclonal antibodies. van Dijk J, Oosterwijk E, van Kroonenburgh MJ, Jonas U, Fleuren GJ, Pauwels EK, Warnaar SO. J Nucl Med 1988 Jun; 29 (6), pp. 1078–82.

Renal Cell Carcinoma (RCC)/Associated Unique Antigens
Radiolabeled G250 Monoclonal Antibody (MAb)

Cellular/Molecular Mechanism of Action: Monoclonal antibodies can be designed to recognize and physically bind to membrane-associated antigens uniquely expressed by RCC.
Background Information: Excellent visualization of tumor lesions can be achieved with [^{131}I]-labeled MAb G250 in cases with RCC. In several cases, [^{131}I]-cG250 immunoscintigraphy can detect tumor lesions that cannot be visualized by radiography or computed tomography (CT).
Application: Improved detection and characterization of RCC lesions through the development of [^{99m}Tc]-labeled MAb G250 preparation and the acquisition of scintigraphic images with increased sensitivity and specificity.
Semi-Synthetic Methods: The G250 MAb can be labeled with [^{99m}Tc] according to 3 methods using (i) S-hydrazinonicotinamide (HYNIC); (ii) S-benzoylmercaptoacetyltriglycine (MAG3); and (iii) a direct labeling method (Schwarz method).
Implementation: Evaluation of the *in vitro* stability, biodistribution, and imaging potential of 3 [^{99m}Tc]-labeled G250 preparations to detect *in vivo* xenographs of RCC. [^{125}I]-G250 and the non-specific MAb [^{131}I]-MN14 can be used as reference control antibodies. Stability of radiolabeled monoclonal antibody preparations can be tested in serum at 37°C over the course of a 48-hour incubation peri-

od. In addition, diethylenetriamine pentaacetic acid, cysteine, and glutathione challenge assays can be performed to acquire supplemental information.

Animal Model: Murine (nude mice with subcutaneous RCC xenografts).

Interpretation and Analysis: All preparations show good stability in serum during 48-hour incubation periods. [^{99m}Tc]-G250 (Schwarz) displays release of the radiolabel at a 100-fold or higher molar excess of cysteine and at a 10,000-fold or higher molar excess of glutathione. [^{99m}Tc]-MAG3-G250 shows release of the radiolabel at a 10,000-fold molar excess of cysteine. [^{99m}Tc]-HYNIC-G250 is stable under all conditions. Tumors can be clearly visualized with all preparations. [^{99m}Tc]-G250 (Schwarz) shows significantly lower blood levels (3.8 % injected dose per gram [%ID/g]) compared with all other preparations (11.2, 13.4, and 13.4 %ID/g for [^{99m}Tc]-HYNIC-G250, [^{99m}Tc]-MAG3-G250, and [^{125}I]-G250, respectively, 48 hours postinjection). At 48 hours postinjection, mean tumor uptake is very high with all G250 MAb preparations: (92.4 = [^{99m}Tc]-HYNIC-G250; 125.9 = [^{99m}Tc]-MAG3-G250; 29.4 = [^{99m}Tc]-G250 Schwarz; and 75.4 = [^{125}I]-G250 %ID/g). Mean tumor uptake of the non-specific [^{131}I]-MN14 MAb is 6.6 %ID/g. Therefore, [^{99m}Tc]-HYNIC-G250 shows excellent *in vitro* stability and tumor targeting. Moreover, this preparation can be labeled with high efficiency (> 95%) at room temperature within 15 minutes. [^{99m}Tc]-HYNIC-G250 seems to be an ideal candidate for radioimmunodetection of RCC.

In vivo and *in vitro* characterizations of 3 [^{99m}Tc]-labeled monoclonal antibody G250 preparations. Steffens MG, Oosterwijk E, Kranenborg MH, Manders JM, Debruyne FM, Corstens FH, Boerman OC. J Nucl Med 1999 May; 40 (5), pp. 829–36.

Renal Cell Carcinoma (RCC)/Metastatic *[^{111}Indium] ([^{111}In])-Labeled Octreotide*

Cellular/Molecular Mechanism of Action: [^{111}In]-labeled octreotide is a radioactive somatostatin analogue that recognizes and binds to its corresponding receptor complex.

Application: Detection and characterization of RCC.

Route of Administration: [^{111}In]-labeled octreotide (intravascular).

Implementation: Evaluation of the biodistribution of labeled octreotide radioactivity in scintigraphic scans (n = 11) and images correlates with diagnostic compted tomography (CT) and/or x-ray images.

Interpretation and Analysis: Positive tumor uptake can be observed in a number of cases (e.g., n = 9/68). Approximately 60% of known tumor lesions can be visualized with octreotide scan (e.g., n = 40/68). Acquisition of a 2nd scan can be applied to detect progression of disease and/or lack of response to therapeutic intervention characterized by scintigraphic images that display intensified lesion octreotide uptake. False-positive lesions (e.g., n = 4) can be observed in a relatively large number of octreotide scintigraphic images. Renal cell carcinoma expresses somatostatin receptors and can be visualized by radiolabeled octreotide scintigraphy. The scintigraphic technique can be used as an instrument for *in vivo* characterization of the disease. Such diagnostic methodologies could function as the basis for future investigations regarding the possible therapeutic effect of octreotide in the management of renal cell cancer.

[^{111}In-DTPA-D-Phe$_1$]-octreotide scintigraphy in the management of patients with advanced renal cell carcinoma. Edgren M, Westlin JE, Kälkner KM, Sundin A, Nilsson S. Cancer Biother Radiopharm 1999 Feb; 14 (1), pp. 59–64.

Sigma$_2$ Receptor Complexes (Sigma$_2$-Opioid)/ Mammary Adenocarcinoma *[^{99m}Technetium] ([^{99m}Tc])-Sigma$_2$ Receptor Ligand (Racemic Formulations) / Rehnium-2 (Re-2)/ Pentazocine/Haloperidol*

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-labeled [(N-[2-((3′-N′-propyl-[3,3,1]aza-bicyclononan-3α yl)(2′′-methoxy-5-methyl-phenylcarbamate)(2-mercaptoethyl)amino)acetyl]-2-aminoethanethiolato] technetium$_V$ oxide), [^{99m}Tc]$_2$, displays specific binding avidity toward sigma$_2$ receptor complexes (sigma-opioid). Note: Pentazocine is an agonist at κ-opioid receptors and a weak agonist at μ-opioid receptors. In this context, it is classified as a mixed opioid because it functions as an agonist at some opioid receptors and an antagonist at others.

Application: Detection and characterization of neoplastic disease states associated with the expression of sigma$_2$-opioid receptor complexes (e.g., human mammary carcinoma).

Route of Administration: Intravenous.

Semi-Synthetic Methods: Preparation of [^{99m}Tc]$_2$ can be achieved by heating the S-protected starting material, 1, in the presence of acid, a reducing agent (stannous glucoheptonate) and sodium [^{99m}Tc]pertechnetate. The lipophilic racemic mixture can be successfully prepared at a 10–50% yield and a radiochemical purity of approximately 98%. Separation of the isomers, peak A and peak B, can be successfully achieved by using a chiralpak AD column eluted with an isocratic solvent (n-hexane/isopropanol; 3:1; v/v). The peak A and peak B appear to co-elute with the isomers of the surrogate, Re-2, under the same high-performance liquid chromatography (HPLC) condition.

Implementation: Evaluate the *in vitro* binding avidity and selectivity of [^{99m}Tc]$_2$ and Re-2 for sigma$_2$ receptors. Further delineation of the potential diagnostic efficacy of the molecular probes can be determined by performing biodistribution studies utilizing *in vivo* tumor-bearing models.

Animal Model: Murine (mouse mammary adenocarcinoma, cell line 66 known to overexpress sigma$_2$ receptor complexes).

Interpretation and Analysis: *In vitro* binding assays show that Re-2 displays excellent binding affinity and selectivity toward sigma$_2$ receptors (K_i = 2,723 and 22 nM for sigma$_1$ and sigma$_2$ receptor, respectively). Biodistribution studies in tumor-bearing mice (mammary adenocarcinoma) reveal that racemic [^{99m}Tc]$_2$ localizes within tumors. Uptake in the tumor is 2.11, 1.30, and 1.11% dose/gram at 1, 4, and 8 hours postinjection, respectively, suggesting good uptake and retention within tumor cells. Tumor uptake is significantly, but incompletely, blocked (about 25–30% blockage) by co-injection of "cold" (+)pentazocine or haloperidol (1 mg/kg). A majority of the radioactivity localized within the tumor tissue can be extracted (> 60%), and HPLC analysis reveals that it is the original compound (racemic [^{99m}Tc]$_2$ purity ≥ 98%). Distribution of purified HPLC peak A and HPLC peak B can be detected in the same tumor-bearing mice at 4 hours postinjection. Tumor uptake is similar for both isomers, but the blood and peripheral tissue content for the isomer in peak B is higher than that for the isomer in peak A. It is evident that the isomer in peak A possesses significantly better tumor/blood and tumor/muscle ratios. The higher rate of *in vivo* metabolism can be confirmed by the higher thyroid uptake values for the peak B isomer when compared to the peak A isomer. Therefore, the [^{99m}Tc]-labeled sigma receptor imaging agent [^{99m}Tc]$_2$ has demonstrated the feasibility of using [^{99m}Tc]-labeled agents for imaging tumor cell expression of sigma receptor complexes.

Development of a [^{99m}Tc]-labeled sigma$_2$ receptor-specific ligand as a potential breast tumor-imaging agent. Choi SR, Yang B, Plossl K, Chumpradit S,

Wey SP, Acton PD, Wheeler K, Mach RH, Kung HF. Nucl Med Biol 2001 Aug; 28 (6), pp. 657–66.

Somatostatin Receptor Complexes/Neuroendocrine Cancers/Gliomas/Mammary Carcinoma/Melanoma/Small Cell Lung Cancer

[^{99m}Technetium] ([^{99m}Tc])-Tricine-(HYNIC-TOC)/[^{99m}Tc]-Ethylenediaminediacetic Acid-(HYNIC-TOC)/[^{99m}Tc]-Tricine-Nicotinic Acid-(HYNIC-TOC)/[^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)-Octreotide/[^{111}In]-DTPA-D-Phe$_1$-Octreotide

Cellular/Molecular Mechanism of Action: Recognizes and physically binds to somatostatin receptor complexes.

Background Information: Indium-111-labeled DTPA-D-Phe$_1$-octreotide ([^{111}In]-DTPA-OC, OctreoScan) has been introduced into clinical routine for the detection of somatostatin receptor (SSTR)-positive tumors, which are predominantly of neuroendocrine origin. Other potential applications in other SSTR-positive cancers (e.g., small cell lung cancer, breast cancer, melanoma) have been limited mainly due to restricted availability and high cost of suitable radionuclides. Previous attempts to introduce [^{99m}Tc]-labeled analogues of octreotide have not been very successful in terms of labeling technique, *in vivo* biodistribution, and/or tumor detection capabilities.

Application: Early, rapid image-based diagnosis of somatostatin receptor positive neoplastic lesions.

Route of Administration: [^{99m}Tc]-HYNIC-D-Phe$_1$-Tyr$_3$-octreotide and [^{111}In]-DTPA-octreotide (intravascular).

Semi-Synthetic Methods: Production of high-specific activity [^{99m}Tc] radiolabeled hydrazinonicotinyl-Tyr$_3$-octreotide (HYNIC-TOC) using tricine, ethylenediaminediacetic acid (EDDA), and tricine-nicotinic acid as coligand systems.

Implementation: Evaluation of the *in vitro* properties and *in vivo* biodistribution of [^{99m}Tc]-tricine-HYNIC-TOC, [^{99m}Tc]-EDDA-HYNIC-TOC, and [^{99m}Tc]-tricine-nicotinic acid-HYNIC-TOC compared to [^{111}In]-DTPA-octreotide. Receptor binding analyses can be performed using AR42J rat pancreatic tumor cell membranes. Complementary investigations can involve the characterization of internalization processes and protein binding studies performed, in addition to establishing *in vivo* biodistribution profiles within pancreatic tumors.

***Ex Vivo* Model:** Rat AR42J pancreatic tumor cell membrane receptor binding analyses.

Animal Model: Murine (AR42J pancreatic tumor-bearing nude mice).

Interpretation and Analysis: The [^{99m}Tc]-labeled HYNIC peptides possess retained somatostatin receptor binding affinities (Kd $<$ 2.65 nM). Protein binding and internalization rates are dependent on the coligand used. Specific tumor uptake between 5.8% and 9.6% of the injected dose per gram (%ID/g) can be appreciated for the [^{99m}Tc]-labeled peptides, compared with 4.3 %ID/g for [^{111}In]-DTPA-octreotide. Tricine applied as a coligand shows higher activity levels in muscle, blood, and liver, whereas tricine-nicotinic acid produces significant levels of activity in the gastrointestinal tract. EDDA displays the most promising overall biodistribution profile, with tumor-to-liver and tumor-to-gastrointestinal tract ratios similar to those obtained with [^{111}In]-DTPA-octreotide, lower ratios in blood and muscle, but considerably higher tumor-to-kidney ratios. Molecular TOC can be radiolabeled to high specific activities using HYNIC as a bifunctional chelator. The high specific tumor uptake, rapid blood clearance, and predominantly renal excretion make [^{99m}Tc]-EDDA-HYNIC-TOC a promising candidate as an alternative to [^{111}In]-DTPA-octreotide for tumor imaging.

[^{99m}Tc]-HYNIC-[Tyr$_3$]-octreotide for imaging somatostatin-receptor-positive tumors: Preclinical evaluation and comparison with [^{111}In]-octreotide. Decristoforo C, Melendez-Alafort L, Sosabowski JK, Mather SJ. J Nucl Med 2000 Jun; 41 (6), pp. 1114–9.

Somatostatin Receptor Complexes/Neuroendocrine Tumors/Gliomas/Mammary Carcinoma/Melanoma/Small Cell Lung Cancer

[^{99m}Technetium] ([^{99m}Tc])-Tricine-HYNIC-TOC/[^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)-D-Phe$_1$-Octreotide

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-tricine-HYNIC-TOC detects and physically binds to somatostatin receptor complexes in a manner highly analogous to [^{111}In]-DTPA-D-Phe$_1$-octreotide.

Background Information: [^{111}In]-labeled DTPA-D-Phe$_1$-octreotide (DTPA-OC, OctreoScan) has been introduced into clinical routine for the detection of somatostatin receptor (SSTR)-positive tumors, which are predominantly of neuroendocrine origin. Other potential applications in other SSTR-positive cancers (e.g., small cell lung cancer, breast cancer, melanoma) have been limited mainly by restricted availability and high cost of suitable radionuclides. Previous attempts to introduce [^{99m}Tc]-labeled analogues of octreotide have not been very successful in terms of the labeling procedure, *in vivo* biodistribution, and/or tumor detection capabilities.

Application: The agent [^{99m}Tc]-HYNIC-D-Phe$_1$-Tyr$_3$-octreotide or [^{99m}Tc]-tricine-HYNIC-TOC can be utilized to detect and characterize neoplastic cell types that express elevated membrane densities of somatostatin receptor complexes (similar to [^{111}In]-DTPA-OC). [^{99m}Tc]-HYNIC-D-Phe$_1$-Tyr$_3$-octreotide appears to provide more rapid (early) image acquisition of SSTR-positive neoplastic lesions.

Route of Administration: [^{99m}Tc]-HYNIC-D-Phe$_1$-Tyr$_3$-octreotide and [^{111}In]-DTPA-octreotide (intravascular).

Implementation: Evaluation of neoplastic lesions proven to be positive for SSTR expression utilizing both the [^{99m}Tc]-labeled analogue HYNIC-D-Phe$_1$-Tyr$_3$-octreotide (HYNIC-TOC = tricine as a coligand) and [^{111}In]-DTPA-octreotide. Serial whole-body scans, spot views, and/or single photon emission tomography studies are then performed and images qualitatively and semi-quantitatively evaluated (e.g., selective regions-of-interest [ROI]).

Human Model: Cases with proven SSTR-positive neoplastic lesions (n = 13 patient scans; n = 12 SSTR-positive tumors; n = 9/13 scanned with both [^{99m}Tc]-tricine-HYNIC-TOC and [^{111}In]-DTPA-OC).

Interpretation and Analysis: Delineation of the biodistribution of [^{99m}Tc]-tricine-HYNIC-TOC reveals high physiological uptake within the kidney, moderate uptake in the liver and spleen, and little uptake within the gastrointestinal system. The tracer shows predominantly renal and negligible hepatobiliary excretion. Known SSTR-positive tumor sites show rapid and intense tracer accumulation. [^{99m}Tc]-tricine-HYNIC-TOC is rapidly taken up by tissues within the 1st hour after injection and has basically no significant clearance ($<$ 20%) from normal or tumor tissue thereafter. Alternatively, [^{111}In]-DTPA-OC shows continuous clearance from normal tissues as well as renal and very little hepatobiliary excretion. Nevertheless, patterns of accumulation of [^{99m}Tc]-tricine-HYNIC-TOC in tumors and normal organs are comparable to those for [^{111}In]-DTPA-OC. Comparison on a lesion-by-lesion basis reveals similar tumor detection capabilities in intrahepatic tumor sites and superior capabilities

of [^{99m}Tc]-tricine-HYNIC-TOC with respect to extrahepatic lesions. In conclusion, [^{99m}Tc]-tricine-HYNIC-TOC shows promise as a tracer for SSTR imaging, given its favorable clinical characteristics (specific and high receptor affinity, good biodistribution, renal excretion, low radiation exposure, high imaging quality), on-demand availability, and cost-effectiveness. [^{99m}Tc]-tricine-HYNIC-TOC allows earlier diagnosis (10 minutes–4 hours) compared with [^{111}In]-DTPA-OC (4–24 hours).

Detection of somatostatin receptor-positive tumors using the new [^{99m}Tc]-tricine-HYNIC-D-Phe$_1$-Tyr$_3$-octreotide: First results in patients and comparison with [^{111}In]-DTPA-D-Phe$_1$-octreotide. Bangard M, Béhé M, Guhlke S, Otte R, Bender H, Maecke HR, Biersack HJ. Eur J Nucl Med 2000 Jun; 27 (6), pp. 628–37.

Somatostatin Receptors/Renal Clearance: Strategies for Modification
[^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)-Met(1)-Octreotide/[^{111}In]-DTPA-Phe$_1$-Octreotide

Cellular/Molecular Mechanism of Action: Octreotide and lanreotide are somatostatin derivatives that specifically bind to somatostatin receptors.
Background Information: Metabolic studies employing [^{111}In]-DTPA-labeled peptides and polypeptides show that the radiolabeled (poly)peptides generate [^{111}In]-DTPA adducts of amino acid that possess long residence times in the lysosomal compartment of tissues where (poly)peptides accumulate. However, some investigations have suggested that metal-chelate-methionine (Met) might possess *in vivo* behaviors different from metal-chelate adducts of other amino acids.
Application: Octreotide or lanreotide are the most frequently used radiopharmaceuticals for detection and characterization of certain neoplastic cell types that express relatively high membrane densities of somatostatin receptors. Different molecular components can be used to modify the rate of renal clearance for biopharmaceutical conjugates containing octreotide or lanreotide.
Semi-Synthetic Methods: [^{111}In]-DTPA-Met(1)-octreotide.
Implementation: Elucidate some of the biological characteristics of Met to determine if they may accelerate the renal elimination rate of [^{111}In]-DTPA-Met into the urine (when using [^{111}In]-DTPA-Met(1)-octreotide as an imaging agent) and compare findings to [^{111}In]-DTPA-L-Phe$_1$-octreotide functioning as a reference control.
Interpretation and Analysis: Both [^{111}In]-DTPA-conjugated octreotide analogues are stable during 3-hour incubation periods in murine serum (37°C). Both [^{111}In]-DTPA-octreotide analogues show rapid radioactive clearance from the blood and similar accumulation of radioactivity in the kidney. No significant differences can be observed between the 2 agents pertaining to renal radioactivity levels from 10 minutes to 24 hours postinjection. Metabolic studies indicate that [^{111}In]-DTPA-Met(1)-octreotide and [^{111}In]-L-Phe$_1$-octreotide generate [^{111}In]-DTPA adducts of Met and Phe, respectively, as the final radiometabolites at similar rates. These findings suggest that the long residence times of tissue radioactivity after administration of [^{111}In]-DTPA-labeled peptides and polypeptides can be attributed to the inherent characteristics of the [^{111}In]-DTPA chelation group.

Significance of [^{111}In]-DTPA chelate in renal radioactivity levels of [^{111}In]-DTPA-conjugated peptides. Akizawa H, Arano Y, Mifune M, Iwado A, Saito Y, Uehara T, Ono M, Fujioka Y, Ogawa K, Kiso Y, Saji H. Nucl Med Biol 2001 May; 28 (4), pp. 459–68.

Somatostatin Receptors/Staging of Malignancy/Predictive Response to Somatostatin-Based Therapeutics/Prognostic/Differential Diagnostic Marker
[^{123}I]-Tyr$_3$-Octreotide/[^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)-D-Phe$_1$-Octreotide

Cellular/Molecular Mechanism of Action: Octreotide functions as a peptide analogue of somatostatin.
Background Information: Octreotides plays an important role in neoplasia because their physiological properties are mediated through specific somatostatin receptor complexes expressed by neoplastic cell populations. Analysis of somatostatin receptors *in vitro* reveals that they are expressed at relatively high densities by various type of human neoplasias and serves as the experimental basis for the development of somatostatin analogues as non-invasive *in vivo* imaging agents. Relevant examples in this regard include the development of [^{123}I]-Tyr$_3$-octreotide and [^{111}In]-DTPA-D-Phe$_1$-octreotide to facilitate gamma camera scintigraphic imaging masses suspected of being tumors that express somatostatin receptor complexes at relatively high membrane densities.
Application: Non-invasive *in vivo* detection and imaging of neoplastic disease states.
Route of Administration: [^{123}I]-Tyr$_3$-octreotide (intravenous) and [^{111}In]-DTPA-D-Phe$_1$-octreotide (intravenous).
Interpretation and Analysis: Lesion uptake of imaging agent above background levels for normal healthy tissue represents a possible positive detection of a neoplastic lesion. The somatostatin receptor imaging strategy may help localize primary tumors and metastases, sufficient for staging certain tumors types, to predict the probability of success with somatostatin-based therapies and as a prognostic or differential diagnostic disease marker. It may also be of use in other pathological disease states for localization of selected inflammatory processes and to monitor anti-inflammatory therapy. Somatostatin receptor imaging represents an example of the clinical use of a small peptide as an efficient *in vivo* diagnostic tool.
Instrumentation: Gamma camera scintigraphic imaging modalities.

Receptor imaging of human diseases using radiolabeled peptides. Reubi JC, Lamberts SJ, Krenning EP. J Recept Signal Transduct Res 1995 Jan–Mar; 15 (1–4), pp. 379–92.

Somatostatin Receptors: Subtype 2 (sst2)/Pituitary Neoplasia/Pancreatic Neoplasia/Adrenal Neoplasia
[^{111}Indium] ([^{111}In])-(DTPA0,Tyr$_3$)-Octreotide/[^{111}In]-(DOTA0,Tyr$_3$)-Octreotide/[^{111}In]-(DTPA0)-Octreotide

Cellular/Molecular Mechanism of Action: Possess physical binding avidity for sst2 complexes.
Application: Detection and characterization of pituitary, pancreatic, and adrenal neoplastic lesions that express an overabundance of sst2 complexes.
Implementation: Evaluate the potential usefulness of radiolabeled (DTPA0,Tyr$_3$)-octreotide and (DOTA0,Tyr$_3$)-octreotide as radiobiopharmaceuticals for somatostatin receptor-targeted scintigraphy and radiotherapy. Detailed analysis can entail the characterization of *in vitro* somatostatin receptor binding complemented by determination of the *in vivo* patterns of metabolism of agents compared to [^{111}In]-DTPA0-octreotide.
Animal Model: Rats (CA20948 tumor-bearing Lewis rats).

Interpretation and Analysis: Comparisons made between different peptide-chelator constructs reveal that (DTPA0,Tyr_3)-octreotide and (DOTA0,Tyr_3)-octreotide have a higher affinity than (DTPA0)-octreotide for sst2 (e.g., mouse AtT20 pituitary tumor cell membranes with all IC_{50} values obtained in the low nanomolar range). Studies *in vivo* in CA20948 tumor-bearing Lewis rats reveal a significantly higher uptake of both [^{111}In]-labeled (DOTA0,Tyr_3)-octreotide and (DTPA0,Tyr_3)-octreotide in sst2-expressing tissues than after the injection of [^{111}In]-(DTPA0)-octreotide, showing that substitution of Tyr for Phe at position 3 in octreotide results in increased affinity for its receptor and in a higher target tissue uptake. Uptake of [^{111}In]-labeled (DTPA0)-octreotide, (DTPA0,Tyr_3)-octreotide, and (DOTA0,Tyr_3)-octreotide in pituitary, pancreas, adrenals, and tumor is decreased to less than 7% of control by pretreatment with unlabeled octreotide/rat (0.5 mg), reflecting specific binding to sst2 complexes. Comparing different radionuclides, [^{90}Y]-(DOTA0,Tyr_3)-octreotide has the highest uptake in sst2-positive organs, followed by the [^{111}In]-(DOTA0,Tyr_3)-octreotide, whereas (DOTA0,[^{125}I]-Try_3)-octreotide uptake is low compared to that of the other radiopharmaceuticals, when measured 24 hours after injection. Renal uptake of [^{111}In]-labeled (DTPA0)-octreotide, (DTPA0,Tyr_3)-octreotide and (DOTA0,Tyr_3)-octreotide is reduced over 50% by the intravenous injection of D-lysine (400 mg/kg), whereas radioactivity in blood, pancreas, and adrenals is not affected.

Preclinical comparison of (DTPA0)-octreotide, (DTPA0,Tyr_3)-octreotide, and (DOTA0,Tyr_3)-octreotide as carriers for somatostatin receptor-targeted scintigraphy and radionuclide therapy. De Jong M, Bakker WH, Breeman WA, Bernard BF, Hofland LJ, Visser TJ, Srinivasan A, Schmidt M, Béhé M, Mäcke HR, Krenning EP. Int J Cancer 1998 Jan 30; 75 (3), pp. 406–11.

Thyroid Cancer
[^{99m}Technetium] ([^{99m}Tc])-Methoxy-Isobutyl-Isonitrile (MIBI)

Background Information: [^{99m}Tc]-MIBI scintigraphy has recently been utilized in clinical scenarios for detecting thyroid cancer metastases. In this capacity, its role has been considered supplementary to serum thyroglobulin (Tg) measurements and radioactive iodine [^{131}I] whole-body scans (WBS).

Implementation: Evaluate the sensitivity and specificity of [^{99m}Tc]-MIBI scans to localize metastatic lesions in conditions of thyroidectomized thyroid cancer. Presence or absence of thyroid cancer can be determined in concert with other diagnostic modes, including serum Tg measurements, [^{131}I] WBS, bone scans, chest x-rays, computed tomography (CT), ultrasonography, histopathology, and evolution of disease assessed during follow-up examinations. All scans are evaluated on a lesion-to-lesion basis for the purpose of detecting neck, lung, and bone metastases and also on a region basis, namely head-neck, chest, and abdomen-pelvis-extremities (ab-p-ex) areas.

Human Model: Cases of thyroidectomized thyroid cancer (n = 68).

Interpretation and Analysis: Sensitivity of detection with [^{99m}Tc]-MIBI is approximately 94.4% (e.g., n = 17/18) for neck, 78.4% (e.g., n = 40/51) for lung, and 92.8% (e.g., n = 64/69) for skeletal lesions. Positive predictive values (PPV) and negative predictive values (NPV) are approximately 96.3% (e.g., n = 26/27) and 97.7% (e.g., n = 43/44) for head-neck; 94.7% (e.g., n = 71/75) and 50.0% (e.g., n = 12/24) for chest; and 100.0% (e.g., n = 25/25) and 93.1% (e.g., n = 54/58) for ab-p-ex regions, respectively. For all scan sites taken together, PPV and NPV are approximately 96.1% (e.g., n = 122/127) and 86.5% (e.g., n = 109/126), respectively. Therefore, [^{99m}Tc]-MIBI can be proposed as a 1st-line diagnostic agent for the follow-up protocol of thyroid cancer patients, although the ability to detect small lung metastases is somewhat limited.

Diagnostic value of [^{99m}technetium]-methoxyisobutyl isonitrile ([^{99m}Tc]-MIBI) scintigraphy in detecting thyroid cancer metastases: A critical evaluation. Alam MS, Kasagi K, Misaki T, Miyamoto S, Iwata M, Iida Y, Konishi J. Thyroid 1998 Dec; 8 (12), pp. 1091–100.

Thyroid Cancer: Differentiated
[^{123}Iodine] ([^{123}I])/[^{131}Iodine] ([^{131}I])

Cellular/Molecular Mechanism of Action: The radioisotopes [^{131}I] and [^{123}I] are recognized as atomic iodine by the thyroid gland.

Application: Detection and characterization of abnormalities affecting the thyroid gland (e.g., differentiated thyroid cancer, hyperthyroidism).

Dosage: Iodine [^{131}I] at 120 MBq/dose (ablation level of activity of [^{131}I] = 4,000 MBq) compared to [^{123}I] at 200 MBq/dose applied as a diagnostic agent.

Considerations: For measurements taken posttherapy, the gamma camera is operated in the high-count rate mode with appropriate correction factors to compensate for any count loss. The resultant radiation dose to the thyroid remnant, as the therapeutic radioiodine is being taken up, may be sufficient to inhibit the uptake process, thus leading to a reduction in maximum uptake when compared with that of the diagnostic activity of radioiodine.

Implementation: Evaluation of radioiodine [^{131}I] administration for diagnostic purposes following recent surgery as a measure of thyroid bed at 3 days. Subsequent thyroid ablation using [^{131}I] is administered 3–38 days later (median 14) and uptake in the thyroid bed measured once or twice, 1–3 days posttherapy. Affected cases can alternatively be given [^{123}I] as the diagnostic agent with the uptake measured at 24 hours. Ablation dosages of radioiodine can then be administered 5–47 (median 19) days later and uptake again measured at 24 hours.

Human Model: Cases that have undergone recent surgery for differentiated thyroid cancer (n = 26 with [^{131}I] and n = 16 with [^{123}I]).

Interpretation and Analysis: Reduced uptake of therapeutically administered [^{131}I] can be observed in the vast majority of cases (n = 26/26 or 100% at $p < 0.001$) that display a median value of 32.8% (range 6–93%) of the uptake in the diagnostic study. In cases given diagnostic [^{123}I], reduced uptake of the ablative radioiodine can be observed in 94% of cases (n = 15/16 at $p < 0.001$), and overall the median value is 58.8% (range 17–130%) of the diagnostic uptake. In a small number of cases, uptake posttherapy is increased (e.g., n = 1/16). The "stunning" observed in case group types given [^{123}I] is significantly less ($p < 0.001$) than in case group types given [^{131}I].

In cases given diagnostic [^{131}I], "stunning" appears to increase in severity the longer the time interval between the diagnostic and therapeutic radionuclides, for intervals up to 25 days. Thereafter, there seemed to be some recovery of uptake capability. Overall there is no evidence of a large rapid loss of radionuclide from the thyroid bed 1–3 days posttherapy. The "stunning" observed using [^{123}I] cannot be explained by errors in the estimation of relative uptake due to different tissue absorption of the [^{131}I] and [^{123}I] photons, nor by the radiation dose delivered by [^{123}I]. However, ablative [^{131}I] itself may cause "stunning" because the cumulated activity, over the 1st few hours of uptake, is not insignificant when compared with all the cumulated activity from a diagnostic administration of [^{131}I]. The resultant radiation dose delivered to the thyroid remnant, as the therapeutic radioiodine is being taken up, may be sufficient to inhibit the uptake process, thus leading to a reduction in maximum uptake when compared with that of the diagnostic activity of radioiodine.

Instrumentation: Computer-integrated nuclear medicine scintigraphic imaging instrumentation (dual-head gamma camera).

Self-stunning in thyroid ablation: Evidence from comparative studies of diagnostic [^{131}I] and [^{123}I]. Hilditch TE, Dempsey MF, Bolster AA, McMenemin RM, Reed NS. Eur J Nucl Med Mol Imaging 2002 Jun; 29 (6), pp. 783–8.

Thyroid Cancer: Differentiated Thyroid Cancer *[^{123}I] Compared to [^{131}I] Posttherapy Radioactive Imaging*

Cellular/Molecular Mechanism of Action: [^{123}I] and [^{131}I] are radioactive analogues of iodine and are physiologically recognized as molecular iodine by the thyroid gland.

Application: Nuclear medicine imaging for detection and characterization of differentiated thyroid cancer.

Route of Administration: Oral.

Dosage: [^{123}I] (185–555 MBq).

Background Information: Radioactive iodine [^{131}I] plays a major role in the diagnosis and management of differentiated thyroid cancer (DTC); however, data on the use of the [^{123}I] isotope in DTC are limited.

Implementation: Evaluation of thyroid cancer conditions utilizing [^{123}I] and comparison with results obtained following posttherapeutic [^{131}I] doses. Scans are evaluated in 3 clinical scenarios including (i) after the 1st [^{131}I] therapy dose; (ii) following the 2nd [^{131}I] therapy dose; and (iii) in cases of elevated Tg and negative diagnostic scan findings.

Human Model: Cases of thyroid cancer (n = 238 diagnostic whole-body scans performed 24 hours postadministration of radioiodine (images acquired with [^{123}I] at 24 hours and 4–5 days post-[^{131}I] therapy).

Interpretation and Analysis: Comparison of whole-body scans performed 24 hours after oral ingestion of [^{123}I] with their corresponding [^{131}I] posttherapy whole-body scans obtained 4–5 days after [^{131}I] therapy reveals a high level of concordance (concordance rate, 93.8%; n = 166 pairs). Some posttreatment scans (e.g., n = 6/166) can show more foci in the thyroid bed than pretreatment scans but display no evidence of uptake in new areas. A small fraction of posttreatment scans (e.g., n = 5/166) will show foci in new locations (e.g., n = 3/166 cervical lymph nodes (CLN); n = 1/166 lung; and n = 1/166 bone metastasis). Following secondary [^{131}I] therapy, a relatively large number of pairs will demonstrate complete concordance (e.g., n = 28/34 pairs; concordance rate, 82.4%). Some discordant pairs show additional foci in areas that have already detected a positive signal on pretreatment scans (e.g., n = 5). A relatively small number of cases may show posttreatment scans with new bone metastasis in a different site from the bone metastases identified on the corresponding pretreatment scan (e.g., n = 1). In pairs of scans in cases with elevated Tg and negative pretreatment scans, a relatively large percentage will remain negative on posttreatment scans (e.g., n = 15/27) while a little more than 1/5 may have posttreatment scans that reveal uptake in the thyroid bed (e.g., n = 6/27) and slightly more than 1/10 posttreatment scans show lung uptake (e.g., n = 3/27) in cases that have computed tomography scans of the chest with evidence of only bronchiectasis (n = 2/3 cases) and lung scarring (n = 1/3 cases) without evidence of lung metastases. A small number of posttreatment scans (e.g., n = 3) may show definite uptake (in thyroid bed, thyroid bed and lung, and CLN) compared with their corresponding pretreatment scans, originally described as being negative but retrospectively thought to have faint uptake. In pretreatment scans (e.g., n = 56), the [^{123}I] diagnostic activity level can be effectively achieved with 185 MBq, and the results show complete concordance in the vast majority of scan-pairs (e.g., n = 54/56). A small number of posttreatment scans will show additional uptake (e.g., n = 2; n = 1 bone; n = 1 CLN). Such observations suggest that pretreatment scanning using [^{123}I] is highly comparable to [^{131}I] posttreatment scans and that [^{123}I]is an excellent diagnostic agent in DTC.

[^{123}I] isotope as a diagnostic agent in the follow-up of patients with differentiated thyroid cancer**:** Comparison with post-[^{131}I] therapy whole body scanning. Alzahrani AS, Bakheet S, Al Mandil M, Al-Hajjaj A, Almahfouz A, Al Haj A. J Clin Endocrinol Metab 2001 Nov; 86 (11), pp. 5294–300.

Thyroid Cancer: Metastatic *[^{99m}Technetium] ([^{99m}Tc]) Furifosmin/[^{99m}Tc]-Methoxy-Isobutyl-Isonitrile (MIBI)/[^{99m}Tc]-SestaMIBI/ [^{99m}Tc] Tetrofosmin/[^{18}F]FDG-PET/[^{201}Thallium] ([^{201}Tl])/[^{131}I]/[^{123}I]*

Many isotopes are available for imaging cases with suspected thyroid cancer recurrence and metastases. Thyroid-stimulating hormone (TSH)-stimulated low-dose [^{131}I] whole-body scanning with serum thyroglobulin either by standard LT4 withdrawal or rhTSH stimulation is the preferred test for monitoring cases without palpable disease or elevated serum thyroglobulin on LT4 therapy. This approach has the advantage of detecting disease states that may be amenable to [^{131}I] therapy, although low-dose [^{131}I] scans are less sensitive than are scans with other imaging agents. [^{123}I] has better imaging characteristics than [^{131}I] and has been shown to be equivalent or superior to low-dose [^{131}I] in recent studies. As the availability of [^{123}I] increases and the cost decreases, this agent may replace [^{131}I] in imaging for recurrent or metastatic thyroid cancer. Other imaging procedures to find suspect disease states may be required when low-dose [^{131}I] imaging procedures are negative in conditions such as those associated with elevated serum thyroglobulin during LT4 therapy or after TSH stimulation. Many diagnostic imaging services perform a sensitive neck ultrasound to look for surgically remediable disease and consider a non-contrast computed tomography (CT) scan of the chest to look for small pulmonary metastases that poorly concentrate low doses of [^{131}I]. Fluoro-18-deoxyglucose positron emission tomography (PET), [^{99m}Tc]-MIBI, [^{201}Tl], and [^{99m}Tc] tetrofosmin are primarily useful in the setting of a negative whole-body [^{131}I] scan and elevated serum thyroglobulin. [^{18}F]FDG-PET seems to have the highest sensitivity in this setting and would be the preferred imaging agent, but availability and cost are major issues. Although some researchers have advocated these radiopharmaceuticals as 1st-line agents replacing [^{131}I], there is little support for this position. This approach to imaging is not cost-effective because positive scans in these cases would most likely require [^{131}I] scintigraphy to determine whether the lesions are amenable to radioiodine therapy. [^{99m}Tc] pertechnetate, [^{99m}Tc] furifosmin, and somatostatin receptor scintigraphy have a limited role in imaging for recurrent or metastatic differentiated thyroid carcinoma. In choosing among [^{99m}Tc]-MIBI, [^{201}Tl], and [^{99m}Tc] tetrofosmin, the technetium label of sestaMIBI and tetrofosmin results in better image quality and faster imaging than [^{201}Tl]. Although [^{99m}Tc]-sestaMIBI and [^{99m}Tc]tetrofosmin have not been compared in a large clinical series, the higher tumor-to-background ratio and consistently high sensitivities of [^{99m}Tc] tetrofosmin suggest that it could potentially have additional value over [^{99m}Tc]-sestaMIBI, but there is still limited experience with [^{99m}Tc] tetrofosmin.

Isotope imaging for metastatic thyroid cancer. Haugen BR, Lin EC. Endocrinol Metab Clin North Am 2001 Jun; 30 (2), pp. 469–92.

Thyroid Carcinoma: Metastatic Medullary Thyroid Carcinoma (MTC)/Lung Carcinoma: Small Cell Type/Cholecystokinin B (CCK-B) Receptor Expression *[^{131}I]-Labeled Gastrin-I (Heptadecapeptide)*

Cellular/Molecular Mechanism of Action: Radiolabeled [^{131}I]-labeled heptadecapeptide gastrin-I functions as a CCK-B receptor binding ligand.

Background Information: The outstanding sensitivity of pentagastrin in detecting the presence of primary, recurrent, or metastatic MTC suggests widespread expression of the corresponding receptor type in human MTC. Indeed, recent autoradiographic studies have demonstrated the presence of cholecystokinin (CCK-B or gastrin) receptors not only in more than 90% of MTCs but also in a high percentage of small cell lung cancers, stromal ovarian cancers, astrocytomas, and several other tumor types.
Application: Detection and characterization of metastatic thyroid carcinoma lesions.
Route of Administration: [^{131}I]-labeled gastrin-I (intravenous).
Dosage: [^{131}I]-labeled gastrin-I (1 μg).
Implementation: Evaluate the potential of radiolabeled gastrin to target CCK-B receptor expressing tumors by determinging the *in vivo* biodistribution of radioiodinated human heptadecapeptide gastrin-I. In this context, both the diagnostic and therapeutic capabilities of radiolabeled CCK-B preparations can be evaluated utilizing [^{131}I]-labeled gastrin-I to investigate their behavior in conditions of metastatic MTC.

Animal Model: Murine (nude mice bearing subcutaneous xenografts of the human MTC cell line, TT).

Human Model: Cases of metastatic MTC.
Interpretation and Analysis: Peptide in amounts of approximately 1 μg produces maximum tumor uptake (8.9 ± 2.9 %ID/g) and can be observed *in vivo* in animal models at 1 hour postinjection, with tumor-to-blood ratios as high as 6.3 ± 1.9. Physiological CCK-B receptors in the stomach, gallbladder, and pancreas are targeted as well (e.g., murine). The major route of excretion is the kidney, but strong evidence for a biliary excretion pathway also exists. Pilot therapy studies with [^{131}I]-labeled gastrin show significant antitumor efficacy compared to untreated reference controls. In accordance with preclinical data, good receptor targeting is observed at tumor sites within the stomach, gallbladder, and pancreas of cases with metastatic MTC. Such observations suggest that gastrin and its analogues may represent a useful new class of receptor binding peptides for the diagnosis and therapy of a variety of tumor types, including MTC and small cell lung cancer. Future preclinical and clinical studies will address in more detail the molecular features that render CCK-B receptor binding agents potentially useful candidates for *in vivo* scintigraphy and radionuclide therapy.

Targeting of cholecystokinin-B/gastrin receptors *in vivo*: Preclinical and initial clinical evaluation of the diagnostic and therapeutic potential of radiolabeled gastrin. Behr TM, Jenner N, Radetzky S, Béhe M, Gratz S, Yücekent S, Raue F, Becker W. Eur J Nucl Med 1998 Apr; 25 (4), pp. 424–30.

Thyroid Carcinoma/Metastatic Medullary Thyroid Cancer (MTC)/Cholecystokinin-B (CCK-B) and Gastrin Receptor Expressing Neoplasias

[^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)-Octreotide/Pentagastrin Stimulation/ CCK [-Trp-Met-Asp-PheNH$_2$]/[^{111}In]-DTPA-D-Glu(1)-Minigastrin

Background Information: Nuclear medicine is engaged with the detection of pathological processes with the help of radionuclides. An interesting approach is to target antigens, symporters, or receptors with diagnostic and therapeutic radionuclides. Different peptide receptors like somatostatin, bombesin/gastrin-releasing peptide (GRP), or vasoactive intestinal peptide (VIP) are (over) expressed on cancer cells, and are therefore an ideal target for diagnosis and therapy with radiolabeled peptides. The somatostatin analogue OctreoScan (octreotide coupled with DTPA) can be labeled with [^{111}In] and is widely used in nuclear oncology for the staging of different tumors (e.g., carcinoids). Other peptides like neurotensin, bombesin/GRP, and VIP have been employed in (pre)clinical investigations. The staging of metastatic MTC with the conventional radiological procedures is sometimes difficult. The high sensitivity of the pentagastrin stimulation test in detecting primary or metastatic MTC indicates the presence of tumor, but its localization is often not possible. This reaction of the tumor cells to the pentagastrin stimulation test suggests a widespread expression of the corresponding receptor type on human MTC. Indeed, autoradiographic studies demonstrated CCK-B/gastrin receptors not only in > 90% of MTCs but in a high percentage of small cell lung cancers, stromal ovarian, and potentially a variety of other tumors, including gastrointestinal adenocarcinomas, neuroendocrine tumors, and malignant glioma.
Considerations: Candidate ligands of interest include a variety of CCK/gastrin-related peptides, all having in common the C-terminal CCK receptor binding tetrapeptide sequence-Trp-Met-Asp-PheNH$_2$ or derivatives thereof. They were members of the gastrin or cholecystokinin families, or possessed characteristics of both, which differ by the intramolecular position of a tyrosyl moiety. Stability of these agents and their affinity has been studied and optimized *in vitro* and *in vivo* along with the determination of their biodistribution and therapeutic efficacy tested in preclinical models.
Implementation: Develop and systematically optimize suitable radioligands for targeting CCK-B receptors *in vivo* and investigate their role in the staging and therapy of MTC and other CCK-B receptor expressing malignancies. Candidate ligands of interest include a variety of CCK/gastrin-related peptides, all having in common the C-terminal CCK receptor binding tetrapeptide sequence-Trp-Met-Asp-PheNH$_2$ or derivatives thereof.

Effectiveness of minigastrin targeting properties can futher be demonstrated in conditions of advanced metastatic disease (e.g., n = 8 cases) involving the injection of a dose-escalation administration protocol with potentially therapeutic activities of [^{90}Y]-labeled minigastrin derivative at 4- to 6-week intervals (30–50 mCi/m^2 per injection for a maximum of 4 injections).
Interpretation and Analysis: Best tumor uptake and tumor-to-non-tumor ratios are obtained with members of the gastrin family, due to their superior selectivity and affinity for the CCK-B receptor subtype. Radiometal-labeled derivatives of minigastrin show excellent targeting of CCK-B receptor expressing tissues in animals and healthy human volunteers. Preclinical therapy experiments in MTC-bearing animals show significant antitumor efficacy. In a related clinical study of 75 MTC cases with metastatic MTC, n = 43/75 suffered of known, and n = 32/75 had occult disease. Scintigraphy for CCK-B receptor expression performed with [^{111}In]-DTPA-D-Glu(1)-minigastrin in such instances reveals normal organ uptake essentially confined to the stomach (and to a lesser extent the gallbladder and, in premenopausal women, to normal breast tissue) as a result of CCK-B receptor specific binding, and to the kidneys as excretory organs. All tumor manifestations known from conventional imaging can be visualized as early as 1 hour postinjection, with increasing tumor-to-background ratios over time; frequently at least 1 lesion will be detected in cases with occult disease (e.g., n = 29/32 cases; patient-based sensitivity 91%). The lesions detected can include local recurrences, lymph node, pulmonary, hepatic, splenic, and bone (marrow) metastases.

In conditions of advanced metastatic disease injected with [^{90}Y]-labeled minigastrin derivative, using a dose-escalating protocol is associated with the development of hematologic and renal abnormalities. Such side effects appear to reflect a dose-limiting toxicity between 40 and 50 mCi/m^2. Some cases will experience partial remission (e.g., n = 2), some cases will stabilize a previously rapidly-progressing disease state (e.g., n = 4). Such findings suggest that

CCK-B receptor ligands may be a useful new class of receptor binding peptides for diagnosis and therapy of a variety of (CCK-B receptor expressing) tumor types. They allow for a sensitive and reliable staging of patients with metastatic MTC. Initial therapeutic results are promising, but nephrotoxicity is a major concern to be solved.

Cholecystokinin-B (CCK-B)/gastrin receptor targeting peptides for staging and therapy of medullary thyroid cancer and other CCK-B receptor expressing malignancies. Béhé M, Behr TM. Biopolymers 2002; 66 (6), pp. 399–418.

Thyroid (Cancer) Metastasis
[^{99m}Technetium] ([^{99m}Tc])-Methoxy-Isobutyl-Isonitrile (MIBI)

Application: Detection of thyroid cancer metastases that can serve as a supplemental diagnostic procedure to the measurement of serum thyroglobulin and radioiodine [^{131}I] whole-body scans in thyroidectomized cases of thyroid cancer.
Route of Administration: Intravenous.
Considerations: The imaging agent [^{99m}Tc]-MIBI has been proposed as a 1st-line diagnostic agent for the follow-up protocol of thyroid cancer, although the ability to detect small lung metastases is somewhat limited.
Implementation: Presence or absence of thyroid cancer can be judged applying several other diagnostic modalities, including serum thyroglobulin measurements, [^{131}I]-whole-body scan, bone scans, chest x-rays, computed tomography (CT), ultrasonography, histopathology, and monitoring the evolution of disease during follow-up periods. All scans are read on a lesion basis for detecting neck, lung, and bone metastases and also on a regional basis, namely head-neck, chest, and abdomen-pelvis-extremities (ab-p-ex) areas.
Interpretation and Analysis: Sensitivity of detection has been reported to be 94.4% (n = 17/18) for neck, 78.4% (n = 40/51) for lung, and 92.8% (n = 64/69) for skeletal lesions. Positive predictive value (PPV) and negative predictive value (NPV) were 96.3% (n = 26/27) and 97.7% (n = 43/44) for head-neck; 94.7% (n = 71/75) and 50.0% (n = 12/24) for chest; and 100.0% (n = 25/25) and 93.1% (n = 54/58) for ab-p-ex regions, respectively. For all scan sites taken together, PPV and NPV are 96.1% (n = 122/127) and 86.5% (n = 109/126), respectively.
Instrumentation: Computer-integrated nuclear medicine scintigraphic camera imaging.

Whole-body scintigraphy with [^{99m}Tc]-MIBI, [^{18}F]-FDG, and [^{131}I] in patients with metastatic thyroid carcinoma. Fridrich L, Messa C, Landoni C, Lucignani G, Moncayo R, Kendler D, Riccabona G, Fazio F. Nucl Med Commun 1997 Jan; 18 (1), pp. 3–9.

Thyroid Remnant and Thyroid Carcinoma Metastasis/Pre- and Postthyroid Abalation Diagnostics
[^{123}Iodine] ([^{123}I])/[^{131}Iodine] ([^{131}I])

Cellular/Molecular Mechanism of Action: Iodine is preferentially assimilated by the thyroid gland.
Application: Postoperative detection of thyroid remnants and/or thyroid carcinoma metastases. The uptakes of thyroablative doses of [^{123}I] by postoperative thyroid remnants and/or thyroid carcinoma metastases following diagnostic surveys with [^{131}I] or [^{123}I] can be compared by visual inspection.
Dosage: [^{131}I] (3–10 mCi) and [^{123}I] (300 μCi).
Implementation: Evaluation of [^{123}I] and [^{131}I] as diagnostic and therapeutic radiopharmaceutical agents (e.g., n = 26 survey cases receiving [^{131}I] at a diagnostic dose level of 3 to 10 mCi; and n = 14 cases receiving [^{123}I] at the usual diagnostic dose of 300 μCi). The age, sex, and tumor type of the 2 groups are not statistically different.
Human Model: Cases with a diagnostic scan demonstrating functioning tissue, remnant, and/or metastasis, following thyroidectomy for differentiated thyroid carcinoma. The age, sex, and tumor type in the 2 groups receiving either [^{123}I] or [^{131}I] are not statistically different.
Interpretation and Analysis: The uptake of the ensuing thyroablative dose of [^{131}I] appears, by visual inspection, to be impaired in slightly less than 80% of cases receiving [^{131}I] group (e.g., n = 20/26) and in none of the [^{123}I] group (e.g., n = 0/14: $p < 0.00003$). In [^{131}I] group cases, there is a suggestion of a dose response (e.g., the higher the administered activity of [^{131}I] for the diagnostic scan, the greater the decline in the subsequent uptake of the thyroablative dose [$p = 0.0007$]). Thyroid remnants or cervical lymph node metastases appear to be affected more frequently than are distant (pulmonary or skeletal) metastases ($p = 0.004$). Therefore, iodine uptake function may be suppressed by the absorbed radiation from the 3–10 mCi "diagnostic" scanning dose of [^{131}I]. In this regard, [^{123}I] may be a better initial diagnostic agent to be used prior to radioablation therapy.
Instrumentation: Computer-integrated nuclear medicine scintigraphic imaging instrumentation.

Superiority of iodine-123 compared with iodine-131 scanning for thyroid remnants in patients with differentiated thyroid cancer. Mandel SJ, Shankar LK, Benard F, Yamamoto A, Alavi A. Clin Nucl Med 2001 Jan; 26 (1), pp. 6–9.

Treatment Response (Radiation Therapy)/Glucose Metabolism/Amino Acid Metabolism/Nucleic Acid Metabolism
[^{18}F]-5-Fluorodeoxyuridine/L-Methyl-[^{14}C]Methionine/6-[^{3}H]Thymidine/2′-Deoxy-5-[^{18}F]Fluorouridine

Cellular/Molecular Mechanism of Action: Reflects neoplastic cell nucleic acid metabolism.
Application: Diagnostic imaging of neoplastic lesions.
Route of Administration: [^{18}F]-5-fluorodeoxyuridine (usually intravascular).
Implementation: Evaluation of metabolic tracers for glucose, amino acid, and nucleic acid metabolisms. 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}FDG), L-methyl-[^{14}C]methionine (^{14}C-Met), [6-^{3}H] thymidine (^{3}H-Thd), 2′-deoxy-5-[^{18}F]fluorouridine (^{18}FdUrd), and the conventional radionuclide [^{67}Ga]-citrate can be used to assess the feasibility of monitoring the effectiveness of tumor radiotherapy using a quadruple tracer technique. In this context, 2 combinations of 4 tracers (^{18}FDG or ^{18}FdUrd, ^{14}C-Met, ^{3}H-Thd, and [^{67}Ga]) can be compared in a time-course study after single-dose irradiation (20 Gy) and also used in a dose-dependency study performed 6 days after 5, 10, 15, or 20 Gy of irradiation.
Animal Model: Rat AH109A tumor model.
Interpretation and Analysis: [^{18}F]-FDG shows a large change in uptake and a steady response to radiotherapy. Fluorodeoxyuridine shows a rapid decrease after radiotherapy, but the range of change in uptake is narrow. [^{67}Ga] cannot detect tumor response early after treatment but shows a marked change in uptake later. 6-^{3}H-Thd and [^{14}C]-Met show a rapid response to irradiation and a high sensitivity for monitoring radiotherapy, suggesting that they may be applicable for PET studies.

Tracer feasibility for monitoring tumor radiotherapy: A quadruple tracer study with fluorine-18-fluorodeoxyglucose or fluorine-18-fluorodeoxyuridine, L-[methyl-14C]methionine, [6-^{3}H]thymidine, and gallium-67. Kubota K, Ishiwata K, Kubota R, Yamada S, Tada M, Sato T, Ido T. J Nucl Med 1991 Nov; 32 (11), pp. 2118–23.

PANCREAS

See neoplastic disease section within this chapter for malignant and benign conditions affecting the pancreas.

Pancreas: Normal/Pancreatitis (Potential Application)
[^{99m}Technetium] ([^{99m}Tc])-Hexamethylpropyleneamine Oxime (HMPAO) Neutrophils

Cellular/Molecular Mechanism of Action: Granulocytes are capable of migration activity in response to chemotactic factors (stimuli).
Route of Administration: [^{99m}Tc]-HMPAO-labeled granulocytes (intravenous).
Dosage: [^{99m}Tc]-HMPAO-labeled granulocytes (mean = 5.42×10^7 cells in a 2 mL volume/cat).
Semi-Synthetic Methods: Autologous granulocytes are labeled *in vitro* with [^{99m}Tc]-HMPAO. Feline granulocytes can be successfully labeled with [^{99m}Tc]-HMPAO at a labeling efficiency of 15 to 42% (average of 27%).
Implementation: Evaluate the biodistribution of autologous [^{99m}Tc]-HMPAO-labeled granulocytes. In this context, autologous-origin [^{99m}Tc]-HMPAO-labeled granulocytes are injected intravenously, followed by the acquisition of whole-body scintigraphic images at 1, 5, 15, and 30 minutes and 1, 2, and 4 hours postadministration. At 12 to 24 hours, computed tomomgraphy (CT) images of the abdomen are taken both precontrast and postcontrast. Following CT imaging, a surgical pancreatic biopsy sample is collected for analysis. Less than 1 minute is often necessary to acquire a 500,000-count image.
Animal Model: Feline (anesthetized for the acquisition of CT images).
Interpretation and Analysis: Granulocytes distribute predominantly to the lung, spleen, and liver in order of decreasing activity. Only background activity can be identified in the region of the pancreas. The pancreas is easily identified on CT images of the abdomen. The pancreas is hypoattenuated relative to both the spleen and liver. Contrast image-enhancing media can improve the visualization of the pancreas with peak efficacy appreciated immediately, then gradually clearing over a 30-minute test period. Following contrast medium administration the pancreas remains hypoattenuated relative to the spleen. Analysis of biopsy specimens using the animal model described usually confirms the absence of pancreatic inflammation and appreciation of no adverse effects as a result of pancreatic biopsy. Both CT and radiolabeled granulocytes appear to hold promise as imaging procedures for the detection of feline pancreatitis. Normal parameters may be altered in the face of inflammation, facilitating the detection of feline pancreatitis. Data from cases of suspect feline pancreatitis are needed to evaluate these methods for clinical utility.

Evaluation of the feline pancreas using computed tomography and radiolabeled leukocytes. Head LL, Daniel GB, Tobias K, Morandi F, DeNovo RC, Donnell R. Vet Radiol Ultrasound 2003 Jul–Aug; 44 (4), pp. 420–8.$eref

RENAL SYSTEM

Glomerular Filtration Function
[^{99m}Technetium] ([^{99m}Tc])-Mercaptoacetyltriglycine (MAG3)/[^{123}I]-Ortho-Iodohippuric Acid (OIH)

Cellular/Molecular Mechanism of Action: Mercaptoacetyltriglycine and OIH are passively excreted into the urine by mechanisms of plasma filtration at the level of the renal glomerulus.
Application: Evaluation of renal glomerular function/perfusion.
Route of Administration: [^{99m}Tc]-MAG3 and [^{123}I]-OIH (intravascular).
Implementation: Evaluation of renal function based on analysis of scintigraphic clearance of [^{99m}Tc]-MAG3 and [^{123}I]-OIH.
Human Model: Cases of impaired renal function (n = 12 patients).
Interpretation and Analysis: MAG3 renal scintigraphy provides better quality and functional information compared to OIH. The MAG3 and OIH renograms show identical relative kidney uptake and T_{max}, but $T_{1/2}$ of MAG3 is longer ($p < 0.001$). The plasma clearance of MAG3 is lower than that of OIH, but they correlate significantly ($r = 0.87$, $p < 0.005$). Also, the plasma clearances of MAG3 and *para*-aminohippuric acid (calculated by the standard clearance technique) show good correlation ($r = 0.77$, $p < 0.05$). Urinary excretion during the 1st 60 minutes after injection is the same for the 2 radiopharmaceuticals. Therefore, [^{99m}Tc]-MAG3 can function as a suitable alternative to [^{123}I]-OIH for renal scintigraphic imaging.

Clinical assessment of [^{99m}Tc]-MAG3 scintigraphy for impaired renal function. Sato M, Hirayama K, Ishikawa N, Takeda T, Wu J, Itai Y, Kobayashi M, Koyama A. Kaku Igaku 1994 Jan; 31 (1), pp. 75–84.

Glomerular Filtration/Glomerular Injury/(Minimal Change Glomerulonephritis)
[^{99m}Technetium] ([^{99m}Tc])- or [^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)/[^{131}I]-Hippuran Alternative/[^{99m}Tc]-Glucoheptonate (GHA) Alternative/[^{99m}Tc]-Dimercaptosuccinate (DMS) Alternative

Cellular/Molecular Mechanism of Action: The agents [^{131}I]-hippuran, [^{99m}Tc]-GHA, [^{99m}Tc]-DMS, and [^{99m}Tc]- and [^{111}In]-DTPA are retained within the intravascular compartment and passively eliminated in the urine by glomerular filtration.
Application: Renal diagnostic agents.
Route of Administration: Intravenous.
Animal Model: Glomerular lesions can be induced by intravenous injection of puromycin aminonucleoside (PA) 9 days before radionuclide studies (model of spontaneous "minimal change" glomerulonephritis in humans).
Interpretation and Analysis: Computer-generated early renal uptake of [^{99m}Tc]-DTPA or GHA correlates with the glomerular filtration rate (GFR) quantitated by biexponential plasma clearance of DTPA administered by single intravenous injection. The early renal uptake of hippuran and DMS correlates poorly with GFR as assessed by DTPA clearance. However, the 2-hour renal retention of DMS correlates well with the DTPA clearance. None of the parameters measured with [^{131}I]-hippuran correlate well with DTPA clearance, probably because of decreases in protein plasma binding of hippuran secondary to hypoproteinemia. None of the agents are superior to labeled DTPA for the detection of glomerular damage in this experimental model.

Detection of diffuse glomerular lesions in rats: I. Comparisons of conventional radioactive agents. McAfee JG, Thomas FD, Subramanian G, Lyons B, Roskopf M. J Nucl Med 1986 Apr; 27 (4), pp. 502–12.

Nephro-Urology Imaging Agent
[^{99m}Technetium] ([^{99m}Tc])-Malic Acid/[^{99m}Tc]-Dimercaptopropionic Acid

Implementation: Evaluation of the fates of thalidomide and norphenylephrine and safety evaluation of some xenobiotics includ-

ing food additives, plasticizers, and domestic chemical products performed in animals using synthesized [^{14}C]- or [^{3}H]-labeled compounds.

Interpretation and Analysis: Of several agents examined, 2 new radiopharmaceuticals, [^{99m}Tc]-malic acid and [^{99m}Tc]-dimercaptopropionic acid, have displayed effectiveness as renal imaging agents when administered at adequate concentrations in animal models and humans. Useful information can be obtained for the diagnosis of renal diseases using both radiopharmaceutical agents.

Application of radionuclides in pharmaceutical fields. Tanaka A. Yakugaku Zasshi 2000 Jun; 120 (6), pp. 506–19.

Renal Artery Stenosis (RAS): Vascular Hypertension/ Renal Ischemia/[RAS > 50%] *[^{99m}Technetium] ([^{99m}Tc])-Mercaptoacetyltriglycine (MAG3)/Captopril Test*

Cellular/Molecular Mechanism of Action: Captopril inhibits the biochemical activity of angiotensin-converting enzyme (ACE inhibitor).

Physiological Mechanism of Action: Angiotensin in its most active form promotes increased vascular tone.

Route of Administration: Captopril (oral).

Dosage: Captopril (50 mg).

Implementation: Evaluation of vascular hypertension utilizing renal scintigraphic imaging facilitated by the application of [^{99m}Tc]-MAG3 1 hour after captopril premedication. Baseline studies are obtained only for those cases showing abnormal findings in provocative studies and after having undergone previous renal arteriography analysis.

Human Model: Cases with vascular hypertension (e.g., n = 82).

Interpretation and Analysis: Sensitivity and specificity for the detection of RAS > 50% is approximately 89% and 91%, respectively. After captopril administration, tracer parenchymal transit time increases significantly in ischemic kidneys (334 ± 93 seconds in baseline conditions vs 468 ± 96 seconds after captopril, $p < 0.001$) but not in kidneys without RAS or RAS < 50% (243 ± 46 seconds vs 271 ± 95 seconds, p = non-significant). False-positive responses are mostly bilateral and associated with marked decreases in blood pressure. [^{99m}Tc]-MAG3 is an effective compound for detecting RAS ≥ 50% with captopril renal scintigraphy. Performing the provocative test as a 1st step considerably reduces the number of scintigraphic studies required.

Use of technetium-^{99m}-MAG3 for renal scintigraphy after angiotensin-converting enzyme inhibition. Dondi M, Monetti N, Fanti S, Marchetta F, Corbelli C, Zagni P, De Fabritis A, Losinno F, Levorato M, Zuccalá. J Nucl Med 1991 Mar; 32 (3), pp. 424–8.

Renal Failure: Acute/Cardiac Failure: Congestive/ Endothelin Receptor Expression: Antagonist Receptor Occupancy/Individualization of Endothelin Receptor Antagonist Dosage *[^{125}I]ET-1/BQ-123/BQ-788/L-749,329*

Cellular/Molecular Mechanism of Action: The agents BQ-123 and BQ-788 bind to and block ETA and ETB type endothelin receptor complexes (receptor antagonists). Conversely, L-749,329 (non-peptide antagonist) has a relatively balanced affinity for each of the 2 endothelin receptor subtypes.

Physiological Mechanism of Action: Endothelin is 1 of the most potent vasoconstrictors known. As a point of reference, prostacycline (PGI_2) is 1 of the most potent vasodilatory agents identified to date and thromboxane TXA2 is a potent vasocontrictive agent.

Background Information: Endothelin has been implicated in various diseases, including acute renal failure and congestive heart failure. Such observations imply that these disease states can possibly be managed with endothelin receptor antagonists. However, establishing the dosages for these antagonists may be difficult because no convenient physiologic indicator of action exists and because of complexities in receptor function. Two receptor subtypes have been identified for which selective antagonists have been reported (e.g., BQ-123 for the ETA receptor and BQ-788 for the ETB receptor). Of the 3 natural peptide hormones (ET-1, ET-2, and ET-3), ET-1 exhibits high affinity for both subtypes of receptor.

Application: Characterization of the relative expression of 2 endothelium receptor subtypes in a manner that provides information for establishing individualized dosage schedules for endothelin receptor antagonists pertaining to the management of various disease states, including acute renal failure and congestive heart failure.

Implementation: Evaluation of the *in vivo* biodistribution of [^{125}I]ET-1 with and without the presence of selective peptide antagonists, and a non-peptide antagonist (L-749,329). The agent [^{125}I]ET-1 possesses binding avidity for endothelin receptor complexes.

Animal Model: Rat.

Animal Model: Primate (rhesus monkey).

Interpretation and Analysis: The agents BQ-123, BQ-788, and L-749,329 inhibit binding consistent with physical interaction with a single receptor site. However, the sum of inhibition by the selective antagonist is greater than 100% (as defined by inhibition with L-749,329), which suggests (i) lower *in vivo* selectivity than determined *in vitro*, and/or (ii) receptor subtype interactions. The latter explanation is supported, in part, by *in vitro* autoradiographic studies as well as investigations in isolated tissues and cell preparations. Synthetic ET-1 radiolabeled with [^{123}I] facilitates the acquisition of images that reflect receptor distribution in 2 *in vivo* animal models. Utilizing planar, non-invasive imaging, it is possible to visualize the occupancy of endothelin receptor by antagonists in both the kidney and lung. [^{123}I]ET-1 can therefore be used to determine appropriate clinical dosages of antagonist needed for receptor saturation.

Radioiodinated endothelin-1: A radiotracer for imaging endothelin receptor distribution and occupancy. Gibson RE, Fioravanti C, Francis B, Burns HD. Nucl Med Biol 1999 Feb; 26 (2), pp. 193–9.

Renal Function: Measurement and Quantification *[^{99m}Technetium] ([^{99m}Tc])-Mercaptoacetyltriglycine*

Background Information: Pharmacokinetic modeling based on compartmentalization has provided a valuable tool to assess the clearance patterns of various glomerular and tubular agents. However, no models have been proposed thus far that combine vascular data and imaging data in order to gain a deeper knowledge about renal pathophysiology and to obtain more diagnostic information of clinical relevance. To this aim, investigations have utilized a 4-pool model for the evaluation of separate renal functions.

Application: Evaluation of renal function in cases of renal disease and in the screening of potential renal transplant donors.

Implementation: Evaluation of a 4-pool model based on the identification of the 2 kidneys as 2 distinct pools. Such an approach makes it possible to integrate the separate kidney contributions derived from *in vivo* imaging data, and allows quantitative measurement of many parameters specific to each kidney.

Human Model: Cases without evidence of disease (n = 10 volunteers) compared to conditions of various types of renal disease (n = 20).

Interpretation and Analysis: The parameters TERR, TERL, MRTR, MRTL, vR, vL, k3R-1, K3L-1 permit the differentiation of normal from abnormal states of renal function, as well as being able to distinguish between monolateral and bilateral renal disease in cases known to have abnormal renal function. The diagnostic approach applied combines the advantages of plasma clearance methods with those derived by gamma camera imaging, while also making it possible to quantitate differential parameteters of renal function. Such features may be clinically relevant in renal transplant donors, where full knowledge of renal pathophysiology could guide the procedure.

A theoretical 4-compartment model to evaluate separate kidney technetium-^{99m}-MAG3 kinetics in humans. Curti G, DeMartini D, Santaniello B, Taddei G, Fresco GF. Kidney Int 1998 Dec; 54 (6), pp. 2029–36.

Renal Glomerular Filtration Rate
[^{99m}Technetium] ([^{99m}Tc])-Diethylenetriamine Pentaacetic Acid (DTPA)/[Creatinine Clearance Test]/ Captopril Test Enhancement

Cellular/Molecular Mechanism of Action: Creatinine and DTPA per se do not exert a molecular or cellular or biological effect. Instead, they function as marker molecules because they are passively excreted into the urinary filtrate from the plasma.
Application: Evaluation of renal glomerular filtration rate especially in scenarios where urine cannot be collected for analysis by conventional analysis procedures (creatinine clearance test [CCT]).
Route of Administration: [^{99m}Tc]-DTPA (intravascular).
Implementation: Computer validation for implementing the Gates' method for calculating parameters associated with radionuclide glomerular filtration rate (RGFR). Accuracy of the original method can be improved by replacing the Tonnesen's formula that estimates renal depth with direct measurement from lateral views to calculate tissue attenuation correction.

Evaluation of renal glomerular filtration rate can be achieved applying both the CCT and dynamic [^{99m}Tc]-DTPA renal scintigraphy (DRS). Radionuclide glomerular filtration rate is quantified from the attenuation-corrected absolute DTPA uptake of the kidneys on DRS between 120 and 180 seconds after injection. Attenuation correction is estimated using the lateral views of the kidneys taking into account the distance from the computed geometric center of the kidneys to the posterior body surface along a line vertical to the collimator surface. CCT and glomerular filtration rate estimates from DRS are then compared by linear regression.

Human Model: Chronic renal failure (n = 38 cases).
Interpretation and Analysis: Radionuclide glomerular filtration rate estimates agree well with CCT, yielding a correlation coefficient of approximately 0.92 (e.g., n = 38 cases) and 0.90 in cases suffering from chronic renal failure (e.g., n = 11). The proposed modifications improve RGFR accuracy to the precision range of blood sample–based methods. The modified method requires little additional work and no extra cost in cases undergoing DRS. Radionuclide glomerular filtration rate calculation may be advantageous in cases when 24-hour urine collection for CCT cannot be obtained, and it should improve the accuracy of the captopril test.

Renal depth estimates to improve the accuracy of glomerular filtration rate. Steinmetz AP, Zwas ST, Macadziob S, Rotemberg G, Shrem Y. J Nucl Med 1998 Oct; 39 (10), pp. 1822–9.

Renal Glomerular Function/Renal Allograft/ Transplant Rejection
[^{99m}Technetium] ([^{99m}Tc])-Acidic Fibroblast Growth Factor 1 (FGF-1)/[^{99m}Tc]-Mercaptoacetyltriglycine (MAG3)

Background Information: Acidic fibroblast growth factor functions as a potent hormonal inducer of *in vivo* wound repair. In addition, involvement of FGF-1 in a number of pathophysiological conditions, including chronic human renal allograft rejection, has been described. Consequently, there is an increasing need to monitor FGF-1 pharmacokinetics and distribution for both therapeutic and diagnostic purposes.
Route of Administration: [^{99m}Tc]-MAG3 and [^{99m}Tc]-labeled FGF-1 (intravenous).
Implementation: Evaluation of renal function facilitated by the application of dynamic [^{99m}Tc]-MAG3 imaging, and subsequent image analysis of [^{99m}Tc]-labeled FGF-1 ([^{99m}Tc]-FGF-1) biodistrubution profiles. Complementary validation investigations entail performing microautoradiography of harvested kidneys to facilitate determination of [^{99m}Tc]-FGF-1 compartmental localization.

Animal Model: Rat (sham-operated, syngeneic renal transplanted, and allogeneic renal transplanted rats).
Interpretation and Analysis: Allogeneic renal transplants appear grossly abnormal on [^{99m}Tc]-MAG3 renal scans. In scenarios of this type, a significant reduction in [^{99m}Tc]-FGF-1 renal binding can be detected by imaging analyses, in contrast to renal binding in sham-operated and syngeneic renal transplanted scenarios, which are not significantly different. Both groups of renal transplants show a redistribution of FGF-1 to the glomerular compartment. Therefore, [^{99m}Tc]-FGF-1 serves as a new radiotracer that can effectively measure *in vivo* targeting of growth factor. Reduced renal binding of [^{99m}Tc]-FGF-1 in the allogeneic transplanted kidney is consistent or correlates with decreases in blood flow. Unique glomerular targeting of [^{99m}Tc]-FGF-1 in the transplanted kidney provides additional evidence supporting a role for this growth factor in the pathogenesis of chronic rejection.
Instrumentation: Anger gamma camera.

Glomerular targeting of acidic fibroblast growth factor-1 in renal transplanted rats. Zinn KR, Kelpke S, Akhi K, Viera L, Chaudhuri TR, Thompson JA. Transplantation 2002 May 15; 73 (9), pp. 1447–54.

Renal Tubular Function/Renal Plasma Flow
[^{99m}Technetium] ([^{99m}Tc])-Labeled Hippurate Analogues

Cellular/Molecular Mechanism of Action: Radiolabled hippurate analogues are rapidly cleared from the blood (rat) by the kidney. Due to their extensive elimination by the kidney, they can serve as imaging agents during periods of peak removal from the plasma.
Application: The 7 [^{99m}Tc]-labeled hippurate analogues: [^{99m}Tc]-hippurate, [^{99m}Tc]-alpha-hydroxyhippurate, [^{99m}Tc]-*m*-hydroxyhippurate, [^{99m}Tc]-*o*-hydroxyhippurate [^{99m}Tc]-salicylglycine ([^{99m}Tc]-SG)], [^{99m}Tc]-*p*-hydroxyhippurate, [^{99m}Tc]-salicylglycylglycine, and [^{99m}Tc]-salicylglycylglycylglycine can all be applied for imaging the kidney.
Route of Administration: Intravenous.
Considerations: The imaging agent [^{99m}Tc]-SG has the same desirable biological properties of the 2 diagnostic agents, [^{99m}Tc]-mercaptoacetyltriglycine ([^{99m}Tc]-MAG3) and [^{99m}Tc]-dimercaptosuccinate ([^{99m}Tc]-DMSA).

Interpretation and Analysis: A fraction of [^{99m}Tc]-SG administered systemically displays a definite transit time within the kidney and is excreted rapidly into the urine, being similar to [^{99m}Tc]-MAG3. The binding ratio to the plasma proteins is 96.0% (91.1% associated with albumin), being higher than that for [^{99m}Tc]-DMSA, at 30 minutes. Lipophilicity analysis reveals far less pH-dependent changes in a range between pH 4.0–7.4. [^{99m}Tc]-SG distributes about 91% into the renal cortex, being similar to that of [^{99m}Tc]-DMSA. The biological properties of [^{99m}Tc]-SG suggest that it is a promising agent for measuring renal plasma flow and renal morphology.

Synthesis and preclinical evaluation of technetium-^{99m}-labeled hippurate analogues. Fukuoka M, Kiyohara T, Kobayashi T, Kojima S, Tanaka A, Kubodera A. Nucl Med Biol 1995 Feb; 22 (2), pp. 181–91.

RESPIRATORY SYSTEM

Adult Respiratory Distress Syndrome (ARDS) *[^{111}Indium] ([^{111}In])-Labeled (aICAM-1)*

Cellular/Molecular Mechanism of Action: Possesses binding avidity for leukocyte cell populations.
Application: Detection of foci associated with sepsis and inflammation.
Considerations: Previous studies utilizing the bleomycin-induced lung injury model suggest that [^{111}In]-labeled anti-intercellular adhesion molecule-1 (e.g., rat aICAM-1) might be a useful ARDS diagnostic agent. Lung inflammation can be detected with [^{111}In]-aICAM-1 very early in the course of the disease. These results support the suggestion that [^{111}In]-aICAM-1 could be a very early, highly specific ARDS diagnostic agent and may be useful to detect a wide range of inflammatory conditions.
Implementation: Evaluation of lung injury as a function of the biodistribution, scintigraphic images, and lung ICAM-1 upregulation following the administration of [^{111}In]-labeled polymorphonuclear leukocytes (rat PMNs), [^{111}In]-aICAM-1, [^{111}In]-labeled normal IgG (murine nmIgG), and [^{111}In]-labeled serum albumin (rat RSA) injected 18–24 hours before animal model termination.

Animal Model: Rat (control and post–oleic acid–induced injury).

Interpretation and Analysis: [^{111}In]-RSA and [^{111}In]-nmIgG localize in inflamed lung by 5 minutes postinjury (PI). [^{111}In]-PMN uptake increases significantly only at 24 hours PI. [^{111}In]-aICAM-1 localization increases significantly (30–60%) at 1 hour PI and remains elevated up to 24 hours PI. Lung/blood ratios (L/B) at 1 and 4 hours PI are very low (< 0.6) for [^{111}In]-nmIgG and [^{111}In]-PMN; however, for [^{111}In]-aICAM-1, they are > 1 and 25–60% higher than those for control samples. A low L/B suggests poor inflammation detection on the images. Images and region-of-interest (ROI) analysis confirm that only [^{111}In]-aICAM-1 can distinguish inflamed lungs at 4 hours PI. ICAM-1 is upregulated at 4 and 24 hours PI.

Early detection of oleic acid–induced lung injury in rats using [^{111}In]-labeled anti-rat intercellular adhesion molecule-1. Weiner RE, Sasso DE, Gionfriddo MA, Thrall RS, Syrbu S, Smilowitz HM, Vento J. J Nucl Med 2001 Jul; 42 (7), pp. 1109–15.

Adult Respiratory Distress Syndrome (ARDS)/ Respiratory Distress Syndrome of Newborns (RDS) *[^{99m}Technetium] ([^{99m}Tc])-Exogenous Natural Surfactant (ENS)*

Cellular/Molecular Mechanism of Action: Pulmonary surfactant is a lipoproteic biological fluid secreted by alveolar Type II cells that prevents collapse of respiratory alveoli by reducing the surface tension imposed on these structures.
Application: Detection of RDS and ARDS.
Dosage: [^{99m}Tc]-ENS (900–1,110 MBq).
Considerations: Surfactant deficiency is the major factor responsible for RDS and ARDS. Since 1980, the exogenous administration of surfactant has been studied for the treatment of these syndromes.
Semi-Synthetic Methods: Exogenous natural surfactant (ENS) applied as a radiopharmaceutical precursor following radiolabeling with [^{99m}Tc] to produce [^{99m}Tc]-ENS.
Implementation: Evaluation of [^{99m}Tc]-ENS for aerial/ventilation lung scintigraphy (3 minute nebulization) compared to [^{99m}Tc]-diethylenetriamine pentaacetic acid (DTPA).

Human Model: Volunteers ($n = 5$; some of whom smoke).

Interpretation and Analysis: [^{99m}Tc]-ENS images are of at least the same quality as [^{99m}Tc]-DTPA images. However, in some smoking volunteers, the [^{99m}Tc]-DTPA images show some regions that seemed to be not well ventilated, although these areas appear well ventilated in [^{99m}Tc]-ENS imaging. Such observations suggest that [^{99m}Tc]-ENS can be used for ventilation scintigraphy to allow the observation of some areas that cannot be visualized using [^{99m}Tc]-DTPA as a ventilation agent.

[^{99m}Tc]-ENS ventilation scintigraphy: Preliminary study in human volunteers. Calmanovici G, Boccio J, Goldman C, Hager A, De Paoli T, Alak M, Degrossi O, García del Río H, Nicolini J, Caro R, Zubillaga M. Nucl Med Biol 2000 Feb; 27 (2), pp. 215–8.

The pulmonary surfactant system: Physiology, pathologies associated with its alteration, and exogenous administration as therapeutic and diagnostic agent. Calmanovici G, Boccio J, Lysionek A, Salgueiro M, Caro R, Hager A, de Paoli T, Zubillaga M. Acta Physiol Pharmacol Ther Latinoam 1998; 48 (4), pp. 175–90.

Amiodarone-Induced Pulmonary Toxicity (AIPT) *[^{99m}Technetium] ([^{99m}Tc])-Diethylenetriamine Pentaacetic Acid (DTPA)/Gallium-67 [^{67}Ga]*

Application: Amiodarone hydrochloride, which is used in life-threatening cardiac tachyarrhythmia, has been known to cause AIPT as a complication.
Route of Administration: Inhalation of aerosolized preparations.
Implementation: Investigate the clinical value of [^{99m}Tc]-DTPA scintigraphy in cases with AIPT in comparison with [^{67}Ga] scan imaging. Both [^{67}Ga] and [^{99m}Tc]-DTPA are delivered as an aerosol prior to scintigraphy imaging, in addition to performing various laboratory tests.

Human Model: Cases of AIPT ($n = 7$; Type A); cases receiving AIPT without pulmomary toxicity ($n = 8$; Type B); and reference control subjects ($n = 11$).

Interpretation and Analysis: [^{67}Ga] scintigraphy is positive in a modest percentage of cases of AIPT (e.g., $n = 4/7$), but no abnormalities may be detected in any Type B cases (e.g., $n = 0/8$). A positive correlation can be found ($r = 0.52$, $p < 0.05$) between k_{ep} values determined by [^{99m}Tc]-DTPA aerosol scintigraphy and the cumulative dose of amiodarone. The mean k_{ep} values are approximately 2.04% ± 0.85%/min, 1.30% ± 0.42%/min, and 0.86% ± 0.19%/min for groups A, B and C, respectively. The mean clearance rate of Type A case is significantly faster than that of normal reference controls ($p < 0.0005$) and Type B cases ($p = 0.028$). In addition, there is a significant difference between Type B and Type C cases ($p = 0.015$). In conclusion, [^{67}Ga] lung scintigraphy is a useful method for the detection of AIPT, but [^{99m}Tc]-DTPA aerosol scintigraphy offers better results

than [^{67}Ga] scintigraphy. Early changes in [^{99m}Tc]-DTPA clearance may be observed in cases receiving amiodarone. The k_{ep} value in cases with AIPT is noticeably increased with respect to normal reference controls. Given its favorable physical properties, low cost, lower radiation burden, and ability to be used as an objective measure for the pulmonary clearance rate, [^{99m}Tc]-DTPA aerosol scintigraphy appears to be promising in cases receiving amiodarone therapy.

Technetium-^{99m}-DTPA aerosol scintigraphy in amiodarone-induced pulmonary toxicity in comparison with Ga-67 scintigraphy. Dirlik A, Erinc R, Ozcan Z, Atasever A, Bacakoglu F, Nalbantgil S, Ozhan M, Burak Z. Ann Nucl Med 2002 Nov; 16 (7), pp. 477–81.

Deposition Estimation/Respiratory Nebulization (Inhalation)
[^{99m}Technetium] ([^{99m}Tc])-Tobramycin-Serum Albumin

Cellular/Molecular Mechanism of Action: The albumin component of [^{99m}Tc]-serum albumin serves to increase the molecular weight of the agent complex in a manner that hinders the rate of diffusion across biological membranes and under certain circumstances encourages uptake by the mononuclear phagocytic system.

Background Information: The ability to predict drug deposition of inhaled drugs used in cystic fibrosis (CF) is important if there is a need to target specific doses of drug to the lungs of individual cases. The gold standard of measuring pulmonary deposition is the quantification of an aerosolized radiolabel either mixed with the drug solution or tagged directly to the compound of interest. Accuracy of the quantification can be assured if there is agreement between the amount of radioactivity before and after administration. Before administration, the radiolabel is concentrated in the well of the nebulizer, whereas after administration, it is distributed throughout the nebulizer, the expiratory filter and connectors, and the upper airway, stomach, trachea, and lung. Not only is the geometry of the distribution that is presented to the gamma camera different, but there are different attenuation factors for the various body tissues.

Application: Preparations of [^{99m}Tc]-tobramycin-serum albumin can be used to evaluate the accuracy of the quantification of pulmonary deposition and compare *in vitro* nebulizer performance with that measured *in vivo* during the deposition study.

Route of Administration: [^{99m}Tc]-tobramycin-serum albumin (nebulized inhalation).

Dosage: [^{99m}Tc]-tobramycin-serum albumin (80 milligrams).

Implementation: Delivery of 80 milligrams of tobramycin and [^{99m}Tc] bound to human serum albumin using a Pari LC Jet Plus (Pari Respiratory Equipment, Inc., Richmond, VA) breath-enhanced nebulizer.

Human Model: Normal (n = 10).

Interpretation and Analysis: Approximately 99 ± 2% of the initial radioactivity can be accounted for *in vivo* postdelivery. The fraction of [rate of lung deposition/total body deposition] is the *in vivo* respirable fraction (0.62 ± 0.07), which closely agrees with *in vitro* measurements of respirable fraction (0.62 ± 0.04). Drug output measured by the change in weight and concentration in the nebulizer systematically overestimates drug output measured by deposition studies. The results indicate that 11.8 of the initial 80 mg is deposited in the lungs. This technique could be adapted to accurately quantify the amount of deposition on any inhaled therapeutic agent, but caution must be used when extrapolating performance of a nebulizer on the bench to expected deposition in clinical cases.

Accounting for radioactivity before and after nebulization of tobramycin to insure accuracy of quantification of lung deposition. Coates AL, Dinh L, MacNeish CF, Rollin T, Gagnon S, Ho SL, Lands LC. J Aerosol Med 2000 Fall; 13 (3), pp. 169–78.

Inflammation of Endothelium: Acute Pulmonary/Bleomycin-Induced Pulmonary Injury
[^{111}Indium] ([^{111}In])-Anti-ICAM-1 Monoclonal Antibody

Cellular/Molecular Mechanism of Action: The antibody component of [^{111}In]-anti-ICAM-1 monoclonal IgG antibody recognizes and physically binds to intracellular adhesion molecule-1 complexes expressed on the exterior surface membrane of "activated" endothelial cells.

Application: Detection of endothelial injury and acute vascular inflammatory responses, including alterations associated with bleomycin-induced pulmonary injury.

Route of Administration: [^{111}In]-anti-ICAM-1 monoclonal IgG antibody (intravenous).

Implementation: Evaluation of alterations in endothelial intracellular adhesion molecule expression following bleomycin-induced pulmonary injury employing [^{111}In]-anti-ICAM-1 monoclonal IgG antibody (e.g., [^{111}In*]-aICAM-1) with findings compared to [^{111}In]-labeled normal mouse IgG (e.g., [^{111}In*]-nmIgG). Tissue samples are harvested 24 hours following image acquisition and their radioactivity measured. Lung tissue can be cryostat-sectioned to detect the presence of ICAM-1 by immunofluorescence, and the upregulation of LFA-1α identified in blood polymorphonuclear leukocytes (PMNs) using fluorescence-activated cell-sorter (FACS) analysis.

Animal Model: Rat (lung injury induced by intratracheal bleomycin injection 4 or 24 hours before humane sacrifice).

Interpretation and Analysis: Evaluation of bleomycin-induced lung injury utilizing [^{111}In]-anti-ICAM-1 monoclonal IgG antibody reveals that the percent injected dose/organ in lungs both at 4 and 24 hours postbleomycin increases significantly in contrast to values observed in either uninjured reference controls or with the administration of [^{111}In]*nmIgG. At 4 and 24 hours postinjury, the target-to-blood (T/B) ratio is 8/1 and 6/1, respectively. For [^{111}In]*nmIgG, the T/B ratio at 4 hours is 0.5/1 and 0.4/1 at 24 hours. [^{111}In]-anti-ICAM-1 monoclonal IgG antibody preparations administered post-bleomycin-induced pulmonary injury produce 4- and 24-hour images that can easily be distinguished from uninjured reference controls. All images with [^{111}In]*nmIgG show only cardiac blood-pool and liver activity with little lung activity. Lung ICAM-1 immunofluorescence intensity increases in the bleomycin-treated pulmonary samples compared to uninjured lungs. Expression of LFA-1α on PMNs increases 19% and 210% at 4 hours and 24 hours postinjury, respectively, compared to control values. Biodistribution and imaging data therefore demonstrate that [^{111}In]-anti-ICAM-1 monoclonal IgG antibody is capable of detecting early acute bleomycin-induced lung injury. Immunofluorescence and FACS data suggest that [^{111}In]-anti-ICAM-1 monoclonal IgG antibody uptake is a specific process. Monoclonal antibody preparations possessing such attributes have potential as an early radionuclide detector of acute inflammations.

Early detection of bleomycin-induced lung injury in rat using indium-111-labeled antibody directed against intercellular adhesion molecule-1. Weiner RE, Sasso DE, Gionfriddo MA, Syrbu SI, Smilowitz HM, Vento J, Thrall RS. J Nucl Med 1998 Apr; 39 (4), pp. 723–8.

Inflammatory Pulmonary Disease/Acute Respiratory Distress Syndrome (ARDS)
[^{111}Indium] ([^{111}In])-Labeled Neutrophils/[^{111}In]-aICAM-1/[^{111}In]-Labeled Normal Mouse IgG (nmIgG)/[^{111}In]-Labeled Rat Serum Albumin (RSA)

Cellular/Molecular Mechanism of Action: The monoclonal immunoglobulin aICAM-1 recognizes and physically binds to ICAM-1 complexes (intracellular adhesion molecule-1). Neutrophils

through mechanisms of chemotactic activity migrate to anatomical regions of inflammation and sepsis.

Background Information: Previous study of the bleomycin-induced lung injury models has revealed that [^{111}In]-labeled anti-rat intercellular adhesion molecule-1 (aICAM-1) might be a useful ARDS diagnostic agent.

Application: Detection and characterization of conditions of inflammation within the lung similar to that associated with ARDS.

Implementation: Evaluation of the biodistribution, scintigraphic imaging profiles, and lung ICAM-1 upregulation utilizing [^{111}In]-labeled polymorphonuclear leukocytes (PMNs), [^{111}In]-aICAM-1, [^{111}In]-labeled nmIgG, and [^{111}In]-labeled RSA injected 18–24 hours before euthanasia.

Animal Model: Rat neutrophils (polymorphonuclear leukocytes or PMNs) and rat *in vivo* radioactive distribution studies following induced injury with oleic acid.

Interpretation and Analysis: [^{111}In]-RSA and [^{111}In]-nmIgG localize in inflamed lung at 5 minutes postinjury (PI). [^{111}In]-PMN uptake increases significantly only at 24 hours PI. [^{111}In]-aICAM-1 localization increases significantly (30–60%) at 1 hour PI and remains elevated up to 24 hours PI. Lung/blood ratios (L/B) at 1 and 4 hours PI are very low (< 0.6) for [^{111}In]-nmIgG and [^{111}In]-PMN; however, for [^{111}In]-aICAM-1, values are > 1 and 25–60% higher than those observed for reference controls. A low L/B suggests poor detection of inflammation on acquired images. Images and region-of-interest (ROI) analysis confirm that only [^{111}In]-aICAM-1 can distinguish inflamed lungs at 4 hours PI. ICAM-1 is upregulated at 4 and 24 hours PI. Under certain circumstances, [^{111}In]-aICAM-1 can detect lung inflammation very early in the course of the disease, which suggests that [^{111}In]-aICAM-1 could be a very early, highly specific ARDS diagnostic agent and may be useful to detect a wide range of inflammations.

Early detection of oleic acid–induced lung injury in rats using [^{111}In]-labeled anti-rat intercellular adhesion molecule-1. Weiner RE, Sasso DE, Gionfriddo MA, Thrall RS, Syrbu S, Smilowitz HM, Vento J. J Nucl Med 2001 Jul; 42 (7), pp. 1109–15.

Pulmonary Metabolism: FMO-II
[^{11}C]-Octylamine

Cellular/Molecular Mechanism of Action: Preferentially sequesters within pulmonary tissues.

Physiological Mechanism of Action: Substantial levels of flavin-containing monooxygenase Type II (FMO-II) can be found associated with microsomal enzyme fractions; it possesses considerably high activities for primary amines like octylamine.

Application: Characterization of metabolizing schemes and as an investigative platform for evaluating candidate pharmaceutical agents.

Route of Administration: [^{11}C]-octylamine.

Dosage: [^{11}C]-octylamine.

Interpretation and Analysis: The initial lung uptake during the 1st minute is $70 \pm 6\%$ of the administered dose, and 40% of the injected dose remains after 15 minutes. Approximately 12% of the administered radioactivity is exhaled as [$^{11}CO_2$] during the 1st 30 minutes.

Metabolism of fluperlapine by cytochrome P450-dependent and flavin-dependent monooxygenases in continuous cultures of rat and human cells. Fischer V, Wiebel FJ. Biochem Pharmacol 1990 Apr 15; 39 (8), pp. 1327–33.

Ventilation Imaging/Pulmonary Embolism/ Ventilation/Perfusion Dual Imaging Evaluation
Xenon ([^{133}Xe])/[^{99m}Technetium] ([^{99m}Tc])-Macroaggregated Serum Albumin

Cellular/Molecular Mechanism of Action: Diffusible radioactive gas, which is inhaled down to the level of the alveolus.

Application: Detection of altered or abnormal ventilation patterns within the pulmonary tree. Examples include neoplastic lesions and septic pulmonary disease.

Route of Administration: Inhalation.

Dosage: Respiratory system inhalation.

Implementation: Evaluation of pulmonary embolism cases as a function of [^{133}Xe] ventilation images in 2 posterior oblique views; [^{99m}Tc]-macroaggregated serum albumin perfusion scans; and angiograms in cases subsequently discharged on anticoagulant therapy. On review, angiograms are considered embolism positive if intraluminal filling defects, a trailing embolus, or a branch occlusion $\geq$ than a segmental branch are detected (n = 29; 73%); and uncertain if reinterpreted as either equivocal or negative or in the presence of a single, small subsegmental filling defect of questionable clinical significance. The ventilation-perfusion scans are read as high (n = 18; 45%), intermediate (n = 10; 25%), or low (n = 12; 30%) probability.

Human Model: Cases that have undergone both nuclear imaging and invasive selective pulmonary angiography (n = 160; n = 40 embolism cases discharged on anticoagulant therapy; average age 57 years; n = 25/40 female).

Interpretation and Analysis: The proportion of cases with diagnostic certainty on angiography in the high-, intermediate-, and low-probability scintigraphic subgroups is, respectively, 100% (n = 18/18), 70% (n = 7/10), and 33% (n = 4/12) (p = 0.004). In patients diagnosed with pulmonary embolism based on selective angiography, a lower probability of pulmonary embolism on ventilation-perfusion scintigraphy correlates with a lesser degree of diagnostic certainty on angiography and a higher incidence of single subsegmental emboli.

Scintigraphic probability and angiographic diagnostic certainty in acute pulmonary embolism. Lette J, Cerino M, Barrette G, Dufresne MP, De Maria S, Eybalin MC, Levasseur A. Clin Nucl Med 2003 Nov; 28 (11), pp. 897–904.

Ventilation Perfusion/Mismatch Profiles/Reverse Ventilation Perfusion Mismatch
[^{99m}Technetium] ([^{99m}Tc])-Diethylenetriamine Pentaacetic Acid (DTPA) Aerosol/[^{99m}Tc]-Macroaggregated Albumin (MAA)

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-DTPA aerosol simply permeates the air inhaled during the ventilation cycle and the radioactive vapor subsequently enters functional alveolei. [^{99m}Tc]-MAA, due to its size, remains primarily within the intravascular compartment following direct infusion.

Background Information: The phenomenon of reverse mismatched ventilation-perfusion on pulmonary scintigraphy is a fairly common occurrence.

Application: [^{99m}Tc]-DTPA aerosol can be applied to detect regional alterations in pulmonary ventilation. Conversely, [^{99m}Tc]-MAA can be utilized to map regional changes in pulmonary vascular perfusion.

Route of Administration: [^{99m}Tc]-DTPA aerosol (inhalation); [^{99m}Tc]-MAA (intravenous).

Implementation: Evaluation of scenarios characterized by alteration in pulmonary ventilation and vascular perfusion.

Interpretation and Analysis: Affected cases can display evidence of declining oxygen saturation and reverse mismatched ventilation-perfusion imaging patterns associated with radiotracer retention in a main bronchus. In such situations, [^{99m}Tc]-DTPA aerosol intake within the lung can reveal tracer retention in the trachea and right main bronchus and absent ventilation in the right or left lung. Alternatively, [^{99m}Tc]-MAA lung images reflect patterns of pulmonary vascular perfusion within either the left or right lung. In this fashion, it is possible to detect regions within the lung that may be perfused but not ventilating, or segments that are ventilating but not being perfused adequately (e.g., ventilation-perfusion mismatch). In contrast, reverse mismatched ventilation-perfusion, or totally absent ventilation with preservation of some perfusion in the right lung, results in functional intrapulmonary shunting, which can explain decreasing oxygen saturation observed in some pulmonary conditions. A concurrent portable thoracic radiograph may show elevations of the right hemidiaphragm, a shift of the mediastinum to the right, deviation of the endotracheal tube, narrowing of the intercostal space of the right thorax, and collapse of the right lower lobe. Radiographic findings of underventilation of the right lung with atelectasis of the right lower lobe can be due to a variety of causes, including mucous obstruction of the right main bronchus.

Reverse mismatched ventilation-perfusion pulmonary imaging with accumulation of technetium-^{99m}-DTPA in a mucous plug in a main bronchus: A case report. Shih WJ, Bognar B. J Nucl Med Technol 1999 Dec; 27 (4), pp. 303–5.

Ventilation Perfusion/[Strategies for Improved Imaging]
[^{133}Xe] Aerosol/[^{99m}Technetium] ([^{99m}Tc]) Intravascular Marker

Background Information: In nuclear medicine, the match and mismatch between the images of lung perfusion and ventilation provide an important criterion for the diagnosis of pulmonary embolism. Usually, for imaging clarity, ventilation scans using [^{133}Xe] are performed before the perfusion scan using [^{99m}Tc]. But the inverse order is preferred clinically, because (i) if the perfusion image is normal, there is no need to do the ventilation scan, and (ii) if the perfusion image is abnormal, the ventilation image can be obtained by focusing on the perfusion abnormalities. However, the quality of the ventilation image is reduced if the ventilation scan is performed after perfusion scans, because the 140 keV photons emitted from [^{99m}Tc] will scatter into the acquisition window of [^{133}Xe] (81 keV).
Implementation: Evaluation of image processing techniques for the purpose of discovering strategies that provide reduced scattering effect from [^{99m}Tc] in a manner that facilitates acquisition of better [^{133}Xe] ventilation images.
Interpretation and Analysis: First, an image sequence in the preferred inverse order is simulated using the images acquired in the normal order. Image processing techniques are used to find an optimized way to reduce the scattering background in the ventilation image. Second, a real image sequence is acquired in the inverse order. An improved ventilation image is then obtained by applying image processing techniques to this image sequence.

Enhancement of Xe-133 ventilation lung scan image acquired after [^{99m}Tc] perfusion scan. Lu H, Farison JB, Dennis MJ. Biomed Sci Instrum 1997; 33, pp. 118–25.

Ventilation Perfusion
[^{99m}Technetium] ([^{99m}Tc])-C and [^{81m}Kr] Aerosol Inhalation/[^{99m}Tc]-Macroaggregated Albumin (MAA)

Background Information: A method of producing aerosols (technegas) that been described, in which [^{99m}Tc] is bound to carbon atoms ([^{99m}Tc]-C), has been evaluated by comparing [^{99m}Tc]-C images with those obtained with [^{81m}Kr] in the same patients.
Route of Administration: [^{99m}Tc]-MAA (intravenously).
Dosage: [^{99m}Tc]-C and [^{81m}Kr] (200 kcounts inhaled) and [^{99m}Tc]-MAA injection (4–7 mCi injected, and 400 kcounts inhaled).
Implementation: Evaluation of ventilation/perfusion patterns. Immediately after the last [^{99m}Tc]-C view, a supine position is maintained and [^{81m}Kr] is inhaled at tidal volume. Immediately after [^{81m}Kr] ventilation views are recorded, MAA preparations are injected (4–7 mCi). The same 4 views (ant, lop, rop, post) are recorded after inhalation of [^{99m}Tc]-C and [^{81m}Kr] and injection of [^{99m}Tc]-MAA (400 kcounts).

Human Model: Cases suffering from 23-fold pulmonary embolism (PE; n = 25).
Interpretation and Analysis: The mean penetration index of [^{99m}Tc]-C (0.91) is lower than that of [^{81m}Kr] (1.04) ($P < 0.03$). The apex to base lung distribution of [^{99m}Tc]-C and [^{81m}Kr] appear to be similar. The mean heterogeneity of [^{99m}Tc] distribution is 23 fold greater than that of [^{81m}Kr] (14) ($p = 10^{-4}$). The [^{99m}Tc]-C ventilation image quality is considered very good (e.g., n = 16/25) or good (e.g., n = 6/25). Significant foci of high bronchial uptake are infrequent. Interpretation of the examinations performed after inhalation of [^{99m}Tc]-C and [^{81m}Kr] is usually concordant (e.g., n = 25/25 or 100%). Usually no cases have an [^{81m}Kr]/[^{99m}Tc]-MAA examination suggestive of PE when [^{99m}Tc]-C/[^{99m}Tc]-MAA indicates a low probability of PE, and vice versa. [^{99m}Tc]-C aerosols enable good quality ventilation images to be obtained in nearly all cases. Thus [^{99m}Tc]-C aerosols could be used in preference to [^{81m}Kr] in ventilation studies for the diagnosis of PE.

Comparison of technetium-^{99m} aerosol and krypton-81m in ventilation studies for the diagnosis of pulmonary embolism. Peltier P, De Faucal P, Chetanneau A, Chatal JF. Nucl Med Commun 1990 Sep; 11 (9), pp. 631–8.

Ventilation/Perfusion (V/Q)
[^{99m}Technetium] ([^{99m}Tc])-Pertechnegas/[^{99m}Tc]-Macroaggregated Albumin (MAA)

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-Pertechnegas is inhaled into the deepest reaches of the respiratory tract similar to gaseous air. [^{99m}Tc]-MAA, due to its large size (molecular mass), largely remains within the intravascular compartment after injection.
Background Information: [^{99m}Tc]-Pertechnegas is a micro-aerosol carbon particle labeled with [^{99m}Tc] radioisotope.
Route of Administration: [^{99m}Tc]-Pertechnegas (inhaled).
Dosage: [^{99m}Tc]-Pertechnegas (1 mCi or 37 MBq); [^{99m}Tc]-MAA (4 mCi or 148 MBq).
Implementation: Evaluation of suspected pulmonary emboli lesions applying ventilation imaging facilitated by the inhalation of [^{99m}Tc]-Pertechnegas in 5 breaths or less. Planar images in multiple projections are recorded for preset counts. A final posterior image can be acquired to evaluate residual lung background activity. Immediately following ventilation imaging, perfusion imaging in the identical projections is performed using [^{99m}Tc]-MAA.

Human Model: Cases suspected of suffering from pulmonary emboli (n = 4).
Interpretation and Analysis: A number of cases may demonstrate matched uniform ventilation and perfusion (e.g., n = 2/4) or segmental ventilation-perfusion "mismatch" defects (e.g., n = 2/4) consistent with pulmonary emboli. In each case, residual Pertechnegas background lung activity (count rate) at the end of the last ventilation image is less than 10% of the initial [^{99m}Tc]-MAA counts (count rate). The advantages of Pertechnegas, in comparison to

aerosolized diethylenetriamine pentaacetic acid (DTPA), include (i) less residual ventilation image activity superimposed on the MAA perfusion images; (ii) reduced demand for patient cooperation due to fewer required breaths (5 or less); and (iii) lack of deposition of activity in the central airways. Therefore, Pertechnegas has properties that make it ideally suited for routine lung ventilation imaging.

Technetium-^{99m} labeled microaerosol "Pertechnegas." A new agent for ventilation imaging in suspected pulmonary emboli. Ashburn WL, Belezzuoli EV, Dillon WA, Mensh BD, Hoogland D, Yeung DW, Coade GE. Clin Nucl Med 1993 Dec; 18 (12), pp. 1045–52.

Ventilation/Perfusion (V/Q)/[High Spatial Resolution]
Xenon ([^{133}Xe])

Cellular/Molecular Mechanism of Action: Xenon is radioactive gas that can be inhaled into the lower respiratory tract.
Route of Administration: Xenon is inhaled as an aerosol so that regions of the lung can be profiled according to their degree of ventilation or lack thereof.
Implementation: Evaluation of pulmonary function by measuring both regional ventilation (V) and perfusion (Q) in which the regional radiodensity (RD) in the lung during [^{133}Xe] wash-in is a function of regional V (increasing RD) and Q (decreasing RD). Four 2.5-mm-thick computed tomography (CT) images are simultaneously acquired immediately cephalad to the diaphragm at end inspiration for each breath during 3 minutes of [^{133}Xe] breathing. Observed changes in RD during [^{133}Xe] wash-in are used to determine regional V and Q.

Animal Model: Sheep (5 anesthetized, pharmacologically paralyzed, mechanically ventilated, supine).
Interpretation and Analysis: For 16 mm^3, Q displays more variance than V: the coefficient of variance of Q (CV_Q) = 1.58 ± 0.23, the CV of V (CV_V = 0.46 ± 0.07, and the ratio of CV_Q to CV_V = 3.5 ± 1.1. CV_Q (1.21 ± 0.37) and the ratio of CV_Q to CV_V (2.4 ± 1.2) is smaller at 1,000-mm^3 scale, but CV_V (0.53 ± 0.09) is not. V/Q distributions also display scale dependence: log SD of V and log SD of Q are 0.79 ± 0.05 and 0.85 ± 0.10 for 16-mm^3 and 0.69 ± 0.20 and 0.67 ± 0.10 for 1,000-mm^3 regions of lung, respectively. V and Q measurements made with CT and [^{133}Xe] also demonstrate vertical orientation and isogravitational heterogeneity as described using other methodologies. Sequential images acquired by CT during [^{133}Xe] breathing can be used to determine both regional V and Q non-invasively with high spatial resolution.

Determination of regional ventilation and perfusion in the lung using xenon and computed tomography. Kreck TC, Krueger MA, Altemeier WA, Sinclair SE, Robertson HT, Shade ED, Hildebrandt J, Lamm WJ, Frazer DA, Polissar NL, Hlastala MP. J Appl Physiol 2001 Oct; 91 (4), pp. 1741–9.

RESPIRATORY BREATH TESTS: PULMONARY AND HEPATIC FUNCTION ASSESSMENT

Asthma Bronchiale/Inhalation Diagnostic Agent
L-[guanidino-[^{15}N]$_2$-Arginine Monohydrochloride/ Spirometry and Oscillometry Alternative

Application: Inhalation breath test.
Route of Administration: Inhalation.
Dosage: Aqueous solution, 20 atom % [^{15}N].
Considerations: Inhalation of a chemical diagnostic agent should provide novel ways of establishing a more specific diagnosis, for instance of inflammatory states of the bronchial and lung mucosa. The amino acid, L-[guanidino-[$^{15}N_2$]arginine monohydrochloride is resorbed and partly metabolized to [^{15}NO].
Implementation: Exhaled air is collected under defined conditions in 10-L breath bags and analyzed for NO using chemiluminescence.
Interpretation and Analysis: Under standardized test conditions, healthy persons (e.g., n = 6) exhale 0.97 ± 0.08 μmol NO/m^3 compared to conditions of asthma (e.g., n = 7) with 1.17 ± 0.14 μmol NO/m^3. A better distinction is expected comparing the [^{15}NO] exhalation. The [^{15}N] abundance of NO is determined using Cryotrap gas chromatography–mass spectrometry instrumentation. Between 30 and 80 minutes after inhaling 700 mg [^{15}N]arginine, a maximum with a plateau of the [^{15}NO] abundance is found in the exhaled air. At this time, healthy and asthmatic subjects exhibit clear differences in their exhaled [^{15}NO] amounts. Under standardized test conditions, healthy cases (n = 6) exhale 102.3 ± 6.7 nmol [^{15}NO]/m^3, whereas asthmatic cases (n = 7) exhale only 76.1 ± 10.9 nmol [^{15}NO]/m^3. The yield of [^{15}NO] after the inhalation of [^{15}N]-labeled arginine could potentially serve as a marker for demonstrating pathophysiological changes in lung epithelium.

[$^{15}N]_2$-arginine as a 1st potential inhaled diagnostic agent to characterize respiratory diseases. Krumbiegel P, Denk E, Russow R, Rolle-Kampczyk U, Metzner G, Herbarth O. Exp Lung Res 2002 Oct–Nov; 28 (7), pp. 535–42.

Hepatic Function: Breath Test
[^{13}C]-Methacetin/[^{15}N]-Methacetin/[^{14}C]-Methacetin

Application: Detection of conditions associated with hepatic disease.
Considerations: Stable isotopes, such as [^{13}C], should be substituted for the long-lived radionuclide [^{14}C] in the [^{14}C] breath test when doing liver function tests in children and pregnant women.
Implementation: Synthesize, evaluate, and compare the capacity of [^{13}C], [^{15}N]-methacetin, and [^{14}C]-methacetin to function as suitable diagnostic agents. Methods have been described for the measurement of [^{14}C] and [^{13}C] in the breath.
Interpretation and Analysis: Following oral administration of radio carbon-labeled methacetin to cases with liver disease and healthy reference controls, a good degree of correlation can be appreciated between [^{13}C]- and [^{14}C]-measurements in the same subject, which is complemented by an effective level of discrimination between controls and affected cases. Measurements of [^{14}C] in the urine support the presumed advantage of [^{13}C]-methacetin over [^{14}C]-methacetin when applied for such purposes.

[^{13}C]-methacetin breath test for monitoring hepatic function in cirrhotic patients before and after liver transplantation. Petrolati A, Festi D, De Berardinis G, Colaiocco-Ferrante L, Di Paolo D, Tisone G, Angelico M. Aliment Pharmacol Ther 2003 Oct 15; 18 (8), pp. 785–90.

Hepatic Metabolic Capacity Breath Test
[^{13}C]-Methacetin/[^{14}C]-Methacetin/[^{15}N]-Methacetin

Background Information: To grade liver damage, the Child-Pugh classification can be used, but these tests do not quantitatively reflect the functional hepatic reserve.
Application: Detection of conditions associated with hepatic disease.
Considerations: Stable isotopes, such as [^{3}C], should be substituted for the long-lived radionuclide [^{14}C] in the [^{14}C] breath test when doing liver function tests in children and pregnant women. For comparison, [^{13}C]-methacetin, [^{15}N]-methacetin, and [^{14}C]-methacetin have been synthesized as suitable diagnostic agents.

Implementation: Evaluation of liver function through assessement of hepatic metabolization of biochemical substrates detected by measurement of [^{14}C] and [^{13}C] in exhaled CO_2 air.

Diagnostic Interpretation and Implications: Following oral administration of radio carbon-labeled methacetin, a good degree of correlation can be appreciated between [^{13}C]- and [^{14}C]-measurements in the same subject, which is complemented by an effective level of discrimination between controls and cases with hepatic disease. Measurements of [^{14}C] in urine support the presumed advantage of [^{13}C]-methacetin over [^{14}C]-methacetin when applied for this particular purpose.

[^{13}C]-methacetin metabolism in patients with cirrhosis: Relation to disease severity, hemoglobin content, and oxygen supply. Zipprich A, Meiss F, Steudel N, Sziegoleit U, Fleig WE, Kleber G. Aliment Pharmacol Ther 2003 Jun 15; 17 (12), pp. 1559–62.

Hepatic Metabolism/Dysfunction and Biochemical Capacity/[Breath Test]
[^{13}C]-Methacetin/[^{13}C]-Phenylalanine

Cellular/Molecular Mechanism of Action: [^{13}C]-methacetin and [^{13}C]-phenylalanine are radioactive analogues of methacetin and the amino acid phenylalanine. Each of these agents is metabolized in the liver and radioactive [$^{13}CO_2$] is ultimately excreted in exhaled air.

Background Information: To grade liver damage, the Child-Pugh classification can be used, but these tests do not quantitatively reflect the functional hepatic reserve.

Considerations: Advantages of the [^{13}C]-phenylalanine breath test and [^{13}C]-methacetin breath test include their large margin of safety and convenient utilization for quantifying functional hepatic reserve.

Application: [^{13}C]-phenylalanine breath test and [^{13}C]-methacetin breath tests can potentially be utilized to quantify functional hepatic reserve in conditions of chronic liver disease.

Dosage: [^{13}C]-phenylalanine (100 mg) and [^{13}C]-methacetin (75 mg).

Implementation: Evaluation of [^{13}C]-phenylalanine and [^{13}C]-methacetin as possible molecular/biochemical probes in breath test protocols for assessing liver function in the form of functional hepatic reserve in conditions of chronic hepatic disease. In this context, breath samples are collected after the administration of [^{13}C]-phenylalanine and [^{13}C]-methacetin. Mass spectrometry analysis is utilized to measure [$^{13}CO_2$] enrichment.

Human Model: Both tests performed in 48 healthy volunteers and 48 chronic liver disease patients.

Interpretation and Analysis: Both tests discriminate between normal and abnormal hepatic function, decreasing results of the [$^{13}CO_2$] enrichment agreeing with the increasing severity in cases of hepatic disease ([^{13}C]-phenylalanine breath test multiple correlation coefficient: 0.72, global $p < 0.001$; [^{13}C]-methacetin breath test: 0.73, $p < 0.001$). Correlation between the [^{13}C]-phenylalanine breath test and [^{13}C]-methacetin breath test is 0.63, $p < 0.001$. In cases where both tests detect pathological changes, the sensitivity for detecting hepatic dysfunction is high (98%), although specificity decreases to 60%. Best results are obtained at 30 minutes with the [^{13}C]-phenylalanine breath test and at 10 minutes with methacetin breath test. Therefore, both [^{13}C]-phenylalanine breath test and methacetin breath test are safe and easy tests to perform and both are able to discriminate the hepatic functional capacity between the different groups studied.

[^{13}C]-phenylalanine and [^{13}C]-methacetin breath test to evaluate functional capacity of hepatocyte in chronic liver disease. Lara Baruque S, Razquin M, Jimenez I, Vazquez A, Gisbert JP, Pajares JM. Dig Liver Dis 2000 Apr; 32 (3), pp. 226–32.

Intestinal Malabsorption and Compromised Mucosal Integrity/[Hydrogen Breath Test]
D-Xylose Absorption

Application: Malabsorptive evaluation in renal failure is difficult because most absorptive testing requires urinary collections. Kinetic analysis of D-xylose absorption and D-xylose breath testing are performed in an effort to establish an effective absorption test in cases that are functionally anephric.

Route of Administration: D-xylose (oral and intravenously).

Dosage: D-xylose 15-g (oral) and 10-g (intravenous) dosage schemes.

Considerations: Serum samples have been used to calculate the kinetic rate constants and extent of D-xylose absorption. After the oral D-xylose, end expiratory breaths are collected every 15 minutes for 3 hours and analyzed for H_2 (e.g., measured by gas chromatography analysis).

Implementation: Evaluation of fasting renal failure cases without diarrhea or symptoms of malabsorption on 2 separate non-dialysis days after receiving D-xylose on day 1 (oral) and day 2 (IV). Serum samples are used to calculate the kinetic rate constants and extent of D-xylose absorption.

Interpretation and Analysis: The mean absorption rate constant (Ka) and bioavailability (F) are similar to published values for normal subjects using the 15-g dose (0.936 min^{-1}; range, 0.227–1.96; and 74%, range 46–99, respectively). Some cases ($n = 12$) have normal 1-hour serum D-xylose concentrations (> 20 mg/dL). There are no clear inverse correlations between the rate constant for absorption or bioavailability and peak breath hydrogen or the area-under-the-curve for breath H_2 vs time. Using 15-g oral D-xylose, mean bioavailability and absorption rate constants are normal in functionally anephric patients with no clinical evidence of malabsorption. Some cases ($n = 3$) have elevated breath peak H_2 concentrations, but there are no clear inverse correlations between bioavailability and breath H_2 values. A 1-hour serum D-xylose concentration > 20 mg/dL may be considered normal in such case groups, similar to patients with normal renal function.

Instrumentation: Gas chromatography (hydrogen gas measurement).

D-xylose kinetics and hydrogen breath tests in functionally anephric patients using the 15-gram dose. Craig RM, Carlson S, Ehrenpreis ED. J Clin Gastroenterol 2000 Jul; 31 (1), pp. 55–9.

Pulmonary Cell Decompensation/Pulmonary Inflammation
[$^{1\text{-}13}$C]-Glucose/([$^{13}CO_2$] Breath Tests)

Cellular/Molecular Mechanism of Action: Following inhalation, agents are absorbed into the systemic circulation where they are subsequently metabolized by the liver and then exhaled as a radiolabeled metabolite (e.g., CO_2).

Application: Breath test (CO_2-based).

Route of Administration: An aerosolized aqueous solution (25%) is inhaled with the aid of an aerosol provocation system (APS).

Dosage: Optimum inhalation dose for [^{13}C]-glucose has been determined to be 205 mg.

Considerations: Many tests designed to evaluate pathophysiological alterations of the gastrointestinal tract involve the oral intake of a biochemical substrate that is metabolized and subsequently measured in exhaled air samples. (e.g., CO_2-based breath test, [^{15}N]-based urine test). Despite this traditional methodology, it seems logical that the study of pathophysiological changes of the bronchial and lung epithelium can be more appropriately evaluated through the inhaled administration of a stable isotope-labeled agent.

Investigators have suggested that the inhalation of suitable [^{13}C]-labeled substrates will pave the way for a new group of [$^{13}CO_2$] breath tests aiding investigations of specific pathophysiological changes in the pulmonary tract, such as inflammations of certain sections and decompensations of cell functions.

Implementation: Evaluation of suitable [^{13}C]-labeled substrates for the detection and quantitative measurement of [$^{13}CO_2$] in exhaled air. Breath samples are collected at 15-minute intervals and analyzed for [$^{13}CO_2$] 75–120 minutes after the end of inhalation.

Interpretation and Analysis: Breath samples collected at 15-minute intervals and analyzed for [$^{13}CO_2$] 75–120 minutes after the end of inhalation demonstrate a well-reproducible maximum delta [^{13}C] value of 6% over baseline (DOB). Speculating that the pulmonary resorption of the [^{13}C]-glucose is the rate-limiting step of elimination, decompensations in the epithelium ought to be reflected as changes in 1-[^{13}C]-glucose resorption rates and altered [$^{13}CO_2$] output.

Instrumentation: Nebulizer (“Medic Aid”; Erich Jaeger Würzburg).

Towards an inhalative [^{13}C] breath test method. Krumbiegel P, Rolle-Kampczyk U, Liebergeld P, Herbarth O, Köbrich R. Isotopes Environ Health Stud 2002 Jun; 38 (2), pp. 65–70.

19 Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT)

General/Artifacts in Positron Emission Tomography (PET)/Computed Tomography (CT) Imaging
Iodinated Contrast Imaging Agents

Implementation: Intravenous and oral iodinated contrast agent administered to oncology cases (e.g., n = 30) combined with whole-body PET/CT. Computed tomography images acquired in the presence of intravenous and oral iodinated contrast agent are used for PET attenuation correction. Positron emission tomography images have been assessed for the presence of artifacts that are defined as a region of high count rate on attenuation-corrected images in accurate co-registration with a contrast-enhanced blood vessel. Intravascular enhancement of thoracic veins is quantified by the application of regions-of-interest (ROI) analyses, and quantities in cases with artifact (Group 1) and without artifact (Group 2) correlated. Body surface area is calculated for all cases.

Interpretation and Analysis: Contrast-induced PET artifact can be appreciated in approximately 13% of cases (e.g., n = 4/30 cases). Mean differences in density within enhanced intravascular images are highly significant ($p < 0.001$) compared to case groups with artifact (2,262 ± 304 Hounsfield units [HU]) and without artifact (1,058 ± 209 HU). Body surface area is significantly lower ($p = 0.035$) in cases with artifact (1.67 ± 0.11 m^2) than in cases without artifact (2.01 ± 0.18 m^2). Contrast-enhanced dual-modality PET/CT examinations may result in a PET artifact that is due to the transient bolus passage of undiluted intravenous contrast agent.

Focal tracer uptake: A potential artifact in contrast-enhanced dual-modality PET/CT scans. Antoch G, Freudenberg LS, Egelhof T, Stattaus J, Jentzen W, Debatin JF, Bockisch A. J Nucl Med 2002 Oct; 43 (10), pp. 1339–42.

Adrenal Gland: Function and Structure
[^{131}I]-6-β-Iodomethyl-Norcholesterol/[^{75}Se]-Selenomethyl-Norcholesterol/[^{131}I]-, [^{123}I]-, and [^{124}I]-Metaiodobenzylguanidine (MIBG)/[^{18}F]-Metafluoro-Benzyl-Guanidine/[^{123}I]-Tyr-Octreotide/[^{111}In]-Diethylenetriamine Pentaacetic Acid (DTPA)-Octreotide/[^{111}In]-DOTA-Octreotide/[^{18}F]-2-Fluoro-d-Deoxyglucose/Metyrapone/Tyrosine Analogs/Low-Density Lipoproteins (LDL Receptors)/Bombesin/Vasoactive Intestinal Polypeptide/Cholecystokinin/Opiate Peptides/[^{11}C]-Acetate

Background Information: Over the past 30 years nuclear medicine imaging of the adrenal gland and its lesions has been achieved by exploitation of a number of physiological characteristics associated with this organ. By seeking and utilizing features that are quantitatively or qualitatively different from those of the adjacent tissues, it is possible to functionally characterize the adrenal gland and associated disease states. In most instances lesions affecting both the cortex and the medulla retain the basic physiology related to their tissue of origin. Agents widely used in clinical practice include (i) uptake and storage of radiolabeled cholesterol analogues via the LDL receptors and cholesterol ester storage pools in the adrenal cortex utilizing [^{131}I]-6-β-iodomethyl-norcholesterol and [^{75}Se]-selenomethyl-norcholesterol; (ii) catecholamine type I, (presynaptic, uptake mechanism and intracellular granule uptake and storage mechanism in the adrenal medulla and extra-adrenal paraganglia utilizing [^{131}I]-, [^{123}I]-, and [^{124}I]-MIBG and [^{18}F]-meta-fluoro-benzyl-guanidine); (iii) cell surface receptor binding of peptides/neurotransmitters/modulators such as those for the family of 5 subtypes of somatostatin receptors ([^{123}I]-tyr-octreotide, [^{111}In]-DTPA-octreotide, [^{111}In]-DOTA-octreotide, and many others); (iv) although not specific for the adrenal gland, increased tumor glycolysis, particularly in the most malignant adrenal-associated cell types, the [^{18}F]-2-fluoro-d-deoxyglucose can be expected to detect certain neoplastic lesions such as malignant pheochromocytomas (particularly the minority of cases that cannot be detected by MIBG) and adrenal incidentalomas (particularly when they occur with known extra-adrenal malignancies).

There is a variety of adrenal tissue characteristics that have the potential for being exploited for various purposes that are not currently employed for clinical uses, but nevertheless have potential as imaging agents. These include (i) inhibitors of adrenal cortical steroid hormone synthesis enzymes (e.g., radiolabeled analogues of metyrapone); (ii) radiolabeled lipoproteins that bind to adrenocortical LDL receptors; (iii) inhibitors of catecholamine biosynthesis enzymes (e.g., radiolabeled analogues of tyrosine and related amino acids); and (iv) cell surface receptors for various peptides and hormones that may be overexpressed by adrenal cortical or adrenal medullary tumors (e.g., radiolabeled analogues of adenocorticotropic hormone (ACTH) for adrenocortical cells of zona fasciculata or zona glomerulosa origin; neurotransmitter/hormone message peptides binding to cell surface receptors such as bombesin, vasoactive intestinal polypeptide, cholecystokinin, and opiate peptides). The adrenal cortex can also synthesize cholesterol from acetate, and preliminary studies with [^{11}C]-acetate positron emission tomography have shown interesting results.

Application: Detection and characterization of the adrenal gland function and dysfunction.

Functional scintigraphy of the adrenal gland. Rubello D, Bui C, Casara D, Gross MD, Fig LM, Shapiro B. Eur J Endocrinol 2002 Jul; 147 (1), pp. 13–28.

CARDIOVASCULAR SYSTEM

Acetylcholine Receptors: Muscarinic/Cardiac Muscarinic Acetylcholinergic Receptors/Central Nervous System (CNS) Muscarinic Acetylcholinergic Receptors/Carcinoma of the Colon
[^{123}I]-PIP/[^{125}I]-PIP

Cellular/Molecular Mechanism of Action: The agent IPIP functions as a ligand for muscarinic acetylcholinergic complexes (mAChR).

Background Information: Prior to approval for *in vivo* use in humans, organ doses for radioiodinated IPIP ligands must 1st be evaluated.

Application: Radioiodinated IPIP ligands can be applied *in vivo* for imaging mAChR using modalities like single photon emission computed tomography (SPECT).

Implementation: Identify the most optimum mAChR ligand-binding properties of 4 different IPIP sterioisomers through the evaluation of their biodistribution pattern. A biokinetic model can be developed for the Z-(S)-IPIP stereoisomer for estimating the internal absorbed dose in humans based on extrapolation from an appropriate rat model.

Animal Model: Rat (IPIP biokinetic model).

Interpretation and Analysis: The thyroid is the critical organ for this radiopharmaceutical, with an absorbed dose estimate of 2.4 mGy/MBq for both males and females, when labeled with [^{123}I]. Even when blocked, the thyroid is still the critical organ, yet it receives a 90% reduction in dose. The heart and brain receive the next highest doses in both males and females. Effective dose estimates for the use of pure [^{123}I]-PIP in humans are 0.16 mSv/MBq for males and 0.14 mSv/MBq for females. The biodistribution studies of the Z-(S)-IPIP stereoisomer show the most promise as a successful agent for imaging muscarinic receptor sites in the heart and brain. IPIP can potentially be applied as a therapeutic radiopharmaceutical for certain colon carcinomas where muscarinic receptor sites are expressed in the tumor cells. These results provide preliminary data for use of IPIP in clinical studies on humans.

In vivo biodistribution of [^{125}I]-PIP and internal dosimetry of [^{123}I]-PIP radioiodinated agents selective to the muscarinic acetylcholinergic receptor complex. Breeden WK 3rd, Hamby DM, Carey JE Jr, Eckerman KF, McPherson DW, Knapp FF Jr. Med Phys 2000 Apr; 27 (4), pp. 778–86.

Acetylcholinesterase
[^{11}C]-Edrophonium

Semi-Synthetic Methods: Tracers can be prepared by N-[^{11}C]-methylation of precursors using [^{11}C]-methyl triflate and isolated by solid-phase extraction (SPE) purification procedure that provides a 50–65% radiochemical yield.

Implementation: Evaluation of [^{11}C]-edrophonium and its analogues as potential positron emission tomography (PET) imaging agents for the detection and characterization of cardiac acetylcholinesterase.

Facile synthesis of [^{11}C]-edrophonium and its analogues as new potential PET imaging agents for heart acetylcholinesterase. Zheng QH, Liu X, Fei X, Wang JQ, Mock BH, Glick-Wilson BE, Sullivan ML, Hutchins GD. Bioorg Med Chem Lett. 2003 May 19; 13 (10), pp. 1787–90.

Angiography/Coronary Artery: Angina Pectoris (Vasospastic)/Presynaptic Myocardial Sympathetic Abnormality
[^{123}I]-Labeled Metaiodobenzylguanidine (MIBG)/ Ergonovine

Cellular/Molecular Mechanism of Action: Ergonovine promotes arterial wall smooth muscle contraction.

Application: Assess the presence and location of presynaptic myocardial sympathetic abnormality in conditions of vasospastic angina.

Route of Administration: [^{123}I]-labeled MIBG given IV. Ergonovine is administered by intracoronary infusion (ICA).

Dosage: [^{123}I]-labeled MIBG (111 mBq).

Considerations: Single photo emission computed tomography (SPECT) with MIBG can permit the non-invasive detection and evaluation of suspected vasospastic angina.

Interpretation and Analysis: In cases of vasospastic angina pectoris (n = 50) previously evaluated by coronary artery angiography following intracoronary artery ergonovine infusion, a 99–100% obstructive spasm is defined as a positive response. Images aquired 20 minutes and 3 hours following the administration of [^{123}I]-MIBG SPECT (111 mBq) can reveal mild reductions in tissue uptake (e.g., n = 5/25). In contrast, images at 3 hours often demonstrate [^{123}I]-MIBG SPECT abnormalities (e.g., n = 20/25). The location of [^{123}I]-MIBG abnormalities can be completely or partially consistent with the spastic coronary regions (e.g., n = 18/25). Alternatively, reduced [^{123}I]-MIBG uptake may be noted in cases with a negative ergonovine provocative test (e.g., n = 4/25). Accordingly, positive and negative predictive values of MIBG SPECT for the provocative test have been estimated to be 83% (e.g., n = 20/24) and 81% (e.g., n = 21/26), respectively.

Instrumentation: Single photon emission computed tomography.

Value of iodine-123 metaiodobenzylguanidine scintigraphy in patients with vasospastic angina. Taki J, Yasuhara S, Takamatsu T, Nakajima K, Tatami R, Ishise S, Matsunari I, Takayama T, Tonami N. Eur J Nucl Med 1998 Mar; 25 (3), pp. 229–34.

Angiography: Coronary Artery/Epicardial Vasculature
[^{99m}Technetium] ([^{99m}Tc])-SestaMIBI/ [^{99m}Tc]-Tetrofosmin/Dipyridamole

Cellular/Molecular Mechanism of Action: SestaMIBI and tetrofosmin function as intravascular carrier molecules for [^{99m}Tc].

Application: Imaging of vascular structures, including the coronary artery and epicardial arteries, utilizing various modalities including nuclear medicine techniques.

Implementation: Evaluation of the anatomical integrity of cardiac vascular structures in conditions of 50 to 90% stenosis of 1 to 2 major epicardial vessels without previous evidence of myocardial infarction (e.g., n = 81 cases) applying dipyridamole single photon emission computed tomography (SPECT) imaging facilitated by the application of either [^{99m}Tc]-sestaMIBI or [^{99m}Tc]-tetrofosmin.

Interpretation and Analysis: [^{99m}Tc]-sestaMIBI can detect reversible perfusion defects in a greater number of segments than is possible with [^{99m}Tc]-tetrofosmin (total 363 and 285, $p < 0.001$, and mean ± standard deviation [SD], 2.2 ± 3.0 and 1.8 ± 2.5 per patient, $p = 0.008$, respectively), it demonstrates a larger extent of perfusion defect than [^{99m}Tc]-tetrofosmin (mean ± SD, 15.8% ± 12.3% and 12.0% ± 11.4%, $p < 0.03$, respectively), and more often correctly identifies disease in more than 1 coronary artery ($p = 0.02$). There is better defect contrast with [^{99m}Tc]-sestaMIBI (defect/normal wall count ratios are 0.60 ± 0.15 vs 0.73 ± 0.14 for [^{99m}Tc]-sestaMIBI and [^{99m}Tc]-tetrofosmin, respectively, $p = 0.01$, for reversible defects in identical segments with both agents; and 0.73 ± 0.16 vs 0.79 ± 0.17, respectively, $p < 0.01$, for reversible defects detected with either agent alone). There is no significant difference in diagnostic sensitivity or image quality. Such differences between 2 commonly used tracers may have significant diagnostic and prognostic implications.

Enhanced detection of reversible perfusion defects by Tc-99m sestaMIBI compared to Tc-99m tetrofosmin during vasodilator stress SPECT imaging in mild-to-moderate coronary artery disease. Soman P, Taillefer R, DePuey EG, Udelson JE, Lahiri A. J Am Coll Cardiol 2001 Feb; 37 (2), pp. 458–62.

Angiography/Cardiac: Myocardial Ischemia/ Coronary Artery Stenosis
[^{99m}Technetium] ([^{99m}Tc])-SestaMIBI/Dipyridamole Stress Test/Levovist (Ultrasound Contrast Media)

Background Information: Power Doppler imaging is a method that can be adapted for myocardial contrast echocardiography

(MCE). It can selectively evaluate the signal coming from an ultrasound contrast agent, thereby facilitating the conduction of myocardial perfusion studies.

Application: Detection and characterization of coronary artery stenosis, myocardial ischemia/perfusion.

Implementation: Evaluation of the relative accuracy of the noninvasive imaging modalities of power Doppler MCE; stress echo wall motion; and nuclear scan imaging (single photon emission computed tomography [SPECT]) to assess coronary artery stenosis and myocardial ischemia/perfusion without and during pharmacological stress testing using coronary angiography as a reference standard. Power Doppler MCE is acquired using continuous intravenous infusion of Levovist contrast agent. Echo wall motion images can also be acquired for application in comparative analyses. At peak stress, [^{99m}Tc]-sestaMIBI is injected and stress SPECT images acquired 30 minutes after injection.

Human Model: Dipyridamole stress test (e.g., n = 25).

Interpretation and Analysis: Power Doppler MCE and SPECT show an 84% concordance (e.g., n = 21/25 cases; kappa = 0.67) for the detection of ischemia. Concordance based on coronary artery territories for normal perfusion vs fixed defects vs reversible defects is 92% (69% of 75; kappa = 0.81), with 100% for left anterior descending, 92% for right coronary artery, and 84% for circumflex artery structures. Power Doppler MCE has a lower sensitivity than SPECT (89% vs 100%) but higher specificity (100% vs 88%) for identification of stenotic (≥ 70%) coronary arteries as assessed by angiography. Echo wall motion analysis displays the lowest sensitivity (68%) with 100% specificity. Accuracy is 94% for both power Doppler MCE and SPECT, and 83% for wall motion analysis. Power Doppler MCE is a sensitive and specific method for identifying myocardial perfusion during pharmacological stress. Accuracy of power Doppler MCE for stenotic coronary arteries appears to be slightly higher than stress echo wall motion and similar to SPECT.

Non-invasive detection of coronary artery stenosis: A comparison among power Doppler contrast echo, [^{99m}Tc]-sestaMIBI SPECT, and echo wall motion analysis. Rocchi G, Fallani F, Bracchetti G, Rapezzi C, Ferlito M, Levorato M, Reggiani LB, Branzi A. Coron Artery Dis 2003 May; 14 (3), pp. 239–45.

Brugada Syndrome (Arryhythmia)/ Presynaptic Sympathetic Innervation Dysfunction *[^{123}I]-Metaiodobenzylguanidine (MIBG)*

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG is an analogue of the adrenergic neurotransmitter norepinephrine.

Background Information: Patients with Brugada syndrome present with characteristic electrocardiogram (ECG) abnormalities (atypical right bundle-branch block and ST-segment elevation) and life-threatening ventricular tachyarrhythmias despite structurally normal hearts. Involvement of the autonomic nervous system is suggested by the occurrence of ventricular tachyarrhythmias and sudden death at rest or during sleep and by changes of typical ECG signs under pharmacological modulation of the myocardial autonomic tone.

Application: Characterization of presynaptic sympathetic innervation to the heart in conditions of Brugada syndrome.

Route of Administration: [^{123}I]-MIBG (intravenous).

Implementation: Evaluation of the presynaptic cardiac neuronal reuptake of norepinephrine (uptake 1) in cases of Brugada syndrome and age-matched control subjects using the norepinephrine analogue [^{123}I]-MIBG in concert with single photon emission computed tomography (SPECT) and quantitative 33-segment bull's-eye analysis.

Human Model: Brugada syndrome (n = 17; n = 10 age-matched controls).

Interpretation and Analysis: Regionally reduced [^{123}I]-MIBG uptake is present in almost half of all cases with Brugada syndrome (e.g., n = 8/17 or 47%) but not in reference controls. Quantitative analysis shows segmental reduction of [^{123}I]-MIBG uptake in the inferior and septal left ventricular wall in patients with Brugada syndrome compared with control subjects ($p < 0.05$). No correlation can be appreciated between findings with [^{123}I]-MIBG SPECT and clinical characteristics of study patients. Abnormal [^{123}I]-MIBG uptake in Brugada syndrome cases indicates presynaptic sympathetic dysfunction exists within the heart. These findings may have a potential impact on the pathophysiology and arrhythmogenesis in cases with Brugada syndrome. Future quantitative investigations of the presynaptic and postsynaptic sympathetic and parasympathetic branches of the cardiac autonomic nervous system may clarify whether these observations represent a primary adrenergic dysfunction or an imbalance between sympathetic and parasympathetic innervation of the heart.

Cardiac autonomic dysfunction in Brugada syndrome. Wichter T, Matheja P, Eckardt L, Kies P, Schäfers K, Schulze-Bahr E, Haverkamp W, Borggrefe M, Schober O, Breithardt G, Schäfers M. Circulation 2002 Feb 12; 105 (6), pp. 702–6.

Cardiac Death: Predictive Outcome Parameters/ Hemodynamic Responses: Heart Rate and Systolic Blood Pressure *Adenosine/[Adjunct Single Photon Emission Computed Tomography (SPECT) Agent]*

Physiological Mechanism of Action: Adenosine phosphate promotes relaxation of vascular smooth muscle and a negative chronotropic effect on cardiac function.

Application: Detection of hemodynamic responses to adenosine that add incremental prognostic value to myocardial perfusion SPECT (MPS) imaging procedures for enhanced detection of cases as risk of cardiac death.

Implementation: Evaluation of outcome in cases subjected to adenosine (no additional exercise) stress MPS. Multivariable Cox proportional hazards analysis can be used to assess the prognostic value of hemodynamic variables in predicting cardiac death (CD).

Human Model: Postevaluation cases of adenosine stress myocardial perfusion studies (n = 3,444 cases; n = 53.5% women with a mean age 74.0 ± 8.4 years) during a 2.0 ± 0.8 year follow-up (monitoring) period.

Interpretation and Analysis: During an extended monitoring period of 2.0 ± 0.8 year, a relatively small percentage of cardiac deaths may be appreciated (e.g., n = 224/3,444 or 6.5%). Review of multivariable analysis of various parameters reveals a higher resting heart rate (HR) and to a lesser extent lower peak HR as viable markers of impending CD. In addition, peak/rest HR ratio is an independent predictor of CD when added to the multivariable model in place of peak and rest HR. Peak/rest HR ratios can also be used to risk stratify cases within each MPS category. A significant interaction can be appreciated between gender and peak systolic blood pressure (SBP), in which there is an increased risk associated with a low peak systolic blood pressure (SBP < 90 mm Hg at end of adenosine infusion) in men but not in women. Patients undergoing adenosine stress MPS with high rest HR and low peak/rest HR ratio have an increased risk of CD, as do male patients with a low peak SBP measurement. Detection of the hemodynamic response to adenosine adds incremental prognostic value to MPS results and enhances identification of patients at risk for CD.

Prognostic impact of hemodynamic response to adenosine in patients older than age 55 years undergoing vasodilator stress myocardial perfusion study.

Abidov A, Hachamovitch R, Hayes SW, Ng CK, Cohen I, Friedman JD, Germano G, Berman DS. Circulation 2003 Jun 17; 107 (23), pp. 2894–9.

Cardiomyopathy and Hypertension/Propanolol β-Adrenergic Receptor Occupancy/Positron Emission Tomography (PET) vs Single Photon Emission Computed Tomography (SPECT) Spatial Resolution/*In Vivo* and *Ex Vivo* Cardiac and Splenic Analyses

(1R,2S)-4-[^{18}F]-Fluorometaraminol/ [^{125}I]-Iodocyanopindolol/ [^{125}I]-Metaiodobenzylguanidine (MIBG)

Cellular/Molecular Mechanism of Action: [^{125}I]-iodocyanopindolol is a radioiodinated analogue of propanolol and is known to have binding avidity for and blocks the activity of β-adrenergic receptor complexes. (1R,2S)-4-[^{18}F]-Fluorometaraminol (4-FM) has similar binding avidity for β-adrenergic receptor complexes. Metaiodobenzylguanidine is an analogue of norepinephrine.

Application: [^{125}I]-Iodocyanopindolol can be used to detect alterations in β-adrenergic receptor expression and propanolol occupancy of β-adrenergic receptor complexes.

Dosage: [^{125}I]-Iodocyanopindolol (1.5 pmol/L *ex vivo* incubation); propranolol (10 μmol/L *ex vivo* incubation).

Implementation: Evaluate the relative uptake, metabolism, and β-receptor affinity of the new positron-emitting uptake-1 tracer (1R,2S)-4-[^{18}F]-fluorometaraminol (4-FM) with those of the SPECT pharmaceutical MIBG. One approach to achieving this objective can involve determining the biodistribution of no-carrier-added 4-[^{18}F]-FM *in vivo* and *in vitro* in retrograde-perfused hearts. Cardiac and extracardiac distribution can then be assessed, in concert with the detection of metabolite formation assessed by thin-layer chromatography techniques. Studies performed *in vivo* are then repeated with no-carrier-added [^{123}I]-MIBG. By means of autoradiography, the β-receptor affinity of 4-FM can be compared with that of MIBG and propranolol (10 μmol/L) through displacement of [^{125}I]-iodocyanopindolol (1.5 pmol/L) in *ex vivo* tissue slices.

Animal Model: Rats (Wistar Kyoto [WKY] rats and spontaneously hypertensive [SHR] rats).

***Ex Vivo* Model:** Rat (cardiac and spleen slices).

Interpretation and Analysis: Cardiomyopathic hearts show heterogeneous 4-[^{18}F]-FM uptake with gradients of up to 3.6 *in vivo* and *in vitro* between different regions of the heart. Control hearts display such gradients in 4-[^{18}F]-FM uptake only *in vitro*. [^{125}I]-MIBG exhibits a less heterogeneous *in vivo* distribution in SHR hearts. Extracardiac differences between WKY and SHR can be appreciated for uptake of 4-[^{18}F]-FM in the spleen (63.3% ± 4% vs 38.8% ± 5.7% of cardiac activity) and for renal uptake of [^{125}I]-MIBG (373% ± 27% vs 81.4% ± 17% of cardiac activity). Metabolites of 4-[^{18}F]-FM are found only in the liver, while those of [^{125}I]-MIBG are found in the liver and kidney with a nearly equal relative fraction in both types of animals of about 20%, 60%, and 30%, respectively. 4-FM suppresses cardiac-specific β-receptor binding of [^{125}I]-iodocyanopindolol in heart and spleen of both types of animals significantly, whereas MIBG has almost no effect. The more heterogeneous cardiac distribution of 4-[^{18}F]-FM suggests that it reflects alterations in uptake 1 better than [^{125}I]-MIBG in addition to the possibility of quantification and higher spatial resolution by PET compared with SPECT. Altered biotransformation in cardiomyopathic diseases may also impair the evaluation of [^{125}I]-MIBG SPECT data. The β-receptor binding of 4-[^{18}F]-FM must be further elucidated.

Relative uptake, metabolism, and β-receptor binding of (1R,2S)-4-[^{18}F]-fluorometaraminol and [^{123}I]-MIBG in normotensive and spontaneously hypertensive rats. Pissarek M, Ermert J, Oesterreich G, Bier D, Coenen HH. J Nucl Med 2002 Mar; 43 (3), pp. 366–73.

Cardiomyopathy (Dilated)/Left Ventricular Ejection Fraction (LVEF)/Predict Improved Cardiac Sympathetic Nerve Activity/Predict Improved Cardiac Function/Predict Improved Symptoms Posttreatment for Dilated Cardiomyopathy

Dobutamine/[^{123}I]-Metaiodobenzylguanidine (MIBG)

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG functions as an analogue of norepinephrine.

Physiological Mechanism of Action: Dobutamine exerts adrenergic/sympathomimetic properties.

Route of Administration: Dobutamine (intravascular).

Dosage: Dobutamine (5, 10, and 15 μg/kg/min).

Implementation: Measure LVEF at rest and in response to gradient increases in the dose of dobutamine infused before therapy in cases of dilated cardiomyopathy (DCM) according to protocols for dobutamine gated blood-pool scintigraphy (DOB-GBP). Examinations are performed before and after 1 year of therapy. The heart/mediastinum count (H/M) ratio and total defect score (TDS) are determined for [^{123}I]-MIBG images from anterior planar image and single photon emission computed tomography (SPECT) images. Left ventricular ejection fraction and left ventricular end-diastolic dimension (LVDd) are determined by echocardiography.

Human Model: Cases of DCM (n = 22).

Interpretation and Analysis: After 1 year of treatment, echocardiographic LVEF improves > 5% (e.g., n = 11/22 cases). Before treatment, TDS, H/M, LVEF, and LVDd are similar in both groups. However, there is a greater increase in the LVEF during dobutamine infusion in cases displaying improvement compared to those that do not improve (21 ± 8% vs 9 ± 3%, $p < 0.001$). If a critical value of 15% for the DeltaLVEF is used to predict the improvement in LVEF after treatment, sensitivity is 91% and specificity is 82%. The TDS, H/M ratio, LVDd, and New York Heart Association functional class improves in cases demonstrating a post-1 year response that is greater than comparable values for cases that do not demonstrate a response. Dobutamine gated blood-pool scintigraphy, therefore, can be used to predict improved cardiac sympathetic nerve activity, cardiac function, and symptoms after treatment in patients with DCM.

Dobutamine gated blood-pool scintigraphy predicts the improvement of cardiac sympathetic nerve activity, cardiac function, and symptoms after treatment in patients with dilated cardiomyopathy. Kasama S, Toyama T, Hoshizaki H, Oshima S, Taniguchi K, Suzuki T, Kurabayashi M. Chest 2002 Aug; 122 (2), pp. 542–8.

Coronary Artery Spasm/Adrenergic Sympathetic Innervation: Characterization of Abnormalities

[^{123}I]-Metaiodobenzylguanidine (MIBG)/Ergonovine

Considerations: It has been suggested that the sympathetic nervous system might play an important role in the development of coronary artery spasm. However, no cardiac imaging modality has been able to demonstrate abnormal sympathetic innervation in patients with coronary artery spasm.

The agent [^{123}I]-MIBG single photon emission computed tomography (SPECT) can non-invasively localize within regions of the coronary arteries affected by spasm. Invasive diagnostic coronary arteriography with ergonovine provocation test may be unnecessary for diagnosis of coronary artery spasm in cases with typical resting pain, negative exercise tests, or normal thallium perfusion scan results but that are showing abnormalities in [^{123}I]-MIBG SPECT.

Implementation: Assess the presence and location of abnormal sympathetic innervation using [^{123}I]-MIBG SPECT and evaluate the clinical efficacy of [^{123}I]-MIBG SPECT as a non-invasive screening test in cases suffering from coronary artery spasm. In this fashion, coronary arteriography and a provocative test with intravenous administration of ergonovine maleate can be utilized.

Human Model: Cases suspected of having a coronary artery spasm (n = 26 patients; 48.2 ± 12.0 years, range 20–67 years; n = 20/26 men). Cases are divided into 2 groups: Group 1 (n = 18 positive provocative test results) and Group 2 (n = 8 negative provocative test results); in addition to normal controls (n = 10).

Interpretation and Analysis: No abnormal MIBG uptake is observed in control subjects. Abnormal sympathetic nervous innervation using [^{123}I]-MIBG SPECT can be observed either as a reduced uptake or a defective pattern in the perfused areas (e.g., n = 13/18) supplied by vessels associated with ergonovine-induced vasospasm. Normal sympathetic innervation, as evidenced by normal [^{123}I]-MIBG uptake, can be noted in all of the 60 segments of normal vessel territories. Reduced uptake of [^{123}I]-MIBG cannot be detected in perfused areas of vasospasm-induced vessels such as perfused regions of left anterior descending coronary artery [LAD] and the right coronary artery [RCA] (e.g., n = 2 and 3 cases, respectively). Sensitivity and specificity of [^{123}I]-MIBG for detecting coronary artery spasm is 72.2% (95% confidence interval [CI] 55% to 89%) and 100%, respectively. The positive predictive and negative predictive values are 100% and 92.3% (95% CI 91% to 93%), respectively.

^{123}I-MIBG myocardial scintigraphy as a non-invasive screen for the diagnosis of coronary artery spasm. Ha JW, Lee JD, Jang Y, Chung N, Kwan J, Rim SJ, Lee YJ, Shim WH, Cho SY, Kim SS. J Nucl Cardiol 1998 Nov–Dec; 5 (6), pp. 591–7.

Cardiovascular/Coronary Artery Disease (CAD): Angina (Stress-Induced)/Myocardial Infarction

Iodophenyl-9-Methyl Pentadecanoic Acid (9MPA)/ [^{201}Tl]/[^{123}I]-β-Methyl Iodophenyl Pentadecanoic Acid ([^{123}I]-BMIPP)

Application: Detection and characterization of coronary/myocardial disease states.

Implementation: Evaluate the ability to detect CAD with a [^{123}I]-labeled branched fatty acid analog, 9MPA, compared to [^{123}I]-BMIPP and [^{201}Tl] utilizing single photon emission computed tomography (SPECT) imaging modalities. Images with 9MPA SPECT are obtained at 15 minutes after injection. Comparisons are performed with analysis from stress-redistribution [^{201}Tl] SPECT (e.g., n = 23 cases) and BMIPP myocardial fatty acid imaging (e.g., n = 9 cases). The regional uptakes of 9MPA, BMIPP, and [^{201}Tl] are scored semi-quantitatively and the segmental agreements are compared among them.

Human Model: Cases affected by effort angina (n = 24) or myocardial infarction (n = 13).

Interpretation and Analysis: In the segment-to-segment comparison, 9MPA shows reduced activity in comparison to stress-redistribution [^{201}Tl] imaging. The defect score of 9MPA is significantly greater than that of redistribution [^{201}Tl] images ($p < 0.001$). In addition, segmental 9MPA uptake is lower than BMIPP and its defect score is significantly greater than that of BMIPP ($p < 0.05$). When coronary angiography is used as the criterion, 9MPA showes higher sensitivity and lower specificity than stress-redistribution [^{201}Tl] ($p < 0.01$). In conclusion, fatty acid metabolic imaging with 9MPA is a sensitive but non-specific detector of CAD.

Detection of coronary artery disease by iodine-123-labeled iodophenyl-9-methyl pentadecanoic acid SPECT: Comparison with thallium-201 and iodine-123 BMIPP SPECT. Fukuchi K, Hasegawa S, Ito Y, Yamaguchi H, Yoshioka J, Uehara T, Nishimura T. Ann Nucl Med 2000 Feb; 14 (1), pp. 11–6.

Coronary Artery Lesions in Unstable Angina/ Myocardial Metabolic Abnormalities *[^{123}I]-β-Methyl Iodophenyl Pentadecanoic Acid ([^{123}I]-BMIPP)*

Application: Detection of atherosclerotic plaques (deposits) responsible for disease states, including stroke, aneurysm, and myocardial infarction. The imaging agent [^{123}I]-BMIPP is a β-methyl-branched fatty acid analogue that has demonstrated an ability to reveal abnormalities in fatty-acid-related metabolism.

Route of Administration: [^{123}I]-BMIPP (intravascular).

Implementation: Evaluation of [^{123}I]-BMIPP ^{201}thallium chloride ([^{201}Tl]Cl) imaging with single photon emission computed tomography (SPECT) for the capacity to detect anatomical and physiological abnormalities in cases with unstable angina.

Human Model: Cases of unstable angina without prior myocardial infarction (n = 100).

Interpretation and Analysis: Coronary arteries with stenosis due to plaque formation (deposits) demonstrate an increased uptake of [^{123}I]-BMIPP.

In cases of unstable angina without prior myocardial infarction (n = 100), evaluation with ^{123}I-BMIPP and [^{201}Tl]Cl imaging with SPECT and coronary and left ventricular cineangiography performed 1 week after the last episode of angina can reveal a variety of abnormalities, including reduced uptake of [^{123}I]-BMIPP imaging (n = 70/100 cases), reduced uptake of [^{201}Tl]Cl (n = 41/100), and/or abnormal left ventricular cineangiography (n = 49/100).

Significant increases in severity scores for [^{123}I]-BMIPP imaging along with increases in the number of stenosed coronary arteries and the severity of stenosis in individual coronary arteries can be appreciated. One month after percutaneous trans-lumination coronary angioplasty, a significant reduction can be appreciated in [^{123}I]-BMIPP severity scores ($p < 0.01$) along with a significant correlation between the severity scores of [^{123}I]-BMIPP and left ventricular cineangiography ($r = 0.579$, $p < 0.001$).

Overall rates of sensitivity and specificity in detecting significant coronary stenosis by [^{123}I]-BMIPP imaging have been found to be 74% and 86%, respectively, whereas rates of sensitivity and specificity in detecting significant coronary stenosis by [^{201}Tl]Cl are approximately 31% and 91%, respectively. [^{123}I]-BMIPP sensitivity increases to 86% if only advanced coronary stenosis of > 90% is included. Therefore, [^{123}I]-BMIPP myocardial imaging is an effective method of predicting coronary artery lesions of unstable angina patients without provocative testing.

Diagnostic usefulness of myocardial SPECT with [^{123}I]-β-methyl-iodophenyl pentadecanoic acid and [^{201}Tl] in unstable angina. Nakazawa Y, Tahara H, Suyama H, Kakio T, Ohue Y, Goto Y, Inoue K, Yamamoto Y, Nakamura N, Masui K, et al. Kaku Igaku 1995 Sep; 32 (9), pp. 953–8.

Myocardial Function and Perfusion/Incremental Cardiac Mortality Predictive Parameters *^{201}Thallium/Adenosine/[^{99m}Technetium] ([^{99m}Tc])-SestaMIBI*

Background Information: Availability of data is limited regarding the incremental value of adenosine stress myocardial perfusion single photon emission computed tomography (SPECT) (MPS) for the prediction of cardiac death in women vs men and the impact of diabetes mellitus on postadenosine MPS outcomes.

Application: Prediction of cardiac-related mortality. Adenosine MPS has comparable incremental value in predicting cardiac death in women and men.

Implementation: Characterization of case history following evaluation by rest thallium-201/adenosine technetium-99m sesta MIBI MPS (e.g., n = 2,656 women; n = 2,677 men).

Interpretation and Analysis: Women have significantly smaller adenosine stress, rest, and reversible defects than men. During 27.0 ± 8.8 month follow-up, cardiac death rates are lower in women than men (2.0%/year vs 2.7%/year, respectively, $p < 0.05$). Before and after risk adjustment, cardiac death risk increases significantly in both men and women as a function of MPS results. Multivariable models reveal that MPS results provide incremental prognostic value over prescan data for the prediction of cardiac death in both genders. Also, while comparative unadjusted rates of early (≤ 60 days posttest) coronary angiography (17% vs 23%) and revascularization (8% vs 12%) are significantly lower in women ($p < 0.05$), after adjusting for MPS, these rates are similar in men and women. Importantly, diabetic women have a significantly greater risk of cardiac death compared with other patients. Also, after risk adjustment, cases with insulin-dependent diabetes mellitus (IDDM) have a higher risk of cardiac death than patients with non-insulin-dependent diabetes mellitus. Attributes of MPS include its ability to influence decisions regarding the appropriateness of subsequent invasive management procedures (males and females). Diabetic women and patients with IDDM appear to have a greater risk of cardiac death than other patients for any MPS result.

Adenosine myocardial perfusion single photon emission computed tomography in women compared with men. Impact of diabetes mellitus on incremental prognostic value and effect on patient management. Berman DS, Kang X, Hayes SW, Friedman JD, Cohen I, Abidov A, Shaw LJ, Amanullah AM, Germano G, Hachamovitch R. J Am Coll Cardiol 2003 Apr 2; 41 (7), pp. 1125–33.

Myocardial Ischemia
[^{18}F]-Deoxyglucose/[^{99m}Technetium] ([^{99m}Tc])-Methoxy-Isobutylisonitrile (MIBI) Alternative

Cellular/Molecular Mechanism of Action: [^{18}F]-Deoxyglucose is a radiolabeled analogue of glucose that enters cells through glucose transport mechanisms.

Application: Detection and characterization of myocardial ischemia conditions.

Dosage: Glucose (25–50 g); [^{18}F]-fluoro-deoxy-glucose (370 MBq (10 mCi)); [^{99m}Tc]-MIBI (925 MBq (25 mCi)).

Implementation: Evaluation of conditions of myocardial ischemia utilizing both positron emission tomography (PET) and single photon emission computed tomography (SPECT) facilitated by the application of [^{18}F]-deoxyglucose and [^{99m}Tc]-MIBI. One investigative protocol that can be applied to achieve such a diagnostic objective entails delineation of rest metabolism/stress perfusion profiles facilitated by dual-isotope simultaneous-acquisition SPECT. In this context, glucose can be administered (25–50 g) followed approximately 60 minutes later by an injection of [^{18}F]-fluoro-deoxy-glucose (370 MBq (10 mCi)). After a 35-minute distribution phase, cases are then subjected to exercise or pharmacologic stress followed by administration of [^{99m}Tc]-MIBI (925 MBq [25 mCi]).

Human Model: Cases of myocardial ischemia (n = 5) evaluated by both PET and SPECT. Additional cases subjected to coronary angiography within 3 months of dual-isotope SPECT (n = 23; mean age = 54 + 6 years, range 30–83; n = 14 men, n = 9 women).

Interpretation and Analysis: Applying a window of 20% for both photopeaks and [^{99m}Tc]/[^{18}F] concentrations of 3.2:1, the "spillover" from [^{18}F] into the [^{99m}Tc] window is < 6% of the total counts in the window in patients with a normal distribution of both radiopharmaceuticals. Phantom images clearly demonstrate cardiac defects measuring 2 × 1 and 2 × 0.5 cm. There is no significant difference in images of cases undergoing both PET and SPECT (e.g., n = 5 cases). A considerable number of imaging studies will have normal results (e.g., n = 21), while some will display mismatched defects (e.g., n = 15); matched defects (e.g., n = 14); or both matched and mismatched defects (e.g., n = 7). In coronary angiography examinations, approximately one-third can yield normal findings (e.g., n = 7/23), in addition to the detection of matched defects (e.g., n = 1/23) and both matched and mismatched defects (e.g., n = 7). When stenosis of > 70% is used as the criterion for a diagnosis of coronary artery disease, dual-isotope SPECT has a sensitivity of 100%, specificity of 88%, positive predictive value of 93%, negative predictive value of 100%, and an accuracy of 96%. Therefore, dual-isotope SPECT may provide an alternative, accurate, cost-effective method to [^{13}N]-nitrogen ammonia/[^{18}F]-fluoro-deoxy-glucose PET or [^{201}Tl] reinjection for identifying injured or dysfunctional but viable myocardium.

Instrumentation: Dual-isotope single-acquisition SPECT protocol using a multihead SPECT camera equipped with an ultra-high energy collimator.

Evaluation of myocardial ischemia using a rest metabolism/stress perfusion protocol with fluorine-18 deoxyglucose/technetium-99m MIBI and dual-isotope simultaneous-acquisition single photon emission computed tomography. Sandler MP, Videlefsky S, Delbeke D, Patton JA, Meyerowitz C, Martin WH, Ohana I. J Am Coll Cardiol 1995 Oct; 26 (4), pp. 870–8.

Myocardial Ischemia/Perfusion-Metabolism Mismatch
[^{123}I]-(ρ-Iodophenyl)-3-R,S-Methylpentadecanoic Acid/Non-Metabolized [^{123}I]-β-Methyl Iodophenyl Pentadecanoic Acid ([^{123}I]-BMIPP) Myocardial Backdiffusion/Long-Chain Fatty Acid BMIPP: Decreased Tissue Uptake

Molecular/Cellular Mechanism of Action: BMIPP is a fatty acid analog.

Background Information: [^{123}I]-BMIPP is a fatty acid analogue for single photon emission computed tomography (SPECT) imaging. This radiopharmaceutical possesses a unique property, in that it produces imaging patterns of perfusion-metabolism mismatch during SPECT analyses in conditions of ischemic heart disease. However, the mechanism responsible for such properties remains unclear.

Application: Detection and characterization of perfusion-metabolism mismatch in cases with ischemic heart disease applying SPECT imaging methodologies.

Route of Administration: [^{123}I]-BMIPP (intravascular).

Implementation: The agent BMIPP is injected into the left anterior descending artery (LAD), and either blood samples are collected from the cardiac vein or abdominal aorta; or serial biopsy specimens are harvested from the LAD region for comparison to reference controls. The catabolites of BMIPP, including backdiffusion of non-metabolized BMIPP, are measured utilizing high-performance liquid chromatography (HPLC in efflux study). Thin-layer chromatography (TLC) techniques can be applied for the analytical evaluation of tissue specimens.

Animal Model: Canine (n = 11 dogs subjected to open-chest thoracotomy under anesthesia to facilitate a system developed for the release of all blood within the great cardiac vein outside without recirculation. In addition, the LAD is occluded for 30 minutes after

reperfusion (n = 6/11 dogs cardiac vein and abdominal aorta blood is collected post-BMIPP injection; n = 5/11 dogs used for collection of serial biopsy specimens from the LAD region).

Interpretation and Analysis: The agent BMIPP is rapidly extracted from the plasma by myocardiocytes (myocardium) and the subsequent retention is unchanged. The early washout (8 minutes) of radioactivity significantly increases (51% ± 12% to 65% ± 7%; $p < 0.05$) with ischemia. The metabolites from the myocardium consist of a backdiffusion of non-metabolized BMIPP, alpha, intermediate, and full oxidation metabolites. Among these metabolites, backdiffusion of non-metabolized BMIPP in blood significantly increases (27.9% ± 7.7% to 42.3% ± 8.1%; $p < 0.05$), especially in the early phase with ischemia. In tissues, radioactivity is concentrated in the triglyceride pool even in the early phase. In addition, BMIPP and alpha-oxidized metabolites significantly decreases in the early phase with ischemia (t = 1 minute after BMIPP injection, 25.9% ± 8.6% to 14.5% ± 2.1%, $p < 0.01$; t = 2 minutes, 8.9% ± 5.0% to 4.5% ± 1.7%, $p < 0.05$). Such findings demonstrate that backdiffusion of non-metabolized BMIPP from the myocardium increases and BMIPP (long-chain fatty acids) in tissue decreases with ischemia, suggesting backdiffusion of non-metabolized BMIPP might play an important role in myocardial perfusion-metabolism mismatch on SPECT images in cases of ischemic cardiac disease.

Myocardial metabolism of [^{123}I]-BMIPP in a canine model with ischemia: Implications of perfusion-metabolism mismatch on SPECT images in patients with ischemic heart disease. Hosokawa R, Nohara R, Fujibayashi Y, Okuda K, Ogino M, Hirai T, Fujita M, Tamaki N, Konishi J, Sasayama S. J Nucl Med 1999 Mar; 40 (3), pp. 471–8.

Myocardium/Ischemic Cardiomyopathy/Diabetes Mellitus

Acipomox/[^{18}F]-Fluoro-Deoxy-Glucose (FDG) Single Photon Emission Computed Tomography (SPECT)

Cellular/Molecular Mechanism of Action: The agent [^{18}F]-FDG functions as an analogue of glucose. Acipimox is a nicotinic acid derivative.

Application: Detection and characterization of alterations in glucose metabolism using [^{18}F]-FDG. Acipimox utilized in combination with [^{18}F]-FDG SPECT metabolic imaging can be used to assess conditions of ischemic cardiomyopathy even in cases suffering from diabetes mellitus.

Implementation: Evaluation of ischemic cardiomyopathy cases applying [^{18}F]-FDG SPECT imaging analysis in combination with acipimox to assess myocardial viability. Complementary image analysis involves performing resting 2-dimensional echocardiography in an effort to identify dysfunctional myocardial tissue. Image quality is then scored visually and quantitatively, while the myocardium-to-background ratio is determined by region-of-interest (ROI) analysis. The plasma concentrations of glucose and free fatty acids determined to evaluate metabolic status before and during [^{18}F]-FDG imaging.

Human Model: Ischemic cardiomyopathy (n = 70; n = 34/70 diabetes mellitus; n = 12/34 insulin-dependent diabetes mellitus, n = 22/34 non-insulin-dependent diabetes mellitus; n = 36/70 non-diabetic).

Interpretation and Analysis: Acipimox significantly lowers plasma levels of free fatty acids in both case category types. Approximately 50% of diabetic cases (e.g., n = 15/34) have plasma glucose levels of > 9 mmol/L, which is lowered successfully in essentially all cases when administered additional insulin. Visual evaluation of [^{18}F]-FDG images reveals they are of variable quality (diabetic/non-diabetic: n = 27/32 good; n = 5/4 moderate; n = 2/0 poor; p = not statistically significant). The myocardium-to-background ratio of [^{18}F]-FDG SPECT images is comparable in cases with and without diabetes mellitus (3.1 ± 1.0 vs 3.5 ± 0.9; p = not statistically significant). The type of diabetes has no influence on [^{18}F]-FDG image quality. Note: Severe side effects usually are not appreciated during [^{18}F]-FDG SPECT procedures. [^{18}F]-FDG SPECT metabolic imaging after acipimox appears to be safe and practical for routine assessment of viability in patients with ischemic cardiomyopathy. Image quality is good, even in patients with diabetes, although additional insulin is sometimes needed.

Effect of diabetes mellitus on myocardial ^{18}F-FDG SPECT using acipimox for the assessment of myocardial viability. Schinkel AF, Bax JJ, Valkema R, Elhendy A, van Domburg RT, Vourvouri EC, Bountioukos MA, Krenning EP, Roelandt JR, Poldermans D. J Nucl Med 2003 Jun; 44 (6), pp. 877–83.

Myocardial Metabolism: Substrate Imaging

[^{11}C]-Palmitate

Application: Characterization of myocardial substrate metabolism in health and disease.

Considerations: Glucose administration in fasted humans can serve as a provocative test of substrate regulation, which can be abnormal in myocardial disease and can be demonstrated in a non-invasive manner.

Implemenation: Determine non-invasively the changes that develop in myocardial substrate metabolism in normal and diseased human myocardium in response to altered substrate availability in blood and disease-related abnormalities. Such forms of evaluation can be achieved with injections of [^{11}C]-palmitate and serial positron emission tomography (PET) imaging performed after an overnight fast (control period) and again 2 hours later after oral glucose (50 g).

Interpretation and Analysis: The combined use of PET and [^{11}C]-palmitate allows non-invasive demonstration of the known response of substrate metabolism of the human heart to alterations in substrate availability. Myocardial [^{11}C] time-activity curves from serial PET images reveal biexponential clearance patterns. An early rapid phase, defined by relative size and clearance half-time, reflects [^{11}C]-palmitate oxidation and the late slow phase tracer deposition in the endogenous lipid pool. During the control period, the tracer fraction entering the early rapid phase averages 47 ± 13% (standard deviation) in normal subjects and 45 ± 12% in affected patients. Corresponding clearance half-times are 19 ± 7 and 20 ± 5 minutes, respectively. Heart rate and blood pressure remain unchanged after glucose administration, but plasma glucose levels increase by 72.5% in normal subjects and by 98.9% in affected patients, while free fatty acid levels decrease by 72% and 42% ($p < 0.001$), respectively. In normal subjects, the tracer fraction in the early rapid phase falls by 43% ($p < 0.005$) and the clearance half-time increases by 46% ($p < 0.01$). In affected patients, the response of [^{11}C]-palmitate tissue kinetics to glucose are variable. In some cases, the pattern is similar to that of normal subjects, while in other patients a "paradoxic" response may be observed. The tracer fraction entering the rapid clearance phase increases after glucose by 30% ($p < 0.05$) associated with a 36% ($p < 0.05$) decline in clearance half-times. The paradoxic response is unrelated to disease etiology or plasma substrate levels but occurs mostly in left ventricles displaying more severely depressed function.

Effects of substrate availability on myocardial C-11 palmitate kinetics by positron emission tomography in normal subjects and patients with ventricular dysfunction. Schelbert HR, Henze E, Sochor H, Grossman RG, Huang SC, Barrio JR, Schwaiger M, Phelps ME. Am Heart J 1986 Jun; 111 (6), pp. 1055–64.

Myocardial Perfusion Assessment
Dipyridamole/^{201}Thallium ([^{201}Tl]) Single Photon Emission Computed Tomography (SPECT)

Application: Detection and characterization of myocardial ischemia conditions.
Route of Administration: Dipyridamole (slow IV).
Considerations: Dipyridamole stress might be considered appropriate as a provocative test when physical exercise cannot be carried out.
Implementation: Evaluate the diagnostic capability of SPECT to detect diseased coronary vessels utilizing dipyridamole in concert with [^{201}Tl] and SPECT.

Assessment of myocardial infusion (± maximal ergometric exercise) is facilitated by utilizing dipyridamol infusion in concert with myocardial imaging techniques employing [^{201}Tl] and SPECT. To evaluate the diagnostic capability of SPECT in the detection of diseased coronary vessels, the left ventricular myocardium is subdivided into 6 segments related to the 3 major coronary arteries. Single photon emission computed tomography images are acquired after dipyridamole infusion (Methodology Type 1 with n = 41 cases) or after tracer injections at maximal ergometric exercise (Methodology Type 2 with n = 162 cases). Based on findings from [^{201}Tl] and dipyridamole evaluations, cases are then subdivided into 2 groups for diagnostic coronary angiography.

Human Model: Cases suffering from cardiovascular disease (n = 203).
Interpretation and Analysis: Sensitivity for the left anterior descending artery is 77% with Methodology Type I and 79% in Methodology Type II; specificity is 80% and 96%, respectively. Sensitivity for the right coronary artery is 91% with Method I and 85% with Method 2; specificity is 81% and 73%. For the left circumflex artery sensitivity is 65% with Method 1 and 67% for Method 2, while specificity is 89% and 88%, respectively. Myocardial SPECT results after ergometric exercise and dipyridamole infusion appear to be almost superimposable.

Diagnostic accuracy of single photon emission computed tomography with thallium-201 for the identification of diseased coronary arteries. Comparison between dipyridamole infusion and exercise stress test. Dondi M, Monetti N, Levorato M, Corbelli C, Zagni P, Tartagni F, Maiello L, Fallani F, Ortolani P. J Nucl Med Allied Sci 1990 Jan–Mar; 34 (1), pp. 19–23.

Cardiovascular: Myocardial Perfusion (Kawasaki Disease [KD])/Myocardial Perfusion/ Regional Hypoperfusion
[^{99m}Technetium] ([^{99m}Tc])-Tetrofosmin (TF)/ Dipyridamole

Physiological Mechanism of Action: Dipyridamole promotes vasodilator activity.
Background Information: The coronary artery abnormalities in KD often cause myocardial ischemia. Previous publications have described the use of ^{201}thallium myocardial perfusion imaging to determine the extent of ischemia in KD conditions. The technetium perfusion agents offer better resolution and may offer additional information about ventricular function in such conditions.
Application: Tetrofosmin myocardial perfusion imaging can detect regional areas of myocardial hypoperfusion (e.g., KD of children).
Implementation: Evaluation of myocardial perfusion in cases of KD applying [^{99m}Tc]-TF in conjunction with performing a dipyridamole vasodilator stress test. Significant coronary artery stenosis is classified as being ≥ a 75% reduction in luminal diameter. After administration of high-dose dipyridamole infusion (0.70 mg/kg), [^{99m}Tc]-TF is injected, followed by a 2nd [^{99m}Tc]-TF injection at rest 4 hours later. Single photon emission computed tomography (SPECT) images are obtained more than 30 minutes after TF injection.

Human Model: Kawasaki disease (n = 86 cases, 11.5 ± 6.4 years; n = 20 age-matched controls without heart disease). Dipyridamole (high-dose = 0.70 mg/kg); [^{99m}Tc]-TF (1st dose = 74–370 MBq; 2nd dose = 148–740 MBq).
Interpretation and Analysis: Significant coronary artery stenosis, coronary aneurysm without stenosis, and an absence of coronary lesions can be appreciated in a considerable number of cases (e.g., n = 20/86, n = 37/86, and n = 29/86, respectively). Regional myocardial hypoperfusion can be observed in a number of cases that have coronary artery stenosis of 75% or greater (n = 18/86, sensitivity 90%, and specificity 100%). A small percentage of cases do not demonstrate ischemic changes in coronary artery stenosis (n = 2) and some of these cases have good collateral blood flow (n = 1 of 2).

Myocardial ischemia in Kawasaki disease: Evaluation with dipyridamole stress technetium 99m tetrofosmin scintigraphy. Fukuda T, Ishibashi M, Yokoyama T, Otaki M, Shinohara T, Nakamura Y, Miyake T, Kudoh T, Oku H. J Nucl Cardiol 2002 Nov–Dec; 9 (6), pp. 632–7.

Myocardium/Pharmaceutical Efficacy Evaluation
[^{18}F]-Fluoro-Deoxy-Glucose (FDG)/Acipimox

Cellular/Molecular Mechanism of Action: Acipimox is a nicotinic acid derivative.
Background Information: Image quality of cardiac FDG studies is highly dependent on the metabolic conditions during the study; hyperinsulinemic euglycemic clamping ensures adequate image quality. However, the approach is time-consuming. Data in a small number of patients suggest that oral administration of a nicotinic acid derivative (Acipimox) results in good image quality.
Application: Acipimox can be applied to enhance myocardial FDG image quality.
Route of Administration: Acipimox (oral).
Dosage: Acipimox (250 mg; Byk, The Netherlands).
Implementation: Evaluation of the safety and image quality of cardiac FDG single photon emission computed tomography (SPECT) studies after acipimox administration. Image quality is assessed visually and quantitatively facilitated by the calculation of heart-to-lung, heart-to-liver, and myocardium-to-background ratios. Blood samples are drawn at baseline and at the time of FDG injection to determine levels of glucose, free fatty acids, and insulin.

Human Model: Cases receiving Acipimox (n = 21 patients, Type 2); compared to hyperinsulinemic euglycemic clamping (n = 69 cases Type 1).
Interpretation and Analysis: Baseline characteristics of Type 1 and Type 2 patients are comparable. Undesirable sequelae are usually only appreciated in Type 2 cases (n = 4/69 or 19% = paroxysmal flushing). Image quality, assessed visually, is good in 100% of Type 1 and 86% of Type 2 cases. All quantitative parameters of image quality (heart-to-lung, heart-to-liver, and myocardium-to-background ratios) are comparable between Type 1 and Type 2 cases. Baseline plasma levels of all substrates are comparable between groups. At the time of FDG injection, plasma levels of glucose and free fatty acids are comparable between Type 1 and 2 cases, and insulin is higher in Type 1 cases. Cardiac FDG SPECT after acipimox is a simple and safe approach that renders image quality comparable to that obtained during hyperinsulinemic euglycemic clamping.

Safety and feasibility of cardiac FDG SPECT following oral administration of acipimox, a nicotinic acid derivative: Comparison of image quality with

hyperinsulinemic euglycemic clamping in non-diabetic patients. Bax JJ, Visser FC, Poldermans D, Van Lingen A, Elhendy A, Boersma E, Sloof GW, Visser CA. J Nucl Cardiol 2002 Nov–Dec; 9 (6), pp. 587–93.

Sympathetic (Adrenergic) Receptor Expression/ Cardiac Arrythmia: Brugada Syndrome/ Sympathetic Innervation Dysfunction (Presynaptic) *[^{123}I]-Metaiodobenzylguanidine ([^{123}I]-MIBG)*

Cellular/Molecular Mechanism of Action: [^{123}I]-Metaiodobenzylguanidine is an analogue of the adrenergic neurotransmitter norepinephrine.

Background Information: Patients with Brugada syndrome present with characteristic electrocardiogram (ECG) abnormalities (atypical right bundle-branch block and ST-segment elevation) and life-threatening ventricular tachyarrhythmias despite structurally normal hearts. Involvement of the autonomic nervous system is suspected by the occurrence of ventricular tachyarrhythmias and sudden death at rest or during sleep and by changes of typical ECG signs under pharmacological modulation of the myocardial autonomic tone.

Application: Characterization of presynaptic sympathetic innervation to the heart in conditions of Brugada syndrome.

Route of Administration: [^{123}I]-MIBG (intravenous).

Implementation: Evaluation of the presynaptic cardiac neuronal reuptake of norepinephrine (uptake 1) in conditions of Brugada syndrome and age-matched control subjects using the norepinephrine analogue [^{123}I]-MIBG in concert with single photon emission computed tomography (SPECT) and quantitative 33-segment bull's-eye analysis.

Human Model: Brugada syndrome (n = 17; n = 10 age-matched controls).

Interpretation and Analysis: Regionally reduced [^{123}I]-MIBG uptake is present in almost half of all cases with Brugada syndrome (e.g., n = 8/17 or 47%) but not in reference controls. Quantitative analysis shows segmental reduction of [^{123}I]-MIBG uptake in the inferior and septal left ventricular wall in patients with Brugada syndrome compared with control subjects ($p < 0.05$). No correlation can be appreciated between the findings from [^{123}I]-MIBG SPECT and clinical characteristics of study patients. Abnormal [^{123}I]-MIBG uptake in Brugada syndrome cases indicates presynaptic sympathetic dysfunction exists within the heart. These findings may have potential impact on the pathophysiology and arrhythmogenesis in cases with Brugada syndrome. Future quantitative investigations of the presynaptic and postsynaptic sympathetic and parasympathetic branches of the cardiac autonomic nervous system may clarify whether these observations represent a primary adrenergic dysfunction or an imbalance between sympathetic and parasympathetic innervation of the heart.

Cardiac autonomic dysfunction in Brugada syndrome. Wichter T, Matheja P, Eckardt L, Kies P, Schäfers K, Schulze-Bahr E, Haverkamp W, Borggrefe M, Schober O, Breithardt G, Schäfers M. Circulation 2002 Feb 12; 105 (6), pp. 702–6.

Thrombosis: Arterial and Venous/Deep Vein Thrombosis/Pulmonary Embolism/Arterial Thromboembolic Disorders/Cerebral Vascular Stroke/Coronary Artery Thrombotic Disorders/Peripheral Artery Thromboses *Platelet GPIIb/IIIa Receptor Antagonist/ [^{99m}Technetium] ([^{99m}Tc])-DMP728/[^{3}H]-DMP728*

Cellular/Molecular Mechanism of Action: The agent DMP728 functions as a GPIIb/IIIa receptor antagonist on the exterior surface membrane of platelets.

Physiological Mechanism of Action: The composition of venous thrombi is predominantly polymerized fibrin monomer with variable degrees of cross-linking (e.g., XIIIa* activity). Conversely, arterial thrombi are composed of a much higher platelet content.

Background Information: Either venous or arterial thrombosis is a potentially life-threatening event and some diagnostic modalities can be inadequate for diagnostic purposes or for determining the actual morphology of the evolving thrombus. Thus, development of a non-invasive imaging agent that can detect clot location remains a critical and unresolved need in nuclear diagnostic medicine.

Application: Detection and characterization of intravascular venous and arterial thromboses.

Implementation: Evaluation of artificially (*in vitro*) created venous and arterial type thrombi using the potent radiolabeled GPIIb/IIIa receptor antagonist [^{3}H]-DMP728. Complementary investigation can involve the *in vivo* application of [^{99m}Tc]-DMP728 to determine the clot/blood distribution ratio (e.g., target:background ratio). In addition, non-invasive single photon emission computed tomography (SPECT) of venous clots in deep vein thrombosis can be performed as well. As a point of comparison, thrombin inhibitors can also be evaluated using these agents as efficacy probes.

Animal Model: Canine (dogs with mixed arterial/venous or venous clots in arteriovenous shunts, in addition to venous clot foci and deep vein thrombi).

Interpretation and Analysis: Radiolabeled preparations of the GPIIb/IIIa receptor antagonist DMP728 in the form of [^{3}H]-DMP728 and [^{99m}Tc]-DMP728 retain marked binding avidity for platelet GPIIb/IIIa receptor complexes. DMP728 demonstrates a relatively greater affinity for activated than for unactivated human platelets, which may be essential for attaining an optimal thrombus/blood (target/background) distribution ratio and optimal detection of small clots (i.e., greater sensitivity). Therefore, [^{99m}Tc]-GPIIb/IIIa receptor antagonists have potential utility in the diagnosis of venous clots in deep vein thrombosis, pulmonary embolism, and arterial thromboembolic disorders, including stroke and coronary and peripheral artery thrombotic disorders. Thrombin inhibitors do not appear to be as effective in this same context.

Novel technetium-99m-labeled platelet GPIIb/IIIa receptor antagonists as potential imaging agents for venous and arterial thrombosis. Mousa SA, Bozarth JM, Edwards S, Carroll T, Barrett J. Coron Artery Dis 1998; 9 (2–3), pp. 131–41.

CENTRAL NERVOUS SYSTEM (CNS)

Acetylcholine Muscarinic Receptors/Cardiac Muscarinic Acetylcholinergic Receptors/Central Nervous System (CNS) Muscarinic Acetylcholinergic Receptors/Carcinoma of the Colon *[^{123}I]-PIP/[^{125}I]-PIP*

Cellular/Molecular Mechanism of Action: The agent IPIP functions as a ligand for muscarinic acetylcholinergic complexes (mAChR).

Background Information: Prior to approval for *in vivo* use in humans, organ doses for radioiodinated IPIP ligands must 1st be evaluated.

Application: Radioiodinated IPIP ligands can be applied *in vivo* for imaging mAChR using various imaging modalities, including single photon emission computed tomography (SPECT).

Implementation: Identify the most optimum mAChR ligand-binding properties of 4 different IPIP sterioisomers through evaluation of their biodistribution pattern. A biokinetic model can be developed for the Z-(S)-IPIP stereoisomer for estimating the internal

absorbed dose in humans based on extrapolation from an appropriate rat model.

Animal Model: Rat (IPIP biokinetic model).

Interpretation and Analysis: The thyroid gland is the critical organ for this radiopharmaceutical, with an absorbed dose estimate of 2.4 mGy/MBq for both males and females, when labeled with [^{123}I]. Even when blocked, the thyroid is still the critical organ, yet with a 90% reduction in absorbed dose. The heart and brain receive the next highest doses in both males and females. Effective dose estimates for the use of pure [^{123}I]-PIP in humans are 0.16 mSv/MBq for males and 0.14 mSv/MBq for females. The biodistribution studies of the Z-(S)-IPIP stereoisomer show it has the most promise as a successful agent for imaging muscarinic receptor sites in the heart and brain. IPIP can potentially be applied as a therapeutic radiopharmaceutical for certain colon carcinomas where muscarinic receptor sites are expressed by tumor cells. These results provide preliminary data for use of IPIP in clinical studies in humans.

In vivo biodistribution of [^{125}I]-PIP and internal dosimetry of 123IPIP radioiodinated agents selective to the muscarinic acetylcholinergic receptor complex. Breeden WK 3rd, Hamby DM, Carey JE Jr, Eckerman KF, McPherson DW, Knapp FF Jr. Med Phys 2000 Apr; 27 (4), 778–86.

Acetylcholine (Muscarinic) Receptor Screening/ Quantitation of Endogenous Acetylcholine Levels *[^{18}F]-Piperidyl Benzilate/[^{18}F]-Pyrrolidyl Benzilate*

Cellular/Molecular Mechanism of Action: Piperidyl benzilate and pyrrolidyl benzilate both can function as ligands that bind to muscarinic acetylcholine receptor complexes.

Background Information: Compound names include N-(2-[^{18}F]fluoroethyl)-piperidyl benzilate and N-(2-[^{18}F]fluoroethyl)-pyrrolidyl benzilate (FEPB).

Application: Function as an *in vivo* probe for measuring endogenous acetylcholine levels.

Route of Administration: Intravenous.

Examples of Clinical Implementation: Retention is moderate in mouse brain utilizing 3-[^{18}F]-FEPB; however, blocking with scopolamine reveals that uptake is not muscarinic cholinergic receptor-mediated. Conversely, mice administered 4-[^{18}F]-FEPB exhibit high, receptor-mediated retention in mouse brain, with significant clearance after 1 hour. These results suggest that 4-[^{18}F]-FEPB could have application as an *in vivo* probe for measuring endogenous acetylcholine levels.

Interpretation and Analysis: The retention of [^{18}F]-piperidyl benzilate and [^{18}F]-pyrrolidyl benzilate occurs at moderate levels in mouse brain (3-[^{18}F]-FEPB). However, blocking with scopolamine indicates that uptake is not muscarinic cholinergic receptor-mediated. Conversely, some animal subjects (4-[^{18}F]-FEPB) exhibit high, receptor-mediated retention in mouse brain, with significant clearance after a 1-hour period. These results suggest that 4-[^{18}F]-FEPB could have application as an *in vivo* probe for measuring endogenous acetylcholine levels.

Chemical Synthesis: Based on the piperidyl or pyrrolidyl benzilate reference molecule, a variety of compounds can be produced from methyl benzilate and 4-piperidinol, (R)- ± 3-piperidinol, or (R)- ± 3-pyrrolidinol. Amine substituents can include alkyl and aralkyl groups. In the evaluation of such compounds, the *in vitro* K_i values usually range from 0.05 nM to > 100 nM. In preliminary investigations, (R)-*N*-(2-fluoroethyl)-3-piperidyl benzilate (3-FEPB, 22, K_i = 12.1 nM) and *N*-(2-fluoroethyl)-4-piperidyl benzilate (4-FEPB, 8, K_i = 1. 83 nM) have been radiolabeled with [^{18}F] and related radiohalogens. Using alkylation with 2-[^{18}F]-fluoroethyl triflate, 3-[^{18}F]-FEPB (42) and 4-[^{18}F]-FEPB (43) can be produced with radiochemical yields of 7 to 9% with levels of radiochemical purity of 97% or greater.

Synthesis, [18F]-labeling, and biological evaluation of piperidyl and pyrrolidyl benzilates as *in vivo* ligands for muscarinic acetylcholine receptors. Skaddan MB, Kilbourn MR, Snyder SE, Sherman PS, Desmond TJ, Frey KA. J Med Chem 2000 Nov 16; 43 (23), pp. 4552–62.

Acetylcholine Receptor Complexes *(R)-N-(2-Fluoroethyl)-3-Piperidyl Benzilate (3-FEPB)/ N-(2-Fluoroethyl)-4-Piperidyl Benzilate (4-FEPB)/ Scopolamine*

Cellular/Molecular Mechanism of Action: Scopolamine recognizes and physically binds to muscarinic cholinergic receptor complexes (e.g., functions as a competitive antagonist of acetylcholine at muscarinic receptor sites).

Application: Measurement of endogenous acetylcholine levels.

Semi-Synthetic Methods: Production of a series of 31 compounds based on the piperidyl or pyrrolidyl benzilate scaffold using methyl benzilate and 4-piperidinol, (R)- ± 3-piperidinol, or (R)- ± 3-pyrrolidinol. Amine substituents include alkyl and aralkyl groups. The agents 3-[^{18}F]-FEPB and 4-[^{18}F]-FEPB can be produced through alkylation reactions facilitated by the application of 2-[^{18}F]-fluoroethyl triflate.

Implementation: Evaluation of the retention of 3-[^{18}F]-FEPB and 4-[^{18}F]-FEPB within the central nervous system (CNS).

Animal Model: Murine (mouse CNS).

Interpretation and Analysis: *In vitro* K_i values range from 0.05 nM to > 100 nM. Optimal properties are associated with the agents (R)-N-(2-fluoroethyl)-3-piperidyl benzilate (3-FEPB, 22, K_i = 12.1 nM) and N-(2-fluoroethyl)-4-piperidyl benzilate (4-FEPB, 8, K_i = 1.83 nM). Radiolabling with [^{18}F] applying alkylation reactions to produce 3-[^{18}F]-FEPB and 4-[^{18}F]-FEPB results in a 7–9% radiochemical yield and a > 97% radiochemical purity. Under *in vivo* conditions, brain retention is moderate for 3-[^{18}F]-FEPB; however, blocking with scopolamine reveals that uptake is not muscarinic cholinergic receptor-mediated. Conversely, 4-FEPB exhibits high, receptor-mediated retention in mouse brain, with significant clearance after 1 hour. These results suggest that compound #43 could have applications as an *in vivo* probe for measuring endogenous acetylcholine levels.

Synthesis, [18F]-labeling, and biological evaluation of piperidyl and pyrrolidyl benzilates as *in vivo* ligands for muscarinic acetylcholine receptors. Skaddan MB, Kilbourn MR, Snyder SE, Sherman PS, Desmond TJ, Frey KA. J Med Chem 2000 Nov 16; 43 (23), pp. 4552–62.

Alzheimer's Disease (AD)/Muscarinic/ Cholinergic Investigative Probe *RS 86 (2-Ethyl-8-Methyl-2,8-Diazaspiro-[4,5]-Decan-1,3-Dion Hydrobromide)*

Cellular/Molecular Mechanism of Action: The agent 2-ethyl-8-methyl-2,8-diazaspiro-[4,5]-decan-1,3-dion hydrobromide (RS 86) recognizes and physically binds to cholinergic muscarinic receptor complexes as a component of the parasympathetic autonomic nervous system.

Application: The agent 2-ethyl-8-methyl-2,8-diazaspiro-[4,5]-decan-1,3-dion hydrobromide (RS 86) appears to be an adequate probe for the clinical examination of the cholinergic hypothesis of AD.

Implementation: The effects of the compound 2-ethyl-8-methyl-2,8-diazaspiro-[4,5]-decan-1,3-dion hydrobromide (RS 86) in a number of *in vitro* and *in vivo* test systems for muscarinic cholin-

ergic activity can be analyzed and compared to those of classical muscarinic receptor agonists.

Animal Model: Guinea pig (*in vitro* ileum preparations).

Animal Model: Rat (superior cervical ganglion).

Interpretation and Analysis: In radioligand binding assays, RS 86 displays high nanomolar apparent affinity only for sites labeled by [^{3}H]-muscarinic receptor agonists, while its apparent affinity for sites labeled by [^{3}H]-muscarinic receptor antagonists, including [^{3}H]-QNB, [^{3}H]-NMS, and [^{3}H]-pirenzepine, is in the micromolar range. RS 86 has no or only low affinity (IC_{50} greater than 10 μM) for other neurotransmitter or drug receptor sites. The compound induces scopolamine-sensitive contractions of isolated guinea pig ileum showing a pD2 of 6 in this model. In the isolated rat superior cervical ganglion RS 86 also functions as an agonist with a pD2 of 6.7. When given to mice or rats by different routes RS 86 induces central and peripheral effects typical of a muscarinic receptor agonist, such as hypothermia, tremor, mydriasis, salivation, lacrimation, diarrhea, and modification of behavior as observed in an open field. In several of these tests RS 86 is about 10 times less potent than oxotremorine but more potent than arecoline, pilocarpine, aceclidine, or the compound (cis) AF-30. The ED_{50} values for some central effects, including the induction of hypothermia and alert non-mobile behavior, are lower than those for tremor and peripheral effects. Some of the effects last for up to 6 hours depending on dose. Finally, RS 86 administration results in modifications of brain acetylcholine turnover and high affinity choline uptake typical of a central muscarinic receptor agonist. Taken together these results demonstrate clearly that RS 86 is a potent, centrally acting, selective muscarinic receptor agonist. RS 86 appears to be an adequate tool for the clinical examination of the cholinergic hypothesis of AD.

The pharmacological assessment of RS 86 (2-ethyl-8-methyl-2,8-diazaspiro-[4,5]-decan-1,3-dion hydrobromide). A potent, specific muscarinic acetylcholine receptor agonist. Palacios JM, Bolliger G, Closse A, Enz A, Gmelin G, Malanowski J. Eur J Pharmacol 1986 Jun 5; 125 (1), pp. 45–62.

Alzheimer's Disease (AD)/Cognitive Decline: Early Evaluation/Improved Diagnostic Accuracy/ Diminished Disease/Treatment-Related Morbidity of Dementia
2-Deoxy-2-[^{18}F]-Fluoro-D-Glucose

Cellular/Molecular Mechanism of Action: 2-Deoxy-2-[^{18}F]-fluoro-D-glucose is an analogue of endogenous glucose.

Background Information: Growing evidence indicates that appropriate incorporation of positron emission tomography (PET) into the evaluation of patients with early symptoms of cognitive decline can improve diagnostic and prognostic accuracy**.**

Application: 2-Deoxy-2-[^{18}F]-fluoro-D-glucose is an analogue of endogenous glucose that can be used as a probe to detect regional alterations in cellular metabolism. In this regard, 2-deoxy-2-[^{18}F]-fluoro-D-glucose can be applied to detect and characterize the early stages of AD in a manner that can improve therapeutic intervention strategies and patient management.

Route of Administration: 2-Deoxy-2-[^{18}F]-fluoro-D-glucose (intravenous).

Implementation: Evaluate the relative value of 2 strategies for assessing whether AD is responsible for cognitive declines in geriatric patients, and in subsequently managing those patients according to the recommended standards of the American Academy of Neurology (AAN). The 1st strategy is based on an approach already endorsed by the AAN, following evidence-based reviews carried out by its quality standards subcommittee. The 2nd approach is based on many of the same AAN recommendations with respect to initial general medical and neurologic examinations, structural imaging, and laboratory tests, as well as ultimate management, but additionally incorporated PET analyses in appropriate cases for the purpose of determining the presence or absence of a pattern of regional cerebral metabolism characteristic of AD conditions. Clinical outcomes accruing to each strategy are calculated using formalized tools of decision analysis.

Interpretation and Analysis: The utilization of the increased diagnostic accuracy of PET yields lower rates of both false negative (from 8.3 to 3.1%) and false positive (from 23.0 to 11.9%) diagnoses for AD compared with conventional strategies. When combined with AAN treatment recommendations for patients having (or not having) non-severe AD, these differences in diagnostic accuracy correspond to approximately a 62% decrease in avoidable months of nursing home care, and a 48% decrease in months of unnecessary drug therapy resulting from inaccurate diagnoses. The benefit in clinical outcome of the proposed strategy is maintained over a wide range of values for sensitivity, specificity, and projected impact on need for nursing home care. Therefore, the use of PET for evaluating early cognitive declines in geriatric cases can add valuable information to the clinical assessment, resulting in a greater number of conditions being accurately diagnosed and properly treated. The use of PET can diminish disease-related and treatment-related morbidity of dementia, through earlier institution of appropriate management.

Added clinical benefit of incorporating 2-deoxy-2-[18F]-fluoro-D-glucose with positron emission tomography into the clinical evaluation of patients with cognitive impairment. Silverman DH, Cummings JL, Small GW, Gambhir SS, Chen W, Czernin J, Phelps ME. Mol Imaging Biol 2002 Jul; 4 (4), pp. 283–93.

Alzheimer's Disease (AD)/Ayloid Beta (Abeta) Amyloid Peptides/Senile Plaques (Sps)/Abeta40-Positive Amyloid Angiopathy (AA) vs Abeta42-Positive AA
[^{125}I]-TZDM / [^{125}I]-IMSB (Styrylbenzene)

Background Information: Alzheimer's disease is linked to increased brain deposition of abeta peptides in SPs, and recent therapeutic efforts have focused on inhibiting the production or enhancing the clearance of abeta in brain. However, it has not been possible to measure the burden of SPs or assess the effect of potential therapies on brain abeta levels in patients.

Application: Detection and characterization of amyloid abeta peptides in senile plaques associated with conditions of AD.

Implementation: Development and evaluation of a radioligand, [^{125}I]-TZDM, that binds to abeta fibrils with high affinity (crosses the blood-brain barrier (BBB), and labels amyloid plaques *in vivo*).

Interpretation and Analysis: Compared to a styrylbenzene probe, [^{125}I]-IMSB, [^{125}I]-TZDM has a 10-fold greater brain penetration and labels plaques with higher sensitivity for *in vivo* imaging. However, this ligand also labels white matter, which contributes to undesirable high background regions of the brain. Interestingly, parallel to their differential binding characteristics onto fibrils composed of 40 (abeta40)- or 42 (abeta42)-amino acid long forms of abeta peptides, these radioligands display differential labeling of SPs in AD brain sections under experimental conditions. It has been observed that [^{125}I]-IMSB labels SPs containing abeta40, AA, and neurofibrillary tangles, whereas [^{125}I]-TZDM detects only SPs and abeta42-positive AA. Because increased production and deposition of abeta42 relative to abeta40 may be crucial for the generation of SPs, [^{125}I]-TZDM and related derivatives may be more attractive probes for *in vivo* plaque labeling. Further structural modifications of TZDM to lower the background labeling will be needed to optimize the plaque-labeling property.

Detection of amyloid plaques by radioligands for abeta40 and abeta42: Potential imaging agents in Alzheimer's patients. Kung MP, Skovronsky DM, Hou C, Zhuang ZP, Gur TL, Zhang B, Trojanowski JQ, Lee VM, Kung HF. J Mol Neurosci. 2003 Feb;20(1):15–24.

Alzheimer's Disease (AD): Amyloid Plaques (Abeta) *[^{11}C] Stilbene Derivatives*

Cellular/Molecular Mechanism of Action: Stilbene derivatives recognize and physically bind to amyloid plaques analogous to those associated with AD.
Application: [^{11}C]4, N-[^{11}C]-methylamino-4′-hydroxystilbene, may be useful as a positron emission tomography (PET) imaging agent for mapping brain abeta plaques in AD conditions.
Route of Administration: Intravenous.
Semi-Synthetic Methods: Synthesis of a series of stilbene derivatives can be successfully achieved by performing a simple Wadsworth-Emmons reaction between diethyl (4-nitrobenzyl)phosphonate and 4-methoxybenzaldehyde. The [^{11}C]-labeled 4-N-methylamino-4′-hydroxystilbene, [^{11}C]4, is prepared by [^{11}C] methylation of 4-amino-4′-hydroxystilbene.
Interpretation and Analysis: 4-N,N-dimethylamino-4′-methyoxy and the corresponding 4-N-monomethylamino-, 4′-hydroxy stilbenes show good binding affinities for abeta aggregates *in vitro* ($K_i < 10$ nM). The [^{11}C]4 analog displays a moderate lipophilicity (log P = 2.36), and shows very good brain penetration and washout from normal rat brain following intravenous injection. *In vitro* autoradiography of transgenic AD mouse brain sections shows a high specific labeling of beta-amyloid plaques, whereas control sections show no binding.

^{11}C-labeled stilbene derivatives as abeta-aggregate-specific PET imaging agents for Alzheimer's disease. Ono M, Wilson A, Nobrega J, Westaway D, Verhoeff P, Zhuang ZP, Kung MP, Kung HF. Nucl Med Biol 2003 Aug; 30 (6), pp. 565–71.

Amyloid: Beta-Sheet Proteins/Amyloid Plaques: Abeta Peptide/Neurofibrillary Tangles: Tau Protein *Thioflavine Derivatives/[N-Methyl-^{11}C]6-Me-BTA-1*

Cellular/Molecular Mechanism of Action: Thioflavine T (ThT) derivatives possess binding avidity for beta-sheet proteins deposited in amyloid plaques (abeta peptide) and neurofibrillary tangles (tau protein).
Background Information: Assessment of the beta-sheet proteins deposited *in vivo* in amyloid plaques (abeta peptide) or neurofibrillary tangles (tau protein) represents a target for the development of Alzheimer's disease (AD) biological marker systems.
Application: Detection and characterization of beta-sheet proteins deposited in amyloid plaques (abeta peptide) and neurofibrillary tangles (tau protein) similar to those associated with the pathogenesis of AD.
Route of Administration: [Intravenous] if eventually applied in a clinical environment.
Semi-Synthetic Methods: Production of ThT derivatives and the carbon-11 labeled compound [N-methyl-^{11}C]6-Me-BTA-1.
Implementation: Evaluation of [N-methyl-^{11}C]6-Me-BTA-1 to determine its capacity to enter into and be cleared from the central nervous system (e.g., brain).
Animal Model: Mouse (Swiss-Webster).
Interpretation and Analysis: These compounds lack the positively charged quaternary heterocyclic nitrogen of ThT and are therefore uncharged at physiological pH levels. They are 600-fold more lipophilic than ThT. Derivatives of ThT bind to abeta(1-40) fibrils with higher affinity (K_i = 20.2 nM) than ThT (K_i = 890 nM). The uncharged ThT derivatives stain both plaques and neurofibrillary tangles in postmortem AD brain, showing some preference for plaque staining.

The compound [N-methyl-^{11}C]6-Me-BTA-1 enters the brain at levels comparable to commonly used neuroreceptor imaging agents (0.223 % injected dose [%ID]-kg/g or 7.61 %ID/g at 2 minutes postinjection) and shows good clearance of free and non-specifically bound radioactivity in normal rodent brain tissue (brain clearance $T_{1/2}$ = 20 minutes). The combination of relatively high affinity for amyloid, specificity for staining plaques and neurofibrillary tangles in postmortem AD brain, and good brain entry and clearance makes [N-methyl-^{11}C]6-Me-BTA-1 a promising candidate as an *in vivo* positron emission tomography (PET) beta-sheet imaging agent.

Uncharged thioflavine T derivatives bind to amyloid beta protein with high affinity and readily enter the brain. Klunk WE, Wang Y, Huang GF, Debnath ML, Holt DP, Mathis CA. Life Sci 2001 Aug 17; 69 (13), pp. 1471–84.

Amyloid (Abeta) Fibrils/Alzheimer's Disease (AD): Senile Plaques and Neurofibrillary Tangles *[^{11}C]-Methoxy-X04 [Congo Red Derivative]/ Chrysamine G*

Cellular/Molecular Mechanism of Action: Methoxy-X04 is a derivative of both Congo red and chrysamine G that has retained binding avidity for amyloid (abeta) fibrils. Exposure of methoxy-X04 to certain wavelengths of light results in the emission of visibly detectable fluorescence.
Background Information: The identification of amyloid deposits in living AD patients is important for both early diagnosis and for monitoring the efficacy of newly developed antiamyloid therapies.
Application: Detection of senile plaques, neurofibrillary tangles, and cerebrovascular amyloid using either multiphoton microscopy to obtain fluorescent images (methoxy-X04) or positron emission tomography (carbon-11-labeled methoxy-X04).
Route of Administration: Methoxy-X04 (intravenous/intra peritoneal in mice).
Dosage: Methoxy-X04 (5–10 mg/kg intravenous or 10 mg/kg intraperitoneal).
Implementation: Administration of methoxy-X04 for the *in vivo* detection of senile plaques, neurofibrillary tangles, and cerebrovascular amyloid.
Animal Model: Mice (PSI/APP).
Interpretation and Analysis: Methoxy-X04 is a derivative of Congo red and chrysamine G that contains no acid groups and is therefore smaller and much more lipophilic than Congo red or chrysamine G. Methoxy-X04 retains *in vitro* binding affinity for amyloid beta (abeta) fibrils (K_i = 26.8 nM) very similar to that of chrysamine G (K_i = 25.3 nM). Methoxy-X04 is fluorescent and stains plaques, tangles, and cerebrovascular amyloid in postmortem sections of AD brain with good specificity. Using multiphoton microscopy to obtain high-resolution (1 μm) fluorescent images from the brains of living PSI/APP mice, individual plaques can be distinguished within 30 to 60 minutes after methoxy-X04 administration. A single intraperitoneal injection of methoxy-X04 also produces high-contrast images of plaques and cerebrovascular amyloid in PSI/APP mouse brain. Complementary quantitative studies using tracer doses of carbon-11-labeled methoxy-X04 show that it enters rat brain in amounts that suggest it is a viable candidate as an *in vivo* positron emission tomography (PET) amyloid-imaging agent.
Instrumentation: Multiphoton microscopy.

Imaging abeta plaques in living transgenic mice with multiphoton microscopy and methoxy-X04, a systemically administered Congo red deriv-

ative. Klunk WE, Bacskai BJ, Mathis CA, Kajdasz ST, McLellan ME, Frosch MP, Debnath ML, Holt DP, Wang Y, Hyman BT. J Neuropathol Exp Neurol 2002 Sep; 61 (9), pp. 797–805.

Amyloid: Abeta Fibrils (Alzheimer's Disease [AD])/ *Ex Vivo* Abeta Senile Plaques *(S)-Naproxen/(R)-Ibuprofen and (S)-Ibuprofen/ Congo Red/Thioflavine T/2-(1-[6-[(2-[^{18}F]-Fluoroethyl) (Methyl)Amino]-2-Naphthyl]Ethylidene)Malononitrile ([^{18}F]-FDDNP)*

Background Information: Epidemiological studies have suggested that the chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) reduces the relative risk of AD. The possible neuroprotection by NSAIDs in AD is generally attributed to anti-inflammatory activity. An additional mode of drug action may involve an antiaggregatory effect on beta-amyloid (abeta) peptides by commonly used NSAIDs.

Implementation: Utilization of *in vitro* competition assays, autoradiography, and fluorescence microscopy to evaluate the binding of the *in vivo* molecular imaging probe [^{18}F]-FDDNP against (S)-naproxen and (R)-ibuprofen and (S)-ibuprofen (but not diclofenac) to abeta fibrils and *ex vivo* abeta senile plaques from AD brain specimens.

Interpretation and Analysis: The *in vivo* molecular imaging probe [^{18}F]-FDDNP demonstrates a concentration-dependent decrease in binding to abeta fibrils and *ex vivo* abeta senile plaques when applied against (S)-naproxen and (R)-ibuprofen and (S)-ibuprofen (but not diclofenac) preparations. Conversely, *in vitro* amyloid dyes Congo red and thioflavine T do not bind to the FDDNP binding site. Both FDDNP and the NSAIDs that share the same binding site also exhibit antiaggregation effects on abeta peptides, suggesting that the shared binding site on abeta fibrils and abeta plaques may be a site of antiaggregatory drug action. Obviously, the binding of select NSAIDs to plaques, specifically to the binding site of the molecular imaging probe [^{18}F]-FDDNP, is of distinct therapeutic significance in the management of AD. Expanding the current understanding about the molecular requirements of FDDNP binding may aid in optimizing the abeta antiaggregation potency of experimental drugs. [^{18}F]-FDDNP has been used to image plaques *in vivo* with positron emission tomography (PET), and investigations into the influence of abeta antiaggregation on the risk-reduction effects of NSAIDs on AD could utilize [^{18}F]-FDDNP and PET in determining the occupancy rate of NSAIDs and experimental drugs in plaques in the living brain of AD patients.

In vitro detection of (S)-naproxen and ibuprofen binding to plaques in the Alzheimer's brain using the positron emission tomography molecular imaging probe 2-(1-[6-[(2-[^{18}F]-fluoroethyl)(methyl)amino]-2-naphthyl]ethylidene)malononitrile. Agdeppa ED, Kepe V, Petri A, Satyamurthy N, Liu J, Huang SC, Small GW, Cole GM, Barrio JR. Neuroscience 2003; 117 (3), pp. 723–30.

Behavior/Depression and Emesis/Substance P (SP)-Neurokinin-1 (NK1) Receptor/ Aprepitant (MK-0869) Efficacy/Dose and Receptor Occupancy Correlations *[^{18}F]-SPA-RQ*

Substance P-NK1 receptor pathways have been implicated in the pathophysiology of emesis and depression. Autoradiographic studies in monkey and human brains have shown a high expression of NK1 receptors in regions important for the regulation of affective behaviors and the neurochemical response to stress. Furthermore, clinical studies have demonstrated that treatment with the SP (NK1 receptor) antagonist (SPA) aprepitant (also known as MK-0869) significantly improves depression symptoms and reduces the incidence of chemotherapy-induced nausea and vomiting. An important objective of all neuroscience drug discovery and development programs is to establish the correlation among dose, receptor occupancy, and the observed clinical effect (the dose-response relationship). These goals can be achieved using radioactive receptor-specific tracers and dynamic non-invasive brain imaging modalities, such as positron emission tomography (PET). In the SPA program, a tracer [^{18}F]-SPA-RQ has been utilized for PET studies on the basis of several criteria, including high affinity for the NK1 receptor, low non-specific binding, and good blood-brain barrier penetration. Positron emission tomography imaging studies in rhesus monkeys and humans have confirmed these tracer features and established the usefulness of this probe for *in vivo* NK1-receptor occupancy studies. Subsequent PET occupancy studies in humans predict that very high levels of central NK1-receptor occupancy ($> 90\%$) are associated with therapeutically significant antidepressant and antiemetic effects. Imaging studies that focus on quantification of NK1-receptor expression in depressed patients, both before and after successful treatment with antidepressants, will create greater knowledge about how to integrate diagnostic imaging capabilities with the design of effective therapeutic protocols.

Imaging substance P receptors (NK1) in the living human brain using positron emission tomography. Hargreaves R. J Clin Psychiatry 2002; 63 Suppl 11, pp. 18–24.

Behavior/Schizophrenia/Presynaptic Dopamine Metabolism *6-[^{18}F]-Fluoro-L-Dopa*

Cellular/Molecular Mechanism of Action: 6-[^{18}F]-Fluoro-L-dopa is an analogue of L-dopa and binds to membrane-associated dopa receptor complexes.

Application: Detection and characterization of alterations in presynaptic dopamine metabolism in conditions of schizophrenia.

Implementation: Evaluation of presynaptic dopamine metabolism in conditions of schizophrenia using 6-[^{18}F]-fluoro-L-dopa ([^{18}F]-dopa) and positron emission tomography (PET). The ratio of specific/non-specific activity can be calculated for 8 specific cortical and subcortical regions-of-interest (ROIs).

Human Model: Cases of schizophrenia ($n = 19$ drug-free cases; $n = 10$ cases on neuroleptics; $n =$ age- and gender-matched normal controls). The neuroleptic-treated patients receive typical neuroleptics ($n = 4$) or the atypical neuroleptic clozapine ($n = 6$).

Interpretation and Analysis: Cases that are medication-free have a significant reduction in [^{18}F]-dopa uptake in the ventral striatum ($p = 0.04$) and a significantly increased amount of uptake in the posterior cingulate ($p = 0.02$) compared with normal control subjects. The [^{18}F]-dopa PET technique is useful and sensitive in detecting *in vivo* changes in dopamine metabolism in schizophrenia and diagnostic results from using [^{18}F]-dopa as an investigative probe can provide evidence of aberrant dopamine systems in conditions of schizophrenia.

6-[18F]-dopa PET study in patients with schizophrenia. Positron emission tomography. Elkashef AM, Doudet D, Bryant T, Cohen RM, Li SH, Wyatt RJ. Psychiatry Res 2000 Nov 20; 100 (1), pp. 1–11.

Behavior: Schizophrenia: Dopamine Receptors/ Striatal D_2 Dopamine Receptor Occupancy *[^{123}I]-IBZM/Haloperidol/Olanzapine*

Dosage: Haloperidol (10 mg/day); olanzapine (10 mg/day).

Implementation: Delineation of (i) the *in vivo* striatal D_2 dopamine receptor occupancy induced by olanzapine and haloperidol in

schizophrenic conditions using a baseline-end point [^{123}I]-IBZM single photon computed emission tomography (SPECT) design; and (ii) assessement of the relationships of striatal D_2 receptor occupancy with clinical efficacy and extrapyramidal symptoms (EPS). The percentage of D_2 receptors occupied can be estimated using basal ganglia (striatum)/frontal cortex IBZM uptake ratios obtained before and after 4 weeks of maintained antipsychotic treatment.

Human Model: Schizophrenia and schizophreniform disorder cases (e.g., n = 27). Haloperidol (n = 13); olanzapine (n = 14). Ratings of clinical status and EPS can be obtained weekly.

Interpretation and Analysis: Olanzapine produces a mean striatal D_2 receptor occupancy of 49% (range 28–69%) and is significantly lower than that induced by haloperidol (mean 64%, range 46–90%). The baseline end point SPECT design reveals antipsychotic D_2 occupancy percentage values that are lower than those reported using other approaches. The degree of striatal D_2 receptor occupancy correlates with EPS, which predominantly appears in patients on haloperidol. No relationship can be appreciated between the striatal D_2 receptor occupancy and clinical improvement. Olanzapine induces a lower striatal D_2 occupancy than haloperidol. This low striatal D_2 occupancy, together with the lower incidence of EPS with olanzapine treatment contributes additional confirmation to observations regarding the atypical behavior of this antipsychotic pharmaceutical. Nevertheless, conclusions based on SPECT-estimated percentages of antipsychotic D_2 occupancy should be done with caution because SPECT design can influence the results. In this regard, SPECT studies, including baseline and end point examinations, should be encouraged.

Double blind olanzapine vs haloperidol D_2 dopamine receptor blockade in schizophrenic patients: A baseline end point. Bernardo M, Parellada E, Lomena F, Catafau AM, Font M, Gomez JC, Lopez-Carrero C, Gutierrez F, Pavia J, Salamero M. Psychiatry Res 2001 Aug 25; 107 (2), pp. 87–97.

Benzodiazepine Receptor/Prediction of Therapeutic Response/Mental Depression Pathogenesis *[^{123}I]-Ro 16-0154*

Cellular/Molecular Mechanism of Action: [^{123}I]-labeled Ro 16-0154 selectively recognizes and binds to benzodiazepine receptor complexes.

Application: Screening agent potentially applicable to predicting responsiveness to therapeutic intervention and possible characterization of the pathogenesis of mental depression.

Route of Administration: [^{123}I]-Ro 16-0154 (intravenous).

Implementation: Non-invasive *in vivo* evaluation of alterations in benzodiazepine receptor expression.

Animal Model: Primate (brain).

Interpretation and Analysis: Maximal brain uptake of [^{123}I]-Ro 16-0154 is reached at approximately 70 minutes after intravenous administration of the radioligand and represents approximately 10% of the total injected dose. The regional distribution of radioactive densities is consistent with the known distribution of benzodiazepine receptors within the brain, with highest uptake localized over the occipital area. Washout of radioactivity is relatively slow with a rate of 3% per hour after the time of peak radioactivity. Rapid decreases of more than 90% of total brain radioactivity associated with [^{123}I]-Ro 16-0154 uptake can be appreciated with benzodiazepine antagonists (e.g., Ro 15-1788: 0.2–0.3 mg/kg IV).

Instrumentation: Single photon emission computed tomography (SPECT).

SPECT imaging of the benzodiazepine receptor in non-human primate brain with [123I]-Ro 16-0154. Innis R, Zoghbi S, Johnson E, Woods S, al-Tikriti M, Baldwin R, Seibyl J, Malison R, Zubal G, Charney D, et al. Eur J Pharmacol 1991 Feb 7; 193 (2), pp. 249–52.

Metabolism of Glucose and Alterations in Disease/ (Alzheimer's, Epilepsy, Schizophrenia, Cranial Trauma, Brain Tumors) *[^{18}F]-Fluoro-Deoxy-Glucose (FDG)*

Cellular/Molecular Mechanism of Action: [^{18}F]-fluoro-deoxy-glucose is absorbed, diffuses across the blood-brain barrier, and is then intracellularly transported by cell populations within the central nervous system (CNS) in a manner highly analogus to endogenous glucose. Due to the fluoride moiety, [^{18}F]-labeled glucose is not rapidly metabolized intracellularly by glycolytic pathways. The exact trapping mechanisms and their relationships with potential clinical applications still remains to be elucidated.

Background Information: In recent years, significant progress has been made in the design and characterization of new CNS neuronal and postsynaptic receptor imaging agents for positron emission tomography (PET) and single photon emission computed tomography (SPECT). The new diagnostic agents are aimed at detecting and measuring localized changes of neuronal function.

Application: Characterization of alterations in glucose metabolism in various neurologic disorders, including Alzheimer's, epilepsy, schizophrenia, and cranial trauma in addition, to the diagnosis and monitoring of space-occupying and ischemic lesions affecting the brain and spinal cord.

Interpretation and Analysis: Agents like [^{18}F]-FDG likely have potential clinical application for the diagnosis of psychiatric disorders that do not involve morphological changes. Imaging studies using [^{18}F]-FDG with PET are currently the most promising diagnostic tool for the evaluation of various disease states, such as Alzheimer's disease, brain tumor, and epilepsy, as a function of alterations in local glucose metabolism.

Instrumentation: There are 2 types of imaging instruments, SPECT and PET, that use radiopharmaceuticals for the diagnosis of brain disorders. Positron emission tomography instrumention can be applied when the imaging agent [^{18}F]-FDG is administered for the diagnosis of various disease states affecting the CNS.

Overview of radiopharmaceuticals for diagnosis of central nervous disorders. Kung HF. Crit Rev Clin Lab Sci 1991; 28 (4), pp. 269–86.

The use of 2-deoxy-2-[18F]-fluoro-D-glucose positron emission tomography (FDG PET) in the routine diagnosis of epilepsy. Swartz BE, Brown C, Mandelkern MA, Khonsari A, Patell A, Thomas K, Torgersen D, Delgado-Escueta AV, Walsh GO. Mol Imaging Biol 2002 May; 4 (3), pp. 245–52.

Metabolism of Glucose/Schizophrenia Treatment Resistance (Clozapine)/Dopamine D1R Genotype/ Individualization of Antipsychotic Agent Dosage: Schizophrenia/Hepatic Cytochrome P450 2D6 (CYP2D6) Metabolizing Capacity *[^{18}F]-Fluoro-Deoxy-Glucose (FDG)/Clozapine*

Cellular/Molecular Mechanism of Action: The agent [^{18}F]-FDG functions as a false glucose analogue that can detect localized alterations in brain glucose metabolism.

Physiological Mechanism of Action: Clozapine potentiates the effects of GABA-mediated presynaptic and postsynaptic inhibitory pathways.

Background Information: A goal of pharmacogenetics is to clarify associations between allelic variation and risk factors in psychiatric illness.

Application: Characterization of different patterns in dopamine alleles that correlated with resistance to therapeutic management in

conditions of treatment-resistant schizophrenia. A close relationship exists between regional brain metabolic activity and clinical response to clozapine that is related to the D_1-receptor genotype.

Implementation: Evaluation of the capacity for positron emission tomography (PET) scans with [^{18}F]-FDG to detect and characterize cases of treatment-resistant schizophrenic subjects after 5 weeks of clozapine treatment (vs placebo).

Interpretation and Analysis: Significant regional brain metabolic effects can be found for D_1-receptor genotypes ($p < 0.05$: adjusted for multiple comparisons). Metabolic decreases for the 2,2 genotype but not the 1,2 genotype can be observed in all major sectors of the brain, with the exception of the ventral parts of the caudate and putamen. Frontal, temporal, parietal, and occipital neocortices show decreases in metabolism, as do the cingulate juxtaallocortex and the parahippocampal allocortex. Decreases can also be observed in the thalamus, amygdala, and cerebellum bilaterally. No significant metabolic differences by genotype have been observed for D_3, 5-HT_{2A}, and 5-HT_{2C} polymorphisms. In terms of clinical response, the DRD1 2,2 genotype significantly improves with clozapine treatment, demonstrating a 30% decrease in the Brief Psychiatric Rating Scale positive symptoms in contrast to a 7% worsening for the 1,2 genotype ($p < 0.05$). Therefore, brain metabolic activity and clinical response to clozapine appears to be related to the D_1-receptor genotype.

D1-receptor alleles predict PET metabolic correlates of clinical response to clozapine. Potkin SG, Basile VS, Jin Y, Masellis M, Badri F, Keator D, Wu JC, Alva G, Carreon DT, Bunney WE Jr, Fallon JH, Kennedy JL. Mol Psychiatry 2003 Jan; 8 (1), pp. 109–13.

Metabolism of Lipids: Fatty Acid Lipids/Central Nervous System [CNS]): Beta-Oxidation/ Incorporation into Membrane Structures
[U-^{14}C]-Palmitate/[1-^{14}C]-Arachidonate/ Methyl 2-Tetradecylglycidate

Cellular/Molecular Mechanism of Action: Both palmitate and arachidonic acid are saturated fatty acids that are a component of the external cell membrane lipid bilayer. In the CNS, [U-^{14}C]-palmitate undergoes beta-oxidation. Methyl 2-tetradecylglycidate (MEP) is an irreversible inhibitor of carnitine palmitoyltransferase I.

Application: In radiolabeled form, palmitate can be utilized as an *in vivo* probe for detecting alterations in brain lipid metabolism.

Route of Administration: [U-^{14}C]-palmitate (intravascular), [1-^{14}C]-arachidonate (intravascular), methyl 2-tetradecylglycidate (oral).

Dosage: Methyl 2-tetradecylglycidate (10–25 mg/kg).

Implementation: Determination of the effect of inhibiting mitochondrial beta-oxidation of free fatty acids on the incorporation of radiolabeled free fatty acids into brain lipids. Saturated fatty acid [U-^{14}C]-palmitic acid or the polyunsaturated fatty acid [1-^{14}C]-arachidonate (AA) are administered 2, 4, and 6 hours after methyl 2-tetradecylglycidate (MEP).

Animal Model: Rat (irreversible inhibition of carnitine palmitoyltransferase I in males induced through the use of methyl 2-tetradecylglycidate).

Interpretation and Analysis: Methyl 2-tetradecylglycidate increases brain [U-^{14}C]-palmitate-associated radioactivity 2-fold and decreases brain aqueous radioactivity 3- to 5-fold relative to control values at all pretreatment times. The effect is due to a prolongation of the plasma integral of [U-^{14}C]-palmitate due to peripheral inhibition of beta-oxidation, and to direct inhibition of beta-oxidation of the tracer within the brain. Conversely, methyl 2-tetradecylglycidate has no effect on brain organic radioactivity after infusion of [1-^{14}C]-arachidonic acid. Increasing the interval between methyl 2-tetradecylglycidate administration and [U-^{14}C]-palmitate infusion from 2 to 6 hours results in a dramatic redistribution of [U-^{14}C]-palmitate within brain lipids. The percentage of radioactivity in phospholipids decreases from 65 to 33%, whereas that in the free fatty acid fraction increases from 10 to 47% and that in triglycerides becomes elevated 2- to 3-fold over reference controls. Methyl 2-tetradecylglycidate may facilitate the use of radiolabeled palmitate as an *in vivo* probe for detecting alterations in brain lipid metabolism using quantitative autoradiography or positron emission tomography.

Instrumentation: Positron emission tomography.

Effect of inhibition of beta-oxidation on incorporation of [U-14C]-palmitate and [1-14C]-arachidonate into brain lipids. Freed LM, Wakabayashi S, Bell JM, Rapoport SI. Brain Res 1994 May 9; 645 (1–2), pp. 41–8.

Metabolism of Lipids: Phosphatidylinositol/Central Nervous System (CNS)
[^{11}C]-Inositol/[^{11}C]-Inositol-1-Phosphate (IP)

Application: Brain diagnostic imaging agent used in positron emission tomography (PET) imaging techniques for characterizing phosphatidylinositol metabolism.

Route of Administration: Intravenous.

Semi-Synthetic Methods: Radiolabeled inositol (Ins) and inositol-1-phosphate (IP) can be derived from radiolabeled glucose by utilizing brief sequential enzyme reactions. Rapid separation and fractionation of enzymes and labeled products from the reaction mixture can be achieved by high-performance liquid chromatography (HPLC) utilizing a gel-permeation chromatography column.

Interpretation and Analysis: Examination of the biodistribution of Ins, IP, and their acetylated analogs suggests that intact Ins labeled with [^{11}C] would be more effective than [^{11}C]-labeled acetylated Ins as a brain diagnostic agent for PET studies focused on determining the metabolism of phosphatidylinositol and its role as a 2nd messenger within the brain.

Instrumentation: Computer-integrated PET instrumentation.

Sequential enzymatic synthesis and biodistribution of radiolabeled inositol and inositol analogs. Sasaki T, Ogihara-Umeda I, Kojima S, Nishigori H. Int J Rad Appl Instrum [A] 1991; 42 (1), pp. 97–101.

Metabolism/Mitosis and Proliferation Rate
3′-Deoxy-3′-[^{18}F]-Fluorothymidine ([^{18}F]-FLT)

Cellular/Molecular Mechanism of Action: Thymidine is rapidly assimilated by proliferating cell populations (e.g., incorporated into nucleic acids).

Application: [^{18}F]-FLT has been designed as a new positron emission tomography (PET) imaging agent for *in vivo* visualizing of cellular proliferation based on the incorporation of thymidine into nucleic acid.

Route of Administration: Variable but most often intravascular.

Semi-Synthetic Methods: A reliable radiosynthesis of [^{18}F]-FLT has been developed based on [^{18}F] 10 mCi (370 MBq) of radiochemically pure [^{18}F] 1 Ci/μmol (37 GBq/μmol) at the end of synthesis (EOS) within 100 minutes and in 13% radiochemical yield (end of bombardment [EOB]; 7% EOS).

Implementation: Evaluation of neoplastic lesions that display relatively rapid rates of proliferation/growth.

Interpretation and Analysis: Detection of increased radioactivity at a specific anatomical location that corresponds with an anatomical region that has a relatively high rate of cellular proliferation.

Radiosynthesis of 3′-deoxy-3′-[^{18}F]-fluorothymidine: [^{18}F]-FLT for imaging of cellular proliferation *in vivo*. Grierson JR, Shields AF. Nucl Med Biol 2000 Feb; 27 (2), pp. 143–56.

Neurotoxicity: Cytarabine-Associated/Acute Leukemia (AL): Childhood Onset/Cerebral Perfusion/Relative Efficacy of Single Photon Emission Computed Tomography (SPECT) vs Magnetic Resonance Imaging (MRI) *[^{99m}Technetium] ([^{99m}Tc])-Hexamethyl-Propylene-Amine Oxime (HMPAO)*

Background Information: Cytarabine (ara-C) is one of the most effective chemotherapeutic agents in conditions of AL. In addition, it exerts a clear dose effect. Use of high-dose ara-C is hampered, however, by a noticeable toxicity, particularly to the central nervous system (CNS).

Application: Assess the usefulness of CNS perfusion imaging with [^{99m}Tc]-HMPAO SPECT concurrent with MRI to specifically assess the effects of standard- and high-dose ara-C in children with AL.

Implementation: Evaluation of cerebral perfusion by [^{99m}Tc]-HMPAO SPECT in cases of childhood acute leukemia after induction therapy consisting of a (i) standard-dose ara-C; (ii) immediately after consolidation with high-dose ara-C; and (iii) later during follow-up periods (range, 6–44 months). The chemotherapy-related adverse events are monitored and correlated to SPECT and MRI.

Human Model: Cases of childhood acute leukemia (n = 12; age range = 4–15 years; n = 26 perfusion studies).

Interpretation and Analysis: After the induction phase, all children are neurologically normal on MRI. On SPECT imaging, a number of children (e.g., n = 4/12) display a slightly heterogeneous perfusion. After high-dose ara-C (4–36 g/m^2), slightly more than a third of affected children have regressive neurologic signs of potential toxic origin (n = 5/12) and of these cases, a small percentage will have an abnormal MRI scan (n = 1/5), whereas all cases display evidence of diffuse cerebral and/or cerebellar heterogeneous perfusion on SPECT. In some cases that are without any neurologic symptoms (e.g., n = 7) and that have normal MRI scans, normal SPECT profiles will also be appreciated (n = 3/7 normal; n = 4/7 abnormal). On follow-up analysis of cases initially presented with clinical neurological toxicity, (i) a relatively large number display SPECT images that improve (n = 3/4); (ii) a small percentage remain unchanged (e.g., n = 1 of 4, or 25%); and (iii) some display delayed abnormalities that can be recognized on MRI scans (e.g., n = 2/4; T_2 white matter hypersignal and cerebellar atrophy). Diffuse heterogeneous brain hypoperfusion is often the sole early objective imaging feature identified by SPECT of high-dose ara-C neurotoxicity, when MRI still demonstrates normal imaging profiles.

Contribution of single photon emission computed tomography in the diagnosis and follow-up of CNS toxicity of a cytarabine-containing regimen in pediatric leukemia. Véra P, Rohrlich P, Stiévenart JL, Elmaleh M, Duval M, Bonnin F, Bok B, Vilmer E. J Clin Oncol 1999 Sep; 17 (9), pp. 2804–10.

Opioid Receptor Expression *[^{18}F]-Fluorophen*

Cellular/Molecular Mechanism of Action: (±)-5,9 α-Dimethyl-2-[2-(4-fluorophenyl)ethyl]-2′-hydroxy-6, 7-benzomorphan (fluorophen) is a fluorinated derivative of the benzomorphan opiate agonist phenazocine, which physically recognizes and binds to opiate receptor complexes. The agent is relatively selective for μ receptors, congruent for δreceptors at a level that is far greater than its binding avidity for κ receptor complexes.

Application: Detection of opiate receptor complexes.

Semi-Synthetic Methods: (±)-5,9 α-Dimethyl-2′-hydroxybenzomorphan is subjected to *N*-acylation utilizing (*p*-fluorophenyl)-acetyl chloride, followed by diborane reduction of the resulting amide.

Interpretation and Analysis: Fluorination produces only a 2-fold opiate receptor affinity loss when measured either by bioassay or receptor binding (selectivity for μ, congruent to δ and greater than kappa). Labeling of the pharmaceutical fluorophen with [^{18}F] creates an imaging agent sufficiently potent to be useful as a non-invasive *in vivo* probe for visualizing opiate receptor complexes.

Instrumentation: Positron emission trans-axial tomography.

Synthesis and pharmacological characterization of (±)-5,9 alpha-dimethyl-2-[2-(4-fluorophenyl)ethyl]-2′-hydroxy-6,7-benzomorphan (fluorophen), a ligand suitable for visualization of opiate receptors *in vivo.* Rice KC, Konicki PE, Quirion R, Burke TR Jr, Pert CB. J Med Chem 1983 Nov; 26 (11), pp. 1643–5.

Opioid Sigma$_1$-Receptor Complexes: Central Nervous System (CNS) Kinetics *[^{3}H]-Pentazocine/[^{11}C]-SA4503/±Pentazocine/Haloperidol*

Cellular/Molecular Mechanism of Action: The agents ±pentazocine and SA4503 have a high and selective affinity for opioid sigma$_1$-receptor complexes. Haloperidol is a sigma-receptor ligand. Pentazocine is a benzomorphan derivative.

Application: Comparison of brain kinetics for sigma$_1$ receptors. The sigma receptor has been speculated to be involved in several diseases affecting the CNS. It is expressed within the endocrine, immune, and other peripheral organ systems, in addition to a variety of human tumor cell types.

Implementation: Characterization of the biodistribution of sigma$_1$ receptors within the brain.

Animal Model: Murine (mouse brain).

Interpretation and Analysis: Murine brain uptake of [^{11}C]-SA4503 is high after intravenous injection followed by a gradual decrease, whereas that of [^{3}H] ±pentazocine rapidly decreases. The brain uptake of the 2 radioligands is dose-dependent reduced, but the reduction of [^{3}H] ±pentazocine is observed at higher doses. Percentage of saturable [^{3}H] ±pentazocine binding is much lower than that of [^{11}C]-SA4503. The brain uptake of [^{3}H] ±pentazocine is substantially reduced by SA4503 at a dose of 2 μmol/kg, while that of [^{11}C]-SA4503 is blocked by ±pentazocine at a dose of equal to or greater than 20 μmol/kg. Exposure of mice to cyclosporin-A (P-glycoprotein modulator) enhances [^{3}H] ±pentazocine uptake but not [^{11}C]-SA4503. Under control and P-glycoprotein-modulated conditions, brain uptake of [^{3}H] ±pentazocine and [^{11}C]-SA4503 is reduced by haloperidol (representative sigma-receptor ligand). Modulation of P-glycoprotein therefore results in different brain kinetic patterns for [^{3}H] ±pentazocine and [^{11}C]-SA4503, and the latter agent is capable of functioning as a suitable *in vivo* probe.

Instrumentation: Single photon emission computed tomography (SPECT) imaging modalities.

Different brain kinetics of 2 sigma$_1$-receptor ligands, [3H] ±pentazocine and [11C]-SA4503, by P-glycoprotein modulation. Kawamura K, Kobayashi T, Matsuno K, Ishiwata K. Synapse 2003 May; 48 (2), pp. 80–6.

The sigma receptor might be involved in several diseases in the central nervous system. It occurs in the endocrine, immune, and other peripheral organ systems and is expressed in a variety of human tumors. Elsinga PH, Kawamura K, Kobayashi T, Tsukada H, Senda M, Vaalburg W, Ishiwata K. Synapse 2002 Mar 15; 43 (4), pp. 259–67.

Opioid Sigma$_1$-Receptor Complexes (Central Nervous System [CNS])
[^{11}C]-SA4503/[^{11}C]1-([4-Methoxy-^{11}C]-3,4-Dimethoxyphenethyl)-4-(3-Phenylpropyl)Piperazine/[^{11}C]-SA5845/1-([4-Methoxy-^{11}C]3,4-Dimethoxyphenethyl)-4-[3-(4-Fluorophenyl) Propyl]piperazine/[^{11}C]-Ethoxy and [^{18}F]-Fluoroethoxy Analogs

Cellular/Molecular Mechanism of Action: Selectively recognize and physically bind to opioid sigma$_1$-receptor complexes.
Application: Detection and characterization of sigma$_1$-receptor complexes utilizing positron emission tomography (PET) imaging modalities.
Semi-Synthetic Methods: Preparation of 1-([4-methoxy-^{11}C]-3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine ([^{11}C]-SA4 503) and its fluorinated analogue 1-([4-methoxy-[^{11}C]3,4-dimethoxyphenethyl)-4-[3-(4-fluorophenyl)propyl]piperazine, ([^{11}C]-SA 5845) and their [^{11}C]-ethoxy and [^{18}F]-fluoroethoxy analogs.
Implementation: Evaluation of [^{11}C]-SA4503 and its fluorinated analogue [^{11}C]-SA5845 and their [^{11}C]-ethoxy and [^{18}F]-fluoroethoxy analogs as potential agents for PET imaging.
Interpretation and Analysis: [^{11}C]-SA4503 is most selective for sigma$_1$ receptors, while the other 5 radioligands show affinities for sigma$_1$ and sigma$_2$ receptors to a variable extent. All radioligands show receptor-specific binding in the brain and visualize similar regional brain distribution patterns when evaluated by *ex vivo* autoradiography. The [^{11}C]-ethoxy analogs are relatively labile due to their vulnerability to metabolic degradation.

Synthesis and evaluation of ^{11}C- and ^{18}F-labeled 1-[2-(4-alkoxy-3-methoxyphenyl)ethyl]-4-(3-phenylpropyl)piperazines as sigma-receptor ligands for positron emission tomography studies. Kawamura K, Elsinga PH, Kobayashi T, Ishii S, Wang WF, Matsuno K, Vaalburg W, Ishiwata K. Nucl Med Biol 2003 Apr; 30 (3), pp. 273–84.

Opioid Sigma$_1$-Receptor Complexes (Central Nervous System [CNS])
Pentazocine/SA4503/Haloperidol/Cyclosporin A

Molecular/Cellular Mechanism of Action: Pentazocine and SA4503 have a high and selective affinity for opioid sigma$_1$-receptor complexes. Haloperidol also functions as a sigma receptor ligand. Cyclosporin A can function as a P-glycoprotein modulator.
Implementation: Comparison of the brain kinetics of radiolabeled ±pentazocine and SA4503.
Animal Model: Murine.
Interpretation and Analysis: Brain uptake of [^{11}C]-SA4503 is high after intravenous injection followed by a gradual decrease, whereas that of [^{3}H] ±pentazocine rapidly decreases. The brain uptake of the 2 radioligands is dose-dependently reduced, but the reduction of [^{3}H] ±pentazocine is recognized at higher doses. Percentages of the saturable binding of [^{3}H] ±pentazocine is much lower than that of [^{11}C]-SA4503. The brain uptake of [^{3}H] ±pentazocine is blocked to a great extent by SA4503 at a dose of 2 μmol/kg, while that of [^{11}C]-SA4503 is blocked by ±pentazocine at a dose of 20 μmol/kg or greater. In the presence of cyclosporin A, the uptake of [^{3}H] ±pentazocine is enhanced, but [^{11}C]-SA4503 uptake is not. Under control and P-glycoprotein-modulated conditions, the brain uptake of both radioligands is reduced by haloperidol to a different extent. Therefore, P-glycoprotein modulation results in the different brain kinetics of the 2 radioligands. The radiolabeled SA4503 is suitable as an *in vivo* probe, but radiolabeled ±pentazocine is not.

Different brain kinetics of 2 sigma$_1$-receptor ligands, [3H]±pentazocine and [11C]-SA4503, by P-glycoprotein modulation. Kawamura K, Kobayashi T, Matsuno K, Ishiwata K. Synapse 2003 May; 48 (2), pp. 80–6.

Parkinson's Disease (PD)
[^{18}F]-Fluoro-Deoxy-Glucose (FDG)

Cellular/Molecular Mechanism of Action: [^{18}F]-FDG is an analogue of glucose that can be used as a metabolic probe agent.
Background Information: In previous studies, [^{18}F]-FDG and PET have been utilized to quantify regional metabolic abnormalities in PD. The disease state is characterized reproducibly by a specific abnormal PD-related pattern (PDRP).
Application: Assessment of the effects of different stimuli and pharmaceutical agents (e.g., levodopa) on resting-state brain metabolism in PD conditions.
Implementation: Quantify the effects of dopamine replacement induced by IV levodopa infusion on regional metabolism and PDRP network activity. The hypothesis guiding such investigations proposes that clinical response to dopaminergic therapy correlates with such changes in metabolism.

In this context, FDG PET can be used to measure resting-state regional brain metabolism in conditions of PD whereby subjects are scanned both off levodopa and during an individually titrated constant-rate IV levodopa infusion. Statistical parametric mapping can then be used to identify significant changes in regional brain metabolism that occur with this form of intervention. Quantification of levodopa-induced changes in PDRP expression are also established.

Human Model: Cases with PD (n = 7; age, 59.4 ± 4.2 years; Hoehn and Yahr stage, 1.9 ± 0.7, mean ± standard deviation).
Interpretation and Analysis: Metabolic changes with levodopa correlate with clinical improvement as measured by changes in Unified PD Rating Scale (UPDRS) motor scores. Levodopa infusion improves UPDRS motor ratings (30.6% ± 12.0%, $p < 0.002$) and significantly decreases regional glucose metabolism in the left putamen, right thalamus, bilateral cerebellum, and left primary motor cortex ($p < 0.001$). Changes in pallidal metabolism correlate significantly with clinical improvement in UPDRS motor ratings ($p < 0.01$). Levodopa infusion also results in a significant ($p = 0.01$) decline in PDRP expression. The changes in PDRP activity mediated by levodopa correlate significantly with clinical improvement in UPDRS motor ratings ($r = -0.78$, $p < 0.04$).

Levodopa reduces brain metabolism in the putamen, thalamus, and cerebellum in PD patients. Additionally, levodopa reduces PD-related pattern activity, and the degree of network suppression correlates with clinical improvement. The response to dopaminergic therapy in patients with PD may be determined by modulation of cortico-striato-pallido-thalamocortical pathways.

Metabolic correlates of levodopa response in Parkinson's disease. Feigin A, Fukuda M, Dhawan V, Przedborski S, Jackson-Lewis V, Mentis MJ, Moeller JR, Eidelberg D. Neurology 2001 Dec 11; 57 (11), pp. 2083–8.

Parkinson's Disease/Adenosine A(2A) Receptor Selective Antagonist
[^{14}C]-KW-6002

Cellular/Molecular Mechanism of Action: The compound KW-6002 or [(E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydro-[^{1}H]-purine-2,6-dione] recognizes and selectively binds to adenosine A(2A) receptor complexes.
Physiological Mechanism of Action: The agent KW-6002 [(E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydro-[^{1}H]-purine-2,6-dione] functions as a selective adenosine A(2A) receptor

antagonist. KW-6002 also modulates the activity of striatopallidal enkephalin-containing neurons but not striatonigral substance P-containing neurons.

Application: Potential template for diagnostic imaging or platform for diagnostic pharmacology based on the capacity of KW-6002 to exert antiparkinsonian activity in rodent and primate models. In this context, orally administered KW-6002 is distributed selectively to the striatum.

Route of Administration: KW-6002 or [(E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydro-[^{1}H]-purine-2,6-dione] can be given per os (orally).

Dosage: KW-6002 or [(E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydro-[^{1}H]-purine-2,6-dione] administered at 0.1 to 0.3 mg/kg BW.

Implementation: Evaluation of the distribution of [^{14}C]-KW-6002 within the forebrain after administration of pharmacologically effective doses. In addition, the influence of [^{14}C]-KW-6002 can be monitored to determine its effects on preproenkephalin (PPE) and preprotachykinin (PPT) gene expression in rat striatum.

Animal Model: Rat.

Interpretation and Analysis: Highest levels of radioactivity are observed in the striatum after oral administration of [^{14}C]-KW-6002, and at 30 minutes after 0.1 and 0.3 mg/kg, the density values in the striatum are 2.45 and 2.43 times higher than those in reference regions (e.g., frontal cortex), respectively. At doses of 3 mg/kg (per os), the ratio is only 1.58 and the compound is distributed more extensively within the brain. The distribution pattern and intensity of radioactivity are maintained even 90 minutes after the administration of [^{14}C]-KW-6002. Oral administration of KW-6002 (0.3 and 3 mg/kg/day) to rats for 14 days reverses increased gene expression of PPE in striatum depleted of dopamine by prior treatment with 6-hydroxydopamine (6-OHDA). Alternatively, KW-6002 does not alter decreased PPT gene expression in 6-OHDA-treated rats.

Distribution of adenosine A(2A) receptor antagonist KW-6002 and its effect on gene expression in the rat brain. Aoyama S, Koga K, Mori A, Miyaji H, Sekine S, Kase H, Uchimura T, Kobayashi H, Kuwana Y. Brain Res 2002 Oct 25; 953 (1–2), pp. 119–25.

Parkinson's Disease (PD)/Dopaminergic Degeneration (Progressive Nigrostriatal)/ Biomarker System for Therapeutic Trials
[^{123}I]β-CIT

Background Information: [^{123}I]β-CIT and single photon emission computed tomography (SPECT) imaging of the dopamine transporter is a sensitive biomarker of PD onset and severity.

Application: In this study, the authors examine the change in [^{123}I]β-CIT uptake in sequential SPECT scans to assess the rate of progression of the dopaminergic terminal loss in patients with PD.

Human Model: Cases of PD (n = 32) and healthy controls (n = 24) recruited from the Yale Movement Disorders Center and subjected to repeat [^{123}I]β-CIT SPECT imaging during a 1- to 4-year period. The primary imaging outcome is categorized as the ratio of specific to non-displaceable striatal activity. Disease severity can be assessed by Hoehn and Yahr staging, and Unified PD Rating Scale after being 12 hours off drug.

Interpretation and Analysis: Sequential SPECT scans in PD subjects demonstrate a decline in [^{123}I]β-CIT striatal uptake of approximately 11.2%/year from the baseline scan, compared with 0.8%/year in healthy controls ($p < 0.001$). Although [^{123}I]β-CIT striatal uptake in PD subjects correlates with clinical severity, the annual percentage loss of [^{123}I]β-CIT striatal uptake does not correlate with the annual loss in measures of clinical function.

The rate of dopaminergic loss in PD is significantly greater than that of healthy controls, and [^{123}I]β-CIT SPECT imaging provides a quantitative biomarker for progressive nigrostriatal dopaminergic degeneration in PD. As new protective and restorative therapies for PD are developed, dopamine transporter imaging offers the potential to provide an objective end point for these therapeutic trials.

[123I]β-CIT SPECT imaging assessment of the rate of Parkinson's disease progression. Marek K, Innis R, van Dyck C, Fussell B, Early M, Eberly S, Oakes D, Seibyl J. Neurology 2001 Dec 11; 57 (11), pp. 2089–94.

Parkinson's Disease (PD)/Dopaminergic Neurotransporter Systems/Dopamine D_2 Receptors
[^{123}I]-Iodobenzamide (IBZM)/[^{123}I]-2-β-Carbomethoxy-3-β-(4-Iodophenyl)Tropane (β-CIT)

Cellular/Molecular Mechanism of Action: [^{123}I]-2-β-carbomethoxy-3-β-(4-iodophenyl)tropane (β-CIT) functions as a dopamine transporter (DAT) ligand, while [^{123}I]-iodobenzamide (IBZM) is a dopamine D_2 receptor (D_2R) ligand.

Application: Characterization of dopaminergic neurotransmission systems and dopamine receptor with the central nervous system (CNS) as potential diagnostic modalities related to PD and other CNS disorders.

Route of Administration: [^{123}I]-2-β-carbomethoxy-3-β-(4-iodophenyl)tropane (β-CIT) is administered intravenously prior to single photon emission computed tomography (SPECT) image analysis.

Implementation: Evaluation of the biodistribution of [^{123}I]2-β-carbomethoxy-3-β-(4-iodophenyl)tropane and [^{123}I]-iodobenzamide utilizing high-resolution SPECT provides a unique capability for imaging the biodistribution of radiolabeled molecules in small laboratory animals.

Animal Model: Marmoset (brain).

Interpretation and Analysis: Following injection of [^{123}I]β-CIT and [^{123}I]-IBZM, images can be obtained at 240 minutes and 60 minutes, respectively, within the striatum, which is a region of the CNS with a high density of dopaminergic synapses. Furthermore, a significantly low accumulation of [^{123}I]β-CIT in the striatum is observed in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated animals compared to reference controls. Similar accumulations in controls can be observed with deprenyl pretreatment in MPTP-treated animals. However, the striatal accumulation of [^{123}I]-IBZM shows no changes among controls, MPTP-treated, and deprenyl-MPTP-treated groups. Results from SPECT images agree well with those of dopamine concentration and motor behavior. Because MPTP destroys nigrostriatal dopamine nerves and produces irreversible neurodegeneration associated with Parkinsonian syndrome, SPECT imaging data demonstrates that deprenyl shows its neuroprotective effect on Parkinsonism by protecting against the destruction of presynaptic dopamine neurons.

In vivo imaging of brain dopaminergic neurotransmission system in small animals with high-resolution single photon emission computed tomography. Saji H, Iida Y, Kawashima H, Ogawa M, Kitamura Y, Mukai T, Shimazu S, Yoneda F. Anal Sci 2003 Jan; 19 (1), pp. 67–71.

Parkinson's and Attention Deficit Hyperactivity Disorder (ADHD)/Typical Early Parkinson's Disease (PD) Detection
[^{123}I]-Altropane Single Photon Emission Computed Tomography (SPECT)/[^{18}F]-6-Flourodopa Positron Emission Tomography (PET)

Cellular/Molecular Mechanism of Action: Altropane is an iodinated form of the *N*-allyl analogue of WIN 35,428, which acts as a dopamine transport inhibitor.

Background Information: Altropane is currently in phase III clinical trials for PD and phase II clinical trials for ADHD.

Application: Altropane can be utilized as a potential radio-imaging agent in SPECT based modalities, for the early diagnosis of PD and ADHD.

Considerations: Positron emission tomography cameras are expensive and scarce, and the tests are non-reimbursable. A less costly and more available test such as SPECT may be helpful in the diagnosis of early or atypical PD if its sensitivity is comparable to PET scans.

Implementation: Evaluation of the biodistribution of [^{123}I]-altropane (SPECT) and [^{18}F]-6-flourodopa (PET) in cases of early PD with the aid of SPECT imaging.

Human Model: Cases of typical early PD (n = 2, ages 54 and 38 years) with imaging modalities performed within a 3-month period of time. Each case has a 1-year history of intermittent right hand tremor with right arm rigidity and hypokinesia and bradykinesia on fine finger movements. A significant and sustained improvement in Parkinsonian symptoms is noted using a dopamine agonist.

Interpretation and Analysis: Single photon emission computed tomography and PET scans show non-diagnostic fluorodopa PET scans with clear unilateral striatal reduction of tracer uptake contralateral to their Parkinsonian side on altropane SPECT scans. Therefore, when radiolabeled with the gamma emitting isotope [^{123}I], altropane serves as a SPECT ligand with high affinity and selectivity for dopamine transporter complexes. In this manner, it is a good marker for dopamine neurons and is useful for detecting the early stages of PD conditions. Further controlled studies are needed to define its potential role in detecting presymptomatic, early and atypical PD cases.

Instrumentation: Positron emission tomography studies can be performed using a PC-4096 scanner (Scanditronix AB, Sweden) with 15 axial slices and resolution of 6 mm FWHM. The SPECT acquisitions are performed on a MultiSPECT gamma camera (Siemens, Hoffman Estates, IL) equipped with fan-beam collimators with an intrinsic resolution of 4.6 FWHM (for Case 1) and a DSI Ceraspect camera with an annular crystal, with resolution of approximately 6.4 mm FWHM (for Case 2).

Altropane (Boston Life Science). Cattabeni F. Curr Opin Investig Drugs 2002 Nov; 3 (11), pp. 1647–51.

Is altropane SPECT more sensitive to fluorodopa PET for detecting early Parkinson's disease? Fernandez HH, Friedman JH, Fischman AJ, Noto RB, Lannon MC. Med Sci Monit 2001 Nov–Dec; 7 (6), pp. 1339–43.

Parkinson's Disease (PD)/Differentiation from Orthostatic Tremors (OTs) *[^{123}I]-2 β-Carbomethoxy-3β-(-4-Iodophenyl)-N-(3-Fluoropropyl)-Nortropane) ([^{123}I]-FP-CIT)/L-Dopa*

Cellular/Molecular Mechanism of Action: [^{123}I]-FP-CIT functions as a dopamine transporter tracer.

Background Information: There is increasing evidence of a potential role of the dopaminergic system in OT; correlations with Parkinsonism and treatment effects of L-dopa and dopamine agonists have been reported.

Application: Characterization of dopamine transporter function and expression in conditions including OT.

Implementation: Evaluation of cases affected by OT using the dopamine transporter tracer [^{123}I]-FP-CIT as dopamine transporter tracer in conjunction with single photon emission computed tomography (SPECT).

Interpretation and Analysis: A marked reduction in striatal tracer binding can be appreciated in cases of OT compared to normal controls ($p < 0.001$; n = 12 vs 12). Tracer uptake, however, is significantly higher and more symmetrical than in PD, and the caudate and putamen structures are equally affected. Challenges with L-dopa in OT cases (e.g., n = 7) reveal a small but non-significant improvement on electromyography (EMG) and a small but significant improvement in clinical parameters (blinded video rating). Two-month open-label L-dopa treatment (600 mg/day) produces a small improvement in approximately 40% (e.g., n = 2/5) of cases but no significant overall change can be appreciated. Olfactory function using the University of Pennsylvania Smell Identification Test is usually normal in most cases. The detection of marked reductions in tracer uptake on dopamine transporter SPECT therefore supports the concept of the probable role the dopaminergic system has in molecular pathogenesis of OT. Lack of evidence of a clinically relevant therapeutic response to L-dopa suggests that other mechanisms must also be involved in this disease process.

[^{123}I]-FP-CIT-SPECT demonstrates dopaminergic deficit in orthostatic tremor. Katzenschlager R, Costa D, Gerschlager W, O'Sullivan J, Zijlmans J, Gacinovic S, Pirker W, Wills A, Bhatia K, Lees AJ, Brown P. Ann Neurol 2003 Apr; 53 (4), pp. 489–96.

Parkinson's Disease (PD): Dopamine (D_2) Receptors *[^{11}C]/[^{123}I]-Epidepride/[^{11}C]/[^{18}F]-Fallypride/[^{11}C]/[^{125}I]-Nalipride [^{76}Br]-Isoremoxipride (FLB-457)/[^{125}I]-Ioxipride/Sulpiride and Remoxipride/Raclopride/Eticlopride*

Cellular/Molecular Mechanism of Action: Epidepride, fallypride, and isoremoxipride are members of a class of high-affinity dopamine D_2 receptor ligands.

Background Information: Starting in the late 1970s, halogenated analogs of (S)-sulpiride were evaluated in binding assays and behavioral studies, leading to the discovery of remoxipride. Remoxipride is 10 times weaker than sulpiride *in vitro* but 50 times more potent *in vivo*. The search for a putative active metabolite of remoxipride led to the discovery of raclopride and eticlopride, the former becoming a useful radioligand as tritium or carbon-11 labeled form for receptor binding and positron emission tomography (PET) studies, respectively. In the United States, the mono-iodine analogue of raclopride, [^{123}I]-iodobenzamide (IBZM), was found to have moderate putamen-to-cerebellum ratio in rat and human brain. Continued search for metabolites of remoxipride led to the discovery of its 3,6-dihydroxy derivative, NCQ-344, with an extremely potent *in vivo* activity in the rat. Structure-activity-relationship (SAR) studies of the metabolites of remoxipride led to the discovery of the 3-methoxy isomer, isoremoxipride (FLB-457), and its corresponding 6-hydroxy analog, FLB-463, both having affinities for the dopamine D_2 receptor in the 20–30 pM range. Later, the 5-[^{123}I]-iodo analogue of FLB-463, [^{123}I]-ioxipride ([^{123}I]-NCQ-298), became a potential single photon emission computed tomography (SPECT) imaging agent. In the meantime, the deshydroxy analogue of IBZM, [^{125}I]-iodopride, showed binding potential in the rat similar to [^{125}I]-IBZM. Epidepride was designed by combining the structure of isoremoxipride with that of iodopride. In 1988, epidepride was independently prepared and radiolabeled in 3 separate laboratories in Stockholm, Berkeley, and Nashville. Evaluation of 7 [^{125}I]-iodine substituted analogs of raclopride, including IBZM, revealed the unusual high striatum-to-cerebellum ratio of 234 of [^{125}I]-epidepride in the rat. Subsequent SPECT images with [^{123}I]-epidepride demonstrated its ability to identify extrastriatal dopamine D_2 receptors in the human brain. Exploration of the structure of epidepride confirmed its exceptional properties, to be exceeded only by its *N*-allyl homolog, [^{125}I]-nalepride. The design by other investigators of a series of potent 5-(3-[^{18}F]-fluoropropyl) substituted analogs of epidepride for PET imaging lead to the discovery of [^{18}F]-fallypride. By elucidating the role of lipophilicity in the substituted benzamides, the excellent

imaging characteristics of [^{11}C]/[^{123}I]-epidepride, [^{11}C]/[^{76}Br]-isoremoxipride, and [^{18}F]-fallypride could not only be explained but predicted with remarkable accuracy. By using the inverse product of receptor affinity (K_D), and the apparent partition constant of the radioligand ($P_{7.4}$), estimates of maximal binding potential of any radioligand for imaging of any neurotransmitter receptor or transporter site seems possible.

Application: [^{11}C]/[^{123}I]-epidepride, [^{11}C]/[^{18}F]-fallypride, and [^{11}C]/[^{76}Br]-isoremoxipride (FLB-457) can be utilized as high-affinity radioligands for SPECT or PET imaging of dopamine D_2 receptors in the human brain. In contrast to previously used imaging agents, [^{11}C]/[^{123}I]-epidepride, [^{11}C]/[^{18}F]-fallypride, and [^{11}C]/[^{76}Br]-isoremoxipride (FLB-457) are capable of identifying extrastriatal dopamine D_2 receptors.

Considerations: The design of substituted benzamides derive their origin from the atypical antipsychotic agent remoxipride.

The discovery of epidepride and its analogs as high-affinity radioligands for imaging extrastriatal dopamine D_2 receptors in human brain. de Paulis T. Curr Pharm Des 2003; 9 (8), pp. 673–96.

Parkinson's Disease (PD)/Dopamine Receptors
[^{18}F]-Dopa

Background Information: Advances in our understanding of the cause and pathogenesis of PD have permitted the rational selection of putative neuroprotective agents for study in PD. However, the list of agents that might provide neuroprotective effects derived from laboratory studies is daunting, and determining which agents to bring to the clinic and how to find the resources (patients and funds) to properly study so many promising therapeutic opportunities is a challenge. Appropriate outcome variables that are not confounded by any symptomatic effect of the drug and are acceptable to clinicians and regulatory authorities also remain to be defined. The 1st clinical trials designed to test the capacity of putative neuroprotective agents to alter the natural history of PD have now been performed and illustrate some of these problems. The DATATOP (Deprenyl and Tocopherol Antioxidant Therapy of PD) study used the time to reach a disease milestone in untreated PD patients (i.e., need for levodopa) as the primary end point. However, interpretation of results was confounded by the drug's symptomatic effect. The SINDEPAR (Sinemet-Deprenyl-Parlodel) study used the change in motor score between initial visit and final visit after washout of all study medications as the primary end point. However, here too there were concerns about confounding symptomatic effects, because antiparkinsonian medications have now been shown to have a long duration response that can persist for weeks and perhaps even months after withdrawal.

To date, no drug has been established to have a neuroprotective effect in PD, and none has been approved for a neuroprotective indication. Furthermore, regulatory agencies have not yet agreed that any of the outcome measures currently used will be acceptable for approval of a new drug. Resolution of these issues is of critical importance to convince pharmaceutical companies to expend the hundreds of millions of dollars necessary to bring a new drug to market. Drugs that already have been approved in PD for their symptomatic effects, such as dopamine agonists or propargylamines (e.g., selegiline), offer the best opportunity for establishing that a drug is neuroprotective in PD in the immediate future, but herein also lies the difficulty of establishing that any benefits observed are not solely because of the drug's symptomatic properties. Currently, this will most likely entail demonstrating that the drug provides benefit for PD patients for both imaging and clinical markers of disease progression.

Implementation: Integrity of nigrostriatal function can be ascertained with the application of surrogate marker systems, including the evaluation of striatal fluorodopa uptake on positron emission tomography (PET) or β-CIT on single photon emission computerized tomography (SPECT).

Interpretation and Analysis: Characterization of nigrostriatal function with surrogate marker systems involving the evaluation of striatal fluorodopa uptake on PET or β-CIT on SPECT can provide information that can be applied as primary outcome measures. Despite their effectiveness, the interpretation of PET and SPECT image analyses is complicated by the (i) lack of confirmation that striatal uptake of isotope agents does in fact correlate with the remaining number of dopamine neurons or terminals; and the (ii) possible confounding pharmacological effect exerted by diagnostic agents, which has not yet been completely excluded.

Neuroprotection in Parkinson's disease: Clinical trials. Stocchi F, Olanow CW. Ann Neurol 2003; 53 Suppl 3, pp. S87–97; discussion S97–9.

Parkinson's Disease (PD): Dopamine D_2 Receptors/Progression of Neurodegeneration/D_2 Receptor Agonist Response
[^{123}I]β-CIT

Application: [^{123}I]β-CIT single photon emission computed tomography (SPECT) is a useful tool for characterizing the progression of dopaminergic degeneration in PD and may provide an objective method of measuring the effectiveness of neuroprotective treatments.

Implementation: Delineation of the validity of [^{123}I]β-CIT SPECT for monitoring the progression of dopaminergic degeneration in PD and to investigate the influence of short-term treatment with D_2 receptor agonists on striatal [^{123}I]β-CIT binding. To this end, early-stage PD cases (e.g., $n = 50$) are examined involving the acquisition of 2 SPECT imaging series that are obtained 12 months apart. The mean annual change in the ratio of specific to non-specific [^{123}I]β-CIT binding to the striatum, putamen, and caudate nucleus is used as an outcome measure.

Interpretation and Analysis: A decrease in [^{123}I]β-CIT binding ratios between the 2 images is found in all regions of interest. The average decrease in [^{123}I]β-CIT binding ratios is about 8% in the whole striatum, 8% in the putaminal region, and 4% in the caudate region. Comparison of scans (e.g., $n = 9$) under 2 different conditions—in the off state and while on drug treatment—shows no significant alterations in the expression of striatal dopamine transporters as measured using [^{123}I]β-CIT SPECT. Power analysis indicates that to detect a significant ($p < 0.05$) effect of a neuroprotective agent with 0.80 power and 30% of predicted protection within 2 years, 216 cases are required in each group when the effects are measured in the whole putamen. Short-term treatment with a D_2 agonist does not have a significant influence on [^{123}I]β-CIT binding to dopamine transporters. If the latter finding is replicated in larger groups of patients, it supports the suitability of [^{123}I]β-CIT SPECT for examining the progression of neurodegeneration in patients being treated with D_2 receptor agonists.

[^{123}I]β-CIT SPECT is a useful method for monitoring dopaminergic degeneration in early stage Parkinson's disease. Winogrodzka A, Bergmans P, Booij J, van Royen EA, Stoof JC, Wolters EC. J Neurol Neurosurg Psychiatry 2003 Mar; 74 (3), pp. 294–8.

Parkinson's Disease (PD): Dopamine Transporter (DAT)/Attention Deficit Hyperactivity Disorder (ADHD)
[^{99m}Technetium] ([^{99m}Tc]) Tropane Ligands: 2nd Generation

Cellular/Molecular Mechanism of Action: Recognize and physically binds to DAT complexes located presynaptically on dopamine neurons.

Physiological Mechanism of Action: In ADHD, DAT density levels are elevated, while in PD the levels become depleted. The depletion of DAT levels also corresponds with the loss of dopamine.
Background Information: An additional feature of PD is a depletion of dopaminergic neurons within the substantia nigra accompanied by development of fibrillar Lewy bodies comprised primarily of α-synuclein.
Application: The DAT complex can function as a marker for PD and ADHD.
Interpretation and Analysis: The agent (*N*-[(2-((3′-*N*′-propyl-(1′′R)-3′′α-(4-fluorophenyl)tropane-2′′beta-1-propanoyl) (2-mercaptoethyl)amino)acetyl)-2-aminoethanethiolato]technetium (V)oxide) possesses improved selectivity and biological stability, allowing sufficient amounts of the agent to enter the brain and *in vivo* label DAT complexes sufficient to provide quantitative measurement of DAT density (non-human primates).

A 2nd-generation 99m technetium single photon emission computed tomography agent that provides *in vivo* images of the dopamine transporter in primate brain. Meltzer PC, Blundell P, Zona T, Yang L, Huang H, Bonab AA, Livni E, Fischman A, Madras BK. J Med Chem 2003 Jul 31; 46 (16), pp. 3483–96.

Parkinson's Disease (PD): Dopamine Transporter (DAT)
[^{99m}Technetium] ([^{99m}Tc])-TRODAT-1/6-[^{18}F]-Fluoro-L-Dopa ([^{18}F]-Fdopa)

Cellular/Molecular Mechanism of Action: ([2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3,2,1]oct-2-yl]methyl](2-mercaptoethyl)amino]ethyl]amino]ethanethiolato(3-)-N2,N2′,S2,S2′] oxo-[1R-(exo-exo)]), also referred to as TRODAT-1, is a potential agent that recognizes DAT complexes.
Application: [^{99m}Tc]-TRODAT-1 is used to detect and characterize DAT complexes when utilized in combination with single photon emission computed tomography (SPECT) imaging modalities. In contrast, [^{18}F]-Fdopa positron emission tomography (PET) is used for the quantitative assessment of presynaptic nigrostriatal dopaminergic function.
Route of Administration: [^{18}F]-Fdopa (intravascular).
Dosage: [^{18}F]-Fdopa (300 MBq).
Implementation: Evaluation of PD utilizing comparative image scans obtained with [^{18}F]-Fdopa and [^{99m}Tc]-TRODAT-1 functioning as *in vivo* molecular probes. Scans are performed on 2 separate occasions. In addition to visual inspection, specific uptake ratios (SURs) of [^{99m}Tc]-TRODAT-1 in the striatum and putamen are measured bilaterally. For PET, test cases receive carbidoma (100 mg), 90 minutes before [^{18}F]-Fdopa (300 MBq) injection. Images are acquired between 120 and 150 minutes after injection, using a whole-body PET scanner with settings identical to those for the SPECT studies. The SURs for PET are calculated similar to those for SPECT. Individual SURs of the striatum or putamen from SPECT are then correlated with the corresponding PET values using linear regression.
Human Model: Confirmed cases of PD (e.g., n = 20).
Interpretation and Analysis: A consistent image pattern between SPECT and PET is achieved by visual inspection except in approximately 15% of cases (e.g., n = 3/20). In a small percentage of cases (e.g., n = 1/20 with Hoehn and Yahr Scale I PD), the SPECT images are more compatible with the patient's clinical findings, whereas PET shows nearly normal uptake. A small percetage of other cases (e.g., n = 2/20, both cases with Hoehn and Yahr Scale II PD), have PET findings that correlate better with the clinical findings. The caudate and putamen nuclei are more discernable with PET imaging. An acceptable correlation of SUR, however, can be appreciated between SPECT and PET in both the striatum and the putamen ($p < 0.01$ for both). The comparability of [^{99m}Tc]-TRODAT-1 SPECT and [^{18}F]-Fdopa PET suggests that [^{99m}Tc]-TRODAT-1 SPECT may provide a reliable alternative to [^{18}F]-Fdopa PET in the evaluation of clinical PD cases.

Crossover study of [^{99m}Tc]-TRODAT-1 SPECT and [^{18}F]-Fdopa PET in Parkinson's disease patients. Huang WS, Chiang YH, Lin JC, Chou YH, Cheng CY, Liu RS. J Nucl Med 2003 Jul; 44 (7), pp. 999–1005.

Prion Plaques/Creutzfeldt-Jacob Disease (Sporadic and Variant CJD)/Gerstmann-Straussler-Scheinker Disease/Alzheimer's Disease (AD)
2-(1-[6-[(2-Fluoroethyl)(Methyl)Amino]-2-Naphthyl] Ethylidene) Malononitrile (FDDNP)/[^{18}F]-FDDNP

Cellular/Molecular Mechanism of Action: The agent FDDNP is a molecule that both emits fluorescent light energy and recognizes/physically binds to (i) plaques created in the central nervous system (CNS) by prion agents; and (ii) senile plaques and neurofibrillary tangles associated with AD.
Background Information: The fluorescent molecular-imaging probe FDDNP is a highly hydrophobic, viscosity-sensitive, solvent-sensitive, fluorescent substance whose radiofluorinated analogue [^{18}F]-FDDNP has recently been successfully used to label senile plaques and neurofibrillary tangles in the living brain of AD patients with positron emission tomography (PET).
Implementation: Evaluate the fluorescent molecular-imaging probe FDDNP for its ability to selectively and reproducibly label prion plaques in fixed, paraffin-embedded cerebellar sections from patients of confirmed Gerstmann-Straussler-Scheinker disease, sporadic CJD with kuru plaques, and variant CJD (vCJD).
Interpretation and Analysis: The agent FDDNP reliably identifies all prion plaques, including small cluster plaques in vCJD. This finding may open alternative *in vivo* diagnostic possibilities for vCJD.

Molecular-imaging probe 2-(1-[6-[(2-fluoroethyl)(methyl) amino]-2-naphthyl]ethylidene) malononitrile labels prion plaques *in vitro*. Bresjanac M, Smid LM, Vovko TD, Petric A, Barrio JR, Popovic M. J Neurosci. 2003 Sep 3; 23(22), pp. 8029–33.

Seizures and Behavior/Paroxysmal Kinesigenic Choreoathetosis/Dyskinetic Movement Attacks/ Choreoathetosis Attack (Impending): Associated Indicators/Contralateral Basal Ganglia Hyperactivity and Perfusion/Ictal Phase Imaging
[^{99m}Technetium] ([^{99m}Tc])-Ethyl Cysteinate Dimer

Background Information: Paroxysmal kinesigenic choreoathetosis is a rare neurologic disorder characterized by sudden attacks of brief involuntary dyskinetic movement that are precipitated by voluntary movement.
Application: Detection and characterization of contralateral basal ganglia perfusion (ictal phase imaging) in conditions of paroxysmal kinesigenic choreoathetosis.
Implementation: Evaluation of hemidystonia using [^{99m}Tc]-ethyl cysteinate dimer and ictal single photon emission computed tomography (SPECT).
Human Model: A case of hemidystonia (n = 1; 14-year-old male with frequent brief attacks triggered by sudden movement). Note: Normal findings from video electroencephalogram and magnetic resonance imaging.
Interpretation and Analysis: Ictal SPECT using [^{99m}Tc] ethyl cysteinate dimer can detect increases in the perfusion of the contralateral basal ganglia associated with the onset of choreoathetosis

attacks. Hyperactivity of the basal ganglia is associated with the dyskinetic attacks in paroxysmal kinesigenic choreoathetosis.

Ictal [99mTc] ECD SPECT in paroxysmal kinesigenic choreoathetosis. Ko CH, Kong CK, Ngai WT, Ma KM. Pediatr Neurol 2001 Mar; 24 (3), pp. 225–7.

Seizures/Epilepsy: Drug-Resistant Focal Type/ Accuracy of Amobarbital Target Delivery/ Temporary Neurologic Deficits/Risk Assessment of Postsurgical Motor Deficits Before Functional Hemispherectomy/Language Area Identification

Amobarbital (Sodium) Wada Test/[^{99m}Technetium] ([^{99m}Tc])-Hexamethyl-Propylene-Amine Oxime (HMPAO)/Electrical Stimulation Mapping

Cellular/Molecular Mechanism of Action: Amobarbital is a barbiturate.

Physiological Mechanism of Action: Amobarbital suppresses neurologic function.

Application: Analyze the role of selective middle cerebral artery (MCA) Wada tests in the presurgical workup of patients with drug-resistant focal epilepsies.

Route of Administration: Amobarbital is administered selectively into arterial branches supplying specific regions of the cerebral cortex using a catheter introduced into the carotid artery.

Implementation: Evaluation of cerebral cortex function in conditions of (i) motor cortex cerebral hemisphere lesions (connatal MCA infarct; unilateral cortical dysplasia; Rasmussen encephalitis); (ii) circumscribed neoplastic or non-neoplastic lesions adjacent to the motor cortex or classical regions; and (iii) sleep electrical status elepticus (ESES). Sodium amobarbital and [^{99m}Tc]-HMPAO are simultaneously injected through a microcatheter into the distal M1 segment, or the inferior MCA trunk, or into MCA branches in order to identify eloquent cortex.

Human Model: Cases of cerebral cortex dysfunction, including (i) hemispheric lesions involving the motor cortex (n = 9 cases, of which n = 5/9 have connatal MCA infarcts, n = 3/9 have unilateral cortical dysplasia, n = 1/9 Rasmussen encephalitis); (ii) circumscribed neoplastic or non-neoplastic lesions adjacent to the motor cortex or classic language areas (n = 5 cases); and (iii) purely electrophysiologic analyses of ESES conditions (n = 2 cases). Total Wada tests performed (n = 20).

Interpretation and Analysis: Co-registered single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI; n = 18) reveals that sodium amobarbital does not reach the target area in some procedures (e.g., n = 3/20). Temporary neurologic deficits can occur in slightly more than half of all Wada procedures performed (e.g., n = 12/20). Cases subjected to surgical intervention require the use of several different surgical approaches, including functional hemispherectomy (n = 3); partial or extended lesionectomy (n = 4); anterior temporal lobectomy (n = 1); and multiple subpial trans-sections (n = 3). Seizure freedom (Engel class I) can be achieved in a modest percentage of cases (e.g., n = 5/16 cases). The MCA Wada tests can therefore contribute to risk assessment concerning postsurgical motor deficits before functional hemispherectomy. Other indications are less clear. The identification of language areas is targeted primarily by electrical-stimulation mapping after subdural grid implantations, and selective MCA Wada tests in ESES patients have yet to be validated in large patient groups.

Selective middle cerebral artery Wada tests as a part of presurgical evaluation in patients with drug-resistant epilepsies. Urbach H, Von Oertzen J, Klemm E, Koenig R, Linke DB, Kurthen M, Schramm J, Elger CE. Epilepsia 2002 Oct; 43 (10), pp. 1217–23.

Seizures/Epileptic Foci/Psychiatric Disorder Receptor Changes/Parkinson's Disease (PD) Differential Diagnosis/Dopamine D_2/D_3 Receptors/ PD Differential/Neurodegenerative Disease/ Dopamine Transporter/PD/Dopamine Transporter

[^{123}I]-Iomazenil/Benzodiazepine (γ-Aminobutyric Acid) Receptors/[^{123}I]-Iodobenzamide/[^{123}I]-Epidepride/[^{123}I]-CIT (2-β-Carboxymethoxy-3-β(4-Iodophenyl)Tropane)/[^{123}I]-FP-β-CIT (N-Propyl-2-β-Carboxymethoxy-3-β(4-Iodophenyl)-Nortropane/[^{99m}Technetium] ([^{99m}Tc])-TRODAT/[^{99m}Tc]-2-[[2-[[[3-(4-Chlorophenyl)-8-Methyl-8-Azabicyclo [3,2,1]Oct-2-yl]-Methyl](2-Mercaptoethyl)-Amino]-Ethyl]-Amino]-Ethanethiolato(3-)]Oxo-[1R-(Exo-Exo)]-)

Background Information: In the past 10 years, significant progress has been made in the development of new brain-imaging agents for single photon emission computed tomography (SPECT). Most of the new radiopharmaceuticals have been designed to bind specific neurotransmitter receptor or transporter sites in the central nervous system. Most of the site-specific brain radiopharmaceuticals are labeled with [^{123}I]. Results from imaging of benzodiazepine (gamma-aminobutyric acid) receptors by [^{123}I]-iomazenil are useful in identifying epileptic seizure foci and changes of this receptor type in psychiatric disorders. Imaging of dopamine D_2/D_3 receptors (e.g., [^{123}I]-iodobenzamide and [^{123}I]-epidepride) and transporters (e.g., [^{123}I]-CIT (2-β-carboxymethoxy-3-β(4-iodophenyl)tropane) and [^{123}I]-FP-β-CIT (*N*-propyl-2-β-carboxymethoxy-3-β(4 iodophenyl)-nortropane) has proven to be a simple but powerful tool for differential diagnosis of Parkinson's and other neurodegenerative diseases. A [^{99m}Tc]-labeled agent, [^{99m}Tc]-TRODAT (technetium, 2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo [3,2,1]oct-2-yl]methyl]-(2-mercaptoethyl) amino]ethyl]amino] ethanethiolato(3-)]oxo-[1R-(exo-exo)]-), for imaging dopamine transporters in the brain, has been successfully applied in the diagnosis of PD. Despite the fact that [^{123}I] radiopharmaceuticals have been widely used in Japan and in Europe, clinical application of [^{123}I]-labeled brain radiopharmaceuticals in the United States is limited because of difficulties associated with supplying such agents. Development of [^{99m}Tc] agents will likely extend the application of site-specific brain radiopharmaceuticals for routine applications in aiding the diagnosis and monitoring treatments of various neurologic and psychiatric disorders.

Radiopharmaceuticals for single photon emission computed tomography brain imaging. Kung HF, Kung MP, Choi SR. Semin Nucl Med 2003 Jan; 33 (1), pp. 2–13.

Seizures/Epilepsy (Intractable): Wada Test Preoperative Foci Localization/Prediction of Residual Postoperative Deficits/Relationship between Hypoperfusion on Single Photon Emission Computed Tomography (SPECT) and Duration of Hemiplegia/Drug-Induced Delta Electroencephalogram (EEG) Activity/Hexamethyl-Propylene-Amine Oxime (HMPAO)-SPECT Assessment of Methohexital Distribution

Methohexital/[^{99m}Technetium] ([^{99m}Tc])-HMPAO

Physiological Mechanism of Action: Methohexital is a barbiturate that functions as a central nervous system (CNS) depressant.

Application: Methohexital can be applied to accurately localize foci or regions within the CNS responsible for intractable epilepsy conditions. Methohexital can also simultaneously function as a means for predicting the potential (anticipated) severity of postoper-

ative deficits. [^{99m}Tc]-HMPAO can be administered intravenously during the procedure to determine the intracerebral distribution of methohexital while performing the Wada test.

Route of Administration: Methohexital (superselective intracarotic delivery into specific predetermined anatomical regions of the CNS).

Implementation: Evaluation of epileptic conditions being considered for surgery for intractable epilepsy utilizing preoperative evaluation with the selective delivery of methohexital administered through a intracarotid catheter system. The lipophilic brain SPECT agent [^{99m}Tc]-HMPAO is administered intravenously during the procedure to determine the intracerebral distribution of methohexital.

The validity of intracarotid sodium methohexital (ISM) tests depends on consistent delivery of the anesthetizing agent to 1 hemisphere, including mesial structures of the temporal lobe. To prevent postoperative language and memory deficits, correct interpretation of the test results supposes a reliable knowledge of which regions of the brain have been anesthetized. Results of HMPAO-SPECT are compared with clinical and EEG video-monitoring data and with results of digital subtraction angiography (DSA) performed during the test in an effort to establish criteria for determining the level and topographical extent of anesthesia.

Human Model: Cases being considered for surgery for intractable epilepsy (n = 25).

Interpretation and Analysis: In the vast majority of cases, the effect of sodium methohexital (SM) is ipsilateral cerebral hypoperfusion on SPECT and crossed cerebellar diaschisis (e.g., n = 25/25). The distribution of HMPAO varies from patient to patient. The delivery of SM to mesial temporal lobe structures is not constant. Using non-parametric tests, it has been demonstrated that a statistically significant relationship exists between hypoperfusion on SPECT and duration of hemiplegia but not with the duration of aphasia. Hypoperfusion on SPECT is also related to the onset and duration of drug-induced delta activity on EEG. Single photon emission computed tomography reveals a statistically different distribution of SM in the brain from that predicted with DSA, while HMPAO-SPECT can be used for mapping the distribution of methohexital's effects during the ISM Wada test. Several investigations have confirmed that SPECT assessment may be an excellent way of determining the distribution of barbiturate during the examination. It increases confidence in interpreting results of speech and memory testing by detecting either contralateral diffusion of the drug due to crossflow between hemispheres or insufficient quantitative delivery to the homolateral hemisphere.

Monitoring sodium methohexital distribution with [99mTc]-HMPAO with single photon emission computed tomography during Wada test. Coubes P, Baldy-Moulinier M, Zanca M, Boire JY, Child R, Bourbotte G, Frerebeau P. Epilepsia 1995 Oct; 36 (10), pp. 1041–9.

Seizures/Epileptogenic Foci
[^{99m}Technetium] ([^{99m}Tc])-Bicisate Ictal Single Photon Emission Computed Tomography (SPECT)

Background Information: Magnetic resonance imaging (MRI) studies frequently fail to reveal an anatomic origin for the seizures, and scalp electroencephalography is often limited to identification of the involved hemisphere. Functional imaging modalities such as positron emission tomography (PET) and SPECT are more promising tools for this application because they reflect the functional pathology associated with the seizure. These changes are more pronounced ictally, but until recently, no radiopharmaceutical was available that could be used routinely for ictal SPECT.

Application: Nuclear medicine scintigraphic imaging of pediatric cases of refractory epilepsy with [^{99m}Tc]-bicisate. Examples of potential applications include (i) characterization of ictal SPECT in pediatric patients with refractory epilepsy; (ii) comparison of ictal and interictal blood flow patterns in cases of refractory epilepsy; and (iii) function as an effective adjunct diagnostic procedure that complements information obtained from other techniques and methodologies.

Route of Administration: Intravenous.

Implementation: Evaluation of anatomical localization information provided by [^{99m}Tc]-bicisate ictal SPECT compared to parameters acquired by other diagnostic modalities.

Human Model: Cases of pediatric epilepsy (n = 10 at 3 to 19 years of age; mean age 10.9 ± 4.3 years; n = 7 females) all of whom have no lesions detected on MRI scans that could responsible for the seizures.

Interpretation and Analysis: Ictal SPECT reveals the presence of focal perfusion abnormalities in a high percentage of affected cases. By comparison, interictal SPECT imaging (e.g., n = 4) detects regional hypoperfusion that corresponds to regions of hyperperfusion in the ictal studies, and some images (e.g., n = 3) show regional hyperperfusion corresponding to hyperperfused regions in the ictal studies. Some interictal studies (e.g., n = 3) reveal no abnormal perfusion patterns. Scalp electroencephalogram (EEG) can provide localization information in some cases (e.g., n = 5). Such observations suggest that ictal SPECT with [^{99m}Tc]-bicisate is a more promising tool for identifying epileptogenic foci than interictal SPECT or scalp EEG in cases without focal abnormalities on MRI.

Comparative study of 99mTc-ECD and 99mTc-HMPAO for peri-ictal SPECT: Qualitative and quantitative analysis. O'Brien TJ, Brinkmann BH, Mullan BP, So EL, Hauser MF, O'Connor MK, Hung J, Jack CR. J Neurol Neurosurg Psychiatry 1999 Mar; 66 (3), pp. 331–9.

Ictal and interictal brain SPECT imaging in epilepsy using technetium-99m-ECD. Grünwald F, Menzel C, Pavics L, Bauer J, Hufnagel A, Reichmann K, Sakowski R, Elger CE, Biersack HJ. J Nucl Med 1994 Dec; 35 (12), pp. 1896–901.

Ictal and interictal technetium-99m-bicisate brain SPECT in children with refractory epilepsy. Packard AB, Roach PJ, Davis RT, Carmant L, Davis R, Riviello J, Holmes G, Barnes PD, O'Tuama LA, Bjornson B, Treves ST. J Nucl Med 1996 Jul; 37 (7), pp. 1101–6.

Serotonin (5-HT$_{1A}$) Autoreceptors: Dorsal Raphe Nuclei (DRN)/Pandolol Receptor Occupancy
[^{11}C]-WAY 100635

Cellular/Molecular Mechanism of Action: Pandolol and [^{11}C]-WAY 100635 possess binding avidity for membrane-associated serotonin (5-HT$_{1A}$) receptor complexes expressed by certain cell types within the central nervous system.

Background Information: Augmentation of selective serotonin reuptake inhibitor (SSRI) therapy by the 5-HT$_{1A}$ receptor agent pindolol may reduce the delay between initiation of antidepressant treatment and clinical response. The hypothesis is based on the ability of pindolol to block 5-HT$_{1A}$ autoreceptors in the DRN and to potentiate the increase in 5-HT transmission induced by SSRIs. However, placebo-controlled clinical studies of pindolol augmentation of antidepressant therapy have reported inconsistent results.

Application: The agent [^{11}C]-WAY 100635 can be applied to evaluate the differential occupancy levels of membrane-associated somatodendritic and postsynaptic 5-HT$_{1A}$ receptor complexes by established or prototype pharmaceutical agents (e.g., pidolol).

Implementation: Evaluate the occupancy of 5-HT$_{1A}$ receptors during treatment with controlled release (CR) pindolol detected with [^{11}C]-WAY 100635 using positron emission tomography imaging techniques. Cases are then studied 4 times: at baseline; following 1

week of pindolol CR 7.5 mg/day (4 and 10 hours postdose); and following 1 dose of pindolol CR 30 mg (4 hours postdose).

Human Model: Healthy subjects (n = 9).

Interpretation and Analysis: Occupancy of DRN is approximately 40 ± 29% on scan 2, 38 ± 26%; and 64 ± 15% (scan 2, scan 3, and scan 4, respectively). The average occupancy in all other regions is significantly lower at each dose (18 ± 5%, 12 ± 3%, and 42 ± 4%; scans 2, 3, and 4, respectively). Blockade within the DRN achieved under clinical conditions (7.5 mg/day) may be too low and variable to consistently augment the therapeutic effect of SSRIs. However, these data indicate that pindolol exhibits *in vivo* selectivity for DRN 5-HT_{1A} autoreceptors. As DRN selectivity is desirable for potentiation of 5-HT function, this observation represents an important proof of concept for the development of 5-HT_{1A} agents in this application.

Differential occupancy of somatodendritic and postsynaptic 5-HT_{1A} receptors by pindolol: A dose-occupancy study with [^{11}C]-WAY 100635 and positron emission tomography in humans. Martinez D, Hwang D, Mawlawi O, Slifstein M, Kent J, Simpson N, Parsey RV, Hashimoto T, Huang Y, Shinn A, Van Heertum R, Abi-Dargham A, Caltabiano S, Malizia A, Cowley H, Mann JJ, Laruelle M. Neuropsychopharmacology 2001 Mar; 24 (3), pp. 209–29.

Serotonin: 5-HT_{1A} Autoreceptor/Occupancy of Serotonin 5-HT_{1A} Autoreceptor: Dorsal Raphe Nuclei (DRN)/Dose Optimization for Serotonin 5-HT_{1A} Autoreceptor Agents *[Carbonyl-^{11}C]-WAY-100635*

Cellular/Molecular Mechanism of Action: The agent [carbonyl-^{11}C]-WAY-100635 or [carbonyl-^{11}C] *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl)cyclohexa necarboxamide abbreviated as [^{11}C]-WAY-100635 possesses binding avidity for 5-hydroxytryptamine(1A) (5-HT_{1A}) receptor complexes.

Background Information: Preclinical studies in rodents suggest that augmentation of serotonin reuptake inhibitor (SSRI) therapy by the 5-HT_{1A} receptor agent pindolol might reduce the delay between initiation of treatment and antidepressant response. This hypothesis is based on the ability of pindolol to potentiate the increase in serotonin (5-HT) transmission induced by SSRIs, an effect achieved by blockade of the 5-HT_{1A} autoreceptors in the DRN. However, placebo-controlled clinical studies of pindolol augmentation of antidepressant therapy have reported inconsistent results.

Application: The binding avidity of [^{11}C]-WAY-100635 for 5-HT_{1A} receptor complexes can be utilized to evaluate occupancy of these membrane-associated sites. In this context, [^{11}C]-WAY-100635 can effectively assess new prototype pharmaceuticals through positron emission tomography (PET) imaging modalities to determine their selectivity for DRN. Such a strategy can be used as an investigative probe to establish appropriate doses for clinical trials.

Implementation: Evaluation of the occupancy of 5-HT_{1A} receptors following treatment with controlled release (CR) pindolol in 9 healthy volunteers with PET. Each subject is then studied 4 times, at (i) baseline (scan 1) following 1 week of oral administration of pindolol CR (7.5 mg/day) at peak levels; (ii) 4 hours after the dose (scan 2); (iii) 10 hours following the dose (scan 3); and (iv) following 1 dose of pindolol CR (30 mg) at peak levels, 4 hours (scan 4). Pindolol occupancy of 5-HT_{1A} receptors is then evaluated in the DRN and cortical regions as a decrease in binding potential (BP) of the radiolabeled selective 5-HT_{1A} antagonist [carbonyl-^{11}C]-WAY-100635.

Human Model: Healthy cases (n = 9).

Interpretation and Analysis: Pindolol dose-dependently decreases [^{11}C]-WAY-100635 BP. Combining all regions of interest, occupancy is 20 ± 8%; 14 ± 8%; and 44 ± 8% (scans 2, 3, and 4, respectively). Pindolol dose-dependently decreases the binding potential for [^{11}C]-WAY-100635 within the brain. Combining all regions of interest, 5-HT_{1A} receptor occupancy is 20 ± 8%; 14 ± 8%; and 44 ± 8% (scans 2, 3, and 4, respectively). Such findings suggest that with pindolol when used in clinical studies at doses capable of exerting an SSRI effect (e.g., 2.5 mg t.i.d.), the occupancy of 5-HT_{1A} receptors is moderate and highly variable between subjects. This factor may explain the variable results obtained in clinical studies. On the other hand, at each dose tested, pindolol occupancy of 5-HT_{1A} receptors is higher in the DRN compared to cortical regions, demonstrating a significant degree of *in vivo* selectivity for DRN 5-HT_{1A} autoreceptors relative to cortico-limbic postsynaptic receptors. Selectivity of this magnitude is necessary for the potentiation of 5-HT transmission, and this finding represents an important proof of concept in the development of 5-HT_{1A} agents for this application.

Positron emission tomography study of pindolol occupancy of 5-HT_{1A} receptors in humans: Preliminary analyses. Martinez D, Mawlawi O, Hwang DR, Kent J, Simpson N, Parsey RV, Hashimoto T, Slifstein M, Huang Y, Van Heertum R, Abi-Dargham A, Caltabiano S, Malizia A, Cowley H, Mann JJ, Laruelle M. Nucl Med Biol 2000 Jul; 27 (5), pp. 523–7.

5-Serotonin 5-HT_{1A} Receptor Complex Expression *[^{11}C]-CPC-222/(N-[2-[4-(2-Methoxyphenyl)-1-Piperazinyl]Ethyl]-N-(2-Pyridinyl)-2-Bicyclo [2,2,2] Octane Carboxamide)*

Cellular/Molecular Mechanism of Action: The radioligand N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)-2-bicyclo [2,2,2]octane carboxamide ([^{11}C]-CPC-222) selectively binds as a ligand to 5-HT_{1A} receptor complexes.

Application: Detection and characterization of 5-HT_{1A} receptors complexes within the central nervous system (CNS), where it can be used as an *in vivo* probe for positron emission tomography (PET).

Route of Administration: [^{11}C]-CPC-222 (intravenous).

Implementation: Evaluation of the distribution for [^{11}C]-CPC-222 within the CNS applying PET over a 90-minute period following intravenous injection of the radioligand.

Human Model: Healthy volunteers (n = 3).

Interpretation and Analysis: The radioligand [^{11}C]-CPC-222 accumulates at high levels in brain regions with a high density of 5-HT_{1A} receptors. The peak cortical concentration is 1.0–2.5% of the injected dose per liter. The ratio of radioactivity in receptor-rich regions to that of the cerebellum reaches a plateau of 2.5 to 4.0 by 45 minutes after injection. Analysis of the plasma reveals no detectable amount of the potential metabolite, radiolabeled WAY-100634. The radioligand [^{11}C]-CPC-222 therefore appears to possess suitable properties for the study of human 5-HT_{1A} receptors applying PET imaging modalities.

Instrumentation: Positron emission tomography.

Imaging 5-HT_{1A} receptors with positron emission tomography: Initial human studies with [11C]-CPC-222. Houle S, Wilson AA, Inaba T, Fisher N, DaSilva JN. Nucl Med Commun 1997 Dec; 18 (12), pp. 1130–4.

Serotonin 5-HT_2 Receptor *[^{18}F]-Altanserin/Clomipramine*

Cellular/Molecular Mechanism of Action: Altanserin recognizes and binds to serotonin 5-HT_2 receptor complexes. Clomipramine functions as a serotonin reuptake inhibitor.

Application: The radioligand [^{18}F]-altanserin can be utilized *in vivo* to characterize the density and distribution of brain serotonin 5-HT_2 receptor complexes (e.g., positron emission tomography [PET] imaging modalities).

Route of Administration: Clomipramine (intravascular).
Dosage: Clomipramine (25 mg).
Implementation: The postsynaptic receptor binding potential of [^{18}F]-altanserin can be employed as a possible ligand indicator for synaptic serotonin content following pharmacological challenge (e.g., serotonergic antidepressive agents). To delineate such properties, serotonergic antidepressive treatment is administered to cases with recurrent major depression. Cases are then evaluated for PET measurements performed twice within 2 or 3 days and the serotonin reuptake inhibitor, clomipramine administered before acquisition of 1 of the PTE images. Data are analyzed using non-linear least-square regression and Logan's graphical method.
Interpretation and Analysis: Binding potential and distribution volume of altanserin decreases between 14% ($p = 0.03$) and 23% ($p = 0.004$) following clomipramine challenge. This effect is mainly seen in the absence of antidepressive medication. Clomipramine challenge probably increases the synaptic serotonin level, which competes with altanserin leading to a lowered binding potential. The paradigm might, thus, be useful to estimate serotonin release *in vivo*. Pretreatment with serotonergic antidepressants reduces the effect of clomipramine

Influence of synaptic serotonin level on [^{18}F]-altanserin binding to 5-HT$_2$ receptors in man. Larisch R, Klimke A, Hamacher K, Henning U, Estalji S, Hohlfeld T, Vosberg H, Tosch M, Gaebel W, Coenen HH, Muller-Gartner HW. Behav Brain Res 2003 Feb 17; 139 (1–2), pp. 21–9.

Serotonin (5-HT$_{1A}$) Receptor Complexes/Clozapine Response (Atypical)/Clozapine Receptor Occupancy *Carbonyl-[^{11}C]-WAY-100635 Ligand/Clozapine*

Application: Characterization of clozapine occupancy of serotonin 5-hydroxytryptamine$_{1A}$ [5-HT$_{1A}$] receptor subtypes through the application of positron emission tomography (PET) imaging.
Route of Administration: Clozapine (oral or intravenous).
Dosage: Clozapine (oral 200–800 mg vs 1.5 mg/kg–6.0 mg/kg IV).
Implementation: Characterization of clozapine occupancy of brain 5-HT$_{1A}$ receptor complexes utilizing intravenous doses capable of generating plasma concentrations that directly correlate with ranges achieved with oral admistration at the 200–800 mg dose range as recommended for clinical management scenarios (humans). Subjects are each examined (t.i.d.) with PET and the radioligand carbonyl-[^{11}C]-WAY-100635. The 1st measurement is performed at baseline conditions, the 2nd after clozapine 1.5 mg/kg, and the 3rd after 6 mg/kg. Two additional monkeys can be examined at baseline and after 15 mg/kg IV. Central 5-HT$_{1A}$ receptor occupancy is calculated using equilibrium-ratio analysis.
Animal Model: Primate (cynomolgus monkey).
Interpretation and Analysis: Occupancy of 5-HT$_{1A}$ receptor complexes ranges between 23 and 34% after clozapine (1.5 mg/kg) and from 36 to 49% after 6 mg/kg in different brain regions (e.g., n = 4 monkeys). Regional receptor occupancy values after 15 mg/kg are between 39 and 51% in some animal subjects (e.g., n = 2 monkeys). There is no evidence of a difference in image profiles for the frontal cortex, the temporal cortex, and the raphe nucleus. Clozapine readily appears to occupy 5-HT$_{1A}$ receptors in the primate brain at clinically relevant plasma concentrations. The 5-HT$_{1A}$ receptor therefore appears to be a candidate target that may explain the atypical drug actions of clozapine.

Occupancy of 5-HT$_{1A}$ receptors by clozapine in the primate brain: A PET study. Chou YH, Halldin C, Farde L. Psychopharmacology (Berl) 2003 Mar; 166 (3), pp. 234–40.

Serotonin 5-HT$_{2A}$ Receptor Complexes/ Paroxetine Response/Depression Disorders (Major) *[^{18}F] Fluoroethylspiperone ([^{18}F] FESP)/ Cortical Binding Index*

Cellular/Molecular Mechanism of Action: Fluoroethylspiperone functions as a 5-HT$_{2A}$ and D$_2$ receptor antagonist. Radiofluorinated [^{18}F] fluoroethylspiperone ([^{18}F] FESP) therefore can function as a positron emission tomography (PET) imaging agent for characterizing brain 5-HT$_{2A}$ receptor complex expression densities and distribution.
Application: Characterization of the regional distribution and expression density of 5-HT$_{2A}$ receptor complexes within the central nervous system.
Implementation: Investigate differences in 5-HT$_{2A}$ receptor binding between healthy volunteers and patients with major depressive disorder (MDD), either never treated before with antidepressants (drug-naive: DN) or responding to paroxetine treatment (drug-treated: DT). In this context, DN MDD cases (e.g., n = 19) and euthymic DT cases (paroxetine 4 weeks; e.g., n = 15) are compared with a group of normal healthy controls (e.g., n = 20) applying PET facilitated by the use of [^{18}F] FESP, a 5-HT$_{2A}$ and D$_2$ receptor antagonist. A "binding-index" (BI) of [^{18}F] FESP to cortical and basal ganglia region can be calculated as the ratio of the activity in these regions to that of the cerebellum. Differences in BI between groups (e.g., control vs DN or DT) can be assessed by ANOVA, with or without age as a covariate (ANCOVA).
Interpretation and Analysis: A significant reduction in BI (e.g., $p = 0.003$ ANOVA, $p = 0.001$ ANCOVA) can be appreciated in DN patients in the frontal, occipital, temporal, and cingulate cortices, but not in the striatum. No significant differences can be appreciated between control and DT patients. The reduction of [^{18}F] FESP BI in cortical areas of DN (depressed) but not euthymic DT cases suggests an association between the occurrence of depressive symptoms and impairment of cortical 5-HT$_{2A}$ receptors. The cortical BI for [^{18}F] FESP may ultimately function as an effective marker for depression.

5-HT$_{2A}$ receptor binding is reduced in drug-naive and unchanged in SSRI-responder depressed patients compared to healthy controls: A PET study. Messa C, Colombo C, Moresco RM, Gobbo C, Galli L, Lucignani G, Gilardi MC, Rizzo G, Smeraldi E, Zanardi R, Artigas F, Fazio F. Psychopharmacology (Berl) 2003 Apr; 167 (1), pp. 72–8. Epub 2003 Mar 11.

Serotonin 5-HT$_{1A}$ Receptor/Robozoltan (NAD-299) 5-HT$_{1A}$ Receptor Occupancy/Depression *Carbonyl-[^{11}C]-WAY-100635/Robalzotan (NAD-229)*

Background Information: The serotonin 5-HT$_{1A}$ receptor has been ascribed a putative role in the pathophysiology and therapeutic management of depression. NAD-299 (Robalzotan) is a new potential antidepressant with high affinity and selectivity for the 5-HT$_{1A}$ receptor.
Application: Characterization of the occupancy of brain serotonin 5-HT$_{1A}$ receptor complexes by Robalzotan (NAD-299) utilizing positron emission tomography (PET) imaging facilitated by the radioligand carbonyl-[^{11}C]-WAY-100635.
Route of Administration: NAD-299 (robalzotan) single oral doses.
Dosage: Single oral doses of NAD-299 (robalzotan) 0.5, 2.5, and 10 mg).
Implementation: Positron emission tomography examination of the extent and time course of brain 5-HT$_{1A}$ receptor occupancy by NAD-299 as a function of plasma concentration generated with

escalating single oral doses. Cases receive 1 or more single oral doses of NAD-299 (0.5, 2.5, and 10 mg) in aqueous solution under fasting conditions. Total and unbound (after ultrafiltration) plasma concentrations of NAD-299 are determined by liquid chromatography-mass spectrometry (LC-MC), over a tentative dosage interval of 8 hours. Regional 5-HT_{1A} receptor occupancy in the brain is calculated by the simplified reference tissue model using the radioligand carbonyl-[^{11}C]-WAY-100635.

Interpretation and Analysis: Robalzotan (NAD-299) doses of 10 mg produce high levels of occupancy in the raphe (62–85%) and neocortical regions (68–75%) at time point C_{max}, but decline considerably (17–44%) by 7 hours postadministration. The new selective 5-HT_{1A} antagonist NAD-299 therefore occupies 5-HT_{1A} receptors in the living human brain in a dose-dependent manner following oral dosage. The curvilinear relationship between NAD-299 drug concentration and 5-HT_{1A} receptor occupancy is established and can be used for dose selection in subsequent clinical patient studies.

Positron emission tomographic analysis of dose-dependent NAD-299 binding to 5-hydroxytryptamine-1A receptors in the human brain. Andree B, Hedman A, Thorberg SO, Nilsson D, Halldin C, Farde L. Psychopharmacology (Berl) 2003 Apr; 167 (1), pp. 37–45.

Serotonin Transporter
N,N-Dimethyl-2-(2-Amino-4-[^{18}F]-Fluorophenylthio) Benzylamine

Application: Serotonin transporter positron emission tomography (PET) imaging agent.

Semi-Synthetic Methods: The 403U76 analogs *N*,*N*-dimethyl-2-(2-nitro-4-bromophenylthio)benzylamine (4) and *N*,*N*-dimethyl-2-(2,4-dinitrophenylthio)benzylamine (8) can be prepared in multisteps synthesis as precursors for the synthesis of a new serotonin transporter imaging agent, *N*,*N*-dimethyl-2-(2-amino-4-[^{18}F]-fluorophenylthio)benzylamine (12a).

Implementation: Central nervous system (CNS) biodistribution image analysis for the identification and profiling of CNS serotonin transporter expression and function.

Interpretation and Analysis: Biodistribution studies in rats show that the uptake of compound 12a in rat brain is high (approximately 1%/g) and the ratio of the uptake of compound 12a in hypothalamus (a serotonin transporter-rich area) and cerebellum (a serotonin-transporter-devoid area) is 6/1 at 1 hour postinjection. Compound 12a may therefore be a potential new serotonin transporter PET imaging agent.

Synthesis of *N,N*-dimethyl-2-(2-amino-4-[^{18}F]-fluorophenylthio)benzylamine as a serotonin transporter imaging agent. Shiue GG, Fang P, Shiue CY. Appl Radiat Isot 2003 Feb; 58 (2), pp. 183–91.

Serotonin Transporters (SERT)/Cerebral Cortex/ Hypothalamus/Striatum/[Parkinson's Disease]/ Hippocampus
[^{125}I]-ADAM/[^{123}I]-ADAM/2-((2-((Dimethylamino) Methyl)- Phenyl)Thio)-5-Iodophenylamine)

Cellular/Molecular Mechanism of Action: The substances 2-((2-((dimethylamino)methyl)-phenyl)thio)-5-iodophenylamine paroxetine and (+)McN5652 function as ligands that selectively recognize and physically bind to serotonin transporter complexes.

Application: The iodinated tracer ADAM (2-((2-((dimethylamino)methyl)-phenyl)thio)-5-iodophenylamine) can be applied to detect and characterize SERT within the central nervous system (CNS).

Route of Administration: [^{125}I]-ADAM (intravenous).

Implementation: Detection of serotonic tranporters within the CNS using single photon emission computerized tomography (SPECT) imaging modalities.

Animal Model: Rat.

Interpretation and Analysis: Scatchard analysis of saturation binding profiles for [^{125}I]-ADAM to frontal cortical membrane homogenates (rat) produces K_d values of 0.15 ± 0.03 nM and B(max) values of 194 ± 65 fmol/mg protein. Biodistribution of [^{125}I]-ADAM within the brain postadministration reveals high specific binding within regions of the hypothalamus, cortex, striatum, and hippocampus, where SERT are concentrated and the specific binding peaks at 120 to 240 minutes postinjection ([hypothalamus-cerebellum]/cerebellum = 4.3 at 120 minutes post-IV injection). Moreover, specific hypothalamic uptake is blocked by pretreatment with SERT selective competing drugs, such as paroxetine and (+)McN5652, while other non-competing drugs, such as ketanserin, raclopride, and methylphenidate, exert no effect. Radioactive material recovered from rat brain homogenates at 120 minutes after [^{125}I]-ADAM injection is primarily the original compound (> 90%) and is a good indication of the *in vivo* stability of the agent within brain tissues. Both male and female rats show similar and comparable patterns of organ distribution and regional brain uptake. Autoradiograms (*ex vivo*) of rat brain sections (120 minutes after IV injection of [^{125}I]-ADAM) detect intense labeling in several regions (olfactory tubercle, lateral septal nucleus, hypothalamic and thalamic nuclei, globus pallidus, central gray, superior colliculus, substantia nigra, interpeduncular nucleus, dorsal and median raphes, and locus coerulus), which parallels known SERT expression densities. These results strongly suggest that the novel tracer ADAM is superior to the congers reported previously (i.e., IDAM). Labeling of ADAM with [^{123}I] to produce [^{123}I]-ADAM should produce an improved and useful SPECT imaging agent for SERT within the brain.

Selective *in vitro* and *in vivo* binding of [^{125}I]-ADAM to serotonin transporters in rat brain. Choi SR, Hou C, Oya S, Mu M, Kung MP, Siciliano M, Acton PD, Kung HF. Synapse 2000 Dec 15; 38 (4), pp. 403–12.

Serotonin Transporter (SERT)/Hypothalamus Region
[^{125}I]-ODAM/[^{125}I]-[5-Iodo-2-(2-Dimethylaminomethylphenoxy)-Benzyl Alcohol]

Background Information: The SERT is the primary binding sites for selective serotonin reuptake inhibitors, commonly used antidepressants such as fluoxetine, sertraline, and paroxetine. Imaging of SERT with positron emission tomography (PET) and single photon emission computed tomography (SPECT) in humans would provide a useful tool for understanding how alterations of this system are related to depressive illnesses and other psychiatric disorders.

Application: [^{125}I]-ODAM [(5-iodo-2-(2-dimethylaminomethylphenoxy)-benzyl alcohol, 9)] can be applied as an imaging agent for detecting and characterizing central nervous system (CNS) SERT.

Route of Administration: [^{125}I]-ODAM (intravenous injection).

Semi-Synthetic Methods: Radioiodination and semi-synthesis of [^{125}I]-ODAM. Reaction schemes have been developed for the preparation of [^{125}I]-ODAM and the corresponding tri-*n*-butyltin derivative (compound 10). A relatively high radiolabling efficiency (e.g., 95%) can be achieved upon reacting 10 with hydrogen peroxide and sodium [^{125}I]-iodide reagent.

Implementation: Characterization of the binding characteristics of ODAM for cortical membrane homogenates reveals good levels

of affinity ($K_i = 2.8 \pm 0.88$ nM). Using LLC-PK(1) cells specifically expressing the individual transporter (i.e., dopamine [DAT], norepinephrine [NET], and SERT, respectively), ODAM shows a strong inhibition of SERT ($K_i = 0.12 \pm 0.02$ nM). Inhibition constants for the other 2 transporters are lower ($K_i = 3.9 \pm 0.7$ μM and 20.0 ± 1.9 nM for DAT and NET, respectively). Biodistribution following intravenous injection of [^{125}I]-ODAM (rat) shows a rapid brain uptake and washout (2.03, 1.49, 0.79, 0.27, and 0.07% dose/organ at 2, 30, 60, 120, and 240 minutes, respectively). Hypothalamus regions that are rich with serotonin neurons exhibit high specific uptake profiles. Ratios of hypothalamus-cerebellum/cerebellum based on percent dose per gram of these 2 regions show values of 0.35, 0. 86, 0.86, 0.63, and 0.34 at 2, 30, 60, 120, and 240 minutes, post-IV injection, respectively. The specific uptake in hypothalamus can be effectively blocked by pretreatment of known SERT ligands and serves to validate that this ligand displays desirable *in vitro* and *in vivo* properties as a potential SERT imaging agent.

Animal Model: Rat (cortical brain homogenates and biodistribution).

A novel serotonin transporter ligand: (5-iodo-2-(2-dimethylaminomethylphenoxy)-benzyl alcohol. Zhuang Z, Choi S, Hou C, Mu M, Kung M, Acton PD, Kung HF. Nucl Med Biol 2000 Feb; 27 (2), pp. 169–75.

Serotonin Transporter (SERT)/Ecstacy-Induced SERT Alterations
[^{11}C]-±McN5652

Background Information: Alterations of the serotonergic system due to ecstasy consumption have been extensively documented in recent literature. However, reversibility of these neurotoxic effects still remains unclear.

Application: Characterization of various physiological and pharmacological stimuli on SERT complexes (e.g., influence of ecstacy on protracted alterations of the serotonin transporter).

Route of Administration: [^{11}C]-±McN5652 (intravascular).

Dosage: [^{11}C]-±McN5652 (500 MBq).

Implementation: Characterization of the potential influence of ecstacy on SERT can be delineated by positron emission tomography (PET) using the SERT ligand [^{11}C]-±McN5652. The technique that can be employed in such studies involves the intravenous administration of [^{11}C]-±McN5652 ligand (500 MBq IV). Scan images are acquired according to a dynamic scan protocol of 90 minutes using a full-ring whole-body PET system. Trans-axial slices are reconstructed using an iterative method. Individual brains are transformed to a template defined earlier. Distribution volume ratios (DVRs) are derived by the application of a reference tissue approach for reversible binding. Gray matter of the cerebellum can serve as a reference control. Serotonin transporter-rich brain regions (e.g., mesencephalon, putamen, caudate, and thalamus) are selected for the evaluation of SERT availability using volumes of interest predefined according to the standard template.

Human Model: Cases classified as ecstasy users (e.g., n = 117 subjects subdivided into 4 groups: actual ecstasy users [n = 30], former ecstasy users [n = 29], drug-naive control subjects [n = 29], and subjects with abuse of psychoactive agents other than ecstasy [n = 29]).

Interpretation and Analysis: Compared with drug-naive control subjects, the distribution volume ratios in actual ecstasy users is significantly reduced in the mesencephalon ($p = 0.004$) and the thalamus ($p = 0.044$). The DVR in former ecstasy users is very close to the DVR in drug-naive control subjects in all brain regions. The DVR in polydrug users is slightly higher than that in the drug-naive control subjects in all SERT-rich regions (not statistically significant). Ecstasy appears to induce protracted alterations of the SERT. However, availability of SERT may be reversible when evaluated by PET imaging but does not imply full reversibility of actual neurotoxic effects.

Long-term effects of "ecstasy" use on serotonin transporters of the brain investigated by PET. Buchert R, Thomasius R, Nebeling B, Petersen K, Obrocki J, Jenicke L, Wilke F, Wartberg L, Zapletalova P, Clausen M. J Nucl Med 2003 Mar; 44 (3), pp. 375–84.

Trauma (Brain/Cranial)/Long-Term Neuropsychological Outcome
[^{99m}Technetium] Ethyl Cystein Dimer Single Photon Emission Computed Tomography ([^{99}mTc] ECD SPECT)

Implementation: Assess the prognostic value of early brain scintigraphy in head injury and correlation to long-term neuropsychological behavior.

Human Model: Cases of cranial head trauma within 1 month of initial injury (e.g., n = 24 patients).

Interpretation and Analysis: Most affected cases (e.g., n = 24) have abnormalities that can be detected on single photon emission computed tomography (SPECT) scans. One year after trauma, slightly greater than half may have residual neuropsychological sequelae (e.g., n = 14/24 or 58%). The brain-to-cerebellum ratios in the left basal ganglia and brain stem are significantly decreased in patients with memory disorders ($p = 0.03$ and $p = 0.02$, respectively). Moreover, SPECT visual analysis indicates that low uptake in the basal ganglia, thalamus, and brain stem is associated with subsequent motor deficit, frontal behavior, and language and memory disorders. Therefore, brain SPECT can be valuable in predicting the neuropsychological behavior of survivors of severe head injury.

Technetium-99m ECD single photon emission computed tomography in brain trauma: Comparison of early scintigraphic findings with long-term neuropsychological outcome. Baulieu F, Fournier P, Baulieu JL, Dalonneau M, Chiaroni P, Eder V, Pottier JM, Legros B. J Neuroimaging 2001 Apr; 11 (2), pp. 112–20.

Vascular Perfusion: Cerebral Blood Flow
H_2[^{15}O]

Background Information: Blood vessels are usually conspicuous on dynamic computed tomography (CT) perfusion images. The presence of large vessels may lead to overestimation of the quantitative value of cerebral blood flow (CBF).

Application: Estimation of cerebral blood flow dynamics.

Route of Administration: H_2[^{15}O] (intravascular).

Dosage: H_2[^{15}O].

Implementation: Evaluation of the efficacy of the vascular-pixel elimination (VPE) method in quantitative CT perfusion imaging, compared to positron emission tomography (PET) pertaining to CBF dynamics. A 4-channel multidetector row CT scanner is used to perform dynamic cine scanning after a bolus injection of intravenous contrast agent. The CT-CBF is calculated by the central volume principle and deconvolution method. Alternatively, PET can be performed after infusion of [^{15}O]-labeled water and PET-CBF parameters calculated using a non-linear least squares method. Average CBF values of the whole section, gray matter, and white matter with both CT and PET are compared after image acquisition. The comparison is performed with and without VPE. In the VPE method, the vascular pixels are defined by the cerebral blood volume value of the pixel. The threshold of VPE is changed from 5 to 20 mL/100 g. Pixel-by-pixel correlation between CT-CBF and PET-CBF and linear regression analysis is then also performed.

Interpretation and Analysis: In the absence of vascular-pixel elimination, CT-CBF is almost invariably overestimated. As the vascular-pixel elimination threshold decreases, CT-CBF decreases and the correlation coefficient increases. The best correlation is observed at a VPE threshold of 8 mL/100 g (e.g., n = 4/5 cases). Average CT-CBF values, without VPE, of the whole section, gray matter, and white matter are 59.01, 66.73, and 42.53 mL/100 g/min, respectively. With VPE (threshold, 8 mL/100 g), average CT-CBF values of the whole section, gray matter, and white matter are 45.56, 52.75, and 30.38, respectively. The corresponding PET-CBF values are 46.86, 50.89, and 38.20 mL/100 g/min, respectively. Vascular pixels should be excluded from the calculation of CT-CBF to avoid overestimation of CBF values. If vascular pixels are excluded, CBF calculation with CT perfusion imaging is considerably accurate.

Quantitative cerebral blood flow measurement with dynamic perfusion CT using the vascular-pixel elimination method: Comparison with $H_2[^{15}]O$ positron emission tomography. Kudo K, Terae S, Katoh C, Oka M, Shiga T, Tamaki N, Miyasaka K. AJNR Am J Neuroradiol 2003 Mar; 24 (3), pp. 419–26.

Vasculature Perfusion: Cerebral/Cerebral Vascular Perfusion Alterations in Psychiatric Disorders/ Transient Ischemia/Stroke
[^{123}I] and [^{99m}Technetium] ([^{99m}Tc])-Brain Perfusion Agents

Background Information: In the past few years significant progress has been made in the design and characterization of new central nervous system (CNS) neuronal and postsynaptic receptor imaging agents for positron emission tomography (PET) and single photon emission computed tomography (SPECT). The new diagnostic agents are aimed at measuring localized changes in neuronal function. It is likely that these types of agents have potential for clinical application, especially in the diagnosis of psychiatric disorders that do not involve morphological changes.
Application: Detection of cerebral vascular abnormalities including stroke and transient ischemia.
Instrumentation: There are 2 types of imaging instruments, SPECT and PET, that use radiopharmaceuticals for the diagnosis of brain disorders. The use of SPECT in concert with brain perfusion imaging agents, labeled either with [^{123}I] or [^{99m}Tc], is useful in the detection of various cerebral vascular abnormalities, such as stroke and transient ischemia.

Overview of radiopharmaceuticals for diagnosis of central nervous disorders. Kung HF. Crit Rev Clin Lab Sci 1991; 28 (4), pp. 269–86.

Vascular Perfusion: Cerebral
[^{15}O]-Labeled H_2O: Positron Emission Tomography (PET) Imaging/Intravenous Contrast Agent: Computed Tomography (CT) Imaging

Background Information: Blood vessels are usually conspicuous on dynamic CT perfusion images. The presence of large vessels may lead to overestimation of the quantitative value of cerebral blood flow (CBF). Investigations have evaluated the efficacy of the vascular-pixel elimination (VPE) method in quantitative CT perfusion imaging, in comparison with PET.
Implementation: Comparison of CT perfusion and PET image analyses. Dynamic cine scanning is performed after bolus injection of an intravenous contrast agent. Computed tomography-CBF is calculated by the central volume principle and deconvolution method. Positron emission tomography is performed after infusion of [^{15}O]-labeled water. The PET-CBF is calculated by using a non-linear least squares method. Average CBF values of the whole section, gray matter, and white matter with both CT and PET are compared after image registration. The comparison is performed with and without VPE. In the VPE method, the vascular pixels are defined by the cerebral blood volume value of the pixel. The threshold of VPE is changed from 5 to 20 mL/100 g. Pixel-by-pixel correlation between CT-CBF and PET-CBF and linear regression analysis is also performed.
Human Model: Normal healthy cases.
Interpretation and Analysis: Computed tomography-CBF is overestimated in most instances without VPE. As the VPE threshold decreases, CT-CBF decreases and the correlation coefficient increases. The best correlation is observed at a VPE threshold of 8 mL/100 g (e.g., n = 4/5 cases). Average CT-CBF values, without VPE, of the whole section, gray matter, and white matter are 59.01, 66.73, and 42.53 mL/100 g/min, respectively. Average CT-CBF values of the whole section, gray matter, and white matter are 45.56, 52.75, and 30.38, respectively, with the application of VPE (threshold, 8 mL/100 g). The corresponding PET-CBF values are 46.86, 50.89, and 38.20 mL/100 g/min, respectively. Vascular pixels should be excluded from the calculation of CT-CBF to avoid overestimation of the CBF values. If vascular pixels are excluded, CBF calculation with CT perfusion imaging is considerably accurate.
Instrumentation: Computed tomography is performed with a 4-channel multidetector row CT scanner.

Quantitative cerebral blood flow measurement with dynamic perfusion CT using the vascular-pixel elimination method: Comparison with $H_2[^{15}O]$ positron emission tomography. Kudo K, Terae S, Katoh C, Oka M, Shiga T, Tamaki N, Miyasaka K. AJNR Am J Neuroradiol 2003 Mar; 24 (3), pp. 419–26.

Vascular Perfusion: Cerebral Blood Flow (CBF)/ Disease Probe/Pharmaceutical Evaluation
$H_2[^{15}O]$

Cellular/Molecular Mechanism of Action: The agent $H_2[^{15}O]$ mimics the diffusion and transport of natural H_2O molecules.
Application: Measurement of CBF.
Implementation: Evaluation of regional CBF (rCBF) during a passive visual stimulus in which pattern-flash frequency is parametrically manipulated. Goggles are used with a grid of red lights embedded into each lens. A total of 5 positron emission tomography (PET) $H_2[^{15}O]$ water scan images are then acquired for each test subject at alternating (left to right eye) flash frequencies of 0, 1, 4, 7, and 14 Hz.
Human Model: Healthy elderly subjects (n = 19; 64 ± 11 years).
Interpretation and Analysis: Biphasic rising and falling rCBF response can be appreciated within the striate cortex (7 Hz peak) and left anterior cingulate (4 Hz peak), 1 Hz activation in left middle temporal gyrus (V5), monotonically increasing rCBF in posterior areas (lateral and inferior visual association areas, Brodmann 18 and 19), and monotonically decreasing rCBF in anterior areas (frontal, cingulate, and superior temporal) predominantly in the right hemisphere. Striate rCBF changes at all frequencies primarily reflect lateral geniculate input, the middle temporal activation at 1 Hz reflects perception of apparent motion, and the posterior extrastriate rCBF monotonic increase represents a neural response to increasing luminance intensity and form and color complexity that occur as pattern-flash frequency increases. Anterior monotonic rCBF decreases may represent active cross-modal functional suppression of brain areas irrelevant for processing the passive visual stimulus. Pattern-flash rCBF responses are highly reproducible (no series effect), more so in

posterior than anterior brain regions. Reproducibility and systematically changing rCBF responses to this passive stimulus suggest that it could be successfully used as a disease probe to evaluate neural function and drug effects in cognitively impaired patients.

Frequency variation of a pattern-flash visual stimulus during PET differentially activates brain from striate through frontal cortex. Mentis MJ, Alexander GE, Grady CL, Horwitz B, Krasuski J, Pietrini P, Strassburger T, Hampel H, Schapiro MB, Rapoport SI. Neuroimage 1997 Feb; 5 (2), pp. 116–28.

Vascular Perfusion: Cerebral Hemisphere (Dural Arteriovenous Fistulas)/Altered Vascular Integrity/ Dural Arteriovenous Fistulas/Single Photon Emission Computed Tomography (SPECT)/ Preserved Vasoreactivity/SPECT/Hypoperfusion/ Venous Infarction/Presistent Symptoms/Prognostic Indicators
Acetazolamide

Cellular/Molecular Mechanism of Action: Acetazolamide functions as a carbonic anhydrase inhibitor.
Physiological Mechanism of Action: Acetazolamide can promote antiseizure activity and prevent absence seizures.
Background Information: Dural arteriovenous fistulas (DAVFs) with disturbed regional cerebral blood flow (rCBF) include retrograde leptomeningeal venous drainage (RLVD).
Application: Acetazolamide can be used as an adjunct diagnostic probe applied in conjuction with SPECT to characterize conditions of dural arteriovenous fistulas in a manner that can detect preservation or alterations in vasoreactivity that can correspond with either a good prognosis or evidience of venous infarction and persistence of symptoms.
Implementation: Characterization of pretreatment magnetic resonance imaging (MRI)/SPECT and posttreatment MRI/SPECT symptoms and response to therapeutic intervention in conditions of DAVFs with disturbed rCBF.
Human Model: Cases with DAVFs with disturbed rCBF and associated with various clinical symptoms (n = 22). Case Group 1 = retrograde leptomeningeal venous drainage (RLVD) into more than 1 venous sinus (n = 11/22 cases); and Case Group 2 = RLVD into a single venous sinus (n = 11/22 cases).
Interpretation and Analysis: In Case Group 1, preoperative T_2-weighted MR images show no hyperintense areas, and angiographic evidence displays flow into more than 1 venous sinus. Case Group 2 cases display preoperative SPECT with hypoperfused areas that coincide with hyperintense areas on T_2-weighted MR images. Following treatment, hyperintense areas disappear, and symptoms improve in a large number of these cases (e.g., n = 7/11 = Case Group 2a), and their preoperative SPECT studies demonstrate preservation of vasoreactivity after an acetazolamide challenge. In Case Group 2b, the hyperintense areas and symptoms persist in response to acetazolamide challenge (n = 4/11 cases) and reoperative SPECT studies reveal marked disturbances of vasoreactivity. Hyperintense MR images are consistently observed in cases with drainage into a single venous sinus. Hypoperfusion on SPECT scans apparently reflects venous congestion, whereas unpreserved vasoreactivity after an acetazolamide challenge on SPECT scans reflects venous infarction. The preservation of vasoreactivity after the challenge appears to be a good prognostic indicator.

Pre- and posttreatment MR imaging and single photon emission CT in patients with dural arteriovenous fistulas and retrograde leptomeningeal venous drainage. Kai Y, Hamada J, Morioka M, Todaka T, Mizuno T, Ushio Y. AJNR. Am J Neuroradiol 2003; 24 (4), pp. 619–25

CENTRAL NERVOUS SYSTEM (CNS): SPECIAL SENSES

Inner Ear: Autoimmune Disease
Heat Shock Protein/Potential Disease Indicator

Background Information: Autoimmune inner ear disease is a form of cochleovestibular disease associated with variable hearing loss and vertigo for which no reliable diagnostic tests are available.
Application: Assessment of autoimmune inner ear disease when applied in combination with heat shock protein 70 (HSP-70).
Implementation: Limited positron emission tomography (PET) of the inner ear for the characterization of conditions of autoimmune inner ear disease before and 4–6 weeks following high-dose tapering course prednisone. Complementary evaluation parameters can include cranial magnetic resonance imaging, audiometric and vestibular studies, and HSP-70 measurements.
Interpretation and Analysis: Of patients with established and stable autoimmune inner ear disease, approximately 80% (e.g., n = 4/5) will display no PET image abnormalities and negative HSP-70 concentrations. Cases with abnormal PET images can over a brief period of time subsequently manifest clinically active disease. Of cases with active autoimmune inner ear disease, serial monitoring reveals that approximately 80% (e.g., n = 4/5) initially have abnormal PET images in at least 1 ear that eventually become normal in the majority of instances following therarpy. As a complementary feature of PET imaging, HSP-70 correlates with disease activity. A small percentage of cases with clinically active autoimmune inner ear disease (e.g., 20% or n = 1/5) have a normal PET image before and after therapy (positive HSP-70 before therapy and negative after the therapy). Approximately 80–100% of normal subjects (e.g., n = 4/5) will have normal PET images. Positive findings in normal subjects may be accompanied by normal audiometric/vestibular studies and positive HSP-70.

The utility of positron emission tomography in the evaluation of autoimmune hearing loss. Mazlumzadeh M, Lowe VJ, Mullan BP, Fabry DA, McDonald TJ, Matteson EL. Otol Neurotol 2003 Mar; 24 (2), pp. 201–4.

GENE EXPRESSION

Gene Expression: Neoplasia/Herpes Simplex Virus Type I Thymidine Kinase (HSV1-tk)
2′-Deoxy-2′-Flouro-5-Methyl-1-β-D-Arabinofuranosyluracil (FMAU)/9-[(3-[^{18}F]-Fluoro-1-Hydroxy-2-Propoxy)Methyl]-Guanine ([^{18}F]-FHPG)/ 9-(4-[^{18}F]-Fluoro-3-Hydroxy-Methylbutyl)Guanine ([^{18}F]-FHBG)

Application: FMAU has demonstrated potential utilization as a positron emission tomography (PET) radiotracer for imaging HSV-TK gene expression.
Interpretation and Analysis: *In vitro* experiments demonstrate that the accumulation of [^{14}C]-FMAU in HSV-tk-expressing cells is 2.4-fold ($p < 0.02$), 4.0-fold ($p < 0.001$), and 5.3-fold ($p < 0.001$) higher than observed with wild-type cells at 1, 3, and 5 hours, respectively. *In vivo* studies reveal that the tumor uptake in HSV-tk-expressing cells is 2.3-fold ($p < 0.001$), 3.0-fold ($p < 0.001$), and 5.5-fold ($p < 0.001$) higher than the control cells at 1, 2, and 5 hours, respectively. FMAU is more sensitive compared to earlier studies using [^{18}F]-FHPG and [^{18}F]-FHBG in the same cell lines, although the specificity is less than FHBG. While FMAU labeled with PET isotopes may be useful for imaging HSV-tk-expressing tumors *in vivo*, multitracer studies across additional tumor models are necessary in order to identify an optimal PET radiotracer.

Evaluation of 2′-deoxy-2′-flouro-5-methyl-1-beta-D-arabinofuranosyluracil as a potential gene imaging agent for HSV-TK expression *in vivo*. Alauddin MM, Shahinian A, Gordon EM, Conti PS. Mol Imaging 2002 Apr–Jun; 1 (2), pp. 74–81.

Gene Expression: Thymidine Kinase Expression/ Neoplastic Disease: DNA Synthesis/Cellular Proliferation Rate

5-[^{125}I]-Iodo-4′-Thio-2′-Deoxyuridine (ITdU)/ 5-[^{125}I]-Iodo-2′-Deoxyuridine (IUdR)

Cellular/Molecular Mechanism of Action: 5-iodo-4′-thio-2′-deoxyuridine is a metabolically stable nucleoside that can function as a substrate for cellular thymidine kinase biochemical activity.
Application: Radioiodinated forms of ITdU and IUdR can be applied to detect elevations in DNA synthesis in cell populations that positively express thymidine kinase activity. In this capacity, both ITdU and IUdR can serve as indicator molecules for measuring alterations in cellular proliferation especially relevant to the characterization of neoplastic disease states.
Route of Administration: Usually ITdU or IUdR would most frequently be administered intravascularly. Theoretically, it may be possible to effectively image lesions if the agents are administered intrathecally for CNS tumors, by direct injection into tumors, or into the arterial network supplying a suspected neoplastic lesion.
Semi-Synthetic Methods: Radiolabeling of ITdU and 5-iodo-1-(4-thio-beta-D-arabinofuranosyl)uracil (ITAU) with [^{125}I] can be performed utilizing a destannylation reaction of the trimethylstannyl precursor of each nucleoside. The products can be isolated in high yields and with a $>$ 99% level of radiochemical purity.
Implementation: Evaluation of ITdU and ITAU for their ability to detect and characterize cellular proliferation rates as a function of thymidine kinase expression.

***Ex Vivo* Model:** Thymidine kinase-expressing L-M cells and thymidine kinase-deficient mutant L-M (TK$^-$) cells.
Interpretation and Analysis: [^{125}I]-ITdU is effectively phosphorylated by cytosolic nucleoside kinases and specifically incorporated into thymidine kinase-expressing L-M cells to a far greater extent than into thymidine kinase-deficient mutant L-M (TK$^-$) cells. In addition, *in vitro* cell metabolism studies employing [^{125}I]-ITdU reveal that [^{125}I]-ITdU is effectively and specifically incorporated into a DNA fraction ($>$ 90% at 60 minutes). Therefore, IUdR or [^{125}I]-ITdU appears to be an effective marker for the detection of DNA synthesis. Conversely, [^{125}I]-ITAU is neither remarkably phosphorylated by cytosolic nucleoside kinases nor notably incorporated into L-M cells relative to L-M (TK$^-$) cells. [^{125}I]-ITdU and [^{125}I]-ITAU show a higher resistance to phosphorolytic cleavage by recombinant thymidine phosphorylase than does [^{125}I]-IUdR. Furthermore, biodistribution of [^{125}I]-ITdU and [^{125}I]-ITAU display better *in vivo* radioiodination stability than do [^{125}I]-IudR preparations. [^{125}I]-ITdU also displays a significantly higher uptake in proliferating organs (thymus, spleen, small intestine, and bone) than in non-proliferating organs (brain, muscle, liver, and lung), as does [^{125}I]-IUdR, at 18 hours postinjection. As indicated by *in vitro* studies, [^{125}I]-ITAU does not show any significant uptake in proliferating organs. Therefore, radioiodinated ITdU is potentially useful as a proliferation-imaging agent, and further studies should clarify the usefulness of this compound as a SPECT tumor-imaging agent.

Rationale of 5-(125)I-iodo-4′-thio-2′-deoxyuridine as a potential iodinated proliferation marker. Toyohara J, Hayashi A, Sato M, Tanaka H, Haraguchi K, Yoshimura Y, Yonekura Y, Fujibayashi Y. J Nucl Med 2002 Sep; 43 (9), pp. 1218–26.

HEPATIC SYSTEM

Hepatic Function

1-[^{11}C]-Octanoate Positron Emission Tomography (PET)

Cellular/Molecular Mechanism of Action: 1-[^{11}C]-Octanoate.
Application: Evaluation of regional hepatic function using PET.
Considerations: The metabolism of 1-[^{11}C]-Octanoate in the liver is influenced by beta-oxidation.
Implementation: Evaluation of 1-[^{11}C]-Octanoate as a radiopharmaceutical for characterizing liver function when combined with PET.

Animal Model: Murine and rat.
Interpretation and Analysis: In biodistribution studies (mice), 1-[^{11}C]-octanoate is rapidly taken up by the liver. In the liver, 1-[^{11}C]-octanoate is present within parenchymal cells and is predominantly metabolized via beta-oxidation followed by a rapid clearance phase. In CCl_4-challenge tests (mice), significantly slower hepatic clearance is observed with 1-[^{11}C]-octanoate compared to negative reference controls. In PET studies (rats), the time-radioactivity curves in the liver show a 2-phase decrease, and compared with negative reference controls, the CCl_4-challenge test produces slower hepatic half-clearance time for the 1st phase that is related to beta-oxidation metabolism. A preliminary PET study of 1-[^{11}C]-octanoate metabolism in normal humans is consistent with findings observed in preliminary (animal) studies.

Evaluation of 1-[^{11}C]-octanoate as a new radiopharmaceutical for assessing liver function using positron emission tomography. Yamamura N, Magata Y, Kitano H, Konishi J, Saji H. Nucl Med Biol 1998 Jul; 25 (5), pp. 467–72.

Hepatic Function

[^{99m}Technetium] ([^{99m}Tc])-Diethylenetriamine Pentaacetic Acid (DTPA)-Galactosyl Human Serum Albumin

Cellular/Molecular Mechanism of Action: Diethylenetriamine pentaacetic acid generally functions as a chelating agent for heavy metal ion isotopes like [^{111}In].
Physiological Mechanism of Action: [^{99m}Tc]-DTPA-galactosyl human serum abumin is removed from the intravascular compartment by viable hepatocytes.
Application: Characterization of hepatic function without collection of blood samples for *in vitro* sample analysis.
Route of Administration: Intravenous.
Implementation: Delineate the clinical utility of a graphical method for estimating liver uptake and blood retention of [^{99m}Tc]-DTPA-galactosyl human serum albumin [^{99m}Tc]-GSA, using dynamic single photon emission computed tomography (SPECT) analysis. When considering the kinetics of [^{99m}Tc]-GSA, if it is assumed that (i) [^{99m}Tc]-GSA distributes only between blood and liver, and (ii) no metabolism of [^{99m}Tc]-GSA occurs during the observation period, a plot of liver counts vs cardiac blood-pool counts should, theoretically, be a straight line. From the slope and y-intercept of a regression line, coefficients for converting count-based liver and blood-pool data to the % injected dose (%ID) can be calculated. Applicability of the method can be delineated for dynamic SPECT data from cases of hepatic dysfunction. To validate this method, plasma concentrations (%ID/ml plasma) at 6, 15, and 30 minutes after the injection are estimated and compared with measured values. To investigate the clinical significance of the % liver uptake, the value obtained by this method is compared with results from conventional liver function tests, including serum albumin, the

hepaplastin test, prothrombin time, and indocyanine green clearance.

Human Model: (n = 30 cases with hepatic disease).

Interpretation and Analysis: In every data set, a plot of liver counts to cardiac blood-pool counts can be represented (fitted) well by a straight line ($p < 0.00001$). Plasma concentrations estimated by this method show good correlation with measured values at 6, 15 and 30 minutes after injection ($r = 0.748, 0.838, 0.875$, respectively; $p < 0.0001$). The liver uptake determined by the method shows good correlation with results from conventional hepatic function tests ($p < 0.002$). The described graphical method could provide an accurate estimate of %ID of [^{99m}Tc]-GSA in blood without the need for blood sampling. The liver uptake determined by this method could be a simple but useful quantitative indicator of hepatic function.

Estimation of fractional liver uptake and blood retention of [^{99m}Tc]-DTPA-galactosyl human serum albumin: An application of a simple graphical method to dynamic SPECT. Shuke N, Aburano T, Okizaki A, Zhao C, Nakajima K, Yokoyama K, Kinuya S, Watanabe N, Michigishi T, Tonami N. Nucl Med Commun 2003 May; 24 (5), pp. 503–11.

Hepatic Metabolism/Beta-Oxidation (Biochemical Capacity)
[^{123}I]-Para-Iodophenylvaleric ([^{123}I]-IPVA)/ [^{123}I]-Para-Iodophenylenanthic ([^{123}I]-IPEA)/ 1-[^{11}C]-Octanoate Positron Emission Tomography (PET) Alternative

Cellular/Molecular Mechanism of Action: Both [^{123}I]-IPVA and [^{123}I]-IPEA are metabolized primarily through biochemically mediated mechanisms of beta-oxidation (e.g., main pathway responsible for adenosine triphosphate [ATP] production in hepatocytes).

Application: Detection of alterations in beta-oxidation as a parameter for assessing hepatic function.

Implementation: The 2 radioiodinated omega-(4-iodophenyl)-medium chain fatty acids, IPVA and IPEA, can be utilized as radiopharmaceuticals for determining hepatocyte viability. Both *in vitro*/*in vivo* metabolite assays and biodistribution studies (normal mice) indicate that these compounds are actively assimilated by the liver and metabolized by beta-oxidation. However, the agent IPEA is more suitable as an imaging agent than is IPVA. Applying these imaging agents provides a methodology for evaluating postoperative prognosis or therapeutic efficacy.

Interpretation and Analyses: Imaging studies performed in cases of hepatitis applying [^{123}I]-IPEA as an imaging agent for single photon emission computed tomography (SPECT) reveal a 2-phase hepatic time-activity curve for the clearance of the radiopharmaceutical combination. However, the clearance is delayed depending on the severity of hepatitis. Such findings reflect a direct correlation that exists between 1st-phase clearance rate and hepatocyte ATP levels. Therefore, hepatocyte clearance rates can function as an index or parameter that reflects hepatocyte energy production capacity. Based on these considerations, [^{123}I]-IPEA can potentially be useful as a radiopharmaceutical for the diagnosis of hepatic viability based on energy metabolism.

Instrumentation: The application of [^{123}I]-IPVA and [^{123}I]-IPEA require the use of computer-integrated SPECT instrumentation, while 1-[^{11}C]-octanoate employs PET. The advantage of SPECT is that it is more widely used/available than is PET instrumentation.

Evaluation of radioiodinated medium chain fatty acids as new diagnostic agents for the determination of hepatic viability. Yamamura N, Magata Y, Konishi J, Saji H. Eur J Nucl Med 1999 Dec; 26 (12), pp. 1597–605.

Hepatic and Renal Cysts/Polycystic Kidney Disease: Autosomal Dominant
[^{18}F]-Fluoro-Deoxy-Glucose (FDG)

Background Information: Infection of a renal or hepatic cyst is a serious complication of autosomal dominant polycystic kidney disease (ADPKD). Although crucial for successful management, early diagnosis is difficult, largely because of non-specific symptoms and limitations of conventional imaging techniques. Because of an increased metabolic rate, inflammatory cells take up large amounts of glucose. [^{18}F]-FDG, therefore, represents a promising agent for detection of cyst infections using positron emission tomography (PET).

Implementation: Performance of [^{18}F]-FDG PET scan imaging in cases of ADPKD suspected of being affected by renal or hepatic cyst infection.

Human Model: Cases of suspected renal or hepatic cyst infection (e.g., n = 7 FDG PET scans in 3 ADPKD cases suspected of renal or hepatic cyst infection; n = 2 PET scans of case A [PET 1 and 2]; n = 1 PET scan of case B [PET 3]; and n = 4 PET scans of case C [PET 4, 5, 6, and 7].

Interpretation and Analysis: [^{18}F]-FDG PET can identify infected cysts in conditions of (i) renal cyst infection; (ii) hepatic cyst infection; and (iii) simultaneous renal and hepatic cyst infection. Evaluation of FDG PET imaging modalities has proven to be very helpful in establishing diagnoses and in excluding renal and hepatic cyst infections. Based on experience with FDG PET, this diagnostic strategy has promise as an imaging technique enabling early identification of renal and hepatic cyst infections in ADPKD patients.

Diagnosis of renal and hepatic cyst infections by 18-F-fluoro-deoxy-glucose positron emission tomography in autosomal dominant polycystic kidney disease. Bleeker-Rovers CP, de Sévaux RG, van Hamersvelt HW, Corstens FH, Oyen WJ. Am J Kidney Dis 2003 Jun; 41 (6), pp. E18–21.

Hepatic Function and Viability/Beta-Oxidation Capacity
p-[^{123}I]-Iodophenylenanthic Acid (IPEA)

Background Information: Radiopharmaceuticals that reflect beta-oxidation in hepatocytes will provide useful information on the prognosis after surgery or on the efficacy of treatment, because beta-oxidation is the main pathway responsible for the generation of adenosine triphosphate in hepatocytes. Previous reports have described 1-[^{11}C]-octanoate as a diagnostic agent for determination of hepatic viability by means of positron emission tomography (PET).

Application: The agent [^{123}I]-IPEA is a potentially useful radiopharmaceutical for the diagnosis of hepatic viability based on energy metabolism. Single photon emission computed tomography (SPECT) has the advantage of being more widely used than PET.

Route of Administration: [^{123}I]-IPEA (intravenous).

Semi-Synthetic Methods: Production of 2 radioiodinated omega-(4-iodophenyl)-medium chain fatty acids, *p*-iodophenylvaleric acid (IPVA) and *p*-iodophenylenanthic acid (IPEA), can be synthesized to determine their applicability as radiopharmaceuticals for determination of hepatic viability.

Implementation: Evaluation of IPVA and IPEA as candidate radiopharmaceuticals for characterizing hepatic viability. Subsequent evaluations can involve utilization of SPECT methodologies to determine the ability of [^{123}I]-IPEA to differentiate between conditions of hepatitis and normal liver function.

Animal Model: Rat (normal and hepatitis model).

Interpretation and Analysis: Metabolite analyses (*in vitro* and *in vivo*) and biodistribution studies in normal mice indicate that both compounds are actively taken up by the liver and metabolized by beta-oxidation. Investigations performed *in vivo,* however, indicate that [^{123}I]-IPEA is more suitable as an imaging agent than [^{123}I]-IPVA. Hepatic [^{123}I]-IPEA time-activity curves reveal a 2-phase clearance of radioactivity in both normal and hepatitis model rats, but the clearance is delayed depending on the severity of hepatitis. Furthermore, the clearance rate of the 1st phase correlates with ATP levels in hepatocytes, which can be used as an index for energy production capacity in hepatocytes. In conclusion, IPEA is metabolized predominantly by beta-oxidation, and the clearance of IPEA from the liver is closely associated with the ATP concentration in the liver. Thus, [^{123}I]-IPEA is a potentially useful radiopharmaceutical for diagnosis of hepatic viability based on energy metabolism.

Evaluation of radioiodinated medium chain fatty acids as new diagnostic agents for the determination of hepatic viability. Yamamura N, Magata Y, Konishi J, Saji H. Eur J Nucl Med 1999 Dec; 26 (12), pp. 1597–605.

Hepatic Function: Cirrhosis and Chronic Liver Disease/Single Photon Emission Computed Tomography (SPECT) Correlation with Hepatic Function Tests
[^{99m}Tc]-Phytate Colloid/Indocyanine Green

Cellular/Molecular Mechanism of Action: Indocyanine is removed from the intravascular compartment by the liver without being metabolized and is subsequently excreted into the bile.
Background Information: Accurate quantitative determination of liver function is critical in cirrhotic patients in order to predict outcome, particularly in cases that undergo hepatic resection or non-hepatic surgery. As colloid uptake by perfused Kupffer cells is proportional to perfused hepatocyte mass, quantitative liver/spleen scans may be used as an index of perfused hepatocyte mass.
Application: Indocyanine green hepatic-associated parameters can be correlated with findings observed with SPECT for the purpose of assessing liver function in various disease states, including hepatic cirrhosis.
Route of Administration: Indocyanine green (intravenous).
Implementation: Evaluate quantitative SPECT of [^{99m}Tc]-phytate colloid uptake by the liver as a test for hepatic function in cirrhotic patients. SPECT can be used to measure liver volume, quantitative colloid uptake by the liver, and percentage of injected dose/ml of liver tissue. Indocyanine green can be applied in concert with quantitative SPECT to assess liver function in conditions of hepatic cirrhosis.

Human Model: Cases of hepatic cirrhosis (n = 75); non-cirrhotic patients with chronic liver disease (n = 52); and patients without liver disease (n = 36).
Interpretation and Analysis: Although liver volume is similar among cirrhotic, non-cirrhotic, and chronic liver disease states, conditions of cirrhosis have significantly lower total quantitative uptake and quantitative uptake/ml compared to hepatic disease group types ($p < 0.001$). Quantitative liver uptake in cirrhotic patients highly correlates with Child-Pugh scores ($r = -0.64$, $p < 0.0001$) and with indocyanine green retention at 15 minutes ($r = -0.84$, $p < 0.0001$). Quantitative SPECT of the liver may be an additional, useful, non-invasive quantitative test for assessment of hepatic function and severity of liver disease in cirrhotic disease states.

Quantitative liver-spleen scan using single photon emission computerized tomography (SPECT) for assessment of hepatic function in cirrhotic patients. Zuckerman E, Slobodin G, Sabo E, Yeshurun D, Naschitz JE, Groshar D. J Hepatol 2003 Sep; 39 (3), pp. 326–32.

IMMUNE SYSTEM

Lymphocytes/Non-Invasive *In Vivo* Visualization/ Immunomodulating Agent Assessment/ Cell-Mediated Immune Responses
Adaptively Transferred Immune T-Lymphocytes/ Positron Emission Tomography (PET) Reporter Gene/ Micro-Positron Emission Tomography (Micro-PET)

Application: Non-invasive, quantitative, tomographic *in vivo* visualization of lymphocyte populations. Such methodologies can be used to assess the effects of immunomodulatory agents intended to potentiate the immune response to cancer, and can also be useful for the study of other cell-mediated immune responses, including autoimmune conditions.
Biological Cell Preparations: Splenic T lymphocytes are harvested and partially purified from animals that have rejected a tumor induced by Moloney murine sarcoma virus/Moloney murine leukemia virus (M-MSV/M-MuLV). A PET reporter gene is then introduced into the T lymphocyte populations and the preparations are then injected into tumor-bearing mice. Imaging analysis is performed by micro-PET using a substrate specific for the reporter gene. Specific localization of immune T cells to the antigen-positive tumor is detected over time, by sequential imaging.
Implementation: Characterization of neoplastic lesions in tumor-bearing mice facilitated by the injection of adoptively transferred immune T-lymphocytes applied in combination with micro-PET facilitated by the use of a reporter-specific substrate. Specific localization (migration) of immune T cells to the antigen-positive tumor is detected over time by sequential *in vivo* imaging.
Interpretation and Analysis: Naive T-lymphocytes do not localize to the tumor site and serves as an indication that preimmunization is a requirement. Autoradiography and immunohistochemistry analysis can be applied to corroborated micro-PET imaging analyses.

Quantitative imaging of the T cell antitumor response by positron-emission tomography. Dubey P, Su H, Adonai N, Du S, Rosato A, Braun J, Gambhir SS, Witte ON. Proc Natl Acad Sci U S A 2003 Feb 4; 100 (3), pp. 1232–7. Epub 2003 Jan 23.

INFLAMMATION AND SEPSIS

Bacterial Infections: Focal
[^{18}F]-2-Deoxy-2-Fluoro-D-Glucose (FDG)/[^{3}H]-FDG/ [^{125}I]-Human Serum Albumin ([^{125}I]-HSA)/L-[^{11}C]-Methionine/L-[^{3}H]-Methionine/[^{3}H]-Thymidine

Application: Detection of focal sites of septic inflammation within extravascular tissues applying positron emission tomography (PET) imaging modalities.
Implementation: Determination of the relative biodistribution of [^{18}F]- or [^{3}H]-FDG, [^{3}H]-thymidine, L-[^{11}C]- or [^{3}H]-methionine, gallium-67 citrate ([^{67}Ga]-citrate), and [^{125}I]-HSA in cases of focal sepsis (e.g., rat left calf muscles infected with *Escherichia coli* suspension). Uptake of [^{3}H]-FDG within infectious foci is evaluated by autoradiography of tissue histological sections.
Interpretation and Analysis: Although [^{18}F]-FDG, [^{67}Ga]-citrate, and [^{125}I]-HSA show comparatively high uptake in infected muscle (percentage activity of injected dose per gram of tissue normalized for weight in kilogram [%ID/g] $\times$ kg at 2 hours postinjection is approximately [^{18}F]-FDG, 0.184 ± 0.026 to 0.218 ± 0.046; [^{67}Ga]-citrate, 0.221 ± 0.016; [^{125}I]-HSA, 0.198 ± 0.019), the in-

fected muscle to blood ratio is much higher for [^{18}F]-FDG than for [^{67}Ga]-citrate or [^{125}I]-HSA ([^{18}F]-FDG, 10.31 ± 0.76 to 14.89 ± 2.26; [^{67}Ga]-citrate, 1.24 ± 0.67; [^{125}I]-HSA, 0.20 ± 0.02). Draining reactive lymph nodes also show higher accumulations of [^{18}F]-FDG than of [^{67}Ga]-citrate or [^{125}I]-HSA. The uptake of [^{3}H]-thymidine and L-[^{11}C]-methionine or [^{3}H]-methionine in the infected muscle is lower than that of [^{18}F]-FDG or [^{3}H]-FDG (at 2 hours postinjection, [^{3}H]-thymidine = 0.039 ± 0.005 and L-[^{3}H]-methionine = 0.063 ± 0.007 (%ID/g) × kg. Autoradiographs show that the highest [^{3}H]-FDG uptake is seen in the area of inflammatory cell infiltration surrounding the necrotic region. In conclusion, [^{18}F]-FDG, which rapidly accumulates in sites of bacterial infection and in reactive lymph nodes with a high target-to-background ratio, appears to be a promising agent for the detection of infection.

Uptake of positron emission tomography tracers in experimental bacterial infections: a comparative biodistribution study of radiolabeled FDG, thymidine, L-methionine, [^{67}Ga]-citrate, and [^{125}I]-HSA. Sugawara Y, Gutowski TD, Fisher SJ, Brown RS, Wahl RL. Eur J Nucl Med 1999 Apr; 26 (4), pp. 333–41.

METABOLISM: HYPOXIA, ISCHEMICA, MITOSIS AND PROLIFERAITON

Metabolism/Hypoxia of Tissues
2-Nitroimidazoles/[^{18}F]-Labeled EF5

Cellular/Molecular Mechanism of Action: The class of pharmaceuticals typified by the 2-nitroimidazoles is bioreductively activated and bind to cell populations in tissues that have developed a hypoxic environment.

Background Information: The measurement of pathologically low levels of tissue pO_2 is an important diagnostic goal for determining the prognosis of many clinically important diseases, including cardiovascular insufficiency, stroke, and cancer.

Application: Potential application as a carrier imaging agent in the detection of ischemic lesions, including those recognized in cardiovascular insufficiency, CNS stroke, and neoplastic disease.

Route of Administration: Intravenous.

Dosage: Dependent upon molecular detector system applied according to conditions of the pathological state.

Interpretation and Analysis: Bioreductively activated pharmaceuticals like the 2-nitroimidazole class compounds bind to cells at a rate that is maximal under conditions of severe hypoxia (e.g., less than 0.05% oxygen) and are inhibited as a function of increasing oxygen concentration (according to Michaelis-Menten kinetics). A number of detection possibilities exist for the drug adducts, including invasive assays that can measure drug adducts in tissue sections at cell-to-cell levels of resolution. Use of such agents in non-invasive assays is important and, to this end, a number of drugs have been conjugated with radioactive isotopes suitable for detection by nuclear medicine techniques. In contrast to invasive assays, resolution and contrast are much more limited with non-invasive assays. Thus, there are many factors contributing to the balance of pros and cons for the non-invasive vs invasive use of 2-nitroimidazole drugs as tissue/organ/lesion hypoxia detectors. Such compounds are suitable for clinical use as molecular platforms for positron emission tomography (PET) imaging (e.g., [^{18}F]-labeled EF5: current clinical trials using invasive assays).

Non-invasive PET and SPECT imaging of tissue hypoxia using isotopically labeled 2-nitroimidazoles. Koch CJ, Evans SM. Adv Exp Med Biol 2003; 510, pp. 285–92.

Metabolism: Hypoxia of Tissues/Cardiovascular Insufficiency/Stroke/Neoplastic Disease
2-Nitroimidazole/[^{18}F]-Labeled EF5

Background Information: A class of bioreductively activated drugs, typified by the 2-nitroimidazoles, has excellent potential for measuring pathologically low tissue pO_2 levels. Such drugs bind to cells at a rate that is maximal under conditions of severe hypoxia (e.g., less than 0.05% oxygen) and are inhibited, with Michaelis-Menten kinetics, as a function of increasing oxygen concentration. A number of detection possibilities exist for the drug adducts, including invasive assays that can measure drug adducts in tissue sections at cell-to-cell levels of resolution. Use of such agents in non-invasive assays is important and, to this end, a number of drugs have been conjugated with radioactive isotopes suitable for detection by nuclear medicine techniques. In contrast with the invasive assays, resolution and contrast are much more limited with the non-invasive assays. Thus, there are many factors contributing to the balance of pros and cons for the non-invasive vs invasive use of 2-nitroimidazole drugs as hypoxia detectors.

Application: Potential utilization for the detection and characterization of pathologically hypoxic regions within tissues and organ systems relevant to the diagnosis and prognosis of disease states related to cardiovascular insufficiency, stroke, and cancer.

Non-invasive PET and SPECT imaging of tissue hypoxia using isotopically labeled 2-nitroimidazoles. Koch CJ, Evans SM. Adv Exp Med Biol 2003; 510, pp. 285–92.

Metabolism: Hypoxia of Tissues/Neoplastic Disease/ Cardiovascular: Myocardial Ischemia Secondary to Infarction/Central Nervous System (CNS): Vascular Stroke
[^{18}F]-Fluoroazomycinarabinofuranoside ([^{18}F]-FAZA)/ [^{18}F]-Fluoromisonidazole ([^{18}F]-FMISO)

Cellular/Molecular Mechanism of Action: [^{18}F]-FAZA, a recently developed hypoxia tracer for positron emission tomography (PET) imaging of tissue hypoxia, with an established tracer [^{18}F]-FMISO, both function as tracers or indicator molecules for tissue hypoxia.

Application: Potential detection of conditions associated with tissue hypoxia such as those recognized with neoplastic disease, CNS vascular stroke, and myocardial ischemia secondary to vascular infarction.

Implementation: Comparison of the uptake of [^{18}F]-FAZA with [^{18}F]-FMISO. Analysis can be performed *in vitro* with carcinoma cell lines or *in vivo* in natural or experimentally transplanted neoplastic lesions.

Animal and Cellular Models: Rat (Walker 256 rat carcinosarcoma cells propagated *in vitro* in tissue culture and *in vivo* in experimental rat tumors 11–12 days after tumor cell implantation).

Interpretation and Analysis: Hypoxia-selective uptake *in vitro* of both [^{18}F]-FAZA and [^{18}F]-FMISO in tumor cells, 20 and 100 minutes postincubation, is of the same magnitude (20 minutes: 1.24 ± 0.4% ([^{18}F]-FAZA); 1.19 ± 0.7% ([^{18}F]-FMISO); 100 minutes: 3.6 ± 1.6% ([^{18}F]-FAZA); 3.3 ± 1.7% ([^{18}F]-FMISO)). Positron emission tomography imaging reflects a similar radiotracer distribution in rat tumors for [^{18}F]-FAZA and [^{18}F]-FMISO 1 hour after radiotracer injection. The concentration of [^{18}F]-FAZA in tumors as measured by PET, however, is lower in comparison to [^{18}F]-FMISO [SUV (FAZA) = 0.61 ± 0.2 vs SUV(FMISO) = 0.92 ± 0.3, $p < 0.05$] although the tumor-to-muscle ratios for [^{18}F]-FAZA and [^{18}F]-

FMISO do not differ in the PET images obtained after 1 hour [SUV(FAZA) = 2.5 ± 0.5 vs SUV(FMISO) = 2.9 ± 0.7]. A comparison of PET data 3 hours postinjection [SUV(FAZA) = 3.0 ± 0.5 vs SUV(FMISO) = 4.6 ± 1.8, $p < 0.05$] demonstrates a lower [^{18}F]-FAZA uptake that indicates a lower sensitivity of [^{18}F]-FAZA compared to [^{18}F]-FMISO for detecting hypoxic regions at a longer time period. However, there is a faster elimination of [^{18}F]-FAZA from blood, viscera, and muscle tissue, via the renal system. The advantage of a faster reduction of nonspecific binding, in light of similar or marginally lower tumor uptake, warrants further investigation of [^{18}F]-FAZA as a marker of regional hypoxia in tumors.

[^{18}F]-fluoroazomycinarabinofuranoside (^{18}FAZA) and [^{18}F]-fluoromisonidazole (^{18}FMISO): A comparative study of their selective uptake in hypoxic cells and PET imaging in experimental rat tumors. Sorger D, Patt M, Kumar P, Wiebe LI, Barthel H, Seese A, Dannenberg C, Tannapfel A, Kluge R, Sabri O. Nucl Med Biol 2003 Apr; 30 (3), pp. 317–26.

Metabolism: Hypoxia of Tissues/Neoplastic Disease/ Design of Cancer Treatment Schedules
Hypoxia-Activated Diagnostic Pharmaceuticals

Conclusive research has shown that regions of acute/chronic hypoxia, which exist within the majority of solid tumors, have a profound influence on the therapeutic outcome of cancer chemotherapy and radiotherapy and are a strong prognostic factor of disease progression and survival. A strong argument therefore exists for assessing the hypoxic fraction of tumors prior to patient treatment, and for tailoring this treatment accordingly. Tumor hypoxia also provides a powerful physiological stimulus that can be exploited as a tumor-specific condition, allowing for the rational design of hypoxia-activated anticancer drugs or novel hypoxia-regulated gene therapy strategies.

Hypoxia and oxidative stress. Tumor hypoxia-therapeutic considerations. Williams KJ, Cowen RL, Stratford IJ. Breast Cancer Res. 2001; 3 (5), pp. 328–31. Epub 2001 Aug 07.

Metabolism: Hypoxia of Tissues/Neoplastic Disease: Prognostic Indicator for Therapeutic Intervention
[2-(2-Nitro-1[H]-Imidazol-1-yl)-N-(2,2,3,3,3-Pentafluoropropyl)-Acetamide]/[^{18}F]-EF5

Background Information: Tumor hypoxia is an important prognostic indicator for cancer therapy outcome. EF5 [2-(2-nitro-1[H]-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)-acetamide] has been employed to measure tumor hypoxia in animals and humans using immunohistochemical methods. EF5 is a lipophilic molecule designed to produce a very uniform biodistribution, a feature of obvious benefit for use in positron emission tomography (PET) imaging. Previous studies have demonstrated that early tissue culture passage 9L tumors are not severely hypoxic and that Q7 tumors are characterized by heterogeneous regions of tumor hypoxia (e.g., Q7 tumors are usually more hypoxic than early passage 9L tumors).
Application: Non-invasive PET imaging of tumors using [^{18}F]-labeled EF5 (rats).
Route of Administration: [^{18}F]-EF5 (intravascular). [^{18}F]-EF5 is excreted primarily via the urinary system.
Dosage: [^{18}F]-EF5 (50–100 μCi in 30 mg/kg carrier non-radioactive EF5). The carrier is used to ensure drug biodistribution comparable to prior studies using immunohistochemical methods.
Implementation: In tumors of a small size, partial volume effects may result in underestimation of concentration of the compound. Validation of PET data can be performed by gamma counting of imaged tissue. Acceptable tumor models include Morris 7777 (Q7) hepatoma (e.g., n = 5) and 9L glioma (e.g., n = 2) grown subcutaneously in rats. Demonstration of non-invasive PET imaging of rat (e.g., n = 7) with tumors using [^{18}F]-labeled EF5 is performed at various time points following [^{18}F]-EF5 administration.
Interpretation and Analysis: Images obtained 10 minutes following drug administration demonstrate that EF5 distributes evenly to all organ systems, including brain. Later images show increased uptake in most Q7 tumors compared with muscle. Liver uptake remains relatively constant over the same time period. Tumor-to-muscle ratios range from 0.82 to 1.73 (based on PET images at 120 minutes postinjection) and 1.47 to 2.95 (based on gamma counts at approximately 180 minutes postinjection). Tumors are easily visible by 60 minutes postinjection when the final tumor-to-muscle ratios (based on gamma counts) are 2 or greater. Neither the 9L tumors nor the smallest Q7 tumor meet this criterion, and therefore these tumors are not seen on the PET images. These preliminary results suggest that [^{18}F]-EF5 is a promising agent for non-invasive assessment of tumor hypoxia. Research investigations are under way to delineate the diagnostic efficacy of such preparations in brain tumor cases.
Instrumentation: HEAD Penn-PET scanner.

Non-invasive imaging of tumor hypoxia in rats using the 2-nitroimidazole [^{18}F]-EF5. Ziemer LS, Evans SM, Kachur AV, Shuman AL, Cardi CA, Jenkins WT, Karp JS, Alavi A, Dolbier WR Jr, Koch CJ. Eur J Nucl Med Mol Imaging 2003 Feb; 30 (2), pp. 259–66. Epub 2002 Nov 23.

Metabolism: Ischemic Lesions/Central Nervous System (Brain)/N-Methyl-D-Aspartate (NMDA) Receptor Activation
N-(1-Naphthyl)-N′-(3-[^{125}I]-Iodophenyl)-N′-Methylguanidine

Cellular/Molecular Mechanism of Action: N-(1-naphthyl)-N′-(3-iodophenyl)-N′-methylguanidine or CNS 1261 is a ligand that interacts with NMDA receptors following their activation.
Application: [^{125}I]-CNS 1261 can potentially function as a radioligand for imaging NMDA receptor activation.
Route of Administration: [^{125}I]-CNS 1261 (intravascular).
Semi-Synthetic Methods: Preparation of [^{125}I]-CNS 1261 can be prepared by radioiodination of *N*-(1-naphthyl)-*N′*-(3-tributylstannylphenyl)-*N′*-methylguanidine using Na[^{125}I] and peracetic acid.
Implementation: Detection of regional areas of cerebral ischemia.
Animal Model: Rat.
Interpretation and Analysis: Uptake of [^{125}I]-CNS 1261 *in vivo* reflects NMDA receptor distribution in normal rat brain, whereas in ischemic rat brain, uptake is markedly increased in areas of NMDA receptor activation. Radiolabeled CNS 1261 appears to be a good candidate for further development as a single photon emission computed tomography tracer in the investigation of NMDA receptor activation in conditions of cerebral ischemia.

Synthesis and binding characteristics of N-(1-naphthyl)-N′-(3-[^{125}I]-iodophenyl)-N′-methylguanidine ([^{125}I]-CNS 1261): A potential SPECT agent for imaging NMDA receptor activation. Owens J, Tebbutt AA, McGregor AL, Kodama K, Magar SS, Perlman ME, Robins DJ, Durant GJ, McCulloch J. Nucl Med Biol 2000 Aug; 27 (6), pp. 557–64.

Metabolism of Lipids: Fatty Acid Lipids/Central Nervous System [CNS]): Beta-Oxidation/ Incorporation into Membrane Structures
[U-^{14}C]-Palmitate/[1-^{14}C]-Arachidonate/Methyl 2-Tetradecylglycidate

Cellular/Molecular Mechanism of Action: Both palmitate and arachidonic acid are saturated fatty acids that are a component of

the external cell membrane lipid bilayer. In the CNS, [U-^{14}C]-palmitate undergoes beta-oxidation. Methyl 2-tetradecylglycidate (MEP) is an irreversible inhibitor of carnitine palmitoyltransferase I.

Application: In radiolabeled form, palmitate can be utilized as an *in vivo* probe for detecting alterations in brain lipid metabolism.

Route of Administration: [U-^{14}C]-palmitate (intravascular), [1-^{14}C]-arachidonate (intravascular), methyl 2-tetradecylglycidate (oral).

Dosage: Methyl 2-tetradecylglycidate (10–25 mg/kg).

Implementation: Determination of the effect of inhibiting mitochondrial beta-oxidation of free fatty acids on the incorporation of radiolabeled free fatty acids into brain lipids. Saturated fatty acid [U-^{14}C]-palmitic acid or the polyunsaturated fatty acid [1-^{14}C]-arachidonate (AA) are administered 2, 4, and 6 hours after methyl 2-tetradecylglycidate (MEP).

Animal Model: Rat (irreversible inhibition of carnitine palmitoyltransferase I in males induced through the use of methyl 2-tetradecylglycidate).

Interpretation and Analysis: Methyl 2-tetradecylglycidate increases brain [U-^{14}C]-palmitate-associated radioactivity 2-fold and decreases brain aqueous radioactivity 3- to 5-fold relative to control values at all pretreatment times. The effect is due to a prolongation of the plasma integral of [U-^{14}C]-palmitate due to peripheral inhibition of beta-oxidation, and to direct inhibition of beta-oxidation of the tracer within the brain. Conversely, methyl 2-tetradecylglycidate has no effect on brain organic radioactivity after infusion of [1-^{14}C]-arachidonic acid. Increasing the interval between methyl 2-tetradecylglycidate administration and [U-^{14}C]-palmitate infusion from 2 to 6 hours results in a dramatic redistribution of [U-^{14}C]-palmitate within brain lipids. The percentage of radioactivity in phospholipids decreases from 65 to 33%, whereas that in the free fatty acid fraction increases from 10 to 47% and that in triglycerides becomes elevated 2- to 3-fold over reference controls. Methyl 2-tetradecylglycidate may facilitate the use of radiolabeled palmitate as an *in vivo* probe for detecting alterations in brain lipid metabolism using quantitative autoradiography or positron emission tomography.

Instrumentation: Positron emission tomography.

Effect of inhibition of beta-oxidation on incorporation of [U-14C]-palmitate and [1-14C]-arachidonate into brain lipids. Freed LM, Wakabayashi S, Bell JM, Rapoport SI. Brain Res 1994 May 9; 645 (1–2), pp. 41–8.

Metabolism of Lipids: Phosphatidylinositol/ Central Nervous System (CNS) *[^{11}C]-Inositol/[^{11}C]-Inositol-1-Phosphate (IP)*

Application: Brain diagnostic imaging agent used in positron emission tomography (PET) imaging techniques for characterizing phosphatidylinositol metabolism.

Route of Administration: Intravenous.

Semi-Synthetic Methods: Radiolabeled inositol (Ins) and inositol-1-phosphate (IP) can be derived from radiolabeled glucose by utilizing brief sequential enzyme reactions. Rapid separation and fractionation of enzymes and labeled products from the reaction mixture can be achieved by high-performance liquid chromatography (HPLC) utilizing a gel-permeation chromatography column.

Interpretation and Analysis: Examination of the biodistribution of Ins, IP, and their acetylated analogs suggests that intact Ins labeled with [^{11}C] would be more effective than [^{11}C]-labeled acetylated Ins as a brain diagnostic agent for PET studies for determining the metabolism of phosphatidylinositol and its role as a 2nd messenger within the brain.

Instrumentation: Computer-integrated PET instrumentation.

Sequential enzymatic synthesis and biodistribution of radiolabeled inositol and inositol analogs. Sasaki T, Ogihara-Umeda I, Kojima S, Nishigori H. Int J Rad Appl Instrum [A] 1991; 42 (1), pp. 97–101.

Metabolism/Mitosis and Proliferation Rate *3′-Deoxy-3′-[^{18}F]-Fluorothymidine ([^{18}F]-FLT)*

Cellular/Molecular Mechanism of Action: Thymidine is rapidly assimilated by proliferating cell populations (e.g., incorporated into nucleic acids).

Application: [^{18}F]-FLT has been designed as a new positron emission tomography (PET) imaging agent for *in vivo* visualizing of cellular proliferation based on the incorporation of thymidine into nucleic acid.

Route of Administration: Variable but most often intravascular.

Semi-Synthetic Methods: A reliable radiosynthesis of [^{18}F]-FLT has been developed based on [^{18}F]10 mCi (370 MBq) of radiochemically pure [^{18}F]1 Ci/μmol (37 GBq/μmol) at end of synthesis (EOS) within 100 minutes and in 13% radiochemical yield (end of bombardment [EOB]; 7% EOS).

Implementation: Evaluation of neoplastic lesions that display relatively rapid rates of proliferation/growth.

Interpretation and Analysis: Detection of increased radioactivity at a specific anatomical location corresponds to a region that has a relatively high rate of cellular proliferation.

Radiosynthesis of 3′-deoxy-3′-[^{18}F]-fluorothymidine: [^{18}F]-FLT for imaging of cellular proliferation *in vivo*. Grierson JR, Shields AF. Nucl Med Biol 2000 Feb; 27 (2), pp. 143–56.

Metabolism/Mitosis and Proliferation Rate *2′-Deoxy-2′-[^{18}F]-Fluoro-β-D-Arabinofuranosyl/ [^{18}F]-FAU, [^{18}F]-FMAU, [^{18}F]-FBAU, [^{18}F]-FIAU*

Semi-Synthetic Methods: Efficient and reliable synthesis of 2′-deoxy-2′-[^{18}F]-fluoro-β-D-arabinofuranosyl nucleosides.

Interpretation and Analysis: Overall decay-corrected radiochemical yields of 35 to 45% of 4 analogs, FAU, FMAU, FBAU, and FIAU, are routinely obtained in $>$ 98% radiochemical purity and with specific activities of greater than 3 Ci/μmol (110 MBq/μmol) in a synthesis time frame of approximately 3 hours. When approximately 220 mCi (8.15 GBq) of starting [^{18}F]-fluoride is used, 25–30 mCi (0.93–1.11 GBq) of product (enough to image 2 patients sequentially) is typically obtained.

Synthesis of 2′-deoxy-2′-[^{18}F]-fluoro-beta-D-arabinofuranosyl nucleosides, [^{18}F]-FAU, [^{18}F]-FMAU, [^{18}F]-FBAU, and [^{18}F]-FIAU, as potential PET agents for imaging cellular proliferation. Synthesis of [^{18}F]-labeled FAU, FMAU, FBAU, FIAU. Mangner TJ, Klecker RW, Anderson L, Shields AF. Nucl Med Biol 2003 Apr; 30 (3), pp. 215–24.

Metabolism/Mitosis and Proliferation Rate *3′-Deoxy-3′-[^{18}F]-Fluorothymidine ([^{18}F]-FLT)*

Cellular/Molecular Mechanism of Action: [^{18}F]-FLT is a nucleoside analogue that enters cells and is phosphorylated by human thymidine kinase 1, but the 3′ substitution prevents further incorporation into DNA.

Application: [^{18}F]-FLT is a positron emission tomography (PET) imaging agent that shows promise for studying cellular proliferation in human cancers.

Implementation: Estimations of radiation dosimetry for [^{18}F]-FLT can be calculated based on data for time-dependent tissue concentrations of radioactivity for blood samples and PET images (e.g., n = 18 cases) after intravenous injection. Radiation-absorbed doses are calculated using the Medical Internal Radiation Dose (MIRD)

Committee methods, taking into account variations that are based on the distribution of activities observed in individual cases. Effective dose equivalent (EDE) is calculated using International Commission on Radiological Protection Publication 60 tissue-weighting factors for the standard man and woman.

Interpretation and Analysis: For a single bladder voiding at 6 hours after [^{18}F]-FLT injection, the [^{18}F]-FLT EDE (mean ± standard deviation [SD]) is 0.028 ± 0.012 mSv/MBq (103 ± 43 mrem/mCi) for a standard male patient and 0.033 ± 0.012 mSv/MBq (121 ± 43 mrem/mCi) for a standard female patient. The organ that receives the highest dose is the bladder (male, 0.179 mGy/MBq [662 mrad/mCi]; female, 0.174 mGy/MBq [646 mrad/mCi]), followed by the liver (male, 0.045 mGy/MBq [167 mrad/mCi]; female, 0.064 mGy/MBq [238 mrad/mCi]), the kidneys (male, 0.035 mGy/MBq [131 mrad/mCi]; female, 0.042 mGy/MBq [155 mrad/mCi]), and the bone marrow (male, 0.024 mGy/MBq [89 mrad/mCi]; female, 0.033 mGy/MBq [122 mrad/mCi]).

Organ dose estimates for [^{18}F]-FLT are comparable to those associated with other commonly performed nuclear medicine tests, and the potential radiation risks associated with [^{18}F]-FLT PET imaging are within accepted limits.

[^{18}F]-fluorothymidine radiation dosimetry in human PET imaging studies. Vesselle H, Grierson J, Peterson LM, Muzi M, Mankoff DA, Krohn KA. J Nucl Med 2003 Sep; 44 (9), pp. 1482–8.

Metabolism/Mitosis and Proliferation Rate
[^{18}F]-FAU ([^{18}F] 1-(2′-Deoxy-2′-Fluoro-β-D-Arabinofuranosyl)Uracil)

Cellular/Molecular Mechanism of Action: The agent FAU or [1-(2′-deoxy-2′-fluoro-beta-D-arabinofuranosyl)uracil] is incorporated into strands of DNA as a "false" or "decoy" nucleotide and functions as an inhibitor of DNA synthesis.

Physiological Mechanism of Action: Functions as an inhibitor of DNA synthesis, thereby inhibiting the synthesis of viable messenger RNA (mRNA/transcription) or biologically functional protein fractions (translation).

Application: Radiofluorination of FAU to produce [^{18}F]-FAU or [^{18}F] 1-(2′-deoxy-2′-fluoro-beta-D-arabinofuranosyl)uracil is suitable as a diagnostic radiopharmaceutical for positron emission tomography (PET) imaging procedures.

Route of Administration: [^{18}F]-FAU.

Implementation: Determination of the pharmacokinetic and relative organ/tissue distribution of [^{18}F]-FAU following injection of the tracer through the combined application of PET imaging (duration = ≥ 4 hours) and high-performance liquid chromatography (HPLC) analysis of collected blood, urine, and tissues/organs.

Animal Model: Canine (e.g., n = 3 normal dogs).

Interpretation and Analysis: In an *in vivo* environment, [^{18}F]-FAU is evenly distributed throughout most of organ systems and tissues. In sharp contrast to thymidine and its analogs, the marrow has less retention of [^{18}F]-FAU than non-proliferating tissues.

Imaging [^{18}F]-FAU [1-(2′-deoxy-2′-fluoro-beta-D-arabinofuranosyl) uracil] in dogs. Sun H, Collins JM, Mangner TJ, Muzik O, Shields AF. Nucl Med Biol 2003 Jan; 30 (1), pp. 25–30.

Metabolism/Mitosis and Proliferative Activity/ Neoplastic Disease: DNA Synthesis/Cellular Proliferation Rate/Thymidine Kinase Expression
5-Iodo-4′-Thio-2′-Deoxyuridine (ITdU)

Cellular/Molecular Mechanism of Action: ITdU is a metabolically stable nucleoside that can function as a substrate for cellular thymidine kinase biochemical activity.

Application: Radioiodinated forms of ITdU and 5-iodo-2′-deoxyuridine (IUdR) can be applied to detect elevations in DNA synthesis in cell populations that positively express thymidine kinase activity. In this capacity, both ITdU and IUdR can serve as indicator molecules for measuring alterations in cellular proliferation especially relevant to the characterization of neoplastic disease states.

Route of Administration: Usually ITdU or IUdR would most frequently be administered intravascularly. Theoretically, it may be possible to effectively image lesions if the agents are administered intrathecally for central nervous system (CNS) tumors, by direct injection into tumors, or into the arterial network supplying a suspected neoplastic lesion.

Semi-Synthetic Methods: Radiolabeling of ITdU and 5-iodo-1-(4-thio-beta-D-arabinofuranosyl)uracil (ITAU) with [^{125}I] can be performed utilizing a destannylation reaction of the trimethylstannyl precursor of each nucleoside. The products can be isolated in high yields and with a > 99% level of radiochemical purity.

Implementation: Evaluation of ITdU and ITAU for their ability to detect and characterize cellular proliferation rates as a function of thymidine kinase expression.

***Ex Vivo* Model:** Thymidine kinase-expressing L-M cell rather than a thymidine kinase-deficient mutant L-M (TK$^-$) cell.

Interpretation and Analysis: [^{125}I]-ITdU is effectively phosphorylated by cytosolic nucleoside kinases and specifically incorporated into thymidine kinase-expressing L-M cells to a far greater extent than into thymidine kinase-deficient mutant L-M (TK$^-$) cells. In addition, *in vitro* cell metabolism studies employing [^{125}I]-ITdU reveal that [^{125}I]-ITdU is effectively and specifically incorporated into a DNA fraction (> 90% at 60 minutes). Therefore, IUdR or [^{125}I]-ITdU appears to be an effective marker for the detection of DNA synthesis. Conversely, [^{125}I]-ITAU is neither remarkably phosphorylated by cytosolic nucleoside kinases nor notably incorporated into L-M cells relative to L-M (TK$^-$) cells. [^{125}I]-ITdU and [^{125}I]-ITAU show a higher resistance to phosphorolytic cleavage by recombinant thymidine phosphorylase than does [^{125}I]-IUdR. Furthermore, biodistribution of [^{125}I]-ITdU and [^{125}I]-ITAU display better *in vivo* radioiodination stability than do [^{125}I]-IudR preparations. [^{125}I]-ITdU also displays a significantly higher uptake in proliferating organs (thymus, spleen, small intestine, and bone) than in non-proliferating organs (brain, muscle, liver, and lung), as does [^{125}I]-IUdR, at 18 hours postinjection. As indicated by *in vitro* studies, [^{125}I]-ITAU does not show any significant uptake in proliferating organs. Therefore, radioiodinated ITdU is potentially useful as a proliferation-imaging agent, and further studies should clarify the usefulness of this compound as a single photon emission computed tomography (SPECT) tumor-imaging agent.

Rationale of 5-[^{125}I]-iodo-4′-thio-2′-deoxyuridine as a potential iodinated proliferation marker. Toyohara J, Hayashi A, Sato M, Tanaka H, Haraguchi K, Yoshimura Y, Yonekura Y, Fujibayashi Y. J Nucl Med 2002 Sep; 43 (9), pp. 1218–26.

MUSCULOSKELETAL SYSTEM

Osteomyelitis (Chronic)/Inflammatory Spondylitis
[^{18}F]-Fluoro-Deoxy-Glucose (FDG)

Cellular/Molecular Mechanism of Action: Fluoro-deoxyglucose functions as a biochemical analogue of endogenous deoxyglucose within various cell populations.

Application: Detection and characterization of septic osteomyelitis and inflammatory spondylitis conditions and their response to therapeutic intervention. Unlike computed tomography and magnetic resonance imaging, FDG positron emission tomography (PET) is not affected by metal implants used for the management of fractures.

In the early postoperative phase, FDG PET seems to be of limited value owing to unspecific tracer uptake.

Implementation: Detection and characterization of acute or chronic osteomyelitis and inflammatory spondylitis (e.g., n = 15 cases). Results from FDG PET image analysis are subsequently correlated with histopathological findings applying tissue samples collected intraoperatively (e.g., n = 15/21).

Interpretation and Analysis: Histopathological analysis is able to detect evidence of osteomyelitis or inflammatory spondylitis in a high percentage of cases (e.g., n = 15/15; n = 7/15 acute osteomyelitis, n = 8/15 chronic osteomyelitis or inflammatory spondylitis). Similarly, image analysis using FDG PET is also highly sensitive in detecting cases of acute or chronic osteomyelitis and inflammatory spondylitis (e.g., n = 15/15). Tracer uptake almost invariably correlates with histopathological findings (e.g., n = 15/15). Bone scintigraphy yields a high percentage of true-positive images (e.g., n = 10/11) with a small probability of false-negative results (e.g., n = 1/11). Monitorization of cases in concert with a normalization of clinical parameters usually corresponds to normal or clearly reduced tracer uptake in situations of resolving osteomyelitis and inflammatory spondylitis (e.g., n = 2/2). In early postoperative monitoring, it is impossible to differentiate between postsurgical reactive changes and further infection using FDG PET. Therefore, acute and chronic osteomyelitis of the peripheral as well as the central skeleton can be detected using FDG PET. Osteomyelitis can be differentiated from soft tissue infection surrounding the bone.

Fluorine-18 fluoro-deoxy-glucose PET in infectious bone diseases: Results of histologically confirmed cases. Kalicke T, Schmitz A, Risse JH, Arens S, Keller E, Hansis M, Schmitt O, Biersack HJ, Grunwald F. Eur J Nucl Med 2000 May; 27 (5), pp. 524–8.

NEOPLASIA

Neoplastic Disease Imaging
[^{18}F]-2-Fluoro-2-Deoxy-D-Glucose (FDG)/ [^{18}F]-2-Fluoro-2-Deoxy-D-Mannose (FDM)

Cellular/Molecular Mechanism of Action: Radiofluorinated [^{18}F] glucose analogs are preferentially assimilated by neoplastic cell types at a higher level than normal cells in part due to differences in biochemical metabolism. [^{18}F]-FDM is a byproduct of [^{18}F]-FDG synthesis.

Application: Detection and characterization of neoplastic disease states applying nuclear imaging techniques (e.g., positron emission tomography [PET]).

Implementation: Evaluation of the relationship between pharmacokinetic profiles and capacity to detect neoplastic disease utilizing [^{18}F]-FDG and [^{18}F]-FDM glucose analogs.

Animal Model: Rat (tumor).

Interpretation and Analysis: Neoplastic lesions can be detected by increased uptake of radiofluorinated glucose analogs utilizing nuclear medicine scintigraphy imaging techniques.

Tissue distribution studies have shown high tumor uptake for both [^{18}F]-FDG and [^{18}F]-FDM reagents. Tumor uptake reaches 2.65 ± 0.61% dose [^{18}F]-FDG/g and 2.65 ± 0.81% dose [^{18}F]-FDM/g at 60 minutes and remains relatively constant until 120 minutes. Blood clearance for both [^{18}F]-FDG and [^{18}F]-FDM is very rapid and tumor-to-blood ratios reach 22.1 and 29.4 at 60 minutes, respectively. Tumor-to-tissue ratios for both radiopharmaceuticals is very high in most organs, especially in the liver, kidney, and pancreas. Positron emission tomography of rabbit tumor with [^{18}F]-FDM clearly can delineate the main tumor, central necrosis, and lymph node metastases.

Experimental data suggest that [^{18}F]-FDM, which is a byproduct of [^{18}F]-FDG synthesis, is also an excellent cancer diagnostic agent, as is [^{18}F]-FDG. This is not only a new feature of [^{18}F]-FDM but also an economical improvement on cancer diagnosis by PET.

Experimental study for cancer diagnosis with positron-labeled fluorinated glucose analogs: [^{18}F]-2-fluoro-2-deoxy-D-mannose: A new tracer for cancer detection. Fukuda H, Matsuzawa T, Abe Y, Endo S, Yamada K, Kubota K, Hatazawa J, Sato T, Ito M, Takahashi T, Iwata R, Ido T. Eur J Nucl Med 1982; 7 (7), pp. 294–7.

Neoplasia: Brain/Astrocytoma (Low Grade): Central Nervous System (CNS)/Intra-Axial Brain Tumors
([^{201}Tl]-Pentetreotide/[^{111}In]-Pentetreotide/ ([Gd]-Enhanced Magnetic Resonance Imaging [MRI])

Background Information: Highly undifferentiated glial tumors do not express somatostatin receptors (SSR), in contrast to low-grade astrocytomas, which contain SSR. To differentiate a malignant glioma from a low-grade astrocytoma and to distinguish an SSR-positive intra-axial brain tumor from an SSR negative 1, [^{111}In]-pentetreotide brain single photon emission computed tomography (SPECT) can be prospectively undertaken.

Application: Detection and characterization of intra-axial brain tumors.

Implementation: Evaluation of intra-axial brain tumors utilizing [^{201}Tl]- and [^{111}In]-pentetreotide brain SPECT performed using a 3-day to 4-day interval before surgery. Results from SPECT analyses are compared with those of [Gd]-enhanced MRI.

Human Model: Cases with intra-axial brain tumors (n = 8; n = 3/8 glioblastoma multiforme, n = 1/8 low-grade astrocytoma, n = 1/8 lymphoma, n = 1/8 medulloblastoma, n = 1/8 neurocytoma, n = 1/8 metastatic tumor).

Interpretation and Analysis: Increased uptake of [^{111}In]-pentetreotide can be appreciated in a high percentage of cases with glioblastoma multiforme (n = 2 grade (−); n = 1 grade (+); positive uptake n = 3/3 or 100% of cases) despite lack of SSR.

Low-grade astrocytomas exhibit minimal uptake of [^{111}In]-pentetreotide (grade +). Remaining tumors have an intense uptake of [^{111}In]-pentetreotide. [^{201}Tl] SPECT reveals similar findings to those observed with [^{111}In]-pentetreotide scintigraphy except in some conditions of glioblastoma multiforme (e.g., n = 2). Rare cases that have a negative [^{201}Tl] scan will usually show increases in [^{111}In]-pentetreotide uptake. Similarly, a small percentage of cases that have minimal uptake of [^{111}In]-pentetreotide will usually display increased thallium uptake. The uptake pattern of both [^{201}Tl] and [^{111}In]-pentetreotide appears to correlate with [Gd]-enhanced MRI. [^{111}In]-pentetreotide scintigraphy is sensitive for the detection of intra-axial brain tumors; however, it has no role in assessing the tumor grading or in the definition of receptor profiles.

Indium-111-pentetreotide imaging in intra-axial brain tumors: Comparison with thallium-201 SPECT and MRI. Lee JD, Kim DI, Lee JT, Chang JW, Park CY. J Nucl Med 1995 Apr; 36 (4), pp. 537–41.

Neoplasia: Brain/Cerebral Metastasis/ Detection of Primary Site of Metastasis
[^{18}F]-Fluoro-Deoxy-Glucose (FDG) Positron Emission Tomography (PET)

Cellular/Molecular Mechanism of Action: Radiofluorinated ([^{18}F]) analogues of glucose ([^{18}F]-FDG) are assimilated by neoplastic cell types at a higher level than normal parenchymal cells in part due to biochemical differences in glucose metabolism.

Background Information: Identification of unknown primary tumors in patients presenting with cerebral metastasis is a continued diagnostic challenge. Despite extensive and lengthy diagnostic

work-up, the primary tumors will often remain obscure in a significant proportion of affected patients.

Application: Detection of unknown primary tumors in patients with cerebral metastases.

Implementation: Evaluate the capacity for whole-body [^{18}F]-FDG PET scanning to identify the anatomical location of primary neoplastic lesions in conditions of metastatic disease affecting the brain. Confirmation of tumor lesions can be determined either by direct histological verification or indirectly by the observation of lesion appearance or lesion growth on structural imaging.

Human Model: Cases with histologically confirmed metastatic brain tumors (n = 16 from 34–74 years of age).

Interpretation and Analysis: Whole-body [^{18}F]-FDG PET may identify pulmonary foci of probable primary tumors in a high percentage of cases (e.g., n = 16/16). Successful confirmation may only be achieved in approximately 50% of affected cases (e.g., n = 8/16). In the remaining 50% of cases (e.g., n = 8/16) appropriate tissue samples may not be available (e.g., death or lost to follow-up; note n = 1/16 pulmonary malignant melanoma metastases). Therefore, whole-body [^{18}F]-FDG PET scanning is a sensitive tool in the search for unknown primary tumors of patients with confirmed cerebral metastases, allowing early and focused histological confirmation from suspicious lesions.

Instrumentation: Positron emission tomography instrumentation.

Detection of unknown primary tumors in patients with cerebral metastases using whole-body ^{18}F-flouoro-deoxy-glucose positron emission tomography. Klee B, Law I, Højgaard L, Kosteljanetz M. Eur J Neurol 2002 Nov; 9 (6), pp. 657–62.

Neoplasia: Brain/Gliomas: Heterogeneous/ Stereotactic Biopsy: Improve Accuracy of Lesion Localization and Sampling *[^{18}F]-Fluoro-Deoxy-Glucose (FDG)*

Background Information: A wide variety of metabolic features of brain tumors can be imaged using positron emission tomography (PET), including glucose metabolism, blood flow, oxygen consumption, amino acid metabolism, and lipid synthesis. Currently, FDG is the most widely available PET tracer for body imaging and brain imaging.

Application: [^{18}F]-FDG.

Route of Administration: [^{18}F]-FDG.

Dosage: [^{18}F]-FDG.

Interpretation and Analysis: Malignant brain tumors, like many other soft tissue tumors, show increased glucose metabolism, which is detectable in FDG PET images. FDG PET imaging of brain tumors provides information on tumor grade and prognosis. Compared with other organ systems, FDG PET imaging of the brain presents unique challenges because of the high background glucose metabolism of normal gray matter structures. Co-registration of the magnetic resonance imaging (MRI) or CT and FDG PET images is essential for accurate evaluation of brain tumors and is performed routinely at several institutions. The heterogeneous nature of gliomas can result in significant sampling errors when patients are biopsied for primary tumor diagnosis or recurrent disease. FDG PET can be used to define the most metabolically active targets for stereotactic biopsy. This in turn can improve diagnostic accuracy and reduce the number of biopsy samples required. FDG PET is also useful for evaluating residual or recurrent tumor following therapy, and can be used to survey patients with low-grade brain tumors for evidence of progression into high-grade malignancy. In the case of suspected tumor recurrence or progression, PET can aid in defining appropriate targets for biopsy. One limitation of FDG PET is the occasional inability to distinguish radiation necrosis from recurrent high-grade tumor. A 2nd limitation is that FDG PET is less sensitive than contrast-enhanced MRI for detecting intracranial metastases, and it is the opinion of some clinicians that brain studies should not be included as part of routine whole-body PET studies. Other tracers, such as [^{11}C]-methionine and [^{18}F]-fluorocholine (FCH), also avidly accumulate in brain tumors and have the advantage of low background cortical activity. The relationship between degree of uptake of these agents and tumor grade has not been established. These tracers may be useful in specific clinical situations, however, such as tumor localization for treatment planning or evaluation of low-grade tumors.

Positron emission tomography imaging of brain tumors. Wong TZ, van der Westhuizen GJ, Coleman RE. Neuroimaging Clin N Am. 2002 Nov; 12 (4), pp. 615–26.

Neoplasia: Brain/Glioma/Temozolomide Pharmacokinetics and Tumor Uptake *[^{11}C]-Temozolomide/Dual Radiolabeled at 3-N-Methyl and 4-Carbonyl*

Background Information: Previous reports have postulated that temozolomide undergoes decarboxylation and ring opening at the 3-4 position to produce the highly reactive methyldiazonium ion that alkylates DNA.

Application: Detection and characterization of neoplastic disease states.

Considerations: Dual radiolabeling of [^{11}C]-temozolomide in the 3-*N*-methyl and 4-carbonyl positions to delineate if temozolomide undergoes decarboxylation and ring opening in the 3-4 position to produce the highly reactive methyldiazonium ion that alkylates DNA. Characterization of such processes can facilitate determination if the [^{11}C] in the C-4 position of [4-[^{11}C]-carbonyl]-temozolomide is converted to [^{11}C]CO_2, which would result in lower accumulation of [^{11}C]-temozolomide within neoplastic lesions.

Semi-Synthetic Methods: Dual radiolabeling strategy involving separate radiolabeling of [^{11}C]-temozolomide in the 3-*N*-methyl and 4-carbonyl positions.

Implementation: Quantitate and confirm the mechanism of *in vivo* metabolic activation of temozolomide in addition to evaluation of tumor lesions, normal tissue, and plasma pharmacokinetics of temozolomide *in vivo*, and establish to what extent pharmacokinetics influence tumor targeting. Dual radiolabeled [^{11}C]-temozolomide at the 3-*N*-methyl or 4-carbonyl position is administered systemically to generate corresponding pharmacokinetic profiles (e.g., men) using positron emission tomography (PET). Paired studies are performed with both forms of [^{11}C]-temozolomide in glioma conditions. Another PET scan with [^{11}C]-radiolabeled bicarbonate can be performed and used to account for metabolites of temozolomide using a detailed analytical approach. Plasma is analyzed for [^{11}C]-temozolomide and [^{11}C] metabolites throughout the scan duration. Exhaled air can also be sampled throughout the scan for [^{11}C]CO_2 content. The percentage ring opening of temozolomide over 90 minutes can subsequently be calculated to evaluate whether there is a differential level of metabolic breakdown among plasma, normal tissue, and tumors.

Human Model: Cases of glioma (n = 6).

Interpretation and Analysis: Both radiolabeled forms of [^{11}C]-temozolomide display a rapid systemic clearance over a 90-minute period (0.2 liter/min/m^2), with [^{11}C]CO_2 being the primary elimination product. Plasma [^{11}C]CO_2 is present in all of the studies with [4-[^{11}C]-carbonyl]-temozolomide and in half the studies with [3-*N*-[^{11}C]-methyl]-temozolomide. The mean contributions to total plasma activity by [^{11}C]CO_2 at 10 and 90 minutes are 12% and 28% with [4-[^{11}C]-carbonyl]-temozolomide, and 1% and 4% with [3-N-[^{11}C]-methyl]-temozolomide, respectively. There is a 5-fold increase in

exhaled [^{11}C]CO_2 sampled with [4-[^{11}C]-carbonyl]-temozolomide compared with [3-*N*-[^{11}C]-methyl]-temozolomide ($p < 0.05$). A decrease in tissue exposure [area-under-the-curve between 0 and 90 minutes (AUC(0–90 minutes))] to [^{11}C]-temozolomide is also observed with [4-[^{11}C]-carbonyl]- temozolomide compared with [3-*N*-[^{11}C]-methyl]-temozolomide. A potential therapeutic advantage is the higher [^{11}C]-radiotracer and [^{11}C]-temozolomide exposure (AUC (0–90 minutes)) in tumors compared with normal tissue. [^{11}C]-temozolomide ring opening over 90 minutes is less in plasma (20.9%; $p < 0.05$) compared with tumor (26.8%), gray matter (29.7%), and white matter (30.1%), with no differences ($p > 0.05$) between tumor and normal tissues. The significantly higher amounts of [^{11}C]CO_2 sampled in plasma and exhaled air, in addition to the lower normal tissue and tumor [^{11}C]-temozolomide AUC(0–90 minutes) observed with [4-[^{11}C]-carbonyl]-temozolomide, suggest that [^{11}C] in the C-4 position of [4-[^{11}C]-carbonyl]-temozolomide is converted to [^{11}C]CO_2, resulting in lower [^{11}C]-temozolomide tumor exposure. The higher tumor [^{11}C]-temozolomide AUC(0–90 minutes) in tumors compared with normal tissue and the tissue-directed metabolic activation of temozolomide may confer potential therapeutic advantage in the activity of this agent.

Metabolic activation of temozolomide measured *in vivo* using positron emission tomography. Saleem A, Brown GD, Brady F, Aboagye EO, Osman S, Luthra SK, Ranicar AS, Brock CS, Stevens MF, Newlands E, Jones T, Price P. Cancer Res 2003 May 15; 63 (10), pp. 2409–15.

Neoplasia: Brain/Gliomas
p-[^{123}I]-Iodo-L-Phenylalanine (IPA)

Cellular/Molecular Mechanism of Action: The agent IPA functions as an analogue of phenylalanine and is preferentially assimilated by certain cell types due to its ability to biologically mimic this amino acid molecule.

Background Information: IPA is a recently described radiopharmaceutical that accumulates in gliomas at relatively high levels.

Application: Detection and characterization of gliomas within the central nervous system.

Dosage: IPA (average effective dose for adults is estimated to be 0.0152mSv*MBq^{-1}, leading to an effective dose of 3.8mSv in a typical brain single photon emission computed tomography [SPECT] investigation with 250 MBq IPA).

Implementation: Evaluate the feasibility of SPECT with IPA to image brain tumors under routine clinical conditions. One approach to achieving this objective can involve delineation of whole-body kinetic profiles and brain SPECT, in addition to plasma, urinary, and dosimetric analysis in glioma cases following intravenous IPA injection. Results obtained by IPA SPECT are then compared with histopathology, magnetic resonance images, and positron emission tomography employing [^{18}F]-fluoro-deoxy-glucose.

Human Model: Cases of glioma (n = 4).

Interpretation and Analysis: Tumor lesions can be clearly demonstrated by IPA SPECT at 30 minutes, 1 hour, and 4.5 hours postinjection, even in cases of low-grade gliomas. In conditions of glioblastoma, excellent visualization of the tumor is possible even at 7 hours postinjection, indicative of high retention of the radiopharmaceutical within cerebral gliomas. Analysis of radioactivity within plasma and urine samples serves to validate the high *in vivo* stability of IPA. Blood clearance of IPA is rapid (> 65% after 10 minutes) and elimination is predominantly facilitated by renal excretion with urinary radioactivity excretion ranging from 27% at 1 hour to 54% of injected doses at 5 hours postinjection. The IPA agent is a potentially valuable brain tumor imaging agent for widespread clinical studies with SPECT. Its high specific tumor uptake and retention even in low-grade gliomas represent a major advantage compared to presently available SPECT radiopharmaceuticals. Moreover, the radiation dose estimates indicate that clinical use of IPA will result in acceptable radiation dose levels in humans.

Instrumentation: Dual- and a triple-headed SPECT camera.

Initial evaluation of the feasibility of single photon emission tomography with *p*-[^{123}I]-iodo-L-phenylalanine for routine brain tumor imaging. Samnick S, Hellwig D, Bader JB, Romeike BF, Moringlane JR, Feiden W, Kirsch CM. Nucl Med Commun 2002 Feb; 23 (2), pp. 121–30.

Neoplasia: Brain/Gliomas/Differentiation of High-Grade Tumor vs Radiation Necrosis
[^{201}Tl]/[^{18}F]-2-Fluoro-2-Deoxy-D-Glucose (FDG)

Background Information: Over the last 2 decades the large volume of research involving the development and assessment of various brain tracers has shed invaluable light on the pathophysiology of cerebral neoplasms. Yet the question remains as to how best to incorporate this newly acquired insight into a clinical context.

Application: Methoxy-isobutylisonitrile (MIBI) has been used to detect and characterize postoperative tumor recurrence. [^{18}F]-FDG positron emission tomography (PET) is capable of predicting tumor grade, and the metabolic activity of brain tumors has prognostic significance but limited value in evaluating postoperative tumor recurrence.

Interpretation and Analysis: Many, but not all, studies have shown a relationship between [^{201}Tl] uptake and tumor grade. Due to the overlap between tumor uptake and histologic grades, [^{201}Tl] cannot be used as the sole non-invasive diagnostic or prognostic tool in brain tumor patients. However, it may help to differentiate a high-grade tumor recurrence rate from radiation necrosis. MIBI is theoretically a better imaging agent than [^{201}Tl] but it has not convincingly been shown to differentiate tumors according to grade. MDR-1 gene expression as demonstrated by MIBI does not correlate with chemoresistance in high-grade gliomas. Currently, the clinical role of MIBI in brain tumor imaging has yet to be defined. IMT, a radiolabeled amino acid analog, may be useful for identifying postoperative tumor recurrence and, in this application, appears to be a cheaper, more widely available tool than PET. However, its ability to accurately identify tumor grade is limited. [^{18}F]-FDG PET predicts tumor grade, and the metabolic activity of brain tumors has prognostic significance. Whether FDG uptake has any independent prognostic value that is superior to histology remains debatable. FDG PET is effective in differentiating recurrent tumors from radiation necrosis for high-grade tumors, but has limited value in defining the extent of tumor involvement and recurrence of low-grade lesions. Amino acid tracers, such as MET, perform better for this purpose and thus play a complementary role to FDG. Given the poor prognosis of patients with gliomas, particularly with high-grade lesions, the overall clinical utility of single photon emission computed tomography (SPECT) and PET in characterizing recurrent lesions remains dependent on the availability of effective treatments. These tools are thus mostly suited to the evaluation of treatment response in experimental protocols designed to improve the patients' outcome.

Imaging gliomas with positron emission tomography and single photon emission computed tomography. Benard F, Romsa J, Hustinx R. Semin Nucl Med 2003 Apr; 33 (2), pp. 148–62.

Neoplasia: Brain/Gliomas (Heterogeneous)/Biopsy/ Stereotactic Biopsy: Improve Accuracy of Lesion Localization and Sampling
[^{18}F]-Fluoro-Deoxy-Glucose (FDG)

Cellular/Molecular Mechanism of Action: [^{18}F]-FDG is an analogue of glucose that is perceived (intracellularly assimilated) by cell populations as endogenous glucose.

Background Information: A wide variety of metabolic features of brain tumors can be imaged using positron emission tomography (PET), including glucose metabolism, blood flow, oxygen consumption, amino acid metabolism, and lipid synthesis. Currently, FDG is the most widely available PET tracer for body imaging and brain imaging.
Application: [^{18}F]-FDG has been utilized to detect and characterize certain neoplastic disease states.
Route of Administration: [^{18}F]-FDG is usually non-selectively or selectively administered intravascularly.
Interpretation and Analysis: Malignant brain tumors, like many other soft tissue tumors, show increased glucose metabolism that can be detected by FDG PET imaging. FDG PET imaging of brain tumors provides information on tumor grade and prognosis. Compared with other organ systems, FDG PET imaging of the brain presents unique challenges because of the high background glucose metabolism of normal gray matter structures. Co-registration of the magnetic resonance imaging (MRI) (or or computed tomography [CT]) and FDG PET images is essential for accurate evaluation of brain tumors and is performed routinely at several institutions. The heterogeneous nature of gliomas can result in significant sampling errors when patients are biopsied for primary tumor diagnosis or recurrent disease. FDG PET can be used to define the most metabolically active targets for stereotactic biopsy. This in turn can improve diagnostic accuracy and reduce the number of biopsy samples required. FDG PET is also useful for evaluating residual or recurrent tumor following therapy, and can be used to survey patients with low-grade brain tumors for evidence of degeneration into high-grade malignancy. In the case of suspected tumor recurrence or progression, PET can aid in defining appropriate targets for biopsy. One limitation of FDG PET is the occasional inability to distinguish radiation necrosis from recurrent high-grade tumor. A 2nd limitation is that FDG PET is less sensitive than contrast-enhanced MRI for detecting intracranial metastases, and it has been the experience of some clinicians that brain studies should not be included as part of routine whole-body PET studies. Other tracers, such as [^{11}C]-methionine and [^{18}F]-fluorocholine (FCH), also avidly accumulate in brain tumors and have the advantage of low background cortical activity. The relationship between degree of uptake of these agents and tumor grade has not been thoroughly established. However, these tracers may be useful in specific clinical situations such as tumor localization for treatment planning or evaluation of low-grade tumors.

Positron emission tomography imaging of brain tumors. Wong TZ, van der Westhuizen GJ, Coleman RE. Neuroimaging Clin N Am 2002 Nov; 12 (4), pp. 615–26.

Neoplasia: Brain/Glioma/Mitotic Rate and Proliferation Activity
[^{99m}Technetium] ([^{99m}Tc])-Methoxy-Isobutylisonitrile (MIBI)/Thallium-201 ([^{201}Tl])/MI/MIB Index/Early and Delayed and Retention Ratios/[Antiglioma Ki-67 Antigen IgG Validation]

Background Information: [^{99m}Tc]-MIBI, like [^{201}Tl], is a highly efficient agent for the detection of glioma tumors and monitoring of their growth. Although [^{201}Tl] uptake is known to be partly associated with proliferative activity, little is known about the correlation between MIBI uptake and glioma proliferation activity.
Application: Detection and characterization of central nervous system gliomas, including estimation of proliferative activity.
Implementation: Evaluate the degree of correlation between MIBI uptake and proliferative activities in gliomas. Validation of results can be estimated utilizing staining methods that employ anti-Ki-67 antigen monoclonal antibody(MIB-1). By comparing results with those of [^{201}Tl], it is possible to determine which tracer is suitable for estimating proliferative activities. In this context, affected cases are given MIBI and [^{201}Tl] single photon emission computed tomography (SPECT). Early (10 minutes after injection) and delayed images (3 hours after injection) are then obtained for both MIBI and [^{201}Tl] scintigraphy. Single photon emission computed tomography parameters, early ratio (ER), delayed ratio (DR), and retention index (RI) values are obtained for both radiopharmaceuticals. Immunostaining of lesion samples obtained by surgical excision is then detected with a monoclonal antibody to Ki-67 antigen (MIB-1) staining method in order to facilitate calculation of the MI/MIB index. To evaluate the relationship between the proliferative activity and SPECT parameters, correlation analysis can then be performed.

Human Model: Cases of presurgical glioma (n = 24; n = 6 low-grade gliomas, n = 5 anaplastic astrocytomas, n = 13 glioblastomas). Surgical excision performed in all cases following initial evaluation with both MIBI and [^{201}Tl] scintigraphy.
Interpretation and Analysis: Values for MI correlate with calculated MIBI uptake ratios (r = 0.75 for ER, and r = 0.7 for DR). Both DR and RI for [^{201}Tl] also correlate with MI, but to a lesser degree (r = 0.6 for DR, and r = 0.59 for RI). There appears to be no significant correlation between the MIB-1 index and other parameters. MIBI-uptake parameters demonstrate a stronger positive correlation with the MIB-1 index than that of [^{201}Tl]. With the use of MIBI SPECT, it is possible to estimate the proliferative activity of gliomas non-invasively.

The correlation between ^{99m}Tc-MIBI uptake and MIB-1 as a nuclear proliferation marker in glioma—A comparative study with [^{201}Tl]. Nagamachi S, Jinnouchi S, Nabeshima K, Nishii R, Flores L 2nd, Kodama T, Kawai K, Tamura S, Yokogami K, Samejima T, Wakisaka S. Neuroradiology 2001 Dec; 43 (12), pp. 1023–30.

Neoplasia/Carcinoid and Endocrine Neoplasias/ Foregut Carcinoid/Endocrine Pancreatic Tumors (Functioning and Non-Functioning)/ Adenocorticotropic Hormone (ACTH)-Producing Bronchial Carcinoids (Small Pancreatic and Thorax Lesions)/Liver and Lymph Node Metastases: Increased Detection/Monitoring Treatment Effects/ Reduced Positron Emission Tomography (PET) Renal Artifact
[^{11}C]-5-Hydroxytryptophan (5-HTP)/Carbidopa/ [^{11}C]-Labeled L-dopa/Harmine

Cellular/Molecular Mechanism of Action: 5-hydroxytryptophan is a precursor in the synthesis of serotonin. Carbidopa is an inhibitor of decarboxylase biochemical activity. [^{11}C]-labeled L-dopa is an analogue of L-dopa that can function as a substrate precursor for the production of adrenergic neurotransmitters. Harmine is an inhibitor of monoamineoxidase (MAO-A) biochemical activity.
Background Information: Positron emission tomography performed with various radiolabeled compounds facilitates the study of tumor biochemistry. If the tumor uptake of an administered tracer is greater than that of surrounding normal tissue, it is also possible to localize the tumor. Previous studies utilizing [^{18}F]-labeled deoxyglucose (FDG) have attempted to visualize tumors because this tracer has been successfully used in oncology based on the increased level of glucose metabolism in neoplastic cell populations. However, this tracer has not been found to have any significant degree of uptake by the neuroendocrine tumors.

Pancreatic endocrine tumors (PETs) are rare neoplasms originating from the amine precursor uptake and decarboxylation (APUD) stem cells.

Application: The agent [^{11}C]-labeled 5-HTP can be utilized to detect and characterize primary and metastatic carcinoid neoplastic lesions. Carbidopa can be applied to diminish renal excretion-associated imaging artifacts and improve tumor visualization. Collective application of these agents can also provide valuable information about response to therapeutic intervention. [^{11}C]-labeled L-dopa has been used to characterize functional endocrine pancreatic tumors, while MAO-A inhibitors can be of value in profiling non-functional endocrine pancreatic neoplastic disease states.

Route of Administration: [^{11}C]-labeled 5-HTP (intravascular); carbidopa (peroral premedication).

Implementation: Evaluation of schemes for (i) detection and treatment monitoring of carcinoid tumors, endocrine pancreatic tumors (functional), foregut carcinoids (thoracic and abdominal) with [^{11}C]-labeled 5-HTP and [^{11}C]-labeled L-DOPA; (ii) endocrine pancreatic tumors (non-functional) with MAO-A inhibitors (e.g., harmine); and (iii) urinary artifact reduction (carbidopa).

Interpretation and Analysis: The serotonin precursor [^{11}C]-labeled 5-HTP displays increased uptake and irreversible trapping within carcinoid tumors. The uptake is selective and the resolution is so high that it is possible to detect a greater number of liver and lymph node metastases with PET than with computed tomography (CT) or octreotide scintigraphy. However, high renal excretion of the tracer is a problem because it produces streaky artifacts in the region/area of interest. Premedication with carbidopa decreases renal excretion approximately 6-fold, while at the same time tumor uptake increases 3-fold in a manner that enhances tumor visualization. Monitoring during treatment periods with PET using 5-HTP as a tracer reveals a $>$ 95% correlation between changes in urinary 5-hydroxyindoleacetic acid (U-5-HIAA) and changes in the 5-HTP transport rate. Therefore, PET can be used to monitor treatment effects.

Elevations in U-5-HIAA are considered to be uncommon in endocrine pancreatic tumors (EPTs). In this context, [^{11}C]-labeled L-dopa can be applied due to speculation that it is another amine important in the APUD system. Utilizing L-dopa for such purposes reveals that it is capable of detecting about half of EPTs that are primarily functional tumors. 5-Hydroxytryptophan can potentially be used as a universal tracer in PET whole-body imaging strategies designed to detect and characterize EPTs and foregut carcinoids that can invade both the thorax and abdomen. Utilizing this approach, small lesions can be visualized within the pancreas and thorax (e.g., ACTH-producing bronchial carcinoids) that cannot be detected by other methods (e.g., octreotide scintigraphy, magnetic resonance imaging [MRI], CT).

Several other tracers, like the agent harmine, have demonstrated promising results in characterizing non-functioning pancreatic endocrine tumors. Several laboratories have explored a wide range of biochemical systems, including enzymes and receptors, both for neurotransmitters and for peptides and proteins for *in vitro* assays, with the potential to use some of the developed tracers for *in vivo* visualization and tumor biological studies. Therefore, it appears that PET is a valuable tool in the diagnosis of neuroendocrine tumors. It can detect small lesions in the thorax and abdomen not detected by other methods, which has been of great value preoperatively in several cases. It detects more lesions in the liver and lymph nodes than is possible with other methods and, furthermore, it can be used to monitor treatment effectiveness.

Use of PET in neuroendocrine tumors. *In vivo* applications and *in vitro* studies. Eriksson B, Bergström M, Orlefors H, Sundin A, Oberg K, Längström B. Q J Nucl Med 2000 Mar; 44 (1), pp. 68–76.

Neoplasia/Colon Carcinoma: Carcinoembryonic Antigen Positive
[^{64}Cu]-DOTA-Anti-CEA Minibody/Anticarcinoembryonic Antigen (Anti-CEA)

Cellular/Molecular Mechanism of Action: Anticarcinoembryonic antigen monoclonal antibody possesses binding avidity for carcinoembryonic antigen.

Background Information: Rapid imaging by antitumor antibodies has been limited by the prolonged targeting kinetics and clearance of labeled whole antibodies. Genetically engineered fragments with rapid access and high retention in tumor tissue combined with rapid blood clearance are suitable for labeling with short-lived radionuclides, including positron-emitting isotopes for positron emission tomography (PET).

Route of Administration: Intravenous.

Semi-Synthetic Methods: Engineering of a "minibody" fragment of high-affinity anti-CEA monoclonal antibody (T84.66). The single-chain variable "minibody" fragment, (Fv)-C(H)3, is then converted to a bivalent 80-kDa dimer. The macrocyclic chelating agent 1,4,7,10-tetraazacyclododecane-N,N′,N′′, N′′′-tetraacetic acid (DOTA) can then be conjugated to anti-CEA minibody for labeling with copper-64, a positron-emitting radionuclide ($t_{1/2}$ = 12.7 hours).

Implementation: Determination of the *in vivo* distribution in animal models for neoplastic disease.

Animal Model: Mice (athymic strain bearing paired LS174T human colon carcinoma [CEA positive]) and rats (C6 rat glioma [CEA negative] xenografts).

Interpretation and Analysis: At 5 hours after injection with [^{64}Cu]-DOTA-minibody, micro-PET imaging shows high uptake in CEA-positive tumors (17.9% injected dose per gram [%ID/g] $\pm$ 3.79) compared with control tumors (6.0% %ID/g $\pm$ 1.0). In addition, significant uptake can be seen in the liver, with low uptake in other tissues. Average target/background ratios are 3–4:1 relative to neighboring tissues. Engineered antibody fragments labeled with positron-emitting isotopes such as [^{64}Cu] provide a new class of agents for PET imaging of tumors.

High-resolution micro-PET imaging of carcinoembryonic antigen-positive xenografts by using a copper-64-labeled engineered antibody fragment. Wu AM, Yazaki PJ, Tsai S, Nguyen K, Anderson AL, McCarthy DW, Welch MJ, Shively JE, Williams LE, Raubitschek AA, Wong JY, Toyokuni T, Phelps ME, Gambhir SS. Proc Natl Acad Sci U S A 2000 Jul 18; 97 (15), pp. 8495–500.

Neoplasia/Colorectal Carcinoma/Prediction of Therapeutic Sensitivity
[^{18}F]-5-Fluorouracil (5-FU)

Application: Radiopharmaceutical biodynamic evaluation of 5-FU chemotherapeutic efficacy (antineoplastic properties).

Considerations: The colon 38 carcinoma cell type is 5-FU sensitive in contrast to R1-rhabdomyosarcoma, which is 5-FU resistant.

Semi-Synthetic Methods: Optimized methods for the synthesis of [^{18}F]-labeled 5-FU have been described.

Implementation: Delineation of the relationship between [^{18}F]-5-fluorouracil cellular pharmacodynamics and chemotherapeutic sensitivity to 5-FU chemotherapy in colorectal carcinoma cell types.

Interpretation and Analysis: Instead of initial tumor uptake, chemotherapeutic sensitivity to 5-FU correlates best with carcinoma [^{18}F] efflux out of lesion sites.

An optimized synthesis of [^{18}F]-labeled 5-fluorouracil and a reevaluation of its use as a prognostic agent. Visser GW, Gorree GC, Braakhuis BJ, Herscheid JD. Eur J Nucl Med 1989; 15 (5), pp. 225–9.

Neoplasia/Colorectal Carcinoma/ Pretargeting: Monoclonal Antibody-Mediated/ Human Xenographs (SW1222) in Mice *[^{64}Cu]-DOTA-Biotin/[^{64}Cu]-DOTA-NR-LU-10*

Background Information: Pretargeting involves administration of a tumor-targeting monoclonal antibody (MAb) covalently linked to a molecule having a high-affinity binding site for a rapidly distributed radiolabeled effector/marker molecule.
Application: Pretargeted [^{64}Cu]-DOTA-biotin should afford markedly superior positron emission tomography (PET) imaging contrast and therapeutic efficacy due to its extremely rapid tumor uptake and blood clearance.
Semi-Synthetic Methods: DOTA-biotin (biotinylated 1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid) and the intact immunoconjugate DOTA-NR-LU-10 are labeled to high specific activities with [^{64}Cu], and the serum stabilities and target binding capabilities of each agent are then assessed (*in vitro*).
Implementation: Evaluate the relative effectiveness of pretargeting strategies and conventionally labeled antibody for tumor targeting of the intermediate-lived radionuclide [^{64}Cu]. In both PET imaging procedures and radioimmunotherapy protocols, [^{64}Cu] has demonstrated measurable attributes. One approach for characterizing "pretargeting" strategies can involve labeling of DOTA-biotin (DOTA = 1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid) and the intact immunoconjugate DOTA-NR-LU-10 with [^{64}Cu] at high specific activities. Efficacy can be evaluated *in vitro* by assessing serum stabilities and target binding capabilities. Evaluation of *in vivo* efficacy can be determined in nude mice bearing SW1222 human colorectal carcinoma xenografts by administering (i) [^{64}Cu]-DOTA-biotin, with and without pretreatment with the MAb-streptavidin conjugate NR-LU-10/SA and the synthetic clearing agent Biotin-GalNAc(16); or injection of (ii) [^{64}Cu]-DOTA-NR-LU-10. Biodistribution profiles for each of the two agents are then obtained from 5 minutes to 48 hours after injection.

Animal Model: Mice (e.g., nude mide bearing SW1222 human colorectal carcinoma xenographs).
Interpretation and Analysis: Both [^{64}Cu]-DOTA-biotin and [^{64}Cu]-DOTA-NR-LU-10 are 100% stable *in vitro* in serum. [^{64}Cu]-DOTA-biotin exhibits a > 98% specific binding for immobilized streptavidin, whereas the immunoreactivity averages nearly 80% for [^{64}Cu]-DOTA-NR-LU-10 preparations. Biodistributions in SW1222-bearing mice show that NR-LU-10/SA-pretargeted [^{64}Cu]-DOTA-biotin attains a peak tumor uptake of 18.9% of the injected dose per gram (%ID/g) at 1 hour, with concomitant rapid disappearance from blood and renal excretion. In the absence of pretargeting, [^{64}Cu]-DOTA-biotin has very similar biodistribution and clearance properties, except with extremely low non-specific tumor uptake. In contrast, [^{64}Cu]-DOTA-NR-LU-10 reaches 80.3 %ID/g in tumor tissue after 48 hours, whereas blood clearance is considerably slower than with pretargeted [^{64}Cu]-DOTA-biotin strategies. Comparison of the time-activity curves for tumor uptake and blood clearance of pretargeted [^{64}Cu] and the [^{64}Cu]-labeled antibody reveal that the maximum tumor accumulations of radioactivity are similar for each agent (e.g., 17.9 percentage injected activity per gram [%IA/g] and 20.7 %IA/g, respectively). However, the tumor-to-blood ratio of areas-under-the-curves is 14 times higher for pretargeted [^{64}Cu]-DOTA-biotin because of the substantial increase in blood clearance of the small effector molecule. The extremely rapid tumor uptake and blood clearance of pretargeted [^{64}Cu]-DOTA-biotin should afford markedly superior PET imaging contrast and therapeutic efficacy, compared with conventionally labeled [^{64}Cu]-DOTA-NR-LU-10. Further comparison of the therapeutic efficacy, toxicity, and dosimetry of these 2 agents is warranted.

In vivo evaluation of pretargeted [^{64}Cu] for tumor imaging and therapy. Lewis MR, Wang M, Axworthy DB, Theodore LJ, Mallet RW, Fritzberg AR, Welch MJ, Anderson CJ. J Nucl Med 2003 Aug; 44 (8), pp. 1284–92.

Neoplasia/Colorectal Cancer: Recurrent/ Carcinoembryonic Antigen/Indications for Laparotomy/Resectable Disease Prediction *2-[^{18}F]-Fluoro-2-Deoxy-D-Glucose (PET)/ [^{99m}Technetium] ([^{99m}Tc])-Labeled Arcitumo Monoclonal Antibody (MAb)/Carcinoembryonic Antigen (CEA) Alternative*

Cellular/Molecular Mechanism of Action: Preferentially binds physically to CEA expressed at relatively higher concentrations on the surface membranes of certain neoplastic cell types. Alternatively, [^{18}F]-FDG functions as a glucose analogue and is assimilated by neoplastic cell types at a higher level than normal cells in part due to biochemical differences in glucose metabolism.
Background Information: An increasing CEA level in the absence of disease on imaging studies can be a diagnostic challenge.
Application: Prediction of cases of colorectal cancer that would likely benefit from a laparotomy.
Route of Administration: Intravenous.
Implementation: Evaluation of 2-[^{18}F]-FDG and positron emission tomography (FDG PET) and CEA scans before 2nd-look laparotomy as a means of localizing recurrent colorectal cancer. Cases undergo computed tomography scan, bone scan, colonoscopy, and magnetic resonance imaging, and those without evidence of disease or resectable disease within the abdomen that are evaluated with FDG PET and CEA scans. At 2nd-look laparotomy, a surgeon blinded to the results of the FDG PET and CEA scans performs an exploration and maps their interpretations. A 2nd surgeon, with knowledge of the FDG PET and CEA scans, then explores the case and all lesions are biopsied or resected for pathology.
Interpretation and Analysis: In cases found to have resectable disease or disease that could be treated with regional therapy (e.g., n = 16), FDG PET scan is capable of accurately predicting the effectiveness of such strategies 81% of the time, in contrast to 13% for CEA scans. FDG PET scans can predict unresectable disease in 90% of patients. CEA scans usually fail to predict unresectable disease. In affected cases explored, disease may be found in 94% (n = 26/28), some of which have unresectable disease (n = 10/26). An FDG PET scan can predict unresectable disease in 90% of cases. A CEA scan fails to predict unresectable disease in the vast majority of cases.

Therefore, FDG PET scan can predict those patients who would likely benefit from a laparotomy. If the FDG PET scan indicates resectable disease, laparotomy can be considered. However, if the findings predict unresectable disease or the absence of disease, the patient should pursue systemic therapy or continued observation.

A prospective study of 2-[^{18}F]-fluoro-2-deoxy-D-glucose/positron emission tomography scan, [^{99m}Tc]-labeled arcitumomab (CEA-scan), and blind 2nd-look laparotomy for detecting colon cancer recurrence in patients with increasing carcinoembryonic antigen levels. Libutti SK, Alexander HR Jr, Choyke P, Bartlett DL, Bacharach SL, Whatley M, Jousse F, Eckelman WC, Kranda K, Neumann RD, Carrasquillo JA. Ann Surg Oncol 2001 Dec; 8 (10), pp. 779–86.

Neoplasia/Colorectal Carcinoma: Recurrent Postresection/Surgical Intervention Decision Making/Nuclear Scan vs Computed Tomography (CT) Scan *[^{111}In]-CYT-103 Monoclonal Antibody (MAb)*

Background Information: Recurrent colorectal cancer is seen in as many as 40% of cases after curative resection. In view of the

limitations of endoscopic and cross-sectional imaging, external immunoscintigraphy has been added to the follow-up regimen in conditions at high risk of recurrent disease.

Application: Detection and characterization of colorectal carcinoma as an aid to deciding the merits of surgical intervention.

Route of Administration: Intravascular.

Considerations: Provocative agents (histamine, glucagon, tyramine) are rarely needed. Preoperative localization of tumor lesions can be done with nephrotomography intravenous pyelogram (IVP), computerized axial tomography, ultrasound, [^{131}I]-19-iodocholesterol scan, arteriography, and venography.

Semi-Synthetic Methods: Labeling of CYT-103 MAb (1.0 mg) with [^{111}In] radioisotope (approximately 4.3 mCi).

Implementation: Evaluation of the utility of immunoscintigraphy in conditions of suspected colorectal carcinoma utilizing MAb (e.g., B72.3 or CYT-103) site-specifically labeled with [^{111}In] conjugate. Planar and single photon emission computed tomography (SPECT) imaging is then performed 2–5 days after infusion of, for example, [^{111}In]-CYT-103 preparations.

Human Model: Cases of suspected recurrences of previous "curative" colorectal carcinoma resections.

Interpretation and Analysis: Local or regional recurrences (4 cases) and liver metastasis (6 cases) may be indicated by physical examination and CT. Alternatively, some cases (e.g., n = 9) may have occult disease with increasing serum carcinoembryonic antigen (CEA) levels and negative conventional workups. Serum CEA levels are elevated (mean, 22 ng/ml) in the vast majority of cases (e.g., 100%). A final diagnosis of recurrence or metastasis can be established in a number of cases by 2nd-look surgery or biopsy (e.g., n = 18). Tumors may potentially be identified at the following locations: pelvis (e.g., n = 12 cases), abdominal wall (e.g., n = 2 cases), retroperitoneum (e.g., n = 1 case), liver (e.g., n = 5 cases), and omentum (e.g., n = 2 cases). Superiority of MAb scans is supported by the capacity to detect pelvic and intra-abdominal recurrences (100%) at a higher success rate than CT scan (43%). Liver metastases are identified equally well by both modalities. [^{111}In]-CYT-103 scan findings may influence the management of approximately 55% of affected cases (e.g., n = 10/18). Surgery may be avoided in conditions with scan-detected disseminated metastases. Correct identification of occult local recurrences can be made in some cases (e.g., n = 6). Isolated liver metastasis may be confirmed in instances of equivocal CT scan results. Finally, additional intra-abdominal lesions may also be detected. Such observations suggest an important and beneficial role for [^{111}In]-CYT-103 MAb imaging in conditions of suspected recurrent colorectal carcinomas, particularly in cases with negative cross-sectional imaging results; such imaging may prevent cases from undergoing unnecessary surgical explorations.

In-111 CYT-103 monoclonal antibody imaging in patients with suspected recurrent colorectal cancer. Doerr RJ, Herrera L, Abdel-Nabi H. Cancer 1993 Jun 15; 71 (12 Suppl), pp. 4241–7.

Neoplasia/Differentiation of High-Grade Tumor vs Radiation Necrosis/Prognostic Predictive Capabilities for Neoplastic Disease/Postoperative Tumor Recurrence

[^{102}Tl]/[^{18}F]-2-Fluoro-2-Deoxy-d-Glucose (FDG)/ Methoxy-Isobutylisonitrile (MIBI)/IMT/MET

Background Information: Over a period of 2 decades, a large volume of research has been devoted to the design and development of various brain tracers that has shed invaluable insight about the pathophysiology of cerebral neoplasms. Yet the question remains as to how best to incorporate this newly acquired knowledge into clinical environments.

Application: MIBI has been used to detect and characterize postoperative tumor recurrence. [^{18}F]-FDG positron emission tomography (PET) is capable of predicting tumor grade, and the metabolic activity of brain tumors has prognostic significance but has limited value in evaluating postoperative tumor recurrence.

Interpretation and Analysis: Many, but not all, studies show a relationship between [^{201}Tl] uptake and tumor grade. Due to the overlap between tumor uptake and histologic grades, [^{201}Tl] cannot be used as the sole non-invasive diagnostic or prognostic tool in brain tumor patients. However, it may help in differentiating high-grade tumor recurrence from radiation necrosis. MIBI is theoretically a better imaging agent than [^{201}Tl] but it has not convincingly been shown to differentiate tumors according to grade. MDR-1 gene expression as demonstrated by MIBI does not correlate with chemoresistance in high-grade gliomas. Currently, MIBI's clinical role in brain tumor imaging has yet to be defined. IMT, a radiolabeled amino acid analog, may be useful for identifying postoperative tumor recurrence and, in this application, appears to be a cheaper, more widely available tool than PET. However, its ability to accurately identify tumor grade is limited. FDG PET predicts tumor grade, and the metabolic activity of brain tumors has a prognostic significance. Whether FDG uptake has an independent prognostic value above that of histology remains debated. FDG PET is effective in differentiating recurrent tumor from radiation necrosis for high-grade tumors but has limited value in defining the extent of tumor involvement and recurrence of low-grade lesions. Amino acid tracers, such as MET, perform better for this purpose and thus play a complementary role to FDG. Given the poor prognosis of patients with gliomas, particularly with high-grade lesions, the overall clinical utility of single photon emission computed tomography (SPECT) and PET in characterizing recurrent lesions remains dependent on the availability of effective treatments. These tools are thus mostly suited to the evaluation of treatment response in experimental protocols designed to improve the patients' outcome.

Imaging gliomas with positron emission tomography and single photon emission computed tomography. Benard F, Romsa J, Hustinx R. Semin Nucl Med 2003 Apr; 33 (2), pp. 148–62.

Neoplasia/Epidermal Growth Factor Receptor Tyrosine Kinase

[^{11}C]-N-Acryl Amides/[^{11}C]-Acryloyl Chloride: Labeling Synthon/[^{11}C]-6-Acrylamido-4-(3,4-Dichloro-6-Fluoroanilino)-Quinazoline

Cellular/Molecular Mechanism of Action: [^{11}C]-acryloyl chloride functions as a labeling synthon for the production of [^{11}C]-N-acryl amides.

Application: Synthetic preparations of [^{11}C]-6-acrylamido-4-(3,4-dichloro-6-fluoroanilino)-quinazoline (ML03) can be used as a biomarker targeting platform in positron emission tomography (PET) imaging studies for the detection of epidermal growth factor receptor tyrosine kinase (EGFr-TK) in certain conditions of neoplastic disease.

Semi-Synthetic Methods: The reagent [^{11}C]-acryloyl chloride can be used in automated formats for the radiosynthesis of [^{11}C]-labeled N-acryl amides (e.g., [^{11}C]-6-acrylamido-4-(3,4-dichloro-6-fluoroanilino)-quinazoline [ML03]) and other anticancer chemotherapeutics.

Interpretation and Analysis: The potency of ML03 to inhibit autophosphorylation of EGFr-TK as evaluated by an ELISA assay indicates a low IC_{50} of 0.037nM.

Radiosynthesis of ML03, a novel positron emission tomography biomarker for targeting epidermal growth factor receptor via the labeling synthon: [^{11}C]-acryloyl chloride. Ben-David I, Rozen Y, Ortu G, Mishani E. Appl Radiat Isot 2003 Feb; 58 (2), pp. 209–17.

Neoplasia/Folate Receptors/Neoplastic Disease
[^{67}Ga]-Folate-DF-Folate-(α)/ [^{67}Ga]-Folate-DF-Folate-(γ)

Cellular/Molecular Mechanism of Action: Folate is used as a carrier targeting molecule for the radioisotopes due to its binding avidity for folate receptors expressed at relatively high concentrations by certain tumor cell types compared to normal cell populations. Deferoxamine (DF) functions as a chelating agent for certain radioisotopes (e.g., [$^{67}Ga^{3+}$]).
Application: Identification of folate receptor-positive neoplastic lesions applying nuclear medicine imaging techniques.
Route of Administration: Intravenous.
Semi-Synthetic Methods: The vitamin folic acid can be covalently linked to the chelating agent DF via an amide bond using a simple carbodiimide coupling reaction. A mixture of 2 isomers, DF-folate-(α) and DF-folate-(γ) can be generated by producing chemical interactions with a carboxyl group at either the α- or γ- position of folic acid, respectively. Isomer separation is feasible applying anion-exchange chromatography and NH_4HCO_3 buffer gradients.
Experimental Interpretation: Competitive binding studies reveal that only the DF-folate-(γ) is recognized by the folate receptor on KB cells and interacts with an affinity comparable to unconjugated folic acid. The DF-folate conjugates can be radiolabeled with the gamma-emitting radionuclide [$^{67}Ga^{3+}$] to test for uptake by cultured KB cells overexpressing the folate receptor.
Interpretation and Analysis: The cellular accumulation of [^{67}Ga]-DF-folate-(γ) tracer exhibits rapid uptake kinetics in cell culture with a $T_{1/2}$ of approximately 3 minutes. The KB cell associated [^{67}Ga]-DF-folate-(γ) radioactivity is competitively blocked by free folic acid, indicating that uptake of the [^{67}Ga]-DF-folate-(γ) is specifically mediated by folate receptors. Because folate receptors are overexpressed on the surfaces of many neoplastic cells, these results suggest that the [^{67}Ga]-DF-folate-(γ) complex might be useful as a diagnostic agent for the non-invasive imaging of folate receptor-positive tumors.
Instrumentation: Computer-integrated nuclear medicine scintigraphy imaging camera.

Preparation of [^{66}Ga]- and [^{68}Ga]-labeled Ga(III)-deferoxamine-folate as potential folate-receptor-targeted PET radiopharmaceuticals. Mathias CJ, Lewis MR, Reichert DE, Laforest R, Sharp TL, Lewis JS, Yang ZF, Waters DJ, Snyder PW, Low PS, Welch MJ, Green MA. Nucl Med Biol 2003 Oct; 30 (7), pp. 725–31.

Neoplasia/Gene Expression/Herpes Simplex Virus Tyrosine Kinase (HSV-tk)
2′-Deoxy-2′-Flouro-5-Methyl-1-β-D-Arabinofuranosyluracil/9-[(3-[^{18}F]-Fluoro-1-Hydroxy-2-Propoxy)Methyl]-Guanine/9-(4-[^{18}F]-Fluoro-3-Hydroxy-Methylbutyl)Guanine

Application: 2′-Deoxy-2′-flouro-5-methyl-1-β-D-arabinofuranosyluracil (FMAU) has demonstrated potential utilization as a positron emission tomography (PET) radiotracer for imaging HSV-TK gene expression.
Interpretation and Analysis: *In vitro* experiments demonstrate that the accumulation of [^{14}C]-FMAU in HSV-tk-expressing cells is 2.4-fold ($p < 0.02$), 4.0-fold ($p < 0.001$), and 5.3-fold ($p < 0.001$) higher than the wild-type cells at 1, 3, and 5 hours, respectively. *In vivo* studies reveal that the tumor uptake in HSV-tk-expressing cells is 2.3-fold ($p < 0.001$), 3.0-fold ($p < 0.001$), and 5.5-fold ($p < 0.001$) higher than control cells at 1, 2, and 5 hours, respectively. FMAU is more sensitive compared to earlier studies using 9-[(3-[^{18}F]-fluoro-1-hydroxy-2-propoxy)methyl]-guanine ([^{18}F]-FHPG) and 9-(4-[^{18}F]-fluoro-3-hydroxy-methylbutyl)guanine ([^{18}F]-FHBG) in the same cell lines, although the specificity is less than FHBG. While FMAU labeled with PET isotopes may be useful for imaging HSV-tk-expressing tumors *in vivo*, multitracer studies across additional tumor models are necessary in order to identify an optimal PET radiotracer.

Evaluation of 2′-deoxy-2′-flouro-5-methyl-1-beta-D-arabinofuranosyluracil as a potential gene imaging agent for HSV-TK expression *in vivo*. Alauddin MM, Shahinian A, Gordon EM, Conti PS. Mol Imaging 2002 Apr–Jun; 1 (2), pp. 74–81.

Neoplasia/Hypoxia with Neoplastic Lesions
N-(2-Hydroxy-3,3,3-Trifluoropropyl)-2-(2-Nitro-l-Imidazolyl) Acetamide/Positron Emission Tomography (PET) and Magnetic Resonance Spectroscopy (MRS) Imaging Modalities

Background Information: Hypoxia occurs to a variable extent in a vast majority of rodent and human solid tumors. It results from an inadequate and disorganized tumor vasculature and hence an impaired oxygen delivery. A probe for the non-invasive detection of tumor hypoxia could find important utility in the selection of patients for therapy with bioreductive agents, antiangiogenic/antivascular therapies, and hypoxia-targeted gene therapy. In addition, tumor hypoxia has been shown to predict treatment outcome following radio- or chemotherapy in human cancers, the underlying mechanism for which may involve hypoxia driving genetic instability and resulting tumor progression. Beyond oncology, utility may also include stroke, ischemic heart disease, peripheral vascular disease, arthritis, and other disorders.
Semi-Synthetic Methods: Relevant design goals include: (i) a nitro group with appropriate redox potential for selective reduction and binding in hypoxic tumor cells; (ii) hydrophilic/hydrogen bonding character in the side chain to limit nervous tissue penetration and prevent neurotoxicity; and (iii) 3 equivalent fluorine atoms to enhance MRS/magnetic resonance imaging (MRI) detection, located in a metabolically stable position.
Implementation: Design, validation, and preclinical development of a fluorinated 2-nitroimidazole, N-(2-hydroxy-3,3,3-trifluoropropyl)-2-(2-nitro-l-imidazolyl) acetamide (SR 4554, CRC 94/17) for the purpose of detecting and measuring tumor hypoxia by MRS. Quantitative MRS can additionally be used to assess the retention of [^{19}F] signal in neoplastic lesions.
Animal Model: Murine (tumor bearing with mouse tumors and human tumor xenographs).
Interpretation and Analysis: Reduction of SR 4554 by mouse liver microsomes is dependent on oxygen content, with a half-maximal inhibition at 0.48 ± 0.06%. Under *in vitro* conditions, SR 4554 undergoes nitroreduction by hypoxic but not oxic tumor cells, and electron energy loss spectroscopic analysis shows selective retention in the hypoxic regions of multicellular tumor spheroids.

Pharmacokinetic analyses reveal that low brain tissue concentrations develop in contrast to excellent tumor levels, as measured by high performance liquid chromatography. The extent of this restricted entry into brain tumor is surprising given the overall octanol/water partition coefficient and can be attributed to the hydrophilic/hydrogen bonding character of the side chain. The [^{19}F] retention index (FRI; ratio of [^{19}F] signal levels at 6 hours relative to that at 45 minutes) range from 0.5 to 1.0 and 0.2 to 0.9 for murine tumors and human xenografts, respectively. The correlation between SR 4554 retention and pO_2 is not linear, but when FRI is > 0.5, the % $pO_2 \leq$ 5 mm Hg is always $> 60\%$, indicating that high FRI is associated

with low levels of oxygenation. Finally, whole-body [^{19}F]-MRI in mice reveals that SR 4554 and related metabolites localize mainly in tumor, liver, and bladder regions. A selective MRS signal is readily detectable in tumors at doses at least 7-fold lower than those likely to cause toxicity in mice.

The agent fluorinated 2-nitroimidazole, N-(2-hydroxy-3,3,3-trifluoropropyl)-2-(2-nitro-l-imidazolyl) acetamide (SR 4554, CRC 94/17) therefore is capable of functioning as a non-invasive MRS/MRI probe for detecting and measuring tumor hypoxia. Potentially, this same molecular probe may have potential application in PET methodologies to facilitate improved detection of tumor hypoxia.

Preclinical development and current status of the fluorinated 2-nitroimidazole hypoxia probe N-(2-hydroxy-3,3,3-trifluoropropyl)-2-(2-nitro-1-imidazolyl) acetamide (SR 4554, CRC 94/17): A non-invasive diagnostic probe for the measurement of tumor hypoxia by magnetic resonance spectroscopy and imaging, and by positron emission tomography. Aboagye EO, Kelson AB, Tracy M, Workman P. Anticancer Drug Des 1998 Sep; 13 (6), pp. 703–30.

Neoplasia: Lymphoma/Hodgkin's Lymphoma and Non-Hodgkin's Lymphoma
Positron Emission Tomography (PET) Imaging

Positron emission tomography has now gained a place in the management of patients with cancer, including those with Hodgkin's disease and non-Hodgkin's lymphoma. Restaging studies and those addressing the monitoring of response to treatment are especially in focus. Most of the knowledge gained has been achieved with dedicated bismuth germinate (BGO)-based PET technology, but there are a number of developments that will impact on the use of this metabolic imaging technique in the investigation of patients with lymphoma. The challenges ahead are determined by the need for high-quality whole-body imaging associated with increased patient throughput and the need to investigate the role of new labeled ligands. The latter are likely to yield new insights into tumor cell characterization, tumor behavior, and tumor outcome assessment. The study of new radiolabeled ligands will impose further demands for rapid dynamic data acquisition and accurate tracer quantification. Current and future developments in PET technology range from the use of new detector materials to different detector geometries and data acquisition modes. The search for alternatives to BGO scintillation materials for PET has led to the development of PET instruments that utilize new crystals such as lutetium orthosilicate (LSO) and gadolinium oxyorthosilicate (GSO). The use of these new detectors and the increased sensitivity achieved with 3D data acquisitions represent the most significant current developments in the field. With the increasing demands imposed on the clinical utilization of PET, issues such as study cost and patient throughput will emerge as significant future factors. As a consequence, low-cost units are being offered by manufacturers through the utilization of gamma camera-based single photon emission computed tomography (SPECT) systems for PET coincidence imaging. Unfortunately, clinical studies in lymphoma and other cancers have already demonstrated the limitations of this technology, with 20% of lesions < 15 mm in size escaping detection. On the other hand, the recent development of combined PET/computed tomography (CT) devices attempts to address the lack of anatomical information inherent with PET images, taking advantage of further improvement in patient throughput and hence cost-effectiveness. Preliminary studies using this multimodality imaging approach have already demonstrated the potential of the technique. Although the potential exists, certain technical issues with PET/CT require refinement of the methodology. Such issues include organ movement (such as respiratory motion), which strongly influences the image fusion of a rapidly acquired CT scan with the slower acquisition of a PET dataset, and the derivation of CT-based attenuation coefficients in the presence of contrast agents or metallic implants. The application of the technology for radiotherapy planning also poses a number of associated challenges. Finally, the development of dedicated PET systems based on planar detector arrangements with new detector components has the potential to improve clinical throughput by over 100%, but clinical trials using such systems still have to be carried out in order to establish the associated whole-body image quality.

Impact of technology on the utilization of positron emission tomography in lymphoma: Current and future perspectives. Visvikis D, Ell PJ. Eur J Nucl Med Mol Imaging 2003 Jun; 30 Suppl 1, pp. S106–16. Epub 2003 May 13.

Neoplasia: Lymphoma/Hodgkin's Lymphoma/ Non-Hodgkin's Lymphoma (NHL)/Benign Fibrotic or Necrotic Tissue Differentiation/Prognosis (Morbidity/Mortality)
[^{18}F]-Fluoro-Deoxy-Glucose (FDG)/[^{67}Ga]Gallium

Background Information: Until recently, gallium-67 scintigraphy (GS) has been the best available functional imaging modality for evaluating patients with NHL and Hodgkin's disease (HD).
Application: Fluorine-18 FDG positron emission tomography (PET) and gallium-67 scintigraphy can be used to detect and characterize Hodgkin's lymphoma and NHL.
Implementation: As [^{67}Ga] is a tumor viability agent, the role of gallium-67 scintigraphy is primarily as a complementary follow-up diagnostic procedure.
Interpretation and Analysis: The diagnostic accuracy of gallium-67 scintigraphy in detecting lymphoma is based on optimization of the imaging protocol, knowledge of potential physiological and benign sites of [^{67}Ga] uptake, and the Ga avidity characteristics of individual lymphoma types. A residual mass persisting on CT after treatment poses a common clinical dilemma: It may indicate the presence of viable lymphoma, which requires further treatment, or it can be benign, consisting of only fibrotic and necrotic tissues. Gallium-67 scintigraphy can successfully differentiate between these conditions. Routine follow-up with gallium-67 scintigraphy may allow early diagnosis of recurrence and early institution of treatment. Reversion of a positive gallium-67 scintigraphy to a negative test, and the rapidity with which this occurs, has a high predictive value for prognosis (morbidity/mortality). Lymphoma lesions that appear normal on gallium-67 scintigraphy early during treatment have a better prognosis than lymphoma with persistence of pathological findings. Other tumor-seeking single photon emitting agents, such as thallium-201, technetium-99m methoxy-isobutylisonitrile (MIBI), and indium-111 octreotide, have been investigated in lymphoma, primarily as an alternative to gallium-67 scintigraphy in specific clinical settings, but are of limited value. The role of radioimmunoscintigraphy is gaining importance in conjunction with radioimmunotherapy. Fluorine-18 FDG imaging of lymphoma using either dedicated or camera-based PET systems is gradually replacing gallium-67 scintigraphy for assessment of lymphoma. Fluoro-deoxy-glucose overcomes some of the limitations of gallium-67 scintigraphy, while sharing its tumor viability characteristics. The extensive clinical knowledge and experience accumulated over 3 decades with gallium-67 scintigraphy in lymphoma provides a solid background as well as a model for the assessment of new functional imaging techniques.

Gallium-67 scintigraphy: A cornerstone in functional imaging of lymphoma. Even-Sapir E, Israel O. Eur J Nucl Med Mol Imaging 2003 Jun; 30 Suppl 1, pp. S65–81. Epub 2003 Mar 18.

Neoplasia/Mammary Carcinoma/Estrogen Receptor Expression
[^{123}I]-Labeled Estradiol Derivatives

Background Information: Radiolabeled estrogen receptor ligands are tracers that can be used for functional receptor characterization. Their specificity towards receptors, together with the fact that only 50–70% of mammary carcinomas are receptor-positive, renders them unsuitable for detection of primary tumors or metastases, and this means that estrogen receptor scintigraphy can be used neither for tumor screening nor for staging. However, both [^{18}F]-labeled and [^{123}I]-labeled estradiol derivatives are suitable for *in vivo* imaging of estrogen receptors.
Application: Detection and characterization of mammary carcinoma lesions that express estrogen receptor complexes.
Route of Administration: [^{123}I]-labeled estradiol derivatives (intravascular).
Interpretation and Analysis: The high specificity of both [^{18}F]-labeled and [^{123}I]-labeled estradiol derivatives *in vivo* in animal experiments and related *in vitro* studies has been reproduced *in vivo* in humans. Tracers with positron radiation emitters are, however, hardly suitable for broad application, owing to the short half-life of [^{18}F], which means that users need to be situated close to a cyclotron and a correspondingly equipped radiochemical laboratory. The number of available positron emission tomography (PET) scanners, on the other hand, has increased over the last few years, especially in Germany, so that this, at least, does not present a limiting factor. All the same, [^{123}I]-labeled estradiol derivatives will find more widespread application because the number of gamma cameras incorporating modern multihead systems is several times greater. The results of studies with [^{123}I]-E2-scintigraphy published to date are very promising, even given the initial technical problems just mentioned. As a method of examination, it could be enhanced by using improved tracers with a higher tumor contrast and less disturbance from overlapping in diagnostically relevant locations, for instance, by selecting tracers with higher activities whose excretion is more renal than hepatobiliary. The use of modern multihead camera systems can also be expected to improve the photon yield.

Estrogen receptor scintigraphy. Scheidhauer K, Scharl A, Schicha H. Q J Nucl Med 1998 Mar; 42 (1), pp. 26–32.

Neoplasia/Mammary Carcinoma
N-(N-Benzylpiperidin-4-yl)-2-[^{18}F]-Fluorobenzamide

Cellular/Molecular Mechanism of Action: The molecular agent *N*-(*N*-benzylpiperidin-4-yl)-2-[^{18}F]-fluorobenzamide is selectively assimilated by human mammary carcinoma cell types.
Background Information: *N*-(*N*-benzylpiperidin-4-yl)-2-[^{18}F]-fluorobenzamide, functioning as a ligand for positron emission tomography (PET) imaging of sigma-receptor complexes, has been found to potentially serve as an agent for breast cancer detection.
Application: Detection and characterization of human mammary carcinoma.
Route of Administration: *N*-(*N*-benzylpiperidin-4-yl)-2-[^{18}F]-fluorobenzamide.
Implementation: Detection of mammary carcinoma neoplastic lesions *in vivo* applying PET imaging modalities.

Animal Model: Mice (severe combined immunodeficient [SCID] mice bearing MDA-MB231 tumor and "wild-type" explants of human mammary carcinoma).
Interpretation and Analysis: *In vivo* studies in SCID mice bearing MDA-MB231 tumors reveal (i) a high uptake of compound 2 in neoplastic lesions (3.8%/g); and (ii) ratios of tumor/muscle and tumor/blood of approximately 6.2 and 7.0, respectively, at 1 hour postinjection. Pretreatment of SCID mice with haldol increases the uptake of compound 2 in blood, muscle, and other well-perfused organs, while decreasing its uptake by tumors. The ratios of tumor/muscle and tumor/blood decreases from 6.2 and 7.0 to 1.3 and 1.1, respectively, at 1 hour postinjection. At 2 hours postinjection, the ratios of tumor/muscle and tumor/blood decrease from 4.9 and 7.8 to 1.4 and 1.4, respectively. The tumor uptake of compound 2 in SCID mice bearing primary tumor explants from human cases of breast cancer can be lower than observed with MDA-MB231 tumors (1.66%/g vs 3.78%/g), and the ratios of tumor/muscle and tumor/blood are 3.5 and 3.7, respectively, at 1 hour postinjection. These results suggest that compound 2 may be a potential ligand for PET imaging of breast cancer.

N-(*N*-Benzylpiperidin-4-yl)-2-[^{18}F]-fluorobenzamide: A potential ligand for PET imaging of breast cancer. Shiue C, Shiue GG, Benard F, Visonneau S, Santoli D, Alavi AA. Nucl Med Biol 2000 Nov; 27 (8), pp. 763–7.

Neoplasia/Mammary Carcinoma/ O(6)-Alkylguanine-DNA Alkyltransferase (AGT)
[^{11}C]-p-O(6)[(Methoxymethyl)Benzyl]Guanines/ [^{11}C]-m-O(6)[(Methoxymethyl)Benzyl]Guanines/ [^{11}C]-o-O(6)[(Methoxymethyl)Benzyl]Guanines

Application: Evaluation of the DNA repair protein AGT expression in conditions of mammary carcinoma applying positron emission tomography (PET) imaging modalities.
Semi-Synthetic Methods: Preparation and radiolabeling of O(6)-benzylguanine derivatives 6-O-[^{11}C]-[(methoxymethyl)benzyl] guanines [^{11}C]-*p*-O(6)-MMBG; [^{11}C]-*m*-O(6)-MMBG, 1b; and [^{11}C]-*o*-O(6)-MMBG, 1c.

Synthesis and preliminary biological evaluation of 6-O-[^{11}C]-[(methoxymethyl)benzyl]guanines, new potential PET breast cancer imaging agents for the DNA repair protein AGT. Liu X, Zheng QH, Fei X, Wang JQ, Ohannesian DW, Erickson LC, Stone KL, Hutchins GD. Bioorg Med Chem Lett 2003 Feb 24; 13 (4), pp. 641–4.

Neoplasia/Mammary Gland Disease Differentiation
Gadolinium/Indocyanine Green (ICG)/Near-Infrared Diffuse Optical Tomography (DOT)

Cellular/Molecular Mechanism of Action: Indocyanine green can function as an albumin-bound absorbing dye within the intravascular compartment.
Application: Indocyanine green is an absorbing dye commonly used as a marker system within the plasma compartment. Under certain conditions, ICG possesses the potential to differentiate disease based on the quantified enhancement of suspicious lesions using magnetic resonance imaging (MRI) concurrently with near-infrared DOT (contrast enhancement properties).
Route of Administration: Intravascular.
Implementation: Near-infrared DOT analysis of the mammary gland applied in combination or concurrently with an MRI. Results are then correlated with histopathological evaluation of suspicious lesions.

Human Model: Cases scheduled for excisional biopsy or surgery.
Interpretation and Analysis: The ICG-enhanced optical images co-register accurately with gadolinium-enhanced magnetic resonance images, thereby validating the ability of DOT to image breast tissue. In contrast to simple transillumination, DOT appears to provide for localization and quantification of exogenous tissue chro-

mophore concentrations. Additionally, ICG has demonstrated a potential to differentiate disease based on the quantified enhancement of suspicious lesions.

Concurrent MRI and diffuse optical tomography of breast after indocyanine green enhancement. Ntziachristos V, Yodh AG, Schnall M, Chance B. Proc Natl Acad Sci U S A 2000 Mar 14; 97 (6), pp. 2767–72.

Neoplasia/Mediastinal Tumors: Primary/ Differentiation of Malignant and Benign/Thymic Carcinoma/Thymoma Invasiveness/Malignant Potential of 1° Mediastinal Masses
[^{18}F]-2-Fluoro-2-Deoxy-D-Glucose (FDG)

Cellular/Molecular Mechanism of Action: Preferential uptake of [^{18}F]-FDG by neoplastic tissues related to the elevated level of glucose metabolism observed in cancer cell types relative to normal cells.
Application: Preoperative detection of malignant primary mediastinal tumors applying positron emission tomography (PET). The mean FDG uptake for malignant tumors is significantly higher than that observed for benign tumors. Both thymic cancer and invasive thymoma show high FDG uptake.
Route of Administration: Intravenous.
Considerations: Malignant neoplastic cell types that preferentially assimilate FDG include thymic carcinoma, lymphomas, invasive thymomas, and certain cases of sarcoidosis. Moderate levels of FDG uptake can be appreciated in cases of myeloma, non-invasive thymomas, and schwannoma, whereas a low uptake of FDG is observed in teratoma and various types of benign cysts.
Interpretation and Analysis: Preferential sequestration of FDG within neoplastic lesions can be detected by positron emission tomography. False-positive and false-negative images can be observed utilizing PET, which can be lower than noted with computed tomography (CT) examinations when attempting to predict tumor invasion. Therefore, the use of FDG with PET is clinically helpful in evaluating the malignant nature of primary mediastinal tumors. Investigations have demonstrated that a high level of FDG uptake reflects the invasiveness of the malignant nature of thymic tumors.
Instrumentation: Computer-integrated PET instrumentation.

PET imaging of primary mediastinal tumours. Kubota K; Yamada S; Kondo T; Yamada K; Fukuda H; Fujiwara T; Ito M; Ido T. Br J Cancer] 1996 Apr; Vol. 73 (7), pp. 882–6.

Neoplasia/Mitosis and Proliferation Rate
3′-Deoxy-3′-[^{18}F]-Fluorothymidine ([^{18}F]-FLT)

Cellular/Molecular Mechanism of Action: Thymidine is rapidly assimilated by proliferating cell populations (e.g., incorporated into nucleic acids).
Application: 3′-Deoxy-3′-[^{18}F]-fluorothymidine has been designed as a new positron emission tomography (PET) imaging agent for *in vivo* visualizing of cellular proliferation based on the incorporation of thymidine into nucleic acid.
Route of Administration: Variable but most often intravascular.
Semi-Synthetic Methods: A reliable radiosynthesis of [^{18}F]-FLT has been developed based on [^{18}F]10 mCi (370 MBq) of radiochemically pure [^{18}F] 1 Ci/μmol (37 GBq/μmol) at end of synthesis (EOS) within 100 minutes and in 13% radiochemical yield (end of bombardment [EOB]; 7% EOS).
Implementation: Evaluation of neoplastic lesions that display relatively rapid rates of proliferation/growth.
Interpretation and Analysis: Detection of increased radioactivity at a specific anatomical location corresponds to an anatomical region that has a relatively high rate of cellular proliferation.

Radiosynthesis of 3′-deoxy-3′-[^{18}F]-fluorothymidine: [^{18}F]-FLT for imaging of cellular proliferation *in vivo*. Grierson JR, Shields AF. Nucl Med Biol 2000 Feb; 27 (2), pp. 143–56.

Neoplasia/Mitosis and Proliferation Rate
2′-Deoxy-2′-[^{18}F]-Fluoro-β-D-Arabinofuranosyl/ [^{18}F]-FAU, [^{18}F]-FMAU, [^{18}F]-FBAU, [^{18}F]-FIAU

Semi-Synthetic Methods: Efficient and reliable synthesis of 2′-deoxy-2′-[^{18}F]-fluoro-β-D-arabinofuranosyl nucleosides.
Interpretation and Analysis: Overall decay-corrected radiochemical yields of 35 to 45% of 4 analogs, FAU, FMAU, FBAU, and FIAU, are routinely obtained in $>$ 98% radiochemical purity and with specific activities of greater than 3 Ci/μmol (110 MBq/μmol) in a synthesis time frame of approximately 3 hours. When approximately 220 mCi (8.15 GBq) of starting [^{18}F]-fluoride is used, 25–30 mCi (0.93–1.11 GBq) of product (enough to image 2 patients sequentially) is typically obtained.

Synthesis of 2′-deoxy-2′-[^{18}F]-fluoro-β-D-arabinofuranosyl nucleosides, [^{18}F]-FAU, [^{18}F]-FMAU, [^{18}F]-FBAU, and [^{18}F]-FIAU, as potential PET agents for imaging cellular proliferation. Synthesis of [^{18}F]-labeled FAU, FMAU, FBAU, FIAU. Mangner TJ, Klecker RW, Anderson L, Shields AF. Nucl Med Biol 2003 Apr; 30 (3), pp. 215–24.

Neoplasia/Mitosis and Proliferation Rate
3′-Deoxy-3′-[^{18}F]-Fluorothymidine ([^{18}F]-FLT)

Cellular/Molecular Mechanism of Action: [^{18}F]-FLT is a nucleoside analogue that enters cells and is phosphorylated by human thymidine kinase 1, but the 3′ substitution prevents further incorporation into DNA.
Application: [^{18}F]-FLT is a positron emission tomography (PET) imaging agent that shows promise for studying cellular proliferation in human cancers.
Implementation: Estimations of radiation dosimetry for [^{18}F]-FLT can be calculated based on data for time-dependent tissue concentrations of radioactivity for blood samples and PET images (e.g., n = 18 cases) after intravenous injection. Radiation-absorbed doses are calculated using the Medical Internal Radiation Dose (MIRD) Committee methods, taking into account variations that are based on the distribution of activities observed in individual cases. Effective dose equivalent (EDE) is calculated using International Commission on Radiological Protection Publication 60 tissue-weighting factors for the standard man and woman.
Interpretation and Analysis: For a single bladder voiding at 6 hours after [^{18}F]-FLT injection, the [^{18}F]-FLT EDE (mean ± standard deviation) is 0.028 ± 0.012 mSv/MBq (103 ± 43 mrem/mCi) for a standard male patient and 0.033 ± 0.012 mSv/MBq (121 ± 43 mrem/mCi) for a standard female patient. The organ that receives the highest dose is the bladder (male, 0.179 mGy/MBq [662 mrad/mCi]; female, 0.174 mGy/MBq [646 mrad/mCi]), followed by the liver (male, 0.045 mGy/MBq [167 mrad/mCi]; female, 0.064 mGy/MBq [238 mrad/mCi]), the kidneys (male, 0.035 mGy/MBq [131 mrad/mCi]; female, 0.042 mGy/MBq [155 mrad/mCi]), and the bone marrow (male, 0.024 mGy/MBq [89 mrad/mCi]; female, 0.033 mGy/MBq [122 mrad/mCi]).

Organ dose estimates for [^{18}F]-FLT are comparable to those associated with other commonly performed nuclear medicine tests, and the potential radiation risks associated with [^{18}F]-FLT PET imaging are within accepted limits.

[^{18}F]-fluorothymidine radiation dosimetry in human PET imaging studies. Vesselle H, Grierson J, Peterson LM, Muzi M, Mankoff DA, Krohn KA. J Nucl Med 2003 Sep; 44 (9), pp. 1482–8.

Neoplasia/Mitosis and Proliferation Rate
[^{18}F]-FAU/[^{18}F] 1-(2′-Deoxy-2′-Fluoro-β-D-Arabinofuranosyl)Uracil

Cellular/Molecular Mechanism of Action: The agent FAU or [1-(2′-deoxy-2′-fluoro-beta-D-arabinofuranosyl)uracil] is incorporated into strands of DNA as a "false" or "decoy" nucleotide and functions as an inhibitor of DNA synthesis.
Physiological Mechanism of Action: Functions as an inhibitor of DNA synthesis, thereby inhibiting the synthesis of viable messenger RNA (mRNA) (transcription) or biologically functional protein fractions (translation).
Application: Radiofluorination of FAU to produce [^{18}F]-FAU or [^{18}F] 1-(2′-deoxy-2′-fluoro-beta-D-arabinofuranosyl)uracil is suitable as a diagnostic radiopharmaceutical for positron emission tomography (PET) imaging procedures.
Route of Administration: [^{18}F]-FAU.
Implementation: Determination of the pharmacokinetic and relative organ/tissue distribution of [^{18}F]-FAU following injection of the tracer through the combined application of PET imaging (duration = ≥ 4 hours) and high-performance liquid chromatography (HPLC) analysis of collected blood, urine, and tissues/organs.
Animal Model: Canine (e.g., n = 3 normal dogs).
Interpretation and Analysis: In an *in vivo* environment, [^{18}F]-FAU is evenly distributed throughout most of organ systems and tissues. In sharp contrast to thymidine and its analogs, the marrow has less retention of [^{18}F]-FAU than non-proliferating tissues.

Imaging [^{18}F]-FAU [1-(2′-deoxy-2′-fluoro-beta-D-arabinofuranosyl) uracil] in dogs. Sun H, Collins JM, Mangner TJ, Muzik O, Shields AF. Nucl Med Biol 2003 Jan; 30 (1), pp. 25–30.

Neoplasia/Neuroendocrine Neoplasias: Catecholamine-Secreting/Neuroblastoma/ Pheochromocytoma
[^{131}I]-Metaiodobenzylguanidine (MIBG)/[^{123}I]-MIBG

Cellular/Molecular Mechanism of Action: Metaiodobenzylguanidine is classified as a norepinephrine analog.
Application: Metaiodobenzylguanidine has been in clinical use for 15 years and has been shown to have high sensitivity (about 85%) and specificity (> 95%) for the location of all types of pheochromocytomas. Similar levels of success have been observed with the diagnostic detection of neuroblastoma. Other neuroendocrine lesions have been imaged at lower levels of sensitivity, including carcinoids, medullary thyroid cancer, and non-secretory paragangliomas.
Interpretation and Analysis: Alternative analogs of radiolabeled somatostatin may have greater utility for improved detection of neuroendocrine lesions, including carcinoids, medullary thyroid cancer, and non-secretory paragangliomas. Metaiodobenzylguanidine scintigraphy may also provide a different *in vivo* diagnostic approach for evaluating the sympathetic autonomic nervous system, particularly with respect to cardiac function.
Instrumentation: Various radiolabels for MIBG and its analogs permit planar scintigraphy, single photon emission computed tomography (SPECT), positron emission tomography (PET), intraoperative probe localization, and radiopharmaceutical therapy.

The current status of metaiodobenzylguanidine and related agents for the diagnosis of neuroendocrine tumors. Shapiro B, Sisson JC, Shulkin BL, Gross MD, Zempel S. Quart J Nucl Med 1995 Dec; 39 (4 Suppl 1), pp. 3–8.

Neoplasia/Otolaryngological Conditions/ Thyrotoxicosis and Solitary Thyroid Nodules/ Thyrotoxicosis/Solitary Thyroid Nodules/ Parathyroid Adenoma/Salivary and Lachrymal Gland/Bone
[^{99m}TcO^{-4}]/[^{123}I]/[^{99m}TcO^{-4}]/[^{201}T1]/[^{99m}TcO^{-4}]/ [^{99m}TcO^{-4}]-MDP

Background Information: Nuclear medicine has a distinct role to play in otolaryngological practice. Accurate diagnosis of endocrine conditions is now possible using precise *in vitro* hormone measurements. Specific clinical questions can be answered using *in vivo* investigations. [^{99m}TcO^{-4}]/[^{123}I] scintigraphy is used to evaluate thyrotoxicosis and solitary thyroid nodules. [^{99m}TcO^{-4}]/[^{201}T1] subtraction scanning is of value in the preoperative localization of parathyroid adenomas, and [^{99m}TcO^{-4}] is particularly useful in assessing salivary and lachrymal gland function and drainage. [^{99m}TcO^{-4}]-MDP bone scanning is useful in the evaluation of osteomyelitis, temporomandibular joint dysfunction, bone graft viability, and some facial fractures. The role of radioisotopes in the management of differentiated and medullary carcinoma of the thyroid is now well established.
Application: Detection of thyrotoxicosis and solitary thyroid nodules by scintigraphic imaging.
Interpretation and Analysis: Although there are many other agents available to image head and neck cancer, few can actually achieve the required diagnostic sensitivity and specificity. The introduction of monoclonal antibodies into routine imaging has been hampered by distinct practical problems and the search is now on for more sensitive non-specific diagnostic agents. It is now possible to evaluate new [^{99m}Tc]-labeled tumor-imaging agents using animal tumor model systems, and the use of radioactivity in all aspects of otolaryngological research adds an extra quantitative dimension. Together with single photon emission computed tomography (SPECT), and the introduction of positron emission tomography (PET) to image the physiology of normal tissues and tumors, the use of radionuclide investigations can lead only to an increase in ear, nose, and throat (ENT) diagnostic sensitivity and specificity and, subsequently, to an overall improvement in the diagnosis, staging, and treatment of cancer affecting the head and neck.

Nuclear medicine in otolaryngology. Watkinson JC. Clin Otolaryngol 1990 Oct; 15 (5), pp. 457–69.

Neoplasia/Parathyroid Adenoma
[^{99m}Technetium] ([^{99m}Tc])/ [^{201}T1] Subtraction Scanning

Application: Preoperative localization of parathyroid adenomas by scintographic imaging.
Considerations: Although there are many other agents available to image head and neck cancer, few can actually achieve the required diagnostic sensitivity and specificity. The introduction of monoclonal antibodies into routine imaging has been hampered by distinct practical problems and the search is now on for more sensitive non-specific diagnostic agents. It is now possible to evaluate new [^{99m}Tc]-labeled tumor-imaging agents using animal tumor models, and the use of radioactivity in all aspects of otolaryngological research adds an extra quantitative dimension.

Together with single photon emission computed tomography (SPECT) and the introduction of positron emission tomography (PET) to image the physiology of normal tissues and tumors, the use of radionuclide investigations can lead only to an increase in ear, nose, and throat (ENT) diagnostic sensitivity and specificity and,

subsequently, to an overall improvement in the diagnosis, staging, and treatment of cancer involving the head and neck.

Nuclear medicine in otolaryngology. Watkinson JC. Clin Otolaryngol 1990 Oct; 15 (5), pp. 457–69.

Neoplasia/Parathyroid Adenoma/Thyroid Nodules/ Improved Accuracy of Surgical Resection *[^{99m}Technetium] ([^{99m}Tc]) SestaMIBI/[^{123}I]/ Subtraction Scanning*

Cellular/Molecular Mechanism of Action: The isotope [^{123}I] is assimilated by the thyroid gland in a manner consistent with nonradioactive iodine.

Application: Parathyroid scanning, based on simultaneous recording of technetium [^{99m}Tc]-sestaMIBI and ^{123}iodine images, is able to identify patients with multiple parathyroid gland disease and is a safe imaging technique for unilateral parathyroid surgery. [^{99m}Tc]-SestaMIBI or [^{99m}Tc]-MIBI or [^{99m}Tc]-tetrofosmin are primarily only applied for detecting certain neuroendocrine tumors (NETs) such as medullary thyroid cancer.

Implementation: Evaluation of scintigraphic criteria to determine eligibility for unilateral surgery with findings compared to observations noted with conventional bilateral surgery. In this context, dual-isotope scanning is performed before performing conventional surgery.

Human Model: Seventy consecutive referral cases with primary hyperparathyroidism.

Interpretation and Analysis: A large number of cases (e.g., n = 41/70) may have scan findings compatible with unilateral surgery, with a single focus of high intensity seen on the anterior and lateral views. A smaller but substantial number of residual cases (e.g., n = 29/70) may have one or more criteria of ineligibility including (i) scan findings pointing to multiple gland disease; (ii) no well-identified focus; (iii) contralateral thyroid nodule requiring surgical management; or (iv) family history of hyperparathyroidism or multiple endocrine disease.

In the assessment of the number of enlarged parathyroid glands at surgical inspection and calcemia follow-up, a very low percentage of cases with a single well-defined focus on the scan image show evidence of multiple parathyroid involvement (e.g., n = 0/41 or 0%). Each parathyroid adenoma can usually be resected from the precise site predicted by subtraction scans. Approximately 13% of cases (e.g., n = 9/41) may have surgical findings of multiple parathyroid gland disease (ineligible for inclusion based on preoperative image findings). Unilateral surgery therefore can be safely offered to 60% of patients with primary hyperparathyroidism, on the basis of simultaneous [^{99m}Tc]-sestaMIBI and [^{123}I] scanning. Such diagnostic practices can reduce the duration of surgical procedures, anesthetic dose delivered, hospital stay, and the risks of hypoparathyroidism and injury to the recurrent laryngeal nerve.

Unilateral surgery for primary hyperparathyroidism on the basis of technetium-99m sestaMIBI and iodine-123 subtraction scanning. Hindié E, Mellière D, Jeanguillaume C, Ure$n$a P, deLabriolle-Vaylet C, Perlemuter L. Arch Surg 2000 Dec; 135 (12), pp. 1461–8.

Neoplasia/Pharmacokinetics and Tumor Uptake/ Temozolomide/Glioma *[^{11}C]-Temozolomide/Dual Radiolabeled at 3-N-Methyl and 4-Carbonyl*

Background Information: Previous reports have postulated that temozolomide undergoes decarboxylation and ring opening at the position 3-4 to produce the highly reactive methyldiazonium ion that alkylates DNA.

Application: Detection and characterization of neoplastic disease states.

Considerations: Dual radiolabeling of [^{11}C]-temozolomide in the 3-*N*-methyl and 4-carbonyl positions can be performed to delineate if temozolomide undergoes decarboxylation and ring opening in the 3-4 position to produce the highly reactive methyldiazonium ion that alkylates DNA. Characterization of such processes can facilitate determination if the [^{11}C] in the C-4 position of [4-[^{11}C]-carbonyl]-temozolomide is converted to [^{11}C]CO_2, which would result in lower accumulation of [^{11}C]-temozolomide within neoplastic lesions.

Semi-Synthetic Methods: Dual radiolabeling strategy involving separate radiolabeling of [^{11}C]-temozolomide in the 3-*N*-methyl and 4-carbonyl positions.

Implementation: Quantitate and confirm the mechanism of *in vivo* metabolic activation of temozolomide in addition to evaluation of tumor lesions, normal tissue, and plasma pharmacokinetics of temozolomide, and establish to what extent pharmacokinetics influence tumor targeting. Dual radiolabeled [^{11}C]-temozolomide at the 3-*N*-methyl or 4-carbonyl position is administered systemically to generate corresponding pharmacokinetic profiles (e.g., men) using positron emission tomography (PET). Paired studies are performed with both forms of [^{11}C]-temozolomide in glioma conditions. Another PET scan with [^{11}C]-radiolabeled bicarbonate can be performed and used to account for metabolites of temozolomide using a detailed analytical approach. Plasma is analyzed for [^{11}C]-temozolomide and [^{11}C] metabolites throughout the scan duration. Exhaled air can also be sampled throughout the scan for [^{11}C]CO_2 content. The percentage of temozolomide ring opening over 90 minutes can subsequently be calculated to evaluate whether there is a differential in metabolic breakdown among plasma, normal tissue, and tumor.

Human Model: Cases of glioma (n = 6).

Interpretation and Analysis: Both radiolabeled forms of [^{11}C]-temozolomide display a rapid systemic clearance over a 90-minute period (0.2 liter/min/m^2), with [^{11}C]CO_2 being the primary elimination product. Plasma [^{11}C]CO_2 is present in all of the studies with [4-[^{11}C]-carbonyl]-temozolomide and in half the studies with [3-*N*-[^{11}C]-methyl]-temozolomide. The mean contributions to total plasma activity by [^{11}C]CO_2 at 10 and 90 minutes are 12% and 28% with [4-[^{11}C]-carbonyl]-temozolomide, and 1% and 4% with [3-N-[^{11}C]-methyl]-temozolomide, respectively. There is a 5-fold increase in exhaled [^{11}C]CO_2 sampled with [4-[^{11}C]-carbonyl]-temozolomide compared with [3-*N*-[^{11}C]-methyl]-temozolomide ($p < 0.05$). A decrease in tissue exposure [area-under-the-curve between 0 and 90 minutes (AUC(0–90 minutes))] to [^{11}C]-temozolomide is also observed with [4-[^{11}C]-carbonyl]- temozolomide compared with [3-*N*-[^{11}C]-methyl]-temozolomide. A potential therapeutic advantage is the higher [^{11}C]-radiotracer and [^{11}C]-temozolomide exposure (AUC(0–90 minutes)) in tumors compared with normal tissue. [^{11}C]-temozolomide ring opening over 90 minutes is less in plasma (20.9%; $p < 0.05$) compared with tumor (26.8%), gray matter (29.7%), and white matter (30.1%), with no differences ($p > 0.05$) between tumor and normal tissues. The significantly higher amounts of [^{11}C]CO_2 sampled in plasma and exhaled air, in addition to the lower normal tissue and tumor [^{11}C]-temozolomide AUC(0–90 minutes) observed with [4-[^{11}C]-carbonyl]-temozolomide, suggest that [^{11}C] in the C-4 position of [4-[^{11}C]-carbonyl]-temozolomide is converted to [^{11}C]CO_2, resulting in lower [^{11}C]-temozolomide tumor exposure. The higher tumor [^{11}C]-temozolomide AUC(0–90 minutes) in tumors compared with normal tissue and the tissue-directed metabolic activation of temozolomide may confer potential therapeutic advantage in the activity of this agent.

Metabolic activation of temozolomide measured *in vivo* using positron emission tomography. Saleem A, Brown GD, Brady F, Aboagye EO, Osman S, Luthra SK, Ranicar AS, Brock CS, Stevens MF, Newlands E, Jones T, Price P. Cancer Res 2003 May 15; 63 (10), pp. 2409–15.

Neoplasia/Pharmacokinetics: Chemotherapeutics/ Receptor Expression/Metabolism of Neoplastic Cells/ Signal Transduction/Proliferation Cycle Characteristics/Cell Death/Resistance to Chemotherapy/Angiogenesis: Neoplastic Cell Blood Supply

Radiolabeled 5-Fluorouracil (5-FU)/Radiolabeled Temozolomide/Radiolabeled N-[2-(Dimethylamino)Ethyl]Acridine-4-Carboxamide (DACA)/16-α-[^{18}F]-Fluoro-17β-Oestradiol (FES)/ [^{18}F]-Fluoro-Deoxy-Glucose (FDG)/ [^{11}C]-Methyl-Choline/[^{11}C]-Thymidine/[^{124}I]-Annexin V/ [^{124}C]-Colchicine/[^{124}I]-Anti-VEGF

Background Information: Positron emission tomography (PET) provides the oncologist with information about tumor diagnosis, key malignant transformation pathways, delineation of chemotherapeutic pharmacokinetics/pharmacodynamics, and strategies for monitoring treatment response. Mathematical modeling of tissue data and online plasma radioactive metabolite profiling enable delineation of important tissue kinetic parameters related to uptake, distribution, and washout as well as arterial input function to be derived. The resultant kinetic data allow for not only diagnosis but also the assessment of therapeutic response end points. These end points can be used to measure specific therapeutic effects. This novel application of PET can provide information that is often difficult to measure in intact *in vivo* systems, including pharmcokinetic profiles, ligand-receptor interactions, pathways involved in metabolism, signal transduction, cell cycle and proliferation, cell death, drug resistance, and angiogenesis.

Application: Characterization of *in vivo* ligand-receptor interactions in neoplastic disease states for the purpose of diagnosis, discovery, and characterization of key malignant transformation pathways, delineation of chemotherapeutic pharmacokinetics/ pharmacodynamics, and strategies for monitoring treatment response. Examples include (i) pharmacokinetics of radiolabeled chemotherapeutics: DACA, temozolomide, and 5-FU; (ii) radiolabeled ligands that recognize receptor complexes such as FES; (iii) probes for profiling neoplastic cell metabolism pathways: [^{18}F]-FDG; (iv) signal transduction: [^{11}C]-methyl-choline; (v) cell-cycle and proliferation: 2-[^{11}C]-thymidine; (vi) cell death: [^{124}I]-annexin V; (vii) drug resistance patterns: [^{124}C]-colchicines; and (viii) angiogenesis: [^{124}I]-anti-VEGF.

Pharmacodynamics of radiolabelled anticancer drugs for positron emission tomography. Hutchinson OC, Collingridge DR, Barthel H, Price PM, Aboagye EO. Curr Pharm Des 2003; 9 (11), pp. 931–44.

Pharmacokinetics of radiolabeled anticancer drugs for positron emission tomography. Hutchinson OC, Collingridge DR, Barthel H, Price PM, Aboagye EO. Curr Pharm Des 2003; 9 (11), pp. 917–29.

Neoplasia: Pulmonary/Non-Small Cell Lung Carcinoma/Surgical Resection Response

[^{18}F]-2-Fluoro-2-Deoxy-d-Glucose (FDG)

Background Information: Surgical resection after preoperative chemotherapy in patients with non-small cell lung cancer might only be best for patients who are responders.

Implementation: Evaluate of positron emission tomography (PET) scanning with FDG compared to computed tomography (CT) scanning to determine the effectiveness of surgical resection of primary tumors (non-small cell lung cancer) and at N1 and N2 lymph nodes.

Human Model: Cases with non-small cell lung cancer that have initial FDG PET scan staging with tissue biopsy, neoadjuvant chemotherapy, repeat FDG PET scanning, and repeat biopsies. Reference population: (i) n = 34 cases (24 men; median age, 64 years); (ii) n = 11 cases with N2 disease; (iii) n = 7 cases with N1 disease; (iv) n = 27 cases receiving chemotherapy; (v) n = 7 cases receiving chemotherapy and radiation therapy; and (vi) all cases except n = 9 cases receiving surgical resection.

Interpretation and Analysis: Statistical analysis reveals that FDG PET scanning is more specific ($p < 0.0001$), has a higher positive predictive value ($p = 0.0018$), and has a higher negative predictive value ($p < 0.0001$) than CT scanning for predicting residual tumor at the primary site. FDG PET scanning is more sensitive ($p < 0.0001$) and more accurate ($p < 0.0001$), has a higher positive predictive value ($p < 0.0001$), and has a higher negative predictive value ($p = 0.0002$) than CT scanning for paratracheal nodes (number 2 and 4 lymph nodes). FDG PET scanning has a higher positive predictive value ($p < 0.0001$) than CT scanning for the other N2 (numbers 5, 6, 7, 8, and 9) lymph nodes. Repeat FDG PET scanning is more specific and has a higher positive predictive value and negative predictive value than CT scanning for detecting residual tumor in the lung of patients with non-small cell lung cancer who have received preoperative chemotherapy. It is more sensitive and accurate for paratracheal N2 nodes as well. However, there is no significant difference in its detection of N1 lymph nodes.

Positron emission tomography scanning with 2-fluoro-2-deoxy-d-glucose as a predictor of response of neoadjuvant treatment for non-small cell carcinoma. Cerfolio RJ, Ojha B, Mukherjee S, Pask AH, Bass CS, Katholi CR. J Thorac Cardiovasc Surg 2003 Apr; 125 (4), pp. 938–44.

Neoplasia/Pulmonary/Non-Small Cell Lung Cancer (NSCLC)/Detection of Elevated Cellular Proliferation Rate/Risk of Recurrence Postresection/Response to Cell Cycle Targeted Chemotherapy

3′Deoxy-3′-[^{18}F]-Fluorothymidine (FLT)

Cellular/Molecular Mechanism of Action: Thymidine is a nucleic acid precursor that is incorporated into nuclear DNA in mitotically dividing cell populations.

Background Information: Tumor proliferation has prognostic value in resected early stage NSCLC and can, therefore, predict which NSCLCs are at high risk for recurrence after resection and would benefit from additional therapy. It may also predict which tumor will respond to cell cycle targeted chemotherapy and help assess the tumor response, besides helping to differentiate benign from malignant lung lesions.

Application: Labeled thymidine (e.g., [^{3}H]-thymidine) can be used to assess the rate of mitotic activity within dividing cell populations. Characterizing the uptake of the positron emission tomography (PET) tracer FLT in a series of suspected NSCLCs correlates with tumor proliferation assessed by Ki-67 immunohistochemistry and flow cytometry.

Route of Administration: FLT (intravenous).

Dosage: FLT (0.07 mCi/kg).

Implementation: Evaluation of cases with biopsy-proven or clinically suspected NSCLC applying FLT as an imaging agent (PET 2-hour dynamic study). Tumor FLT uptake is quantitated with the

maximum pixel standardized uptake value (maxSUV), the partial volume corrected maxSUV (PV-corr-maxSUV), the average SUV over a small region-of-interest (aveSUV), and with Patlak analysis of FLT flux (aveFLTflux). Lesion diameter from computed tomography is used to correct the maxSUV for PV effects using recovery coefficients determined for the PET scanner. Immunohistochemistry can be performed to identify Ki-67 expression (proliferation index marker; e.g., n = 11 tissue specimens) and flow cytometry analysis to identify S-phase fraction (SPF; e.g., n = 10 cases). Tissue specimens are reviewed for histology and cellular differentiation (poor, moderate, well). Lesions can range from 1.6 to 7.7 cm.

Human Model: Cases of suspected or biopsy-proven NSCLC (n = 11; n = 10/11 imaged by PET).

Interpretation and Analysis: A small percentage of cases may be inflammatory in nature (e.g., n = 2/11), while a relatively large percentage of cases will be positively identified as NSCLC (e.g., n = 9/11). Excellent correlation exists between SUV measures of FLT uptake and Ki-67 scores [percentage of positive cells; maxSUV vs Ki-67: $\rho = 0.78$, $p = 0.0043$ (n = 11); PV-corr-maxSUV vs Ki-67: $\rho = 0.83$, $p = 0.0028$ (n = 10); aveSUV vs Ki-67: $\rho = 0.84$, $p = 0.0011$ (n = 11)]. Correlation between Ki-67 proliferation scores and Patlak measures of FLT uptake are also strong: aveFLTflux vs Ki-67: $\rho = 0.94$, $p < 0.0001$ (n = 11). The correlation between the SPF and all indices of FLT uptake is weaker and reaches statistical significance for only 2 uptake indices [maxSUV vs SPF: $\rho = 0.69$, $p = 0.03$ (n = 10); PV-corr-maxSUV vs SPF: $\rho = 0.36$, $p = 0.35$ (n = 9); aveSUV vs SPF: $\rho = 0.67$, $p = 0.03$ (n = 10); aveFLTflux vs SPF: $\rho = 0.46$, $p = 0.18$ (n = 10)]. FLT PET may be used noninvasively to assess *in vivo* proliferation rates of lung masses. Therefore, FLT PET may play a significant role in the evaluation of indeterminate pulmonary lesions, in the prognostic assessment of resectable NSCLC, and possibly in the evaluation of NSCLC response to chemotherapy.

Instrumentation: General Electric Advance PET scanner.

In vivo validation of 3′deoxy-3′-[^{18}F]-fluorothymidine ([^{18}F]-FLT) as a proliferation imaging tracer in humans: Correlation of [^{18}F]-FLT uptake by positron emission tomography with Ki-67 immunohistochemistry and flow cytometry in human lung tumors. Vesselle H, Grierson J, Muzi M, Pugsley JM, Schmidt RA, Rabinowitz P, Peterson LM, Vallieres E, Wood DE. Clin Cancer Res 2002 Nov; 8 (11), pp. 3315–23.

Neoplasia/Thyroid Cancer: Metastatic/Recurrent or Metastatic Disease

[^{99m}Technetium] ([^{99m}Tc])-Furifosmin/[^{99m}Tc]-Methoxy-Isobutylisonitrile (MIBI)/[^{99m}Tc]-SestaMIBI/[^{99m}Tc]-Tetrofosmin/[^{18}F]-Fluoro-Deoxy-Glucose (FDG) Positron Emission Tomography (PET)

Many isotopes are available for imaging patients with suspected thyroid cancer recurrence and metastases. Thyroid-stimulating hormone (TSH)-stimulated low-dose [^{131}I] whole-body scanning with serum thyroglobulin either by standard LT4 withdrawal or rhTSH stimulation is the preferred test for monitoring patients without palpable disease or elevated serum thyroglobulin on LT4 therapy. This approach has the advantage of finding disease that may be amenable to [^{131}I] therapy, although low-dose [^{131}I] scans are less sensitive than are scans utilizing other imaging agents. [^{123}I] has better imaging characteristics than [^{131}I] and has been shown to be equivalent or superior to low-dose [^{131}I] in recent studies. As the availability of [^{123}I] increases, and cost decreases, this agent may replace [^{131}I] in imaging for recurrent or metastatic thyroid cancer. Patients who have an elevated serum thyroglobulin on LT4 therapy or after TSH stimulation but have a negative low-dose [^{131}I] scan require other imaging procedures to find the suspected disease. Many diagnostic imaging services perform a sensitive neck ultrasound to look for surgically remediable disease and consider a non-contrast computed tomography (CT) scan of the chest to look for small pulmonary metastases that poorly concentrate low doses of [^{131}I]. Fluoro-18-deoxy-glucose PET, [^{99m}Tc]-MIBI, [^{201}Tl], and [^{99m}Tc]-tetrofosmin are primarily useful in the setting of a negative whole-body [^{131}I] scan and elevated serum thyroglobulin. [^{18}F]-FDG PET seems to have the highest sensitivity in this setting and would be the preferred imaging agent, but availability and cost are major issues. Although some researchers have advocated these radiopharmaceuticals as 1st-line agents replacing [^{131}I], there is little support for this position. This approach to imaging is not cost-effective because positive scans in these patients would most likely require [^{131}I] scintigraphy to determine whether the lesions are amenable to radioiodine therapy. [^{99m}Tc]-pertechnetate, [^{99m}Tc]-furifosmin, and somatostatin receptor scintigraphy have a limited role in imaging for recurrent or metastatic differentiated thyroid carcinoma. In choosing among [^{99m}Tc]-MIBI, [^{201}Tl], and [^{99m}Tc]-tetrofosmin, technetium-labeled sesta-MIBI and tetrofosmin result in better image quality and faster imaging than [^{201}Tl]. Although [^{99m}Tc]-sestaMIBI and [^{99m}Tc]-tetrofosmin have not been compared in a large trial series, the higher tumor-to-background ratio and consistently high sensitivities of [^{99m}Tc]-tetrofosmin suggest that it could potentially have additional value over [^{99m}Tc]-sestaMIBI, but to date, experience with [^{99m}Tc]-tetrofosmin is limited.

Isotope imaging for metastatic thyroid cancer. Haugen BR, Lin EC. Endocrinol Metab Clin North Am 2001 Jun; 30 (2), pp. 469–92.

Neoplasia/Topoisomerase I and II/Adenocarcinoma (HT29)/Gliobastoma (U87MG)/Melanoma (A375M Antigen Positive)

[^{11}C]-9-Methoxyphenazine-1-Carboxamide/[^{11}C]2-(4-Pyridyl)Quinoline-8-Carboxamide/[^{11}C]-DACA/[Topoisomerases I and II]

Cellular/Molecular Mechanism of Action: The tricyclic carboxamide *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA) is a DNA-intercalating agent capable of inhibiting both topoisomerase I and II enzyme fractions.

Background Information: The tricyclic carboxamide DACA is currently in phase II clinical trial. Many related analogs have been developed, but despite their potent *in vitro* cytotoxicities, they exhibit poor extravascular distribution.

Application: Delineation of rational drug selection.

Semi-Synthetic Methods: Each candidate compound shares a common structural feature, *N*-dimethyl side chain, which can be radiolabeled with the positron-emitting radioisotope carbon-11.

Implementation: Evaluate the biodistribution and metabolite profiles of the prototype compound, DACA, with 3 analogs to aid rational drug selection in order to identify agents that are "minimal intercalators" that have lower DNA association constants. In this fashion, promising candidates will be identified in emerging preclinical studies in animals that can ultimately be evaluated in humans using positron emission tomography (PET).

Animal Model: Murine (mice bearing human tumor xenografts).

Interpretation and Analysis: Acridine DACA, phenazine SN 23490, pyridoquinoline SN 23719, and dibenzodioxin SN 23935 are cytotoxic when evaluated with *in vitro* assays utilizing HT29, U87MG, and A375M cell lines (IC50 of 1.4 to 1.8 μM, 0.4 to 0.6

μM, 1.3 to 1.6 μM, and 24 to 36 μM, respectively). *Ex vivo* biodistribution studies with carbon-11 radiolabeled compounds in mice bearing human tumor xenografts show rapid clearance of [^{11}C]-radioactivity (parent drug and metabolites) from blood and major organs. Rapid hepatobiliary clearance and renal excretion can also be observed. There is low (< 5% of injected dose/gram [%ID/g]) and variable uptake of [^{11}C]-radioactivity in 3 tumor types for all of the compounds. Tumor (U87MG) to blood [^{11}C]-radioactivity for [^{11}C]-DACA, [^{11}C]-(9-methoxyphenazine-1-carboxamide (SN 23490), [^{11}C]2-(4-pyridyl)quinoline-8-carboxamide (SN 23719), and [^{11}C]-dibenzo[1,4]dioxin-1-carboxamide (SN 23935) at 30 minutes is approximately 2.9 ± 1.1, 2.3 ± 0.6, 2.6 ± 0.6, and 0.7 ± 0.2, respectively. For SN 23719, the distribution of [^{11}C]-radioactivity in normal tissues and tumors determined *ex vivo* is in broad agreement with that determined *in vivo* by whole-body PET scanning. [^{11}C]-DACA is rapidly and extensively metabolized to several plasma metabolites and a major tumor metabolite. In contrast, [^{11}C]-SN 23935, [^{11}C]-SN 23490, and [^{11}C]-SN 23719 shows less extensive metabolism. In tumor samples, parent [^{11}C]-DACA and [^{11}C]-SN 23935 is present at between 0.3 and 1.5 %ID/g, whereas [^{11}C]-SN 23490 and [^{11}C]-SN 23719 is present at between 1.5 and 2.8 %ID/g. Therefore, by using a strategy employing [^{11}C]-labeling, it is possible to determine the tissue distribution and metabolic stability of novel tricyclic carboxamides.

Given the objective of identifying analogs with potentially improved *in vivo* activity against solid tumors, the agents SN 23490 and SN 23719 have more favorable distribution and metabolic stability compared with DACA and SN 23935. Such observations suggest that further development and evaluation is warranted. The radiolabeling strategy used allows *ex vivo* and *in vivo* evaluation of promising anticancer agents in animals and offers the potential of rapid translation to studies in humans using PET.

Comparative biodistribution and metabolism of carbon-11-labeled N-[2-(dimethylamino)ethyl]acridine-4-carboxamide and DNA-intercalating analogues. Osman S, Rowlinson-Busza G, Luthra SK, Aboagye EO, Brown GD, Brady F, Myers R, Gamage SA, Denny WA, Baguley BC, Price PM. Cancer Res 2001 Apr 1; 61 (7), pp. 2935–44.

Neoplasia/Treatment Response Prediction/ 5-Fluorouracil
[^{18}F]-2-Fluoro-2-Deoxy-D-Glucose (FDG)/ [^{18}F]-2-Fluoro-2-Deoxy-D-Mannose (FDM)

Background Information: There is a clinical need for early detection of tumor response to therapy.
Route of Administration: FDG (intraperitoneal).
Dosage: FDG (1.4 mmol/kg [rat]).
Implementation: Evaluation of the ability for metabolites of FDG to detect responses of solid tumors to 5-fluorouracil chemotherapy applying [^{19}F] magnetic resonance spectroscopy (^{19}F MRS). Following FDG injection, uptake and metabolism are monitored for 2 hours in RIF-1 tumors. Mice treated with 5-fluorouracil (130 mg/kg) receive a repeat dose of FDG 48 hours later.
Animal Model: Murine (mouse *in situ* solid tumor).
Interpretation and Analysis: FDG is detectable immediately and 10 minutes after injection (2nd broad peak detected 5–6 ppm upfield). [^{19}F] MRS *in vitro* analysis of cell and tumor extracts shows that the upfield peak (≥ 15% of total detectable [^{19}F] signal) consists of the epimer α-fluorodeoxymannose (FDM) and various conjugates. The change in the rate of FDM formation, but not FDG or total [^{19}F] signal, correlates significantly with response to 5-fluorouracil ($p = 0.032$), suggesting that [^{19}F] MRS of FDM metabolism *in vivo* may be a novel means of predicting tumor response. Since FDG and FDM employ a fluoride atom, it is possible that they could also be labeled with [^{18}F] for position emission investigations.

Metabolites of 2′-fluoro-2′-deoxy-D-glucose detected by [^{19}F] magnetic resonance spectroscopy *in vivo* predict response of murine RIF-1 tumors to 5-fluorouracil. McSheehy PM, Leach MO, Judson IR, Griffiths JR. Cancer Res 2000 Apr 15; 60 (8), pp. 2122–7.

Neoplasia/Treatment Response (Radiation Therapy)/Glucose Metabolism/Amino Acid Metabolism/Nucleic Acid Metabolism
[^{18}F]-5-Fluorodeoxyuridine/L-Methyl-[^{14}C]-Methionine/ 6-[^{3}H]-Thymidine/2′-Deoxy-5-[^{18}F]-Fluorouridine

Cellular/Molecular Mechanism of Action: Reflects neoplastic cell nucleic acid metabolism.
Application: Diagnostic imaging of neoplastic lesions.
Route of Administration: [^{18}F]-5-fluorodeoxyuridine (usually intravascular).
Implementation: Evaluation of metabolic tracers for glucose, amino acid, and nucleic acid metabolisms (2-deoxy-2-[^{18}F]-fluoro-D-glucose (^{18}FDG), L-methyl-[^{14}C]-methionine ([^{14}C]-Met), 6-[^{3}H]thymidine (^{3}H-Thd), and 2′-deoxy-5-[^{18}F]-fluorouridine (^{18}FdUrd)), and the conventional radionuclide [^{67}Ga]-citrate can be used to assess the feasibility of monitoring the effectiveness of tumor radiotherapy using a quadruple tracer technique. In this context, 2 combinations of 4 tracers ([^{18}F]-FDG or ^{18}FdUrd, [^{14}C]-Met, [^{3}H]-Thd, and [^{67}Ga]) can be compared in a time-course study after single-dose irradiation (20 Gy) and also used in a dose-dependency study performed 6 days after 5, 10, 15, or 20 Gy of irradiation.
Animal Model: Rat AH109A tumor model.
Interpretation and Analysis: [^{18}F]-FDG shows a large change in uptake and a steady response to radiotherapy. Fluorodeoxyuridine shows a rapid decrease after radiotherapy, but the range of change in uptake is narrow. [^{67}Gallium] cannot detect tumor response early after treatment, but shows a marked change in uptake later. 6-[^{3}H]-Thd and [^{14}C]-Met show a rapid response to irradiation and a high sensitivity for monitoring radiotherapy, suggesting that they may be applicable for PET studies.

Tracer feasibility for monitoring tumor radiotherapy: A quadruple tracer study with fluorine-18-fluoro-deoxy-glucose or fluorine-18-fluorodeoxyuridine, L-[methyl-^{14}C]-methionine, [6-3H]thymidine, and gallium-67. Kubota K, Ishiwata K, Kubota R, Yamada S, Tada M, Sato T, Ido T. J Nucl Med 1991 Nov; 32 (11), pp. 2118–23.

Neutrophil Metabolism
Deoxyglucose/Formyl-Methionyl-Leucyl-Phenylalanine (fMLP)/Tumor Necrosis Factor-Alpha (TNF-α)

Cellular/Molecular Mechanism of Action: The chemotactic agent *f*MLP stimulates respiratory burst activity in neutrophil populations.
Background Information: Neutrophil granulocytes play a key role in the pathogenesis of a wide variety of pulmonary diseases. In many such conditions, the injury observed reflects the activation status rather than the total number of inflammatory cells present. The metabolic activity of neutrophils can now be assessed non-invasively using PET to measure the regional uptake of [^{18}F]-FDG after intravenous injection.
Application: Characterization of neutrophil dexoxyglucose metabolism as a model for understanding *in vivo* [^{18}F]-FDG uptake pertaining to viable neutrophil populations.

Implementation: Utilization of tritiated deoxyglucose (DG) in neutrophils isolated from human peripheral blood to discover and characterize the mechanisms responsible for increases in [^{18}F]-FDG signals detected by PET under *in vivo* conditions.

Interpretation and Analysis: The chemotactic agent *f*MLP stimulates (neutrophil) respiratory burst activity and [^{3}H]-DG uptake. Each of these processes is temporally dissociated from 1 another. The neutrophil-priming agent TNF-α causes an identical increase in [^{3}H]-DG uptake compared with *f*MLP but without affecting respiratory burst activity. The stimulation of TNF-α primed cells with *f*MLP causes a major upregulation of superoxide anion generation (O_2^-) in the absence of any incremental increase in [^{3}H]-DG uptake. Furthermore, direct activation of reduced nicotinamide adenine dinucleotide phosphate oxidase activity with the ester phorbol 12-myristate 13-acetate (PMA) results in a concentration-dependent loss of cell-associated [^{3}H]-DG, and preincubation of neutrophils with the phosphoinositide 3-kinase inhibitor wortmannin, which abolished both agonist-stimulated superoxide anion generation and degranulation, but has no effect on TNF-α-stimulated or *f*MLP-stimulated [^{3}H]-DG uptake. In contrast, *f*MLP-stimulated changes in neutrophil shape is not influenced by priming or wortmannin, and this appears to correlate with the functional response of [^{3}H]-DG uptake. In summary, deoxyglucose uptake occurs in both primed and activated neutrophils. It does not correlate with respiratory burst or secretory activity but may reflect the polarization and migrational status of these cells.

Dissociation between respiratory burst activity and deoxyglucose uptake in human neutrophil granulocytes: implications for interpretation of [^{18}F]-FDG PET images. Jones HA, Cadwallader KA, White JF, Uddin M, Peters AM, Chilvers ER. J Nucl Med 2002 May; 43 (5), pp. 652–7.

Renal and Hepatic Cysts/Polycystic Kidney Disease: Autosomal Dominant
[^{18}F]-Fluoro-Deoxy-Glucose (FDG)

Background Information: Infection of a renal or hepatic cyst is a serious complication of autosomal dominant polycystic kidney disease (ADPKD). Although crucial for successful management, early diagnosis is difficult, largely because of non-specific symptoms and limitations of conventional imaging techniques. Because of an increased metabolic rate, inflammatory cells take up large amounts of glucose. [^{18}F]-FDG, therefore, represents a promising agent for detection of cyst infections using positron emission tomography (PET).

Implementation: Performance of [^{18}F]-FDG PET scan imaging in cases of ADPKD suspected of being affected by renal or hepatic cyst infection.

Human Model: Cases of suspected renal or hepatic cyst infection (e.g., n = 7 FDG PET scans in 3 ADPKD cases suspected of renal or hepatic cyst infection; n = 2 PET scans of case A [PET 1 and 2]; n = 1 PET scan of case B [PET 3]; and n = 4 PET scans of case C [PET 4, 5, 6, and 7]).

Interpretation and Analysis: [^{18}F]-FDG PET can identify infected cysts in conditions of (i) renal cyst infection; (ii) hepatic cyst infection; and (iii) simultaneous renal and hepatic cyst infection. Evaluation of FDG PET imaging modalities has proven to be very helpful in establishing diagnoses and in excluding renal and hepatic cyst infections. Based on experience with FDG PET, this diagnostic strategy has promise as an imaging technique enabling early identification of renal and hepatic cyst infections in ADPKD patients.

Diagnosis of renal and hepatic cyst infections by 18-F-fluoro-deoxy-glucose positron emission tomography in autosomal dominant polycystic kidney disease. Bleeker-Rovers CP, de Sévaux RG, van Hamersvelt HW, Corstens FH, Oyen WJ. Am J Kidney Dis 2003 Jun; 41 (6), pp. E18–21.

20 Radiographic Image-Enhancing Agents

CONVENTIONAL RADIOGRAPHY

Adverse Reactions: Allergic Reactions to Iodinated Contrast Agents/Nephrostolithotomy/Antegrade Pyelography with Nephroureteral Stent/ Nephrostogram with Nephroureteral Stent
Gadodiamide [Gd]

Application: Contrast imaging agent that can be applied as an alternative to iodinated preparations in situations where allergy/ hypersensitivity reactions exist against iodinated contrast agents.
Implementation: Utilized as a contrast agent to facilitate diagnostic procedures, including percutaneous access for nephrostolithotomy, antegrade pyelography with nephroureteral stent placement, and diagnostic nephrostogram with nephroureteral stent exchange. In cases with histories of severe allergic reactions to iodinated contrast or marked renal insufficiency, interventional uroradiologic procedures can performed using full-strength [Gd] as a contrast agent in place of iodinated contrast material.
Interpretation and Analysis: Fluoroscopy can be applied to visualize [Gd] for the generation of "satisfactory" digital radiographs without allergic reaction or worsening renal function. In this context, [Gd] can be useful in guiding interventional uroradiologic procedures when iodinated contrast agents are contraindicated.

Interventional uroradiologic procedures performed using gadodiamide as an alternative to iodinated contrast material. Spinosa DJ, Angle JF, Hagspiel KD, Hartwell GD, Jenkins AD, Matsumoto AH. Cardiovasc Intervent Radiol 2000 Jan–Feb; 23 (1), pp. 72–5.

Adverse Effects: Cardiovascular Alterations
Iodinated Contrast Agents

The ideal intravascular contrast agent would be biologically inert and have no pharmacologic actions. Pharmacologic actions of currently used radiographic contrast agents are determined principally by 3 physicochemical properties of the iodine-bearing molecule and its formulation: osmolality, sodium concentration and calcium-binding properties. Within this framework, the calcium-binding 1.5 ratio agents have the most marked effects, and the 3.0 ratio non-ionic agents the least, while the non-calcium-binding formulations of 1.5 ratio agents and ioxaglate (the only 3.0 ratio ionic agent) are in between. Differences in hemodynamic effects are predominantly related to osmolality with the 3.0 agents causing less hemodynamic disturbance. The magnitude of difference is small enough that the 3.0 ratio agents have no important clinical advantage when used in patients with good or moderately impaired left ventricular function. However, the difference may be important in patients with severely impaired circulatory performance. The principal electrophysiologic differences are between the calcium-binding 1.5 ratio agents (which are associated with a clear-cut greater frequency of ventricular fibrillation during coronary injection than the non-calcium-binding 1.5 ratio agents) and the 3.0 ratio agents. There is no justification for the use of calcium-binding 1.5 ratio agents, because non-calcium-binding formulations of the same molecule are available at the same price. The circulatory reserve of most patients makes the differences between 3.0 ratio agents and non-calcium-binding 1.5 ratio agents clinically unimportant. In view of the substantial price disparity between 1.5 ratio and 3.0 ratio agents, non-calcium-binding 1.5 ratio agents are appropriate for patients with good circulatory performance and 3.0 ratio agents are best reserved for patients with impaired circulatory performance.

Cardiovascular effects of iodinated contrast agents. Hirshfeld JW Jr. Am J Cardiol 1990 Oct 26; 66 (14), pp. 9F–17F.

Adverse Reactions: Cardiovascular/Cardiac Angiography Imaging
Iodixanol/Iohexol

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Iodixanol and iohexol are iodinated positive contrast image-enhancing agents that can be applied during diagnostic cardiac angiography procedures. Iodixanol is a ratio 6 non-ionic iodinated contrast agent with an osmolality equivalent to serum.
Implementation: Comparison of iodixanol with iohexol in elective diagnostic cardiac angiography procedures (e.g., n = 101 iodixanol studies; and n = 99 iohexol studies).
Interpretation and Analysis: There are no differences that can be detected between iodixanol and iohexol pertaining to mean changes in systolic or diastolic blood pressure or heart rate during or immediately after any angiography procedure. However, significantly more cases will develop decreases in diastolic blood pressure of > 20 mm Hg during left coronary angiography with iodixanol. The only significant differences in any electrophysiologic parameters is a slightly more prolongation of the PR interval during left coronary angiography with iodixanol and more ST-segment depression with iohexol during coronary angiography. Neither of these alterations can be considered to be clinically significant. Injection-associated discomfort occurs with both agents, but moderate to severe discomfort is more prevalent with iohexol (52%) than with iodixanol (17%) ($p < 0.001$). Potentially serious adverse events, such as ventricular fibrillation can rarely be observed with iohexol (e.g., n = 1/99). Overall, angiographic quality is equal with all angiograms being assessed as good or excellent in both groups ($p = 0.885$). In this low-risk population undergoing cardiac angiography, iodixanol is safe and effective without clinically important differences from iohexol. Additional studies in patients at high risk for complications should help further define the role of iodixanol in cardiac angiography.

Iodixanol, a new isosmotic non-ionic contrast agent compared with iohexol in cardiac angiography. Hill JA, Cohen MB, Kou WH, Mancini GB, Mansour M, Fountaine H, Brinker JA. Am J Cardiol 1994 Jul 1; 74 (1), pp. 57–63.

Adverse Effects: Coronary Microvasculature *Ex Vivo* Vasoactive Responses
Diatrizoate Meglumine

Background Information: Iodinated contrast media can cause a number of well-described acute hemodynamic and vascular effects, including vascular spasm, hypotension, and arrhythmias.
Route of Administration: Diatrizoate meglumine (intravascular).
Dosage: Diatrizoate meglumine (5 ml/injection with a cumulative dose of 60 mL).
Implementation: Examine the endothelium-dependent and endothelium-independent vasodilator responses of the microvessels after previous contrast media administration in a clinically relevant setting. Left coronary angiography (e.g., n = 6 pigs) using high-dose, diatrizoate meglumine (cumulative dose of 60 mL [5 mL/injection]) as a high-osmolar contrast agent. After 1 hour of reperfusion, epicardial coronary microvessels are studied *in vitro* in a pressurized, no-flow state with video microscopy. The vasodilators bradykinin, calcium ionophore A23187, and sodium nitroprusside are sequentially applied extraluminally after preconstriction. Serotonin and the thromboxane A2 analogue U46619 are studied without preconstriction.

Animal Model: Swine (n = 6).
Interpretation and Analysis: Microvessels exposed to diatrizoate meglumine have normal relaxation responses to the endothelium-dependent vasodilators bradykinin and calcium ionophore A23187 when compared to control vessels. The vasoconstrictor responses to U46619 and serotonin are not significantly altered compared to control vessels. Responses to the endothelium-independent vasodilator sodium nitroprusside are not reduced or are slightly enhanced after exposure to contrast media. In summary, coronary resistance vessel response to the endothelium-dependent vasodilators bradykinin and calcium ionophore A23187 are not diminished after previous exposure to diatrizoate meglumine. The vasoconstrictor responses to U46619 and serotonin are similarly unaffected by previous exposure to contrast media. Such observations suggest that, when used in clinically relevant amounts, diatrizoate meglumine does not cause functional endothelium or vascular smooth muscle impairment.

Coronary microvascular responses after exposure to iodinated contrast media. Piana RN, Banitt PF, Nunez BN, Dai HB, Sellke FW. Invest Radiol 1994 Oct; 29 (10), pp. 877–81.

Adverse Reactions: Cardiovascular and Hemodynamic Effects
Gadolinium Diethylenetriamine Pentaacetic Acid ([Gd]-DTPA)/Diatrizoate/Iopamidol

Cellular/Molecular Mechanism of Action: Gadolinium and iodinated compounds possess radiopaque properties. The agent DTPA functions as a chelating agent for either radioactive or non-radioactive heavy metal ions.
Application: Conventionally [Gd] in the form of [Gd]-DTPA, diatrizoate (ionic), and iopamidol (non-ionic) can be used as positive contrast image-enhancing agents for the detection of anatomical anomalies affecting the cardiovascular system (conventional radiography and magnetic resonance imaging).
Route of Administration: [Gd]-DTPA, diatrizoate, and iopamidol can all be given intravascularly as a bolus injection for the detection of anatomical anomalies affecting the cardiovascular system.
Dosage: Gadopentate dimeglumine ([Gd]-DTPA [Magnevist] at high dose = 0.6 mmol/kg or 1.2 ml/kg in swine). Diatrizoate (Urografin 76% at high dose = 0.6 mmol/kg or 1.2 ml/kg in swine). Iopamidol (Solutrast 370% at high dose = 0.6 mmol/kg or 1.2 ml/kg in swine).
Implementation: Evaluation of the hemodynamic reactions induced by high-dose [GD]-DTPA compared with the side effects of high-dose ionic (diatrizoate: Urografin 76%) and non-ionic (iopamidol, Solutrast 370) radiographic contrast media.

Study Design: Assessement of pressure and flow of the systemic and pulmonary circulation following intracardiac bolus injections delivered over a time period of 2 to 4 seconds.

Animal Model: Porcine (e.g., n = 18 swine).
Interpretation and Analysis: Gadolinium, diatrizoate, and iopamidol all decrease the aortic pressure transiently ([Gd]-DTPA and diatrizoate: −25%, iopamidol: −10%; $p < 0.01$). Pulmonary artery pressure, cardiac output, and stroke volume remain increased for several minutes, while vascular resistance declines. Diatrizoate induces stronger and longer-lasting side effects ($p < 0.01$) than [Gd]-DTPA and iopamidol. Despite similar osmolalities, [Gd]-DTPA induces weaker side effects than equivalent volumes of diatrizoate. Other than osmolality, additional factors such as viscosity and chemotoxicity also influence the occurance of contrast mediated–associated side effects.

Gadopentetate dimeglumine and iodinated contrast media. Hemodynamic side effects after bolus injections in pigs. Kuhtz-Buschbeck JP, Ehrhardt K, Kohnlein S, Radtke W, Heintzen P. Invest Radiol 1997 Feb; 32 (2), pp. 111–9.

Adverse Effects: Cardiovascular: Vasodilatory Properties/Nitric Oxide (NO) Release
Iopromide/Iohexol/Iodixanol

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties. Iopromide and iohexol are triiodinated monomers, while iodixanol is a hexaiodinated dimer.
Application: Iohexol and iodixanol can be used as positive contrast imaging agents (e.g., conventional radiography).
Route of Administration: Anticipated to be intravascular with potential applications for direct infusion into body fluid compartments.
Dosage: Iodixanol (320 mg/ml in *ex vivo* perfused arteries) and iopromide (300 mg/ml in *ex vivo* perfused arteries).
Implementation: Method I: Evaluation of the vasodilator effects of iohexol (300 mg/ml) and iodixanol (320 mg/ml) utilizing *ex vivo* endothelium-intact rings (rabbit and dog). Method II: Evaluation of the vasodilator effects of iohexol (300 mg/ml) and iodixanol (320 mg/ml) utilizing isolated perfused arteries (rabbit and dog).

Animal *Ex Vivo* Model: Canine and rabbit (*ex vivo* perfused artery preparations).
Interpretation and Analysis: Increasing concentrations of iohexol or iodixanol cause concentration-dependent relaxations of detector tissue that are insensitive to 100 μM NG-nitro L-arginine methyl ester (L-NAME) and 10 μM indomethacin, whereas viscosity-associated relaxations induced by the "inert" agent dextran (MW 80,000; 1–4%) are attenuated by inhibition of NO synthesis. Relaxations of endothelium-intact rings by exposure to acetylcholine (ACh) are attenuated by preincubation with iohexol or iodixanol, whereas relaxations to sodium nitroprusside (SNP) in endothelium-denuded rings are unaffected. Inhibitory activity does not correlate with either molarity or iodine concentration. Mannitol causes an inhibition of both ACh- and SNP-induced responses. In isolated perfused arteries the depressor response to iodixanol (320 mg/ml) and iopromide (300 mg/ml) administered as a closed arterial bolus attains a plateau with maximal dilatations of approximately 25% and approximately 60%, respectively. Addition of 100 μM NG-nitro L-arginine (L-NOARG) and/or 10 μM indomethacin to the perfusate

does not effect the responses to either contrast agent. Iodinated radiographic contrast media therefore exerts a direct effect on the endothelium in a manner that inhibits NO production rather than an action on vascular smooth muscle. Shear stress-induced stimulation of NO production by iodinated radiographic contrast media is unlikely to contribute to their vasodilator activity *in vivo* when administered during angiography despite high intrinsic levels of viscosity.

Iodinated radiographic contrast media inhibit shear stress- and agonist-evoked release of NO by the endothelium. Hutcheson IR, Griffith TM, Pitman MR, Towart R, Gregersen M, Refsum H, Karlsson JO. Br J Pharmacol 1999 Sep; 128 (2), pp. 451–7.

Adverse Effects: Cardiovascular: Vasodilation Postinjection/Comparison Study
Iodixanol/Iopromide

Cellular/Molecular Mechanism of Action: Iodixanol and iopromide are iodinated organic compounds that possess radiopaque properties.
Application: Iodixanol and iopromide can be applied as radiopaque image contrast-enhancing agents in conventional radiographic methodologies.
Route of Administration: Iodixanol and iopromide (intravenous).
Dosage: Formulation of iodixanol and iopromide at 320 mg I/ml and 300 mg I/ml, respectively.
Implementation: Assess the correlation between flow-dependent and contrast-related vasodilatation associated with the administration of iodixanol or iopromide and evaluate the impact of plaque formation on vasodilatation in coronary arteries. Such an objective can be achieved by evaluating controlled randomized paired cross-over comparisons between iodixanol and iopromide. Vessel area, endosonics and blood flow velocity measurements, and cardiometrics are recorded simultaneously at an identical position, at baseline, after intracardiac bolus injection of 10 ml physiologic saline (flow-dependent vasodilatation), and after randomized application of either iodixanol or iopromide contrast agents.

Human Model: Human cases evaluated by diagnostic intracoronary artery angiography procedures ($n = 10$).
Interpretation and Analysis: The action of iodixanol and iopromide on the vascular wall does not differ and is equal to local flow-dependent vasodilatation induced by a saline bolus (correlation 0.95–0.98). The increase in local luminal area after injection of saline, iodixanol and iopromide in morphologically normal vessels (approximately 2.5 mm^2) is absent in atherosclerotic segments. Both contrast agents and saline demonstrate nearly identical flow increases. If iodixanol or iopromide are used as contrast agents, contrast-related vessel area increases *in vivo* seem to be endothelium-dependent.
Instrumentation: (i) Visions Five-64 F/X intravascular ultrasound-catheter; (ii) 0.014 inches Doppler guide wire.

Is contrast-related vasodilatation after intra-coronary iodixanol and iopromide *in vivo* endothelium-dependent? Wellnhofer E, Dreysse S, Fleck E. Eur J Echocardiogr 2001 Dec; 2 (4), pp. 285–91.

Adverse Effects: Coronary Artery: Decreased Resistance
Ioxaglate (Low Osmolar)/Iohexol (Non-Ionic)/ Diatrizoate Meglumine (Ionic)

Cellular/Molecular Mechanism of Action: L-nitro-arginine is a competitive inhibitor of nitric oxide (NO) synthase (NOS). L-arginine is a substrate for NOS. Nitric oxide is considered to be one of the endogenous mediators of inflammatory responses.
Physiological Mechanism of Action: Meglumine sodium ioxaglate, iohexol, diatrizoate meglumine, and ditrizoate sodium promote vasodilatory responses.
Application: Meglumine sodium ioxaglate, iohexol, diatrizoate meglumine, and ditrizoate sodium promote vasodilatory responses and are traditionally used as positive contrast image-enhancing agents during the course of a variety of diagnostic procedures, including angiography procedures.
Route of Administration: Meglumine sodium ioxaglate, iohexol, diatrizoate meglumine, and ditrizoate sodium can all be delivered intravascularly.
Implementation: Evaluation of changes in coronary blood flow and coronary vascular resistance induced in response to the intracoronary artery injection of saline solution (0.5 mol/L, isosmolar with plasma) and 3 different contrast agents, including meglumine sodium ioxaglate (Hexabrix), a low-osmolar ionic contrast agent; iohexol (Omnipaque 300), a non-ionic contrast agent; and diatrizoate meglumine 66%, diatrizoate sodium 10% (MD-76), an ionic contrast agent. Measurements are made during 3 experimental conditions, including coronary artery infusion of (i) saline solution, control; (ii) L-nitro-arginine (LNNA; 10^{-3} mol/L and 10^{-2} mol/L), a competitive inhibitor of NO synthase; and (iii) L-arginine 10^{-1} mol/L, a substrate for nitric oxide (NO) synthesis.

Animal Model: Anesthetized, open-thorax swine on assisted ventilation.
Interpretation and Analysis: The infusion of L-nitro-arginine (LNNA) produces an increase in baseline coronary vascular resistance ($p < 0.001$), but it does not attenuate the vasodilatory response to the infusion of the contrast-enhancing imaging agents. Both the high and low-osmolar ionic and non-ionic contrast media cause decreases in baseline coronary vascular resistance. For all 3 conditions, diatrizoate sodium (MD-76: highest osmolality preparation) produces the greatest decrease in coronary vascular resistance. The vasodilatory response of the coronary vasculature to contrast agents is directly related to osmolality and is not mediated by NO generation.

Effect of contrast media on coronary vascular resistance: contrast-induced coronary vasodilation. Baile EM, Pare PD, D'yachkova Y, Carere RG. Chest 1999 Oct; 116 (4), pp. 1039–45.

Adverse Effects: Central Nervous System (CNS)/ Synaptic Transmission/Weak Transcient Excitation/ Epileptogenic Activity/Inhibitory Activity
Iomeprol/Ioversol/Iopentol/Ioxaglate/Diatrizoate

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Physiological Mechanism of Action: Certain iodinated contrast agents influence normal neurophysiological processes.
Background Information: All contrast agents should be neurologically safe because although some are not indicated for procedures, such as myelography, just the same they may come in contact with nervous tissue during contrast-enhanced imaging. In part, this is because even when they are injected intravascularly, the presence of undiagnosed injury to the blood-brain barrier may allow penetration across such protective anatomical structures.
Application: Positive contrast image enhancement agent for application within the intravascular system and other body fluid compartments.
Implementation: Delinate the neurologic safety of iomeprol and other widely used radiographic contrast agents by *in vitro* studying of

its potential effects on CNS synaptic transmission. One approach to addressing this objective includes the evaluation of CNS synaptic transmission in terms of evoked field potentials recorded from the pyramidal region of hippocampal slices to determine the influence of radiographic contrast media on CNS function. Field potentials can be evoked by electrical stimulation of the Schaffer collateral pathway. Effects and influences of individual contrast agents can be established in terms of the number and amplitude of population spikes (PS) and as the maximal slope of the excitatory postsynaptic potentials (EPSP). The contrast agents are tested at final concentrations of 3, 10, and 30 mg (iodine)/mL in iso-osmolar conditions with respect to artificial cerebrospinal fluid (CSF).

Animal Model: Rat (measurement of CNS synaptic transmission in the form of evoked field potentials recorded from the pyramidal region of hippocampal slices).

Interpretation and Analysis: Iomeprol, like ioversol, principally exerts a mild inhibitory effect on CNS synaptic transmission, an effect that is preceded by a weak, transient excitation phase. Iopentol exerts a rapid and complete inhibition of synaptic transmission without showing any excitatory effects. Iobitridol, though belonging to the non-ionic monomeric class, exerts, surprisingly, an epileptogenic action at the highest concentration, whereas its inhibitory action is slow and mild. Diatrizoate exerts an epileptogenic activity even at low concentrations, followed by a marked inhibitory action. Ioxaglate, because it is an ionic though dimeric contrast agent, exerts an epileptogenic action at an intermediate concentration, whereas it barely demonstrates an inhibitory effect at all. The effects associated with iopentol, iobitridol, diatrizoate, and ioxaglate tend to reverse with CNS parenchyma tissue washout.

Even taking into account the limitation because of the use of an *in vitro* approach and high contrast agent concentrations, it can be conclude that the positive neuro-tolerability of iomeprol has been further confirmed, and is apparently devoid of epileptogenic activity. Similarly, when compared among other contrast agents exhibiting inhibitory action, iomeprol is the contrast agent with the least amount of activity. In addition, contrary to reports generally described in the literature, non-ionic, low osmolal contrast agents are not all identical in their neuro-tolerability when assessed in the rat hippocampal slice model.

Effect of iomeprol on rat hippocampal slice synaptic transmission: Comparison with other x-ray contrast agents. Frigeni V, Samuelli G, Miragoli L, Grotti A, Lorusso V. Invest Radiol 2002 Apr; 37 (4), pp. 222–31.

Adverse Effects: Central Nervous System (CNS) Sensory Function (Taste, Heat, Pain/Cardiovascular) *Iomeprol/Iohexol/Iomeprol/Iopamidol/Iopromide*

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.

Background Information: Unlike older ionic agents, iomeprol has low chemotoxicity, osmolality, and viscosity and high water solubility. Compared with other non-ionic contrast media, the osmolality and viscosity are lower and the water solubility is reportedly higher with iomeprol.

Application: Iomeprol functions as a non-ionic, monomeric iodinated positive contrast image-enhancing agent for use in conventional radiology.

Route of Administration: Iomeprol (formulated at 150 to 400 mg/ml of iodine).

Interpretation and Analysis: Most radiographs (about 67 to 100%) obtained with iomeprol (containing 150–400 mg/ml of iodine) are of good or excellent quality in non-comparative and comparative trials undergoing various radiographic procedures (e.g., trail size n = 40–6,127 cases). The diagnostic efficacy of iomeprol does not differ significantly from that of other non-ionic agents (iopamidol, iopromide, iohexol, and iotrolan). Iomeprol (containing 150–400 mg/ml of iodine) is well tolerated in clinical trials. Most adverse events are transient and of mild to moderate intensity and similar to those observed with other contrast media. The overall incidence of adverse events range from 3.0% to 49.7% and mainly include focal pain (≤6%) and heat sensations (8–45%), taste disturbances (3–27%) and various pseudoallergic reactions (≤ 20% for each type of event). The incidence of heat or pain and taste disturbances with iomeprol are similar to that observed with iopromide and iopamidol. Pain (but not heat sensations) is reported to be significantly less frequently and taste disturbances are reported to be significantly more frequent with iomeprol than with iohexol in comparative trials. Pseudoallergic reactions (such as nausea, vomiting, skin reactions, dizziness, headache) are significantly less common with iomeprol than with ioxaglate and occur at a similar frequency relative to iopromide and iopamidol. Cardiovascular events are rarely observed with iomeprol. Currently available iomeprol solutions contain a range of iodine concentrations (150 to 400 mg/ml) and are approved for a wide variety of diagnostic procedures. Iomeprol solutions are chemically stable, which negates the need for chelating agents. Formulations of this agent were therefore the 1st not to contain edetic acid (EDTA). Iomeprol shows equivalent diagnostic efficacy, and a similar adverse event profile, to that of other non-ionic contrast media. The availability of a range of iodine concentrations enables iomeprol to be used in a variety of diagnostic procedures. Iomeprol, like others in its class, is suitable for use in diagnostic imaging.

Iomeprol: A review of its use as a contrast medium. Dooley M, Jarvis B. Drugs 2000 May; 59 (5), pp. 1169–86.

Adverse Effects: Coagulation Alterations: Anticoagulant Properties *Iopiperidol/Ioxaglate/Iodixanol*

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.

Background Information: Iopiperidol, a non-ionic, triiodinated contrast agent.

Implementation: Characterization of the *in vitro* and *in vivo* anticoagulant effects of iopiperidol following single or repeated intravenous injections (rats) can be established by the measurement of prothrombin time, activated partial thromboplastin time, thrombin time, thrombin activity, thrombin generation, and fibrinogen tests. Complementary evaluations include analysis of the effect of iopiperidol on adenosine diphosphate- and collagen-induced platelet aggregation both *in vitro* and *in vivo* after single or repeated intravenous administrations (rat).

Animal Model: Rat.

Interpretation and Analysis: Anticoagulant properties of iopiperidol *in vitro* are similar or superior to those of the ionic contrast agent ioxaglate. Iopiperidol also inhibits collagen-induced platelet aggregation significantly more than does iodixanol or ioxaglate ($p < 0.05$). No significant *in vivo* differences between iopiperidol and ioxaglate can be appreciated after either single or repeated administrations. The *in vitro* anticoagulant effect of iopiperidol is similar or even superior to that of ioxaglate; and its *in vivo* effect is similar to that of reference non-ionic contrast media.

Iopiperidol: Non-ionic iodinated contrast medium with promising anticoagulant and antiplatelet properties. Valenti R, Motta A, Merlini S, Morisetti A, Tirone P. Acad Radiol 1999 Jul; 6 (7), pp. 426–32.

Adverse Reactions: Coagulation Alterations/ Antithrombotic Properties: Iodinated Contrast Agents/Synergistic Antiplatelet Aggregatory Properties
Iohexol/Iodixanol/Ioxaglate

Route of Administration: Iohexol and iodixanol (intravenous)
Dosage: Iohexol and iodixanol (both administered at 1,600 mg iodine/kg).
Implementation: The antithrombotic potential of low-osmolar ionic (ioxaglate Na/meglumine) or non-ionic contrast media (iohexol and iodixanol) can be assessed by measuring the time to occlusion (TTO) of the carotid artery and the thrombus weight (TW). Isotonic saline and iso-osmolar (280 mOsm/kg) and hyperosmolar (560 mOsm/kg) solutions of meglumine hydrochloride, meglumine ioxaglate (560 mOsm/kg), sodium ioxaglate (600 mOsm/kg) and sodium and meglumine ioxaglate (commercial solution) can be tested under similar conditions. Interaction with clopidogrel is evaluated by injecting a lower dose of iodinated contrast media (960 mg iodine/kg) 2 hours after clopidogrel (2 mg/kg per os).

Animal Model: Rats with carotid thrombosis induced extravascularly with $FeCl_3$ (35% vol/wt soaked filter paper) applied topically utilizing a soaked section of filter paper. Ultrasound flow imaging is then performed distal to the site of topical $FeCl_3$ application,
Interpretation and Analysis: (i) Ioxaglate prolongs the TTO parameter when compared with saline (30.0 ± 1.1 minute vs 19.6 ± 2.4 minutes, $p < 0.001$), whereas iohexol has no effect (21.3 ± 1.3 minutes). Ioxaglate's effect is associated with a reduction in thrombus-weight with ioxaglate relative to saline (2.6 ± 0.4 mg and 4.7 ± 0.7 mg, respectively, $p < 0.05$) whereas thrombus-weight remains unchanged with iohexol group (4.2 ± 0.4 mg). The non-ionic dimer iodixanol induces a direct vasoconstrictor effect on the carotid artery. (ii) Neither iso-osmolar nor hyperosmolar solutions of meglumine have any effect on TTO, whereas both sodium and meglumine salts of ioxaglic acid prolong the TTO parameter, suggesting that the antithrombotic effect of ioxaglate is mediated by the ioxaglic acid moiety alone because neither meglumine, osmolality, or sodium play a significant role. (iii) A synergistic effect on TTO can be appreciated when ioxaglate is associated with clopidogrel whereas no such effect is appreciated with iohexol. A greater *in vivo* antithrombotic potential can be expected with the application of the ionic contrast medium ioxaglate compared to iohexol (non-ionic contrast media). In addition, a synergistic *in vivo* effect appears to exist between contrast medium and platelet antiaggregatory pharmaceutical agents.

In vivo comparative antithrombotic effects of ioxaglate and iohexol and interaction with the platelet antiaggregant clopidogrel. Labarthe B, Idee JM, Burnett R, Corot C. Invest Radiol. 2003 Jan; 38 (1), pp. 34–43.

Adverse Reactions: Coagulation Alterations/ Anticoagulant Properties/Antiplatelet Properties
Ioxaglate/Iohexol/Iodixanol

Background Information: Thromboembolic complications have been attributed to the use of radiographic contrast media (CM) during interventional procedures for arterial revascularization. However, due to the low frequency of adverse events, comparisons between different CM have been difficult to perform, although it has been suggested that ionic (vs non-ionic) CM may be associated with fewer thrombotic events.
Implementation: Comparison of different CM under physiologically relevant and controlled conditions of blood flow, exposure time, and agent concentration (e.g., ioxaglate, iohexol, and iodixanol). Individual CMs are locally infused into the proximal segment of femoral arteriovenous shunts. Palmaz-Schatz stents (4 mm i.d.) and expanded tubular segments (9 mm i.d.), that exhibit venous-type flow recirculation and stasis characteristics, are deployed into shunts distally. Saline can be infused in identical control studies. Contrast media concentrations within the flowfield can be predicted using computational fluid dynamic techniques.

Animal Model: Primate (baboon thrombosis). Blood flow is maintained at 100 ml/min. Thrombosis phenomenon are measured over a blood exposure period of 2 hours by gamma camera imaging with [^{111}In] platelets and by gamma counting of deposited [^{125}I] fibrin.
Interpretation and Analysis: At infusion rates of 0.1 and 0.3 ml/minute, the low-osmolar ionic CM ioxaglate reduces both platelet and fibrin deposition on the stents by 75% to 80% ($p < 0.005$), while both iohexol and iodixanol reduce platelet deposition by 30% to 50% ($p < 0.05$). In the regions of low shear flow, ioxaglate (0.3 ml/min) also reduces platelet deposition significantly (by 52% vs control results; $p < 0.05$). Thus the 3 contrast agents, ioxaglate, iohexol, and iodixanol, all produce anticoagulant and antiplatelet effects and *in vivo* inherently exert antithrombotic properties. The most striking effects can be appreciated with low osmolarity, ionic contrast agents like ioxaglate.

Antithrombotic effects of ionic and non-ionic contrast media in non-human primates. Markou CP, Chronos NA, Hanson SR. Thromb Haemost 2001 Mar; 85 (3), pp. 488–93.

Adverse Reactions: Coagulation Alterations/ Hemostatic Effects (Hemostatic and Platelet)
Ioxaglate/Iopromide

Cellular/Molecular Mechanism of Action: Ioxaglate (ionic) and iopromide (non-ionic) are iodinated compounds that possess radiopaque properties.
Application: Diagnostic angiography and other procedures requiring the use of a radiopaque tracer contrast image-enhancing agents.
Route of Administration: Intravascular when used in diagnostic angiography procedures.
Implementation: Evaluation of the influence of ioxaglate and iopromide on platelet function (platelet reactivity and serotonin concentration) and expression of coagulation markers [thrombin-antithrombin III complexes, prothrombin fragments (F1+2) and the D-dimers].

***Ex Vivo* Model:** Human blood collected from coronary angiography cases ($n = 40$).
Interpretation and Analysis: The use of an ionic x-ray contrast agent (XCA) (ioxaglate) in diagnostic cardiac catheterisation angiography is associated with lower thrombin generation and lower activation potential of platelets than is associated with the application of iopromide (non-ionic XCA). Such observations confirm the results of various *in vitro* studies and animal investigations.
Instrumentation: Platelet aggregometer and coagulation evaluation instrumentation.

Effect of an ionic compared to a non-ionic x-ray contrast agent on platelets and coagulation during diagnostic cardiac catheterisation. Jung F, Spitzer SG, Pindur G. Pathophysiol Haemost Thromb 2002 May–Jun; 32 (3), pp. 121–6.

Adverse Reactions: Coagulation Alterations/ Thrombogenic Properties
Iopromide

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Iopromide is a non-ionic, iodinated, monomeric, radiographic contrast (imaging enhancing) agent that can be applied in arteriography procedures.
Background Information: Non-ionic contrast media have been postulated to have a higher risk of thrombosis compared with ionic contrast media.
Route of Administration: Iopromide (intravascular).
Dosage: Iopromide.
Implementation: Characterization of the interaction of iopromide with thrombin in order to determine the (i) rate, extent, specificity, and reversibility of the thrombin inhibition promoted by iopromide; (ii) integrity of the thrombin-iopromide complex; (iii) inhibitory potency of iopromide using a validated assay methodology; and (iv) determination of the specificity of iopromide mediated inhibition of thrombin protease activity. Methodologies that can be applied to achieve this objective involve the mixing of iopromide with purified thrombin or pooled serum. The final concentrations of iopromide in the presence of estimated physiologic concentrations of thrombin (1 nmol/L) are 0–184 mmol/L. After incubation for defined time intervals, the activity of thrombin is determined by adding substrate and measuring the absorbance of the generated chromophores at 405 nm. A positive reference controls should include a known thrombin inhibitor (e.g., Thromstop) in order to assess the relative potency of iopromide.
Interpretation and Analysis: The inhibition of thrombin by iopromide is immediate, rapidly reversible, and proportionate to iopromide concentration. The minimum inhibitory concentration of iopromide is 50 mmol/L. At an iopromide concentration of 184 mmol/L, the mean inhibition of thrombin activity is 44.5% approximately. The mean concentration of iopromide associated with a 50% inhibition was 206 mmol/L. The inhibitory potency of iopromide is 4×10^6 times smaller than Thromstop. The inhibition of thrombin by iopromide is specific, because trypsin is not inhibited by iopromide. The results indicate that *in vitro* iopromide at clinically relevant concentrations partially inhibits thrombin activity. However, some *in vitro* models do not consider other factors that may be relevant for the overall coagulation response *in vivo*.

Inhibition of thrombin by iopromide *in vitro*. Graf LL, Young DA, Kressin DC, Marlar RA, Jacob GB, Hinderling PH. Ther Drug Monit 2001 Apr; 23 (2), pp. 93–9.

Adverse Reactions/Lethal Effects/Comparative Median Lethal Dose/Physiological Tolerability
Iomeprol/Iopamidol/Iohexol

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Implementation: Evaluation of the toxicology of pharmaceutical formulations of iomeprol (non-ionic iodinated radiographic contrast agent).
Animal Model: Rodent (murine, rat, guinea pig) and non-rodent (rabbit, canine) animal species.
Interpretation and Analysis: When injected intravascularly, the acute toxicity of iomeprol, both in terms of median lethal dose and symptoms, is comparable to that of analogous triiodinated non-ionic contrast media. Intravenous daily dosing for 4 weeks reveals that iomeprol is well tolerated at doses as high as the maximum dose anticipated for clinical use. Moreover, the compound does not possess reproductive, developmental, or genetic toxicity. Tissue tolerability is completely superimposable on those of reference contrast media reagents, including iopamidol and iohexol. Finally, no antigenic potential can be detected either in mice or guinea pigs. These favourable toxicological characteristics bode well for iomeprol as an intravascular radiographic contrast agent.

Toxicological safety assessment of iomeprol, a new x-ray contrast agent. Morisetti A, Tirone P, Luzzani F, de Haen C. Eur J Radiol 1994 May; 18 Suppl 1, pp. S21–31.

Adverse Reactions: Nephrotoxicity
Gadolinium/Digital Subtraction Angiography (DSA)

Background Information: To prevent iodinated contrast medium-induced nephrotoxicity, gadolinium has been used increasingly for magnetic resonance angiography (MRA) or conventional DSA to visualize arterial anatomy in patients undergoing vascular surgery that are considered to be at high risk because of chronic renal insufficiency.
Application: Postive contrast imaging agent in DSA and magnetic resonance imaging procedures.
Dosage: Gadolinium-based contrast agents ($\geq$ 0.25 mmol/kg)
Implementation: Contrast enhancement of images acquired by magnetic resonance and DSA procedures.
Experimental Model: Single-center retrospective study evaluating the extent of nephrotoxicity induced during the course of MRA procedures (e.g., n = 218) and cases evaluated by DSA (e.g., n = 42) applying gadolinium as the sole contrast agent for diagnostic procedures performed between December 1999 and January 2001. Relevant variables recorded include patient comorbid conditions, indications for vascular imaging, contrast dose, urine output, baseline and postprocedure serum creatinine concentration (SCr), and outcome. Each of these features can provide insight into the relative toxicity of gadolinium contrast media and biological/physiological factors that may contribute to the development of sequelae.
Interpretation and Analysis: In cases with preimage baseline chronic renal insufficiency, approximately 3.5% will develop acute renal failure following administration of gadolinium-based contrast preparations (e.g., n = 7/195; n = 3/153 MRA; n = 4/42 DSA). In cases classified as having baseline chronic renal insufficiency, serum creatinine concentrations can average approximately 38.2 $\pm$ 1.6 mL/min/1.73 m^2, in contrast values of 32.5 $\pm$ 7.8 mL/min/1.73 m^2 for conditions of acute renal failure (p = 0.33). Doses for gadolinium in cases demonstrating renal failure postinjection can range from 0.31 to 0.41 mmol/kg for MRA and 0.27 to 0.42 mmol/kg for DSA procedures. Interestingly, acute renal failure rarely develops in cases with normal baseline serum creatinine levels (e.g., n = 0/65).

Despite reports of negligible nephrotoxicity, rarely gadolinium-based contrast agents can cause acute renal failure in instances where underlying chronic renal insufficiency exists. Estimation of creatinine clearance alone does not enable accurate prediction of which cases are likely to develop acute renal failure. Cases at high risk should be identified, and prophylactic measures exercised to reduce the risk of induced nephrotoxicity.

Safety of gadolinium contrast angiography in patients with chronic renal insufficiency. Sam AD 2nd, Morasch MD, Collins J, Song G, Chen R, Pereles FS. J Vasc Surg 2003 Aug; 38 (2), pp. 313–8.

Adverse Reactions: Nephrotoxicity: Contrast-Induced/Renal Transplant Conditions/Renal Artery Stenosis/Cyclosporin/Diabetes Meletus Contrast Agent

Background Information: Intravenous administration of radiographic contrast agents is an important cause of acute renal failure, accounting for one-third of the cases of hospital-acquired acute renal failure in patients with native kidneys. The safety of intravenous contrast has not been studied in renal allograft recipients because of the availability of cyclosporine as a maintenance immunosuppressive therapy.

Considerations: Cases with renal transplantation may be at a higher risk of contrast-induced nephrotoxicity (CIN) due to concomitant use of cyclosporine and due to a higher prevalence of coexisting diabetes and renal insufficiency.

Implementation: Evaluation of functioning renal allografts with intravenous or intra-arterial contrast agent studies (ICS: allograft angiography with angioplasty for renal artery stenosis).

Examples of relevant intra-arterial contrast studies performed include (i) coronary angiogram (e.g., n = 6); (ii) computed tomography (CT) scan with intravenous contrast (e.g., n = 11); (iii) angiogram for evaluation of peripheral vascular disease (e.g., n = 11); (iv) allograft angiogram with angioplasty (e.g., n = 5); (v) pulmonary angiogram (e.g., n = 1); and (vi) intravenous pyelogram (e.g., n = 1).

Human Model: Renal allograft cases (e.g., n = 44). Pre- and postintravascular contrast study renal function tests (e.g., n = 35/44). Case parameters include (i) mean age equals 42 ± 2.1 years; (ii) mean serum creatinine 2.3 ± 0.25 mg/dl (mean ± standard error of measurement [SEM]); (iii) diabetes (n = 14/35 or 40%); (iv) chronic rejection (n = 9/35 or 25.7%); and (v) cyclosporin administration at the time of ICS (n = 33/35 or 94%).

Interpretation and Analysis: Approximately 25% of renal allowgraft cases will experience a rise in serum creatinine from baseline following intravascular contrast studies (e.g., n = 9/35; diabetes = 5/7; chronic rejection 2/7). Prophylaxis with IV hydration does not guarantee intravenous contrast-induced nephrotoxicity will be avoided (e.g., n = 4/7). The baseline serum creatinine values are not different in patients who have no changes in renal function to those who develop intravenous contrast-induced nephrotoxicity (1.97 ± 0.20 vs 1.54 ± 0.17 mg/dl, p = 1.5, mean ± SEM). More than a 50% increase in baseline serum creatinine can be appreciated in many of these cases (e.g., n = 3/7; diabetes n = 2/3; chronic rejection/congestive heart failure n = 1/3; hydration prophylaxis n = 0/3). Dialysis may not be required in affected cases (e.g., n = 0). Conversely, some cases will display > 25% decreases in baseline serum creatinine after ICS (n = 3).

The incidence of intravascular contrast-induced nephrotoxicity in renal allograft recipients applying a broader classification of ≥ 25% increase in baseline serum creatinine is approximately 21.2% (n = 7/33 cases). The incidence of intravascular contrast-induced nephrotoxicity is 15.3% lower in cases that receive intravenous hydration (e.g., n = 4/26), compared to an incidence of 42.8% (e.g., n = 3/7) in cases receiving no prophylaxis prior to intravascular contrast-induced nephrotoxicity.

Contrast-induced nephrotoxicity in renal allograft recipients. Ahuja TS, Niaz N, Agraharkar M. Clin Nephrol 2000 Jul; 54 (1), pp. 11–4.

Adverse Reactions: Nephrotoxicity (Geriatric Vulnerability)
Ioversol 300

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.

Background Information: Ioversol 300 is a low-osmolality iodinated contrast agent.

Application: Iodinated contrast media in fluid form are applied to detect anatomical abnormalities within body fluid compartments.

Route of Administration: Ioversol 300 (intravascular).

Dosage: Ioversol 300 (300 mg I/ml: mean dose of 1.36 ± 0.06 ml/kg; range 1–2 ml/kg BW).

Implementation: Ioversol is injected as a bolus for computed tomography (CT) imaging procedures. Evaluation parameters include serum creatinine levels and creatinine clearance calculated at 24, 48, and 72 hours after examination with values and compared to a reference serum creatinine value taken before CT imaging. Findings are compared with a control group

Human Model: Cases subjected to CT imaging procedures using iversol contrast media (e.g., n = 44 cases that are age-matched to control subjects).

Interpretation and Analysis: No significant increase in serum creatinine (+0.6 mmol/l) or in creatinine clearance (+0.7 ml/min) can be detected during the 72 hour period following ioversol injection. Rarely, geriatric patients may develop elevations in serum creatine (e.g., n = 1/47). Some geriatric patients may therefore have subclinical renal impairment detected as decreases in initial creatinine clearance. The use of low-osmolality ioversol makes it possible to perform examinations with an iodinated contrast agent without increasing the incidence of nephrotoxicity in elderly subjects.

Effects of the low-osmolality contrast medium ioversol (Optiray) on renal function in a geriatric population. Louvel JP, Primard E, Henry J, Houlette C, Weinstein A, Janvresse A. Acta Radiol 1996 Nov; 37 (6), pp. 950–3.

Adverse Reactions: Neurotoxicity as a Function of Hydrophobicity
Ioversol/Iopromide/Iohexol/Iopamidol

Cellular/Molecular Mechanism of Action: Iodinated organic contrast agents are relatively radiodense and serve as liquid marker preparations to detect anatomical alterations within fluid compartments when applied with conventional radiographic imaging techniques.

Physiological Mechanism of Action: Ideally, iodinated organic agents are physiologically inert.

Background Information: The neurotoxicity of an x-ray contrast medium appears inversely related to hydrophilicity characteristics.

Application: In a clinical scenario, non-ionic x-ray contrast agents (ioversol, iopromide, iohexol, and iopamidol) can be applied to detect and evaluate anatomical abnormalities affecting the cerebral spinal fluid space surrounding the spinal cord.

Implementation: Delineation of the effect of 4 non-ionic x-ray contrast agents, differing in hydrophilicity (ioversol, iopromide, iohexol, and iopamidol), following injection at the cisternal magna.

Animal Model: Rats (ether-anesthetized).

Interpretation and Analysis: Iopromide has an acute median lethal dose of 122 mg I/kg. Other signs of toxicity include convulsions, apnea, dyspnea and hypoactivity. Conversely, ioversol, iohexol and iopamidol cause no deaths when administered intracisternally, up to a dose of 1000 mg I/kg. Animals administered these non-ionic agents display signs of convulsions, apnea, dyspnea, chewing and hypoactivity. Iopromide possesses a hydrophilicity (e.g., water to octanol partition coefficient) approximately 8.5 times smaller than ioversol, 4.6 times smaller than iohexol, and 2.3 times smaller than iopamidol. Based on these observations, triiodinated x-ray contrast

materials with smaller degrees of hydrophilicity appear to produce greater toxicity to the central nervous system.

Neurotoxicity of non-ionic x-ray contrast media after intracisternal administration in rats. Wible JH Jr, Barco SJ, Scherrer DE, Wojdyla JK, Adams MD. Eur J Radiol 1995 Mar; 19 (3), pp. 206–11.

Adverse Reactions: Nephrotoxicity: Renal Tubular Function Alterations (Relative)/Pharmacokinetics *Iodixanol/Iohexol/Ioxaglate*

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Iodixanol, iohexol, and ioxaglate can be applied as positive contrast image-enhancing agents. Iodixanol is a dimeric, ratio 6 radiographic contrast medium formulated to be isotonic relative to plasma in all concentrations fasciliated by the balanced addition of electrolytes.
Implementation: Evaluation of the clinical-chemical parameters and tolerability of iodixanol.
Interpretation and Analysis: Increases in femoral blood flow following administration of iodixanol is significantly smaller than that seen with most other non-ionic contrast media. Iodixanol appears to have less impact than other non-ionic media on renal tubular function. Unlike iohexol and ioxaglate, the rate of adverse events after iodixanol administration is essentially the same for normal cases compared to scenarios of increased risk for negative reactions (cases with previous adverse reactions to contrast administration). The risk following administration of iodixanol also appears to be similar in normal controls and in cases with other risk factors, including those with a history of congestive heart failure, renal insufficiency or disease (e.g., asthma, diabetes, hypertension, vascular disorders). A significant reduction in the sensation of injection-associated heat and pain has been noted for iodixanol relative to ioxaglate. Cardiac electrophysiological and contractility measurements reveal minimal interference associated with iodixanol administration. Iodixanol is a safe and effective non-ionic, isotonic contrast medium that may offer several clinical advantages.

Safety, tolerance, and pharmacokinetics of iodixanol injection, a non-ionic, isosmolar, hexaiodinated contrast agent. Fountaine H, Harnish P, Andrew E, Grynne B. Acad Radiol 1996 Sep; 3 Suppl 3, pp. S475–84.

Adverse Reactions: Neophrotoxicity/Renal Morphological Changes *Iobitridol/Iohexol*

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Route of Administration: Iopamidol (intravascular), iobitridol (intravascular), iohexol (intravascular)
Dosage: Iopamidol (3 g iodine/kg: rat), iobitridol (3 g iodine/kg: rat), iohexol (3 g iodine/kg: rat).
Implementation: Detection of renal morphologic changes induced by intravenously injected contrast media (CM) following 24 hours of water deprivation. Renal specimens are collected and fixed by perfusion for light and electron microscopy 2 hours postinjection of iopamidol, iobitridol, or iohexol (controls receive physiologic saline). In the 2nd part of the study, fixation is performed 48 hours after the injection of the corresponding dose of iobitridol or iohexol.
Animal Model: Rat (n = 40 Wistar strain deprived of water 24 hours prior to contrast injection).
Interpretation and Analysis: Semi-quantitative evaluation of renal structural changes reveals moderate changes in lysosomes within proximal convoluted tubular cells 2 hours after iopamidol injection. Iobitridol and iohexol induce prominent lysosomal alterations with signs of cytoplasmic injury. After 48 hours, the changes induced by iobitridol have almost disappeared, whereas the iohexol group still shows statistically significant levels of vacuolization. Although the general physicochemical properties of iobitridol and iohexol appear similar (*in vitro*), the different lysosomal alteration may reflect differences in their *in vivo* characteristics.

Structural changes in the renal proximal tubular cells induced by iodinated contrast media. Tervahartiala P, Kivisaari L, Kivisaari R, Vehmas T, Virtanen I. Nephron 1997; 76 (1), pp. 96–102.

Adverse Reactions: Prevention of Ioxaglate-Mediated Effects/Protein Extravasation Prophylaxis/Pulmonary Edema Prophylaxis/Decreased Arterial Partial Oxygen Prevention *Gabexate Mesilate/Nafamostate Mesilate*

Cellular/Molecular Mechanism of Action: Gabexate mesilate and nafamostate mesilate function as synthetic protease-inhibitor anticoagulant agents. Iodinated compounds, including ioxaglate, possess radiopaque properties.
Application: Gabexate mesilate and nafamostate mesilate are both prophylactically effective in preventing ioxaglate-induced protein extravasation and pulmonary edema. Ioxaglate is an iodinated positive contrast image-enhancing agent that can be used in conventional diagnostic radiography procedures.
Route of Administration: Ioxaglate (intravenous); gabexate mesilate (intravenous); nafamostat mesilate (intravenous).
Dosage: Gabexate mesilate (10 and 50 mg/kg at 5 minutes prior to ioxaglate injection) or nafamostat mesilate (3 and 10 mg/kg); ioxaglate (4 g iodine /kg).
Implementation: Evaluation of the potential ability of ioxaglate to promote marked protein extravasation, pulmonary edema and decreases in the arterial partial oxygen pressure (e.g., rats).
Interpretation and Analysis: Pretreatment with gabexate mesilate (10 and 50 mg/kg, 5 minutes prior to injection) and nafamostat mesilate (3 and 10 mg/kg) can both prevent all of these reactions induced by ioxaglate associated with protein extravasation and pulmonary edema. In this context, injection of nafamostat mesilate at a dosage of 10 mg/kg results in complete inhibition.

One of the effects evoked by ioxaglate is an enhancement of nafamostat-sensitive protease activity in the extracellular fluid of rat peritoneal mast cell suspensions. An enzyme fraction that gabexate mesilate and nafamostat mesilate inhibit includes the biochemical activity associated with purified human lung tryptase, although nafamostate mesilate is far more potent in this regard. Physiologically, tryptase enhances the permeability of protein through the monolayer of cultured human pulmonary arterial endothelial cells. Ioxaglate, when applied in combination with rat peritoneal mast cells, also creates endothelial barrier dysfunction. These effects of tryptase and ioxaglate are reversed by nafamostat mesilate. Furthermore, immunofluorescence morphological analysis reveals that tryptase or ioxaglate in combination with mast cells increases actin stress fiber formation, while decreasing VE-cadherin immunoreactivity. Both of these actions of tryptase and ioxaglate are reversed by nafamostat mesilate. Therefore, the liberation of tryptase from mast cells plays a crucial role in ioxaglate-induced pulmonary dysfunction. In this respect, nafamostat mesilate may become a useful agent for the cure or prevention of severe adverse reactions to radiographic contrast media.

A potent tryptase inhibitor nafamostat mesilate dramatically suppressed pulmonary dysfunction induced in rats by a radiographic contrast medium.

Sendo T, Itoh Y, Goromaru T, Sumimura T, Saito M, Aki K, Yano T, Oishi R. Br J Pharmacol 2003 Mar; 138 (5), pp. 959–67.

Adverse Reactions/Prophylactic Inhibition of Contrast Media Nephropathy
Acetylcysteine

Cellular/Molecular Mechanism of Action: Acetylcysteine functions as a reducing (antioxidant) agent. Such properties are mediated by its free sulfhydryl group, which donates hydrogen ion to oxidized disulfide bond structures.
Background Information: Radiographic contrast media are used at an increasing rate for a variety of diagnostic and therapeutic applications. As a result, the incidence of contrast agent-induced nephropathy is likely to become a more important sequellae. Efforts to discovery and validate strategies for the prevention of radiographic contrast-induced nephropathy is mandatory. The pathogenesis of radiographic contrast agent-induced nephropathy is considered to be associated with vasoconstriction-mediated renal medullary ischemia and direct toxic damage to renal tubular epithelial cells. These effects may be partly mediated by the generation of reactive oxygen species. Data from experimental studies indicate that antioxidants like acetylcysteine, may prevent radiocontrast-induced nephropathy.
Application: Prophylactic agent to prevent the development of renal dysfunction and injury associated with contrast agent administration especially in cases already suffering from variable degree and nephropathy/dysfunction.
Route of Administration: Aetylcysteine (oral/per os).
Dosage: Acetylcysteine (600 mg b.i.d.).
Considerations: In theory, other antioxidant reagents, including DMSO and vitamin E and vitamin E/selenium, might complement the prophylactic properties associated with acetylcysteine related to renal nephropathy precipitated by the intravascular administration of positive contrast media.
Implementation: Administration of acetylecysteine in cases suffering from moderate renal insufficiency to determine its capacity to function as a prophylactic agent to prevent compromised renal function precipitated by radiocontrast administration.
Interpretation and Analysis: Prophylatic oral administration of acetylcysteine combined with hydration prevents the reductions in renal function following radiocontrast administration. The use of acetylcysteine together with hydration is one of the treatments of choice to protect against radiographic contrast media–induce nephropathy.

Acetylcysteine and contrast media nephropathy. Tepel M, Zidek W. Curr Opin Nephrol Hypertens 2002 Sep; 11 (5), pp. 503–6.

Adverse Reactions/Prophylaxis of Contrast Media (CM)-Induced Nephropathy
Acetylcysteine/Endothelin Blockers/Fenoldopam

Cellular/Molecular Mechanism of Action: Acetylcysteine functions as a reducing (antioxidant) agent. This property is mediated by its free sulfhydryl group, which donates hydrogen ion to oxidized disulfide bond structures. Endothelin blockers/inhibitors (bosentan) prevent endothelin-induced activation of protein kinase-C, thereby suppressing the overproduction of oxidative species that contribute to the mechanisms of endothelial dysfunction. Fenoldopam functions as an intrarenal vasodialator agent mediated through dopamine D1-receptor complexes.
Background Information: Contrast media–associated nephropathy has become a major cause of iatrogenic acute renal failure, especially with the increasing use of radiographic CM in both diagnostic and interventional procedures. Contrast media–associated nephropathy is the 3rd most common cause of iatrogenic acute renal failure, and is associated with increased morbidity and in-hospital mortality.
Application: Acetylcysteine, endothelin blockers, and fenoldopam can be applied as prophylactic agents to prevent contrast agent–induced nephropathy, especially in scenarios of pre-existing renal dysfunction/pathology. Acetylcysteine has also been used as an antioxidant mucolytic agents. Fenoldopam has been successfully applied to prevent renal dysfunction associated with hypotension and hypothermia evoked during cardio-bypass procedures.
Interpretation and Analysis: Contrast media–associated nephropathy typically presents as an acute rise in serum creatinine levels, usually within 48 hours after exposure to CM. Renal medullary ischemia secondary to contrast-induced vasoconstriction is now believed to be the most likely cause of CM-associated nephropathy, although direct renal tubular cytotoxicity does appear to also play a role. The occurrence of CM-associated nephropathy is directly related to the number of coexisting clinical risk factors. Among the many risk factors, pre-existing renal impairment, the presence of diabetes mellitus and the volume of the contrast agent administered are the most important. The most effective means of reducing the incidence of CM-associated nephropathy is through prevention, by 1st identifying the risk factors and then attempting to correct for them before the administration of contrast material. Although it was the earliest and most well tested preventive measure, intravenous hydration continues to be the most effective way to prevent CM-associated nephropathy, recent studies have provided many new preventive modalities. The growing use of these new agents, such as acetylcysteine, endothelin blockers, and most recently fenoldopam, has increased the options available for the prevention of CM-associated nephropathy.

Contrast-associated nephropathy. Soma VR, Cavusoglu E, Vidhun R, Frishman WH, Sharma SK. Heart Dis 2002 Nov–Dec; 4 (6), pp. 372–9.

Adverse Reactions/Pulmonary Artery Pressure Alterations/Cardiopulmonary Angiography Procedures
Iohexol/Ioxaglate

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Iohexol and ioxaglate can both be applied as positive imaging enhancing contrast agents to facilitate the conduction of angiography procedures.
Route of Administration: Iohexol (intravascular); ioxaglate (intravascular).
Dosage: Iohexol (40 ml); ioxaglate (40 ml) within 2 seconds at 600 psi (0.17 kg/m^2).
Implementation: Evaluation of alterations in pulmonary artery pressure induced by iodine-based positive contrast imaging agents (e.g., iohexol, ioxaglate) in cases of suspected pulmonary emboli (e.g., n = 99). Mean pulmonary arterial pressure, pulse rate, and blood pressure are recorded before, immediately after, and 2, 5, and 10 minutes following injection.
Interpretation and Analysis: Pulmonary arterial pressure increases to a maximum at 2 minutes and is higher in patients with pulmonary emboli ($p = 0.06$). Usually, no significant differences can be appreciated between iohexol vs ioxaglate contrast media. The systolic blood pressure and pulse rate in patients with pulmonary emboli increase significantly more with ioxaglate ($p = 0.03$ and 0.04, respectively). In approximately 90% of the images acquired with both contrast image-enhancing agents, the quality of the resulting radiographs is of an excellent quality. Both contrast agents are

safe for pulmonary angiography and yield similar image quality. There appears to be a positive inotropic effect of ioxaglate.

Hemodynamic effects and image quality of low-osmolar ionic and non-ionic contrast media during pulmonary angiography. Smit EM, van Beek EJ, Bakker AJ, Reekers JA. Acad Radiol 1995 Jul; 2 (7), pp. 609–13.

Adverse Reactions/Safety and Diagnostic Effiacy of Contrast Agents/ Excretory Urography
Iodixanol/Iohexol

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Physiological Mechanism of Action: Iodixanol is a non-ionic, dimeric, iodinated contrast agent, whereas iohexol is a monomeric agent.
Application: Iodixanol and iohexol are contrast agents that can be applied to perform excretory urography diagnostic imaging procedures.
Route of Administration: Iodixanol (intravascular).
Dosage: Iodixanol (270 mg I/ml and 320 mg I/ml) compared to iohexol (300 mg I/ml). The dose for every case is 1 ml/kg of body weight (human).
Implementation: Utilization of iodixanol and iohexol to perform excretory urography procedures. Evaluation of the efficacy and safety of the agents can be assessed by recording image quality, alterations in vital signs, laboratory value variations, and adverse reactions. Radiographic images are also interpreted and evaluated for enhancement of image quality.

Human Model: Cases subjected to excretory urography procedures (e.g., n = 75 cases; randomized, double blind, parallel comparison design).
Interpretation and Analysis: Evaluation of each phase of the radiographic study reveals almost uniform good-to-excellent opacification, with no significant differences appreciated among the 3 agents. No significant differences can be detected in vital signs, hematology, blood and urine chemistry, and injection discomfort for iodixanol (270 mg I/ml), iodixanol (320 mg I/ml) or iohexol (300 mg I/ml). Deaths or serious reactions are extremely rare (e.g., n = 0/75) and there are no statistically significant differences in the number of adverse events. Iodixanol at both 270 and 320 mg I/ml is a safe and effective contrast material for intravenous urography. The profile for iodixanol is essentially indistinguishable from that of iohexol at 300 mg I/ml.

Comparison of iodixanol with iohexol in excretory urography. Siegle RL, Gavant ML. Acad Radiol 1996 Sep; 3 Suppl 3, pp. S524–7.

Adverse Reactions/Safety of Contrast Agents (Relative)
Iotrolan/Iohexol

Route of Administration: Iotrolan (selective intracoronary artery delivery); iohexol (selective intracoronary artery delivery).
Dosage: Iotrolan (concentration = 320 mg I/ml); iohexol (concentration = 350 mg I/ml).
Implementation: Evaluate selective coronary angiography and left ventriculography with iotrolan compared to iohexol (e.g., n = 120 cases). Relevant variables that can be measured include maximum increase of the left ventricular end-diastolic pressure up to 6 minutes after ventriculography; hemodynamic and electrocardiographic variables; arrhythmogenicity; clinical laboratory parameters; tolerance; adverse events and diagnostic efficacy.
Interpretation and Analysis: Iotrolan produces a smaller change in left ventricular end-diastolic pressure compared to iohexol, but the difference is not statistically significant. Transient changes in left ventricular systolic pressure, intra-arterial systolic pressure, intra-arterial diastolic pressure, and in electrocardiographic parameters, occur after injections, but they are not clinically significant. Changes in clinical laboratory markers from baseline values are comparable between the 2 groups and confirm good levels of renal and hepatic tolerance. During the acquisition of ventriculograms, iotrolan evokes fewer sequelae compared to iohexol ($p = 0.002$). Adverse events, that are mild or moderate in most cases, are not statistical different between the 2 agents. The contrast quality of both agents is good and not statistically different. No significant difference exists between iotrolan 320 and iohexol 350 with regard to cardiovascular safety or patient tolerance, except for a minor difference in the intensity of induced heat/warmth sensations.

Comparison of iotrolan 320 and iohexol 350 in cardiac angiography: A randomised double blind clinical study. Mezilis N, Salame MY, Dyet JF, Arafa SO, Oakley GD, Cumberland DC. Eur J Radiol 1998 Sep; 28 (2), pp. 171–5.

Adverse Reactions/Safety and Tolerance/ Radiographic Enhancement/Angiography Contrast Agents
Iodixanol/Ioxaglate

Cellular/Molecular Mechanism of Action: Iodixanol is an iodinated compound that has radiopaque properties. Iodixanol is a new isosmotic hexaiodinated non-ionic contrast agent being evaluated for intravascular use.
Background Information: Iodixanol is a hexaiodinated non-ionic compound formulated as an isosmotic liquid that has been designed to provide improved *in vivo* safety and tolerance.
Application: Iodixanol is an iodinated preparation that is utilized as a radiopaque contrast agent in angiography (aortography) diagnostic procedures.
Route of Administration: Iodixanol (intravascular).
Implementation: Delineation of the relative (i) safety and tolerance of iodixanol compared with ioxaglate; and (ii) radiographic enhancement provided by iodixanol compared to ioxaglate by evaluation of their efficacy in renal angiography, visceral angiography, and aortography procedures (e.g., n = 44 cases). Parameters assessed include adverse events, injection-associated discomfort, changes in laboratory parameters, and variations in vital signs. Efficacy is measured by the overall quality of angiographic image enhancement.
Interpretation and Analysis: During the vast majority of diagnostic procedures, no serious adverse events can be appreciated (e.g., n = 0/44). Mild-to-moderate adverse events are more common in cases receiving ioxaglate ($p = .041$). Injection-associated pain is reported in a small percentage of cases receiving ioxaglate (e.g., n = 3/44) but is much more infrequent with iodixanol (e.g., n = 0/44; $p = .093$). Clinical laboratory and vital-sign data show no difference between groups, and there is no difference in the overall quality of angiographic visualization ($p = 0.711$). Iodixanol is associated with improved safety and patient tolerability, while it provides images of equivalent diagnostic efficacy compared with ioxaglate.

Comparison of iodixanol and ioxaglate for adult aortography and renal/visceral angiography: A phase III clinical trial. Siegel EL, Rosenblum JD, Eckard DA, Leef J, Bergh J, Parsa MB, Redick ML. Acad Radiol 1996 Sep; 3 Suppl 3, pp. S507–13.

Adverse Reactions/Safety and Tolerability
Iodixanol/Ioxaglate

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.

Background Information: Iodixanol is an iso-osmolar non-ionic dimeric hydrophilic contrast agent that has a level of diagnostic efficacy comparable to other iodinated contrast media. Because of its physical properties, iodixanol would be expected to produce a lower incidence of adverse events than other non-dimeric contrast media. Indeed, pharmacodynamic studies indicate that iodixanol has fewer cardiovascular effects, causes less renal damage and is associated with similar or smaller changes to the blood-brain barrier and neurological function when compared with non-dimeric non-ionic contrast media.
Application: Iodinated radiographic contrast agents are applied to detect anatomical abnormalities within fluid body compartments.
Interpretation and Analysis: In clinical trials, iodixanol has demonstrated a similar tolerability profile relative to other non-ionic contrast media but induces fewer adverse events than ioxaglate. Iodixanol appears to have an advantage over other contrast media in that it generally causes less frequent and less intense sensations of discomfort following administration by injection. However, in common with other newer and most non-ionic contrast media, iodixanol is expensive. Studies investigating other non-ionic contrast media (but not iodixanol) have shown that it is not cost-effective to replace older, usually ionic, contrast media with the more costly newer alternatives. Nonetheless, in selected patients who are considered at increased risk of contrast medium-associated adverse events, non-ionic agents should be used. Iodixanol, with its lower intensity (and possibly frequency) of discomfort, may be a preferred option under such conditions.

Iodixanol. A review of its pharmacodynamic and pharmacokinetic properties and diagnostic use as an x-ray contrast medium. Spencer CM, Goa KL. Drugs 1996 Dec; 52 (6), pp. 899–927.

Adverse Reactions/Toxicological Profile: Contrast Media
Iobitridol

Cellular/Molecular Mechanism of Action: Iodinated contrast media are radiodense and are traditionally applied as passive liquid marker systems that enhance the accuracy and sensitivity of radiographic imaging techniques.
Application: Iobitridol is a new non-ionic low-osomolality contrast medium utilized to detect anatomical abnormalities within body cavities and fluid compartments.
Route of Administration (Research): Iobitridol (intravenous [mouse, rat, dog]; oral [mouse]; intracisternal [rats]).
Implementation: Characterization of the toxicity of iobitridol following acute or repeated intravenous administration in several different species (mouse, rat, dog); single oral administration in the mouse and intracisternal injection in rats. Complementary evaluations include delineation of teratogenicity and mutagenicity in rats and rabbits in concert with potential local perivenous toxicity in rabbits.
Animal Model: Murine, rat and canine.
Interpretation and Analysis: Acute toxicity of iobitridol in mice is equivalent to iohexol (reference agent). Chronic administration (daily intravenous injections over 4 weeks) in the rat and dog do not demonstrate any particular toxicity for iobitridol. Unlike iohexol, iobitridol does not provoke any vacuolization of the renal tubular cells in the rat following repeated injections. Furthermore, iobitridol does not induce any detectable teratogenic or mutagenic properties. The typical local inflammatory signs observed following perivenous injection in the rabbit are low in intensity and reversible. In summary, the toxicologic profile of iobitridol appears to be favorable and does not show any particular risk for clinical use under the usual indications of water-soluble iodinated contrast agents.

Toxicologic profile of iobitridol, a new non-ionic low-osmolality contrast medium. Donandieu AM, Idee JM, Doucet D, Legros A, Penati S, Nain-Dit-Ducret M, Marmion F, Bonnemain B. Acta Radiol Suppl 1996; 400, pp. 17–24.

Adverse Reactions/Trans-Placental Contrast Media Transport/Fetal Omphalomesenteric Duct Cysts
Iohexol

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Iohexol is an iodinated, low-molecular weight postive contrast imaging enhancing media that can be utilized in angiography procedures.
Route of Administration: Iohexol can be administered intravascularly.
Interpretation and Analysis: Iohexol can be identified in the intestines of twin neonates after administration to pregnant mothers during the course of angiography procedures. Non-ionic contrast agents cross the human placenta and enter the fetus in significant concentrations to the extent that they can facilitate the identification of fetal omphalomesenteric duct cysts.

Transplacental passage of iohexol. Moon AJ, Katzberg RW, Sherman MP. J Pediatr. 2000 Apr; 136 (4), pp. 548–9.

Agents: General Concept/Contrast Agent Properties and Tissue Concentration
Iohexol/[^{14}C]-Labeled Inulin

Background Information: Computed tomography (CT) provides accurate measurement of blood iodine concentration *in vivo*, as well as in phantoms simulating tissue; however, its ability to measure radiopaque agents in biologic tissues in comparison with a standard technique has not been well demonstrated.
Application: Validate the performance of CT imaging for *in vivo* quantification of contrast media in a variety of biologic tissues by evaluating comparisons between CT imaging with an iodinated contrast agent (iohexol) utilizing a reference tracer quantification technique (storage-phosphor autoradiography with [^{14}C]-labeled inulin).
Implementation: Intravenous injection of a cocktail containing iohexol and quantification of [^{14}C]-labeled inulin formulated at different dose ratios. Sacrifice (e.g., rabbit) is performed immediately following injection in order arrest blood flow at different stages of tissue tracer distribution [Baseline = no iohexol-inulin]. Liver, spleen, kidneys, testis, and heart are excised and rapidly frozen. Each organ is subsequently scanned with CT (1-mm contiguous sections) to determine tissue iodine distribution. Twenty-micrometer tissue slices are prepared in planes that correspond with the orientation of CT images. Storage-phosphor screen autoradiography is performed to quantify [^{14}C]-labeled inulin distribution. Digital image analysis of CT images and autoradiograms is performed on spatially matched regions, and resultant tracer concentrations compared.
Animal Model: Rabbit (n = 6 New Zealand White breed).
Interpretation and Analysis: Tracer concentrations are highly correlated, with resultant R2 values exceeding 0.9 in all tissues. The high correlation of results for iodinated tracer quantification in tissues for CT compared to those obtained with the reference technique validate the performance of CT as an accurate means of measuring concentration of radiopaque agent in tissue, independent of tracer dose.

Indicator measurement in tissues: CT with iohexol vs storage-phosphor autoradiography with carbon-14-labeled inulin. Whiteman KR, Wolf GL. Acad Radiol 1998 Nov; 5 (11), pp. 799–803.

Agents: General Concepts/Contrast Agent Properties/Hydrophobicity/Stability/Toxicity/ Function of Molecular Configuration and Chemical Composition
Iobitridol

Background Information: The chemotoxicity of iodinated contrast agents is essentially related to the spatial accessibilty of lipophilic and polarizable iodine atoms.
Laboratory Analysis: Spatial accessibility of lipophilic and polarized iodine atoms in iobitridol can be delineated applying high-performance liquid chromatography (HPLC) analysis and ^{1}H- and ^{13}C-NMR spectroscopy with the static distribution of the hydrophilic groups around the triiodinated benzene ring, and especially the dynamic modulation of this distribution both taken into account. The purpose of addressing the dynamic modulation of distribution parameter is to prevent distortion of the hydrophilic sphere of non-ionic agents when exposed to a hydrophobic environment.
Interpretation and Analysis: Iobitridol is characterized by 2 tertiary carbamoyl substituents whose high rotation barriers (deltaG*353 = 27.6 kcal for E/Z-rotation and deltaG*345 = 17.3 kcal for syn/antirotation) stabilize the hydrophilic sphere. The 3rd dihydroxylated anilide substituent does not undergo SMILES rearrangement and provides iobitridol with its even hydrophilic distribution. Iobitridol possesses remarkable chemical solubility (> 140% m/v) and stability. The iobitridol molecule has been specifically designed with the aim of stabilizing the hydrophilic sphere around the triiodinated benzene ring, therefore permanently masking access to the iodine atoms. This new concept represents a further step forward toward the synthesis of new iodinated contrast agents, which should be totally inert vis-à-vis biological membranes and proteins.

Stabilization of the hydrophilic sphere of iobitridol, a new non-ionic iodinated contrast agent. Meyer D, Petta M, Fouchet MH, Vadel M, Schaefer M, Dugast-Zrihen M, Guillemot M. Acta Radiol Suppl 1996; 400, pp. 8–16.

Agents: General Concepts/Contrast Imaging Agents Properties/ Pharmacokinetics/Renal Clearance (Age-Related Differences)
Iodixanol

Application: Iodixanol is an iso-osmolar iodinated radiocontrast agent.
Route of Administration: Iodixanol (intravascular).
Dosage: Iodixanol.
Implementation: Iodixanol is administered by cardiac catheterization and then plasma samples collected at 0.75 to 1.25 hours, 2 to 4 hours, 8 to 12 hours, and 16 to 32 hours postinjection (e.g., n = 41 cases: newborn to < 2 months; 2 to < 6 months; 6 months to < 1 year; 1 to < 3 years; and 3 to ≤ 12 years). Plasma concentrations of iodixanol can subsequently be determined using high performance liquid chromatography. The primary pharmacokinetic end point to be applied is the terminal elimination rate constant (K_{el}).
Interpretation and Analysis: Mean K_{el} is significantly lower in newborns ≤ 2-month-old cases in contrast to older subjects: (h-1, mean ± standard deviation [SD]): newborn to < 2 months 0.185 ± 0.060, 2 to < 6 months, 0.256 ± 0.046, 6 months to < 1 year 0.299 ± 0.042, 1 to < 3 years 0.322 ± 0.058, and 3 to ≤ 12 years 0.307 ± 0.071; with increasing age, excretion is more rapid, as shown by each age group's elimination half-life ($T_{1/2}$ beta; 4.14 ± 1.41 hours, 2.79 ± 0.55 hours, 2.36 ± 0.37 hours, 2.23 ± 0.51 hours, and 2.36 ± 0.52 hours, respectively). Elimination of iodixanol in children > 6 months of age is comparable to normal adults. Prolonged elimination in children < 6 months of age is related to renal immaturity.

Iodixanol pharmacokinetics in children. Johnson WH Jr, Lloyd TR, Victorica BE, Zales VR, Epstein ML, Leff RD, Ardinger RH Jr, Slovis TL, Johnson JA, Marsters PA. Pediatr Cardiol 2001 May–Jun; 22 (3), pp. 223–7.

Agents: General Concepts/Contrast Imaging Agents/Pharmacokinetics/Iodine Concentrations/ Plasma Clearance
Iohexol

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Iodine can be used as a postive radiographic contrast imaging agent.
Dosage: Iohexol (300 mg iodine/kg in dog/cats).
Laboratory Analysis: Analysis of iodine contained in iohexol preparations utilizing plasma atomic emission spectroscopy. Measurement of iodine at 178.276 nm on the phosphorus 178.287 nm channel of the polychromator by utilization of spectrum shifter offset software, while correcting for P with the sequential P 214.914 nm emission line.
Implementation: Evaluation of glomerular filtration rate using an intravenous injected bolus of iohexol with plasma samples collected at T = 0.0 hours, 3.0 hours, and 7.0 hours.
Animal Model: Canine and feline.
Interpretation and Analysis: Determination of iodine on a polychromator provides excellent precision in the measurement of serum iodine, even with interelement correction of P with a sequential P line. Total imprecision (CV) (n = 13) is 16% (at 13.7 mg/L I), 8.6% (28.7 mg/L), 3.6% (59.0 mg/L), 2.6% (120.5 mg/L), 1.7% (237.8 mg/L), 1.2% (478.7 mg/L), and 1.8% (597 mg/L). The linear range is approximately 15–600 mg/L. Iohexol added to serum (mg/L iodine) equivalent to 15, 30, 60, 120, 240, 480, and 600 produce percent recoveries of approximately (91.3%), (95.7%), (98.3%), (100.4%), (99.1%), (99.7%), and (99.5%), respectively.

A single intravenous iohexol injection is sufficient to measure glomerular filtration rate using a single compartment model for plasma clearance calculated using plasma samples drawn at T = 0.0 hours, 3.0 hours, and 7.0 hours (dog and cat). Using this experimental protocol, correlation coefficients are > 0.99 for the beta phase of the plasma disappearance curve.
Instrumentation: Plasma-atomic emission spectroscopy.

Measurement of serum iohexol by determination of iodine with inductively coupled plasma-atomic emission spectroscopy. Braselton WE, Stuart KJ, Kruger JM. Clin Chem 1997 Aug; 43 (8 Pt 1), pp. 1429–35.

CARDIOVASCULAR SYSTEM

Angiography: Arterial and Venous
Carbon Dioxide (CO_2)

Cellular/Molecular Mechanism of Action: Carbon dioxide functions as a compound with radioleucent properties.
Application: Contrast agent in angiogam procedures.
Route of Administration: Intravascular.
Implementation: Evaluation of the capacity and limitations of CO_2 to function as a contrast agent to guide vascular interventional procedures in scenarios limited by pre-existing renal insufficiency or allergic reaction. Examples of arterial procedures capable of being performed include renal angioplasty or stent (e.g., n = 6), iliac angioplasty or stent (e.g., n = 5), infrainguinal angioplasty (e.g., n = 5), arterial bypass graft angioplasty (n = 3), and thrombolysis (e.g., n = 2). Examples of venous procedures include trans-jugular intrahepatic portosystemic shunt recanalization (e.g., n = 3), angioplasty

of the venous anastomosis of a thigh dialysis graft (e.g., n = 1), and angioplasty of the inferior vena cava (e.g., n = 1).

Human Model: Clinical angiography procedures (e.g., n = 26; n = 21/26 arterial; n = 5/26 venous) in cases with pre-existing renal insufficiency (e.g., n = 21/26; mean creatinine level, 2.8 mg/dl), or allergic reaction (e.g., n = 1/26).

Interpretation and Analysis: The rate of success applying CO_2 facilitated angiography is relatively high (e.g., n = 25/26). Approximately 31% of cases (e.g., n = 8/26) require no iodinated contrast material, while about 42% (e.g., n = 11/26) require less than or equal to 20 ml of contrast material. Alternatively, CO_2 is inadequate in approximately 27% of diagnostic procedures performed (e.g., n = 7/26). Iliac artery angioplasty or stent placement requires an average of 9 ml of iodinated contrast material; infrainguinal angioplasty requires an average of 22 ml of iodinated contrast material. Therefore in the application of CO_2, it can be successfully used as a contrast agent in a variety of vascular interventional procedures. Such diagnostic methods can usually be performed in the iliac and infrainguinal arteries using minimal supplemental iodinated contrast material. However, CO_2 fails to provide satisfactory guidance in approximately 50% of intra-abdominal procedures.

Carbon dioxide as a contrast agent to guide vascular interventional procedures. Eschelman DJ, Sullivan KL, Bonn J, Gardiner GA Jr. AJR Am J Roentgenol 1998 Nov; 171 (5), pp. 1265–70.

Angiography/Contrast Media Comparison (Iodine vs Gadolinium-Based Formulations)/ Digital Subtraction Procedures

Gadolinium Diethylenetriamine Pentaacetic Acid ([Gd]-DTPA)

Application: [Gd]-DTPA can function as an alternative contrast agent for digital subtraction angiography (DSA) in patients with renal insufficiency or previous anaphylactic reaction to iodinated contrast agents.

Route of Administration: [Gd]-DTPA (intravascular).

Dosage: [Gd]-DTPA 0.5-M solution without exceeding a total amount of 0.4 mmol/kg body weight (Magnevist, Schering, Berlin, Germany). Injection rate is identical for iodinated contrast reagents.

Implementation: Angiography procedures performed with power-injected or hand-injected [Gd]-DTPA (e.g., n = 34 digital subtraction angiography studies). Application of this diagnostic contrast agent's provides the flexibility of being able to be utilized as the sole contrast reagent (e.g., n = 18/34) or in combination with carbon dioxide (CO_2: n = 9/34). Validation can be achieved by performing restricted non-ionic contrast medium injections.

Interpretation and Analysis: Vascular anatomical structures that can be successfully imaged include cerebral and carotid arteries, superior limbs, abdominal aorta, renal arteries, renal transplants, iliac arteries, and inferior limbs. Additional examples include endovascular intervention procedures (e.g., n = 10/34) such as transjugular intrahepatic percutaneous stent shunts (e.g., n = 3/10). The incidence of side effects is very low to non-existent. Diagnostic angiographic images can be obtained in a large percentage of cases with the exception of some distal run-off studies (e.g., n = 5/8). Gadolinium-based contrast can produce clinically useful angiograms in patients with a contraindication to iodine who must undergo angiography.

Gadolinium dimeglumine: An alternative contrast agent for digital subtraction angiography. Hammer FD, Goffette PP, Malaise J, Mathurin P. Eur Radiol 1999; 9 (1), pp. 128–36.

Angiography/Contrast Imaging Agents: Relative Effectiveness

Gadolinium Chelates/Iodinated Contrast Imaging Agents/Carbon Dioxide Contrast Agent

Application: Imaging of vascular structures applying digital subtraction angiography (DSA) analyses.

Dosage: Gadolinium chelates (0.5 mol/L solutions; 34 ± 19 mL/case). Carbon dioxide (CO_2; 212 ± 226 mL).

Implementation: Diagnostic angiographies (e.g., n = 15) and percutaneous trans-luminal angioplasties (e.g., n = 15). Digital subtraction angiography performed in cases with contraindications to iodinated contrast media (e.g., n = 30 images; n = 22 cases, n = 5/22 females, n = 17/22 males; renal insufficiency: n = 28; hyperthyroidism: n = 1; contrast allergy: n = 2; mean age 64.9 years). Gadolinium chelates can be applied as the sole contrast agent (e.g., n = 17 images) or applied in combination with CO_2. Some cases will allow the combined applications of these image-enhancing agents with the restricted use of non-ionic iodinated contrast (mean 12.8 ± 4.7 mL; e.g., n = 6 images).

Interpretation and Analysis: The use of gadolinium chelates allows the acquisition of diagnostic angiographic images in the vast majority of cases (e.g., n = 30/30). However, the quality of angiograms is inferior compared with that obtained with iodinated contrast agents and superior compared with CO_2 as the contrast material. Adverse events are rarely recognized. Mean serum creatinine is approximately 2.6 ± 1.5 mg/dL before and 2.3 ± 1.0 mg/dL after DSA and contrast-induced nephropathy is rarely detected. Gadolinium chelates produce an x-ray DSA intermediate in image quality between iodinated contrast and CO_2. Digital subtraction angiography with intra-arterial gadolinium chelate administration may offer an alternative to iodinated contrast material in situations where iodine-containing agents are contraindicated.

The use of gadolinium chelates for x-ray digital subtraction angiography. Wagner HJ, Kalinowski M, Klose KJ, Alfke H. Invest Radiol 2001 May; 36 (5), pp. 257–65.

Angiography/Contrast Media Relative Effectiveness/Vasculature Architecture Malformation/Collateral Circulation/Arteriovenous Shunts/Residual Postembolization Disease

Iodinated Contrast Imaging Agent/Carbon Dioxide (CO_2) Contrast Imaging Agent

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.

Application: Angiography procedures.

Implementation: Evaluation of the diagnostic usefulness of CO_2 as a radiologic contrast medium in pre- and postembolization of hemodynamically active vascular malformations (HAVM).

Pre- and postembolization angiographies using sequentially iodinated contrast medium and CO_2 are performed in cases of HAVM (e.g., n = 8 studies in 5 cases). Images are then assessed for relative uptake by malformations based on the parameters of quality; filling in of the contrast agent in afferent artery of the HAVM; caliber and number of vessels; and existence of pathologic communications in HAVM. Such characteristics are evaluated both before and after embolization.

Interpretation and Analysis: The CO_2 angiographic results are compared to those obtained using iodinated contrast material. Relative to CO_2, iodinated contrast media provide superior image quality. The filling of arterial afferents of HAVM is well defined with both contrast media. In essentially all instances with CO_2 imaging, the

malformations reveal a larger number of vessels. No immediate or subsequent complications can usually be appreciated with CO_2 injections. Exceptions include the development of an intense sensation of cramps in the lower limbs.

As a diagnostic and assessment agent, CO_2 appears to be useful before and especially during the embolization of hemodynamically active vascular malformations. It improves quantification and uptake of the malformation's vascular architecture, detecting collateral circulation and arteriovenous shunts. It also detects residual postembolization disease when iodinated contrast agent is unsuccessful.

Instrumentation: Procedures can be performed aided by the use of Philips Integris V equipped with specific software to process images obtained with CO_2 as contrast medium and with a purpose-built gas injector pump.

CO_2 as a contrast medium in endoluminal treatment of high flow vascular malformations. Gorriz E, Carreira JM, Reyes R, Gallardo L, Pulido JM, Romero A, Maynar M. Eur J Radiol 1999 Sep; 31 (3), pp. 182–7.

Angiography/Contrast Agent Comparative Radiographic Image Enhancement
Iodipamide Ethyl Ester (IDE)

Cellular/Molecular Mechanism of Action: Iodinated compounds have radiopaque properties.

Application: The iodinated particulate contrast agent IDE can be applied in diagnostic angiography procedures. Because it is excreted slowly in bile and is isotonic, iodipamide ethyl ester may prove useful in cases with renal insufficiency, diabetes, multiple myeloma, or severe coronary disease.

Route of Administration: Iodinated particulate contrast agent IDE can be infused intravascularly for angiography procedures.

Dosage: Iodipamide ethyl ester (40–100 mg I/kg).

Implementation: Iodipamide doses are diluted to a total volume of 5 to 20 mL and injected to perform digital subtraction angiography. Reference procedures are performed applying iohexol as a water-soluble contrast medium. All images are reviewed blinded and graded subjectively on a 5-point scale.

Animal Model: Canine (n = 9 anesthetized dogs given iodipamide; and n = iohexol and water-soluble contrast media reference controls).

Interpretation and Analysis: Angiographic studies of multiple vascular territories performed with Iodipamide ethyl ester yield images of slightly lower overall quality compared with images obtained with iohexol ($p = 0.14$, Mann-Whitney U test). Arterial phase images are subjectively superior with iohexol when compared with iodipamide ethyl ester ($p < 0.0001$, chi^2).

Depiction of the corresponding veins during the venous phase on iodipamide ethyl ester angiograms is superior to iohexol angiograms in a large percentage of cases (e.g., n = 12/21), but is not statistically significant ($p > 0.05$ chi^2). Images of the renal vein and portal vein acquired with iodipamide ethyl ester are of a superior grade compared to a large percentage of those performed utilizing iohexol (e.g., n = 8/10). Angiography procedures are feasible with iodipamide ethyl ester as a contrast image-enhancing agent. Compared with iohexol, iodipamide ethyl ester produces images that are of a lesser quality during the arterial phase, but of equal or superior quality in the venous phase depending on the type of vessel imaging procedure.

Preclinical evaluation of an iodinated particulate contrast agent for use during angiography: Work in progress. Sproat IA, Lee FT Jr, Rappe AH, Chosy SG. J Vasc Interv Radiol 1996 Nov–Dec; 7 (6), pp. 889–95.

Angiography/Contrast Imaging Agents/ Pharmacokinetic Profiles/Coagulation Pathways: Potential Influences/Histamine and Leukotriene B_4: Influence on Release
P743 (Monodispersed Iodinated Contrast Agent)/ Iobitridol (Non-Ionic Contrast Agent)

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.

Application: Iodinated contrast imaging agent that can potentially be used in angiography procedures.

Route of Administration: P743 (intravascular).

Dosage: P743 (300 mg I/kg).

Implementation: Synthesis, molecular modeling, and pharmacokinetic profiling of an iodinated macromolecule (P743). Complementary evaluations include delineation of acute toxicity (mice), renal tolerance (normal rabbits), and renal tolerance in uninephrectomized conditions with superimposed dehydration and evaluated by selective intrarenal injection (rats). Permeability effects can also be evaluated *in vitro* applying isolated mastocytes populations along with evaluation of alterations in coagulation pathway function. Computed tomography vascular image analysis are performed after intravenous injection of P743 (300 mg I/kg in rabbits) and compared with the non-specific non-ionic agent iobitridol.

Animal Model: Murine, rat, and rabbit.

Interpretation and Analysis: The monodispersed macromolecular iodinated contrast agent P743 displays pharmacokinetic profiles in both rabbits and rats consistent with that of a rapid-clearance blood-pool agent. Diffusion *in vitro* through the endothelium is low, thus confirming early confinement of this macromolecule, unlike non-specific contrast media. In both species, P743 is excreted by glomerular filtration. Acute toxicity produces no mortality at the highest volume that could be injected into mice, leading to a calculated median lethal dose of greater than 8.9 g I/kg. Renal tolerance is good in euvolemic rabbits and uninephrectomized, dehydrated rats. No histamine or leukotriene B4 release can be detected in populations of isolated RBL-2H3 mastocytes. Coagulation pathways appear to be unaffected by P743 when evaluated at clinically relevant concentrations. Imaging experiments confirm that P743 remains within the vascular compartment for a longer period of time compared to iobitridol, thus allowing vascular enhancement that is twice as high as that of iobitridol during the recirculation phase. The pharmacokinetic and imaging profiles of P743, a new, monodisperse, macromolecular blood-pool iodinated contrast medium, are consistent with those of a rapid-clearance blood-pool agent. Its initial safety profile is satisfactory. Further experimental imaging studies are required to define the clinical interest in such molecules.

Preclinical profile of the monodisperse iodinated macromolecular blood-pool agent P743. Idee JM, Port M, Robert P, Raynal I, Prigent P, Dencausse A, Le Greneur S, Tichkowsky I, Le Lem G, Bourrinet P, Mugel T, Benderbous S, Devoldere L, Bourbouze R, Meyer D, Bonnemain B, Corot C. Invest Radiol 2001 Jan; 36 (1), pp. 41–9.

Angiography/Portal Vein Imaging/Postintrahepatic Injection Pathology/Trans-Jugular Intrahepatic Portosystemic Shunt Placement (TIPS)/ Percutaneous Trans-Hepatic Cholangiography (PTC)
Iohexol (Non-Ionic)/Ditrizoate (Ionic)

Application: Iohexol and CO_2 for direct hepatic injection imaging procedures.

Route of Administration: Iohexol (300 mg I, non-ionic) and ditrizoate (60% ionic) can be injected directly into the pyrenchyma of a hepatic lobe (intrahepatic).
Dosage: Iohexol (300 mg I, non-ionic) and ditrizoate (ionic) each injected at a volume of between 2 and 5 ml (intrahepatic).
Implementation: Delineation of approaches for optimum contrast agent selection and technique for localization of portal veins during TIPS placement or PTC. Parameters evaluated include comparisons of gross anatomic and histologic appearance associated with effects caused by parenchymal injections of iodinated contrast agents and carbon dioxide.

Contrast agents iohexol (300 mg I, non-ionic) or ditrizoate (ionic) are injected into 1 hepatic lobe in combination with injection of the same volume of CO_2 into another lobe. Gross pathology and histologic evaluation of liver tissue is performed at days 2 to 7 postinjection.

Animal Model: Rabbit (e.g., n = 18 New Zealand White rabbits).
Interpretation and Analysis: At the time of administration, anatomical sites of diatrizoate and iohexol injection show persistent dark discoloration, whereas CO_2 sites showed minimal visible changes. On postmortem gross examination, all diatrizoate sites show severe scarring and also commonly show areas of necrosis. Iohexol and CO_2 injection sites show only minimal discoloration and needle-puncture scars ($p < 0.0001$). The histologic grade for injury caused at diatrizoate sites is significantly more severe than for paired CO_2 sites ($p < 0.016$). Iohexol sites have mild histologic changes similar to paired CO_2 sites ($p = 0.375$). Iohexol and CO_2 therefore appear to produce less severe hepatic damage and are preferred to meglumine diatrizoate for direct hepatic injection procedures.

Comparison of hepatic damage from direct injections of iodinated contrast agents and carbon dioxide. Culp WC, Mladinich CR, Hawkins IF Jr. J Vasc Interv Radiol 1999 Oct; 10 (9), pp. 1265–70.

Angiography/Portal Venography/Fine-Needle Percutaneous Trans-Hepatic Parenchymal Portal Venography/Fluoroscopic and B-Mode Ultrasonography (US) Guided Catheter Delivery
Carbon Dioxide (CO_2)

Application: Magnetic resonance colonography.
Implementation: Negative contrast imaging agent for characterization of anatomical features of the portal vein facilitated by fine-needle percutaneous trans-hepatic parenchymal portal venography using injected CO_2 preparations. Under fluoroscopic and B-mode US guidance, right or left lobes of liver are punctured with a 22-G Chiba needle, through which CO_2 is injected with a dedicated CO_2 injector at an injection rate of 20 ml/s for 20 ml, 40 ml/s for 40 ml, 40 ml/s for 60 ml, and 40 ml/s for 80 ml, respectively. The portal venograms can be obtained by digital subtraction angiography (DSA) system with animals maintained in a supine position. Reference controls used for comparisons and validation include the administration of iodinated contrast media to perform trans-arterial portal venography.

Animal Model: Porcine (e.g., n = 3 Belgian landrace pigs).
Interpretation and Analysis: The portal vein can almost invariably be visualized during venography imaging procedures. Optimal images of portal tree structure up to 4-order branches can be obtained in all those receiving injected CO_2 at a rate of 40 ml/sec and appear to be of a much better quality than those obtained by cranial mesenteric arteriography with iodinated contrast agent. Extravasation of CO_2, liver laceration, or any other complication usually does not occur during diagnostic procedures. The technique described demonstrates optimal portography and appears to be safe, minimally invasive, less time-consuming, cost-effective, and easy to perform, with great potential for clinical application.

Fine-needle percutaneous trans-hepatic parenchymal portal venography by using carbon dioxide: A pilot study in pigs. Sun F, Hernandez J, Crisostomo V, Pineda LF, Lima JR, Uson J, Maynar M. Eur Radiol 2003 Feb; 13 (2), pp. 244–50. Epub 2002 May 09.

Angiography/Renal Artery Imaging
Carbon Dioxide (CO_2) Contrast/Nephrotoxicity: Iodinated Contrast Media

Background Information: Use of iodinated contrast for vascular imaging can be associated with nephrotoxicity and hypersensitivity reactions. Renal injury following conventional angiography is more likely to manifest in conditions of pre-existing renal dysfunction. In the setting of suboptimal renal allograft function, these considerations are particularly relevant. Recently, CO_2 has received attention as a non-toxic, injectable, rapidly absorbed gas that is a cost-effective alternative to standard contrast agents in high-risk patients, such as renal transplant recipients.
Implementation: Angiographs and percutaneous trans-luminal angioplasty using CO_2 in cases of transplant renal artery stenosis (serum creatinine 2.8 mg/dl).
Interpretation and Analysis: Vascular imaging of cases with suboptimal renal function requiring definitive vascular imaging or therapy can be achieved with CO_2 as a contrast agent.

CO_2 angiography—A technique for vascular imaging in renal allograft dysfunction. Kuo PC, Petersen J, Semba C, Alfrey EJ, Dafoe DC. Transplantation 1996 Feb 27; 61 (4), pp. 652–4.

Angiography/Renal Function (Compromised)/ Contraindicated Iodinated Contrast Media
Gadopentetate Dimeglumine ([Gd]-DTPA)/Carbon Dioxide (CO_2)

Background Information: Hemodialysis grafts and native fistulas are frequently evaluated angiographically utilizing iodinated contrast material to determine the cause of malfunction. Occasionally, patients are not able to receive iodinated contrast material due to a history of previous severe allergic reaction or concern that iodinated contrast material could worsen existing conditions of altered renal function requiring premature initiation of permanent dialysis.
Application: Diagnostic administration of [Gd]-DTPA with or without CO_2 as imaging enhancing angiography contrast agents, especially in cases with renal abnormalities where iodinated contrast agents are contraindicated (hemodialysis grafts and native fistulas).
Implementation: Characterization of malfunctioning hemodialysis graft and native fistula using carbon dioxide and [GD]-DTPA. An analogous approach involves the application of balloon angioplasty using [GD]-DTPA alone as the alternative contrast agent.
Interpretation and Analysis: In conditions of a malfunctioning hemodialysis graft and native fistula, a high percentage of cases can be successfully evaluated and treated through the diagnostic application of [GD]-DTPA either alone or in combination with CO_2 angiography (e.g., 100% or n = 6/6). Approximately 66% of affected cases are successfully diagnosed using carbon dioxide in combination with [GD]-DTPA as angiographic contrast agents (e.g., n = 5/6). Angiograms using [GD]-DTPA identifies arterial anastomosis lesions and more clearly identifies stenotic lesions and venous outflow anatomy compared to carbon dioxide angiograms. Gadopentetate dimeglumine is useful as an alternative contrast agent in conjunction with

CO_2 in patients with malfunctioning hemodialysis grafts and fistulas, that have a contraindications for the administration of iodinated contrast material.

CO_2 and gadopentetate dimeglumine as alternative contrast agents for malfunctioning dialysis grafts and fistulas. Spinosa DJ, Angle JF, Hagspiel KD, Schenk WG 3rd, Matsumoto AH. Kidney Int 1998 Sep; 54 (3), pp. 945–50.

Gadopentetate dimeglumine as a contrast agent in peripheral angioplasty. A case report. Murphy JM, O'Hare NJ, Smiddy P, Molloy MP. Acta Radiol 1998 Sep; 39 (5), pp. 576–8.

Gadopentetate dimeglumine: A possible alternative contrast agent for CT angiography of the aorta. Pena CS, Kaufman JA, Geller SC, Waltman AC. J Comput Assist Tomogr 1999 Jan–Feb; 23 (1), pp. 23–4.

Angiography:/Renal Function/Diabetic-Induced Pathology
Gadopentetate Dimeglumine ([GD]-DTPA)/ Gadolinium-Based Contrast Medium

Application: Contrast imaging agent for performing angiography image analyses using digital subtraction instrumentation. Gadopentetate dimeglumine is an alternative and safe radiographic contrast agent for angiography and interventional procedures in patients with severe pre-existing renal impairment. In this population with a high risk for contrast-induced acute renal failure, it is less nephrotoxic than iodinated contrast media.
Route of Administration: Gadopentetate dimeglumine (intravenous).
Dosage: Gadopentetate dimeglumine mean dose = 0.34 ± 0.06 mmol/kg body weight.
Implementation: Injection of [GD]-DTPA as a radiographic contrast agent in conditions of severe renal insufficiency. Gadopentetate dimeglumine can be applied either alone (e.g., n = 20/32) or in conjunction with carbon dioxide (n = 12/32). Examples include (i) diagnostic procedures such as selective renal arteriographies (e.g., n = 11), angiographies of the iliac arteries and lower extremities (e.g., n = 6), and venous angiographies of the upper extremity and central veins (e.g., n = 7); in addition to (ii) interventional procedures, including percutaneous trans-luminal renal angioplasties with stenting (e.g., n = 2), percutaneous peripheral vascular interventions (e.g., n = 4), and balloon angioplasties of a dialysis fistula (e.g., n = 2).

Human Model: Cases with pre-existing severe renal insufficiency subjected to angiographic procedures (e.g., n = 32 angiograms, n = 24/32 diagnostic angiographies, n = 8 interventional angiographies; n = 29 cases, n = 17/29 diabetics; mean serum creatinine = 3.6 ± 1.4 mg/dl).
Interpretation and Analysis: Development of postprocedure contrast material–induced renal failure (e.g., n = 1/29 with serum creatinine > 0.5 mg/dl within 72 hours) and other types of complications are relatively rare in occurrence. Cases that experience alterations in renal function sometimes display increases in serum creatinine after percutaneous trans-luminal renal angioplasty and stenting that are probably due to cholesterol embolism. Gadopentetate dimeglumine has a level of radiographic density that is sufficient to allow adequate diagnostic visualization with digital subtraction equipment in all of the diagnostic procedures described.

Gadolinium as an alternative contrast agent for diagnostic and interventional angiographic procedures in patients with impaired renal function. Rieger J, Sitter T, Toepfer M, Linsenmaier U, Pfeifer KJ, Schiffl H. Nephrol Dial Transplant 2002 May; 17 (5), pp. 824–8.

Angiography/Vascular Permeability: Contrast Imaging Agents/Relative Contrast Imaging Agent Endothelial Permeability
Triiodinated Non-Ionic Monomer (Iversol)/Iron Oxide Nanoparticle (Sinerem)/Iodinated Dextran Polymer (P604)

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Background Information: The efficiency of contrast agents in medical imaging depends on their distribution into vascular and interstitial compartments.
Application: Radiopaque contrast imaging agent.
Implementation: Compare the *in vitro* endothelial permeability of different classes of contrast agents with various vascular persistence properties (e.g., triiodinated non-ionic monomer [ioversol]; an iodinated dextran polymer [P604]; and an iron oxide nanoparticle [Sinerem]). Permeability studies are performed through collagen-coated filters (with or without porcine aortic endothelial cell monolayer) by placing each filter-ring luminal chamber into a beaker containing culture medium (abluminal chamber). Contrast media, diluted in the culture medium, is then added to the luminal chamber. Aliquots are sampled from the abluminal chamber for contrast agent measurements. The volume that is cleared of the compound is calculated from the luminal side to the abluminal side. Parallel permeability tests for $[^3H]$-H_2O and Evans blue albumin are performed as reference controls. Finally, the modulatory effect of bradykinin on endothelial permeability to albumin or to contrast agents can also be evaluated.
Interpretation and Analysis: The volume cleared of ioversol, P604, and Sinerem through membrane filters is decreased by 19.6%, 32.1%, and 52.0%, respectively, in the presence of a cell monolayer. Bradykinin (10^{-6} M) significantly increases permeability to albumin, ioversol, and Sinerem. Ioversol and Sinerem induce a significant decrease in albumin permeability. A relation appears to exist between contrast agent molecular size and their endothelial permeability when evaluated in certain *in vitro* models.

In vitro evaluation of vascular permeability to contrast media using cultured endothelial cell monolayers. Martin-Chouly CA, Youmine H, Saiag B, Hentsch AM, Corot C, Legrand A. Invest Radiol 1999 Nov; 34 (11), pp. 663–8.

Angiography/Vena Cava (Inferior) Cavography/ Inferior Vena Cava Diameter/Assessment Prior to Filter Placement/Carbon Dioxide (CO_2) Negative Contrast
Iodinated Postive Contrast Media/ CO_2 Negative Contrast

Cellular/Molecular Mechanism of Action: Carbon dioxide possesses radioleucent properties that can be detected when infused into a liquid medium.
Application: Carbon dioxide can be applied as a negative contrast image-enhancing agent.
Route of Administration: Carbon dioxide (intravascular).
Implementation: Inferior vena cavography applying both CO_2 (negative) and iodinated (positive) contrast materials performed prior to filter placement. The diameter of the IVC is measured and compared in the same 4 locations for both contrast agents. The diameter is corrected for magnification and "pin-cushion" distortion. Ability of CO_2 to correctly classify inferior vena caval (IVC) diameter as ≤ 28 mm or > 28 mm, based on the IVC diameter with iodinated contrast material is established. Blood pressure and arterial oxygen saturation are measured immediately before and after CO_2 injection.

Interpretation and Analysis: There is no significant difference in the measured diameter of the IVC with CO_2 compared to iodinated contrast material after correction for magnification and pincushion distortion (e.g., n = 30 cases). A low percentage of cases may be misclassified as having an IVC $\leq$ 28 mm with CO_2 when, in fact, the IVC diameter is $>$ 28 mm based on iodinated contrast material. Such considerations could be clinically significant for certain IVC filters. Nearly half of renal veins identified on contrast venography can be identified by CO_2 vena cavography (e.g., 47%). No significant differences can be appreciated in blood pressure or oxygen saturation values measured before and after CO_2 injection. A small percentage of cases with pulmonary artery hypertension experience transient, symptomatic hypotension after CO_2 injection (e.g., n = 1/30). Vena cavography with CO_2 in most instances accurately evaluates IVC diameter prior to filter placement.

Discrepancy in measurements between CO_2 and iodinated contrast material can be clinically significant in some cases (e.g., 3.3%) depending on the type of filter placed. Carbon dioxide (CO_2) is less accurate than iodinated contrast material in identifying renal veins. Although CO_2 vena cavography is safe in the majority of patients, it should be used with caution in patients with pulmonary hypertension.

Accuracy and safety of carbon dioxide inferior vena cavography. Boyd-Kranis R, Sullivan KL, Eschelman DJ, Bonn J, Gardiner GA. J Vasc Interv Radiol 1999 Oct; 10 (9), pp. 1183–9.

Angiography/Vena Cava (Inferior) Cavography
Carbon Dioxide (CO_2)

Background Information: The use of iodinated contrast in the critically ill trauma patient has been associated with the development of acute renal failure. The low incidence of nephrotoxicity associated with CO_2 makes it an ideal contrast agent for cavography. However, the use of CO_2 has been limited, because it reportedly underestimates the diameter of the inferior vena cava (IVC).
Application: Angiography, including cavography of the IVC.
Implementation: Delineation of the luminal diameter of the IVC and renal veins utilizing CO_2 and iodinated medias as contrast agents for cavagram procedures.

Human Model: Trauma cases examined over a period of 6 months that required IVC filter placement (e.g., n = 25).
Interpretation and Analysis: The diameter of the IVC and anatomy of renal vein structures can be successfully achieved utilizing injected CO_2 as a negative contrast agent in most diagnostic examinations (e.g., n = 25). Images acquired applying CO_2 cavography compared with those obtained with iodinated positive contrast media reveal very small variations in IVC diameter estimates (e.g., $<$ 1 mm).

Therefore, CO_2 cavagrams appear to accurately reflect the diameter of the IVC and the renal vein anatomy. Additionally, CO_2 cavagrams can be safely performed in the intensive care unit during bedside placement of IVC filters.

Comparison of carbon dioxide and iodinated contrast for cavography prior to inferior vena cava filter placement. Holtzman RB, Lottenberg L, Bass T, Saridakis A, Bennett VJ, Carrillo EH. Am J Surg 2003 Apr; 185 (4), pp. 364–8.

CENTRAL NERVOUS SYSTEM

Myelography/Image Quality/Adverse Reactions: Relative Frequency/Pharmakinetics
Iomerprol/Iohexol/Iotrolan

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Iomeprol is a non-ionic iodinated contrast medium that can be applied to perform myelogram procedures (conventional radiography and myelo-computed tomography [CT]). Other iodinated compounds like iohexol and iotrolan can be applied in a similar fashion.
Route of Administration: Iomeprol (intrathecal).
Dosage: Iomeprol.
Implementation: Evaluation of pharmacokinetic, tolerability, safety, and efficacy of iomeprol following intrathecal injection (e.g., n = 600 cases).
Interpretation and Analysis: Pharmacokinetic study results reveal that after intrathecal administration iomeprol is completely absorbed from the cerebrospinal fluid compartment (once absorbed into the systemic circulation, it is rapidly excreted, unmetabolized, by glomerular filtration). Dose-finding studies show that the opacification produced by iomeprol depends on the injected dose. Although a dose-dependent increase in efficacy is observed, no differences in neurotolerability and safety are detected between the doses tested. Doses of iomeprol greater than 3 g iodine do not cause a greater incidence of adverse events and produce significantly better contrast efficacy. Comparative clinical trials demonstrate that iomeprol and iopamidol, iohexol, and iotrolan always provide adequate opacification of the subarachnoid space, both in conventional myelography and myelo-CT, with adequate delineation of normal structures and pathologic processes. No serious adverse events occur up to a total dose of 4.5 g of iodine. Almost invariably, no differences between the agents with regard to tolerance, adverse events, and effects on vital signs, physical examination, and neurologic examination can be appreciated. Therefore, iomeprol is safe and effective and can be recommended for myelography and myelo-CT.

Clinical experience with iomeprol in myelography and myelo-CT: Clinical pharmacology and double blind comparisons with iopamidol, iohexol, and iotrolan. Katayama H, Heneine N, van Gessel R, Taroni P, Spinazzi A. Invest Radiol 2001 Jan; 36 (1), pp. 22–32.

GASTROINTESTINAL SYSTEM

Esophogeal Leakage/Postoperative Complications/ Esophagogastrectomy/Gastrectomy/Total Laryngectomy
Barium Sulfate/Iodinated Contrast Media

Cellular/Molecular Mechanism of Action: Barium containing preparations and iodinated compounds possess radiopaque properties.
Application: Can be utilized as positive contrast image-enhancing agents in conventional radiography procedures for the detection of esophageal "leaks" in cases subjected to esophagogastrectomy, total gastrectomy, or total laryngectomy.
Implementation: Identify and review radiographic imaging procedures performed with water-soluble contrast agent alone or a water-soluble contrast agent followed by barium that demonstrate "leaks" in cases that have undergone esophagogastrectomy, total gastrectomy, or total laryngectomy (e.g., n = 46; single institution radiology database search between 1998 and 2001).
Interpretation and Analysis: Approximately 50% of the "leaks" detected on radiographic images can be identified with the aid of water-soluble contrast agents (e.g., n = 23/46), while the other 50% can be detected only with high-density barium (e.g., n = 23/46). Of the 23 leaks visualized with water-soluble contrast media, 26% (n = 67/23) can be characterized by blind-ending tracks, 61% (n = 14/23) by sealed-off collections, and 13% (n = 3/23) by extravasations of free contrast material into the mediastinum or neck.

Of the 23 leaks visualized only with high-density barium, 83% (n = 19/23) can be characterized by blind-ending tracks and 17% (n = 4/23) by sealed-off collections. Thus, leaks detected only on images obtained with high-density barium are significantly more likely to be characterized by blind-ending tracks than those detected on images obtained with a water-soluble contrast agent (p = 0.0007). Of patients with clinical follow-up (e.g., n = 33), the findings seen on these imaging studies ultimately affects management in 86% (e.g., n = 12/14) of patients with leaks depicted by water-soluble contrast media and in 53% (e.g., n = 10/19) with leaks depicted only by high-density barium. Observations noted in this report support the use of high-density barium as part of the routine postoperative radiographic examination when no leaks are detected on images obtained with a water-soluble contrast agent.

Usefulness of high-density barium for detection of leaks after esophagogastrectomy, total gastrectomy, and total laryngectomy. Swanson JO, Levine MS, Redfern RO, Rubesin SE. AJR Am J Roentgenol 2003 Aug; 181 (2), pp. 415–20.

Gastrointestinal (GI) Tract
17 (1,3,5-Tri-n-Hexyl-2,4,6-Triiodobenzene)/ Barium Sulfate Alternative

Cellular/Molecular Mechanism of Action: The compound 17 (1,3,5-tri-n-hexyl-2,4,6-triiodobenzene) possesses radiopaque properties.
Background Information: Examination of the GI tract has been performed for decades using barium sulfate. Although this agent has many recognized limitations, including extreme radiopacity, poor intrinsic affinity for the GI mucosa, and very high density, no alternative contrast agents have emerged that produce comparable or better contrast visualization. In fact, the various techniques of the GI radiologic examination (i.e., single contrast, double contrast, biphasic) were developed to compensate for its limitations. Each of these techniques requires complex patient manipulation to achieve adequate mucosal coating or compression to overcome the marked radiopacity of barium sulfate in order to obtain a diagnostically useful examination.
Application: Detection and characterization of anatomical and physiological abnormalities affecting the gastrointestinal tract.
Route of Administration: Gastrointestinal tract.
Implementation: Administration of the compound 17 (1,3,5-tri-n-hexyl-2,4,6-triiodobenzene) to determine how well it is tolerated following intraperitoneal injection.

Animal Model: Rodent (mice, hamsters, rats) with 17 (1,3,5-tri-n-hexyl-2,4,6-triiodobenzene) administered intraperitoneally at 4 times (4X) the anticipated human clinical dosage.

Animal Model: Canine intestinal imaging studies.

***Ex Vivo* Model:** Hepatic microsomes harvested from rat, hamster, canine, monkey and human hepatic tissues applied to delineate how extensively 17 (1,3,5-tri-n-hexyl-2,4,6-triiodobenzene) is metabolized by liver microsomal enzyme systems.
Interpretation and Analysis: The class of radiopaque oils known as the 1,3, 5-trialkyl-2,4,6-triiodobenzenes can improve the efficacy, stability, and safety of barium formulations. The compound 17 (1,3,5-tri-n-hexyl-2,4,6-triiodobenzene), formulated as an oil-in-water emulsion, is well tolerated in rodents (mice, hamsters, rats) following acute oral and/or intraperitoneal administrations at 4 times the anticipated human clinical dose. No metabolism of 17 (1,3,5-tri-n-hexyl-2,4,6-triiodobenzene) can be detected in rat, hamster, dog, monkey, or human hepatic microsomes, suggesting the lack of oral toxicity is a consequence of poor absorption. Image analysis in dogs reveals that emulsions of 17 (1,3,5-tri-n-hexyl-2,4,6-triiodobenzene) provides excellent mucosal coating and improved radiodensity relative to barium sulfate suspensions. On the basis of the preliminary imaging and toxicity data, compound 17 (1,3,5-tri-n-hexyl-2,4,6-triiodobenzene) is selected as a potential development candidate.

1,3,5-Trialkyl-2,4,6-triiodobenzenes: Novel x-ray contrast agents for gastrointestinal imaging. Estep KG, Josef KA, Bacon ER, Illig CR, Toner JL, Mishra D, Blazak WF, Miller DM, Johnson DK, Allen JM, Spencer A, Wilson SA. J Med Chem 2000 May 18; 43 (10), pp. 1940–8.

Pharyngeal Perforation
Iodinated Aqueous Contrast Agents/Barium Suspension (50% Weight/Volume)/Barium Suspension (100% Weight/Volume)

Cellular/Molecular Mechanism of Action: Iodinated compounds and barium preparations possess radiopaque properties.
Application: Diagnostic imaging of gastrointestinal system.
Route of Administration: Oral.
Implementation: Evaluate visual *in vitro* comparisons of an iodinated aqueous contrast agent, a 50% weight/volume barium suspension, and a 100% weight/volume barium suspension in procedures including pharyngography. In cases of detectable pharyngeal perforation, clinical monitoring is performed to exclude complications associated with barium administration.
Interpretation and Analysis: As opposed to the 100% weight/volume barium suspension, *in vitro* comparison between the aqueous contrast agent and the 50% weight/volume barium suspension reveals no substantial differences. Pharyngeal perforations can be detected with the aqueous contrast agent, but in approximately 59% of such cases, diagnostic visualization is superior with 100% weight/volume barium suspension (e.g., n = 10/17) and a small percentage of pharyngeal perforation cases cannot be detected with the latter preparation (e.g., n = 2/17). Because of a higher radiopacity, 100% weight/volume barium suspensions may more sharply delineate perforations. However, in contrast to aqueous contrast media, narrow pharyngeal perforations can be missed. Thus, the use of a 100% weight/volume barium suspension does not improve the detection of pharyngeal perforation.

Detection of pharyngeal perforation: Comparison of aqueous and barium-containing contrast agents. Keberle M, Wittenberg G, Trusen A, Hoppe F, Hahn D. AJR Am J Roentgenol 2000 Nov; 175 (5), pp. 1435–8.

HEPATOBILIARY SYSTEM

Cholangiopancreaticography/Iodine Load
Iopamidol

Background Information: Hyperthyroidism induced by contrast agents is a major problem in patients with pre-existing thyroid disease, particularly in patients with functional thyroid autonomy.
Application: Iopamidol is a non-ionic contrast media in procedures including endoscopic retrograde cholangiopancreaticography.
Dosage: Iopamidol (total iodine load = 57.4 ± 22.8 mmol [7.3 ± 2.9 g]).
Implementation: Determination of the extent iodinated contrast media administered during endoscopic retrograde cholangiopancreaticography influences total and free iodine concentrations within the plasma compartment and excreted in the urine (e.g., n = 15 cases; day 0–day 21).
Interpretation and Analysis: Endoscopic retrograde cholangiopancreaticography procedures utilizing iodinated contrast media promote highly significant increases in total serum iodine levels from 0.8 ± 0.5 to 85.2 ± 116.9 μmol/l at 4 hours postadministration

(e.g., iopamidol). In parallel, serum iodide levels increase from 0.06 ± 0.04 to 5.42 ± 6.09 μmol/l, and urinary iodine excretion increases from 71.1 ± 35.7 μmol/mol creatinine to 621,620.9 ± 636,492.2 μmol/mol creatinine. Peak concentrations of serum iodine are closely related to the total amount of iodine administered ($p < 0.05$). During follow-up, iodine levels return to pre-exposure levels within 2 to 3 weeks. Levels of thyrotropin, free thyroxine, and free triiodothyronine remain unchanged during follow-up monitoring periods. The full clinical significance of elevations in total and free iodine and their urinary excretion associated with the application of iodinated contrast agents in endoscopic procedures remains to be fully established.

Systemic iodine absorption during endoscopic application of radiographic contrast agents for endoscopic retrograde cholangiopancreaticography. Mann K, Rendl J, Busley R, Saller B, Seybold S, Hoermann R, Sauerbruch T, Borner W. Eur J Endocrinol 1994 May; 130 (5), pp. 498–501.

MONOCYTES AND MACROPHAGES

Macrophage Imaging Agent/Liver and Spleen/ Vascular Compartment Imaging Agent
Cyclic Carbonate of Ioxilan (IXC)/Ioxilam Nanoparticles/Ioxilam Microparticles/ Biodegradable Radiopaque Particles

Cellular/Molecular Mechanism of Action: Cyclic carbonate of ioxilan is a biodegradable radiopaque particle preparation that is a prodrug of the non-ionic water-soluble contrast agent ioxilan.
Application: Cyclic carbonate of ioxilan is the prodrug form of non-ionic water-soluble ioxilan contrast agent. This contrast agent enhances liver attenuation and is cleared from the body as ioxilan.
Route of Administration: Ioxilan (intravenous: rats n = 2).
Dosage: Ioxilan (50 mg I/kg body weight).
Implementation: Determination of the influence of particle physical characteristics on IXC particle biodistribution. Formulations of IXC nanoparticles (average diameter = 290 nm) and IXC microparticles (average diameter = 1.7 mm) are prepared and characterized prior to intravenous injection (rats n = 2). The sensitive, reproducible analytic methods, inductively coupled plasma-mass spectrometry (ICP-MS) and high-performance liquid chromatography (HPLC) can be applied to quantify tissue iodine and ioxilan concentrations.
Animal Model: Rat (n = 2).
Interpretation and Analysis: Ioxilan nanoparticles and microparticles are both assimilated by the liver and spleen. The IXC nanoparticles remain in the liver at high concentrations for 6 hours and are then slowly eliminated. Each agent also produces high blood iodine concentrations within the 1st 5 minutes following intravenous injection, suggesting their potential use as a blood-pool imaging agent. Unlike nanoparticles, the microparticles have a significantly lower uptake by the kidney. Because of reduced renal uptake, microparticles are a preferred macrophage imaging agent. Biodegradable radiopaque particles may be used either as blood-pool imaging agents or as macrophage imaging agents depending on their size and distribution characteristics. The ICP-MS and HPLC methods are useful for biodistribution studies of iodinated contrast agents.
Instrumentation: Inductively coupled plasma-mass spectrometry and HPLC.

Biodistribution of cyclic carbonate of ioxilan: A radiopaque particulate macrophage imaging agent. Li C, Yu D, Kan Z, Yang DJ, Tansey W, Kuang LR, Wallace S. Acad Radiol 1996 Jun; 3 (6), pp. 500–6.

RENAL SYSTEM

Renal Clearance (Age-Related Differences)/ Pharmacokinetics
Iodixanol

Application: Iodixanol is an iso-osmolar iodinated radiocontrast agent.
Route of Administration: Iodixanol (intravascular).
Dosage: Iodixanol.
Implementation: Iodixanol is administered by cardiac catheterization and then plasma samples collected at 0.75 to 1.25 hours, 2 to 4 hours, 8 to 12 hours, and 16 to 32 hours postinjection (e.g., n = 41 cases: newborn to < 2 months; 2 to < 6 months; 6 months to < 1 year; 1 to < 3 years; and 3 to ≤ 12 years). Plasma concentrations of iodixanol can subsequently be determined using high performance liquid chromatography. The primary pharmacokinetic end point to be applied is the terminal elimination rate constant (K_{el}).
Interpretation and Analysis: Mean K_{el} is significantly lower in newborns ≤ 2-month-old cases in contrast to older subjects: (iodixanol/hr, mean ± standard deviation [SD]): newborn to < 2 months 0.185 ± 0.060, 2 to < 6 months, 0.256 ± 0.046, 6 months to < 1 year 0.299 ± 0.042, 1 to < 3 years 0.322 ± 0.058, and 3 to ≤ 12 years 0.307 ± 0.071; with increasing age, excretion is more rapid, as shown by each age group's elimination half-life ($T_{1/2}$ beta; 4.14 ± 1.41 hours, 2.79 ± 0.55 hours, 2.36 ± 0.37 hours, 2.23 ± 0.51 hours, and 2.36 ± 0.52 hours, respectively). Elimination of iodixanol in children > 6 months of age is comparable to normal adults. Prolonged elimination in children < 6 months of age is related to renal immaturity.

Iodixanol pharmacokinetics in children. Johnson WH Jr, Lloyd TR, Victorica BE, Zales VR, Epstein ML, Leff RD, Ardinger RH Jr, Slovis TL, Johnson JA, Marsters PA. Pediatr Cardiol 2001 May–Jun; 22 (3), pp. 223–7.

Renal Failure
Iodinated Contrast Media/Gadolinium

Renal failure due to iodinated contrast media is a serious complication of peripheral angiography in patients with impaired renal function. Gadolinium-based agents that are widely used in magnetic resonance imaging have no adverse renal toxicity at recommended doses. These agents have sufficient radiographic density to be seen using digital subtraction equipment. In this context, undiluted [GD]-DTPA can be applied as a radiographic contrast agent (e.g., n = 2 case reports).

Gadopentate dimeglumine as an alternative contrast agent for use in interventional procedures. Hatrick AG, Jarosz JM, Irvine AT. Clin Radiol 1997 Dec; 52 (12), pp. 948–52.

Renal Failure/Diabetes/Metformin: Contrast Media Drug Interaction

Intravascular administration of iodinated contrast media to patients who are receiving metformin, an oral antidiabetic agent, can result in lactic acidosis. However, this rare complication occurs only if the contrast medium causes renal failure, and the patient continues to take metformin in the presence of renal failure. Because metformin is excreted primarily by the kidneys, continued intake of metformin after the onset of renal failure results in a toxic accumulation of this drug and subsequent lactic acidosis. To avoid this complication, metformin must be withheld after the administration of the contrast agent for 48 hours, during which the contrast-induced renal failure becomes clinically apparent. If renal function is normal at 48 hours,

the metformin can be restarted. There is no scientific justification for withholding metformin for 48 hours before administration of the contrast medium, as currently recommended in the package insert. Reviews have been described regarding the pharmacology of metformin and institutional policies for managing patients with diabetes who receive metformin and who require intravascular administration of iodinated contrast media.

Metformin and contrast media: Where is the conflict? Rasuli P, Hammond DI. Can Assoc Radiol J 1998 Jun; 49 (3), pp. 161–6.

Urography: Excretory/Comparison: Image Quality, Vital Signs, Clinical Pathology, Reactions
Iodixanol/Iohexol

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties. Iodixanol is a non-ionic dimeric iodinated contrast agent, while iohexol is a monomeric agent.
Application: Iodixanol and iohexol can be used as positive contrast agents for enhancing radiographic imaging during excretory urography procedures.
Route of Administration: Intravascularly.
Implementation: Evaluation of image quality, vital signs, laboratory values, and adverse reactions (e.g., n = 75 cases). Iodixanol at a dose of 1ml/kg consisting of preparations formulated at either 270 mg I/ml, 320 mg I/ml, or 300 mg I/ml (e.g., n = 25 cases for each concentration). In situations where comparisons are being made between different groups, no important differences in demographics should exist. The radiographs are interpreted and evaluated for overall quality.
Study Design: Randomized, double blind, parallel comparison.
Interpretation and Analysis: Evaluation of each phase of the radiographic study reveals almost uniform good-to-excellent opacification, with no significant differences among the 3 agents. No significant differences exist for the 3 groups in terms of vital signs, hematology, blood and urine chemistry, and injection discomfort. Death or serious reactions are overall a very rare occurrence. There is no statistically significant difference between the numbers of adverse events for each of the 3 contrast imaging agents. Iodixanol at both 270 and 320 mg I/ml has proven to be a safe and effective contrast material for intravenous urography and its profile is essentially indistinguishable from that of iohexol at 300 mg I/ml.

Comparison of iodixanol with iohexol in excretory urography. Siegle RL, Gavant ML. Acad Radiol 1996 Sep; 3 Suppl 3, pp. S524–7.

Urography/Image Enhancement
Iopromide/Iotrolan/Urea/Iloprost/Nifedipine/ Papaverine/Sodium Nitroprusside

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties. Certain molecular additives influence the excretion rate of iodinated contrast agents during urography imaging procedures.
Application: Enhancement of the diagnostic quality of urography images produced with iodinated contrast-enhancing agents, including iopromide and iotrolan (conventional radiography and computed tomography [CT]).
Route of Administration: Iopromide (300 mg/mL) and iotrolan preparations (280 mg/mL) are each administered intravenously.
Dosage: Iopromide (plain films = 300 and 600 mg iodine/kg) and iotrolan preparations (plain films = 300 and 600 mg/kg) in dogs and rabbit. Alternatively, iopromide (CT scans = 50 mg iodine/kg) and iotrolan preparations (CT scans = 50 mg/kg) in rabbit.
Implementation: Conventional plain-film radiography urograms utilizing urea (52 mg/mL), iloprost (10, 20, 50, and 100 ng/mL), nifedipine (0.02 mg/mL), papaverine (0.625 mg/mL) and sodium nitroprusside (0.05 μg/mL) added to commercial iopromide (300 mg/mL) and iotrolan preparations (280 mg/mL). Images are obtained at different time points after contrast injection of iodine preparations (e.g., rabbits and dogs). Image analysis is then repeated using CT and iopromide or iotrolan plus urea (52 mg/mL) or iloprost (20 ng/mL) and iodine doses of 50 mg/kg body weight (rabbits).
Interpretation and Analysis: Urea and iloprost at low concentrations (10 and 20 ng/mL) increase image quality both in plain film and in CT urography. Iloprost at high concentrations (50 and 100 ng/mL), nifedipine, papaverine and sodium nitroprusside decreases image quality. An increase in image quality is accompanied by faster renal elimination of the contrast agent. Therefore, image quality in plain film and CT urography can potentially be improved by additives to the contrast medium.

Use of additives to contrast media to improve imaging in plain film and computed tomography urography. Krause W, Schromm M. Invest Radiol 2003 Jan; 38 (1), pp. 17–26.

REPRODUCTIVE SYSTEM

Hysterosalpingography
Hexabrix/Diatrizoate Meglumine

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Hexabrix is applied as a monoacidic dimeric iodinated contrast material.
Route of Administration: Diatrizoate meglumine (Renografin 60) and Hexabrix can both be infused directly for hysterosalpingography procedures.
Interpretation and Analysis: Serious adverse reactions are relatively rare with the application of either Hexabrix or diatrizoate meglumine. Discomfort during and after the procedure is not statistically different between cases administered either contrast reagent. Furthermore, no differences in radiographic quality can usually be appreciated despite the reduced osmolality and increased viscosity of Hexabrix. Both contrast agents appear to be safe and acceptable contrast agents for hysterosalpingography imaging.

Hexabrix as a contrast agent for hysterosalpingography. Winfield AC, Maxson WS, Harding DR, Diggs J, Wentz AC. Radiology 1984 Jul; 152 (1), pp. 232–3.

COMPUTED TOMOGRAPHY (CT) RADIOGRAPHY IMAGE-ENHANCING AGENTS

Adverse Effects/Sequellae: Bronchospam Induction
Iomeprol (Non-Ionic and Triiodinated)/Iopamidol (Non-Ionic and Triiodinated)/Iohexol (Non-Ionic and Triiodinated)/Diatrizoate (Ionic and Triiodinated)/ Iotrolan (Non-Ionic and Hexaiodinated)/Iocarmate (Ionic and Hexaiodinated)/Ioxaglate (Ionic and Hexaiodinated)/ Lysine Acetylsalicylate/Indomethacin

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Administered intravenously as contrast agents to enhance computed tomography (CT) angiography imaging analyses.

Route of Administration: Preparations of iomeprol (400 mg I/ml), iopamidol (370 mg I/ml), iohexol (350 mg I/ml), diatrizoate (370 mg I/ml), iotrolan (300 mg I/ml), iocarmate (280 mg I/ml), and ioxaglate (320 mg I/ml) can each be delivered intravenously (IV) as iodinated contrast agents for CT angiography imaging.
Dosage: Administration of the iodinated contrast agents (i) non-ionic, triiodinated iomeprol (400 mg I/ml), iopamidol (370 mg I/ml), and iohexol (350 mg I/ml); (ii) ionic, triiodinated diatrizoate (370 mg I/ml); (iii) non-ionic, hexaiodinated iotrolan (300 mg I/ml); and (iv) ionic hexaiodinated iocarmate (280 mg I/ml) and ioxaglate (320 mg I/ml) at a dose of 2 g I/kg. (Note: Anesthetized guinea pigs.)
Implementation: Evaluation of alterations in pulmonary airway resistance following intravenous administration of pharmaceutical formulations of iomeprol (400 mg I/ml), iopamidol (370 mg I/ml), and iohexol (350 mg I/ml), diatrizoate (370 mg I/ml), iotrolan (300 mg I/ml), iocarmate (280 mg I/ml), and ioxaglate (320 mg I/ml). Changes in pulmonary airway resistance are evaluated by measuring intratracheal pressure at the moment of maximum insufflation, or maximal insufflation pressure (MIP).

Animal Model: Guinea pig (anesthetized and subjected to forced ventilation).
Interpretation and Analysis: All contrast media except ioxaglate cause mean increases in MIP of no more than 20%. By contrast, ioxaglate causes a marked bronchoconstrictor effect, and increases in MIP of 242% ± 46%. In the evaluation of pharmaceuticals for antagonistic action on increases in MIP, salbutamol inhibits almost completely the increase in MIP for the 1st 40 minutes post-treatment. Similarly, lysine acetylsalicylate and indomethacin consistently reduce MIP after contrast media administration to levels only 30% and 14% above those of baseline precontrast media, respectively. Promethazine has only a minor inhibitory effect, and the response to prednisolone varies. There is no apparent relationship between the size of the increase in airway resistance and the charge or molecular weight of contrast agents or the pharmaceutical formulation. The increase induced by ioxaglate must be attributed to inherent molecular toxicity mediated through a direct action on the production of bradykinin and/or the prostanoid products of the cyclo-oxygenase pathway, rather than through a direct action on the release of histamine.

Effects of iodinated contrast media on pulmonary airway resistance in anesthetized guinea pigs. Cipolla P, Castano M, Kirchin MA, de Haen C, Tirone P. Acad Radiol 1995 Apr; 2 (4), pp. 306–12.

Adverse Effects/Sequellae Incidence/Discomfort and Sensations/Relative Safety
Iodixanol/Iohexol

Cellular/Molecular Mechanism of Action: Non-ionic iodinated compound that has radiopaque properties.
Background Information: Non-ionic iodinated contrast media have proved effective for computed tomography (CT) of the head and have demonstrated greater tolerability than their ionic counterparts. Phase I safety, tolerance, and pharmacokinetic studies have indicated that iodixanol may be associated with fewer adverse effects than iohexol, while it provides equivalent diagnostic information.
Application: Liquid non-ionic iodinated contrast media applied in CT to detect anatomical abnormalities in body fluid compartments. Iodixanol is also formulated as an isosmolar preparation, which has a distinct advantage over contrast agents like iohexol (iodixanol 50% the osmolality of iohexol). Each of these agents can be applied as an intracranial contrast agent to enhance CT imaging analysis.
Dosage: Iodixanol 270 mg I/ ml (IOD-270) and at 320 mg I/ml (IOD-320) compared to iohexol 300 mg I/ml (IOH-300).
Implementation: Comparison of the safety and diagnostic efficacy of iodixanol to iohexol.
Interpretation and Analysis: No statistically significant differences in the quality of images or in the occurrence of adverse events can be appreciated between the applications of iodixanol and iohexol (270 mg I/ ml & 320 mg I/ml) or iohexol (300 mg I/ml). Pairwise comparisons of injection-associated discomfort reveals that significantly more sensations of discomfort are associated with iohexol (300 mg I/ml) compared to iodixanol (270 mg I/ ml or 320 mg I/ml). Iodixanol at 270 or 320 mg I/ml therefore appears to be safe and effective when administered intravenously at a volume of 100 ml for intracranial CT scans in adult patients.

A randomized comparison of iodixanol and iohexol in adult intracranial computed tomography scanning. Grossman RI, Modic MT. Acad Radiol 1996 Sep; 3 Suppl 3, pp. S488–94.

Agents: General Concepts/Artifact Reduction
Lanthanice-Based Compounds: Gadolinium Diethylenetriamine Pentaacetic Acid ([Gd]-DTPA)/ Iodinated Contrast Media Comparison

Background Information: Recent experiments using computed tomography (CT) an transmission radiography show that [Gd] agents can increase image contrast agent by up to a factor of 2 relative to more commonly used iodinated agents on an equimolar basis. The use of [Gd] has been proposed to reduce beam hardening artifacts.
Application: Lanthanide-based compounds such as [Gd]-DTPA are currently used as contrast agents in magnetic resonance imaging (MRI).
Implementation: Analysis of 3 different CT scanners using a circular water equivalent phantom with contrast-filled tube inserts.
Interpretation and Analysis: Artifacts are a factor of 1.3 to 1.8 more pronounced with an iodinated contrast agent compared with [Gd]-DTPA. A theoretical model that uses an experimentally derived photon energy spectrum is proposed that relates the strength of beam hardening artifacts to the variance (over the energy spectrum) of the attenuation coefficient of the contrast agent. This allows easy assessment of the relative magnitudes of the artifact for different contrast agents

A comparison of beam-hardening artifacts in x-ray computerized tomography with gadolinium and iodine contrast agents. Med Phys 1995 Dec; 22 (12), pp. 1977–1982.

Agents: General Concepts/Contrast Imaging Agents/Computed Tomography (CT) Applications

Background Information: Computed tomography is a reliable, widely available imaging modality with high spatial resolution. Except for trauma detection, in practically all cases contrast agents are administered for improving image quality. The presently available agents are non-specific compounds that distribute into the extracellular space. Specificity (i.e., high contrast in selected tissues) is only possible when the pharmacokinetic behavior (peak levels at the region of interest) is followed on a time scale that counts in seconds. If the peak time is missed, a 2nd injection of contrast material is necessary. The objective of extensive research efforts has therefore been the search for tissue-specific contrast agents that should result both in higher and in longer-lasting local concentrations. For the tissue targets, liver, spleen, lymph nodes, and the blood-pool, efficient contrast-enhancing compounds have been described utilizing different

active transport systems or passive diffusion-limiting molecular sizes. The agents include water-soluble metal chelates, iodinated polymeric compounds and contrast agent-carrying liposomes. The remaining task for the future will be to improve the tolerability of the contrast agents to such an extent that the side-effect incidence and severity is low enough to allow for the use of these agents in patients.

Delivery of diagnostic agents in computed tomography. Krause W. Adv Drug Deliv Rev 1999 Apr 5; 37 (1), pp. 159–73.

Agents: General Concepts/Contrast Imaging Agents/Renal Clearance: Age-Related Differences
Iodixanol

Application: Iodixanol is an iso-osmolar iodinated radiocontrast agent.
Route of Administration: Iodixanol (intravascular).
Implementation: Iodixanol is administered by cardiac catheterization, and then plasma samples are collected at 0.75 to 1.25 hours, 2 to 4 hours, 8 to 12 hours, and 16 to 32 hours postinjection (e.g., n = 41 cases: newborn to < 2 months; 2 to < 6 months; 6 months to < 1 year; 1 to < 3 years; and 3 to ≤ 12 years). Plasma concentrations of iodixanol can subsequently be determined using high-performance liquid chromatography. The primary pharmacokinetic end point to be applied is the terminal elimination rate constant (K_{el}).
Interpretation and Analysis: Mean K_{el} is significantly lower in newborn to < 2-month-old cases vs older subjects: (iodixanol, mean ± standard deviation [SD]): newborn to < 2 months 0.185 ± 0.060, 2 to < 6 months 0.256 ± 0.046, 6 months to < 1 year 0.299 ± 0.042, 1 to < 3 years 0.322 ± 0.058, and 3 to ≤ 12 years 0.307 ± 0.071; with increasing age, excretion is more rapid, as shown by each age group's elimination half-life ($T_{1/2}$ beta; 4.14 ± 1.41 hours, 2.79 ± 0.55 hours, 2.36 ± 0.37 hours, 2.23 ± 0.51 hours, and 2.36 ± 0.52 hours, respectively). Elimination of iodixanol in children > 6 months of age is comparable to normal adults. Prolonged elimination in children < 6 months of age is related to renal immaturity.

Iodixanol pharmacokinetics in children. Johnson WH Jr, Lloyd TR, Victorica BE, Zales VR, Epstein ML, Leff RD, Ardinger RH Jr, Slovis TL, Johnson JA, Marsters PA. Pediatr Cardiol 2001 May–Jun; 22 (3), pp. 223–7.

CARDIOVASCULAR SYSTEM

Angiogenesis: Neoplastic Disease

Background Information: Tumor angiogenesis has significant implications in the diagnosis and treatment of various solid tumors. With the advent of fast, multislice computed tomography (CT) scanners, CT imaging techniques capable of qualitative and quantitative analysis of tumor angiogenesis have been the subject of extensive investigation for several decades. The fundamental basis for CT imaging of angiogenesis includes transport by blood flow of intravenously administered iodinated contrast material to tissue and subsequent exchange by diffusion of contrast molecules between the intravascular space and the extravascular interstitial space. With current fast CT scanners both tissue and vascular enhancement can be measured and traced over time at small time intervals to allow detailed modeling of the distribution of contrast agent in tissue. Both compartmental and distributed parameter models for contrast transport and exchange have been developed to quantify from the CT data the following angiogenesis related parameters: tissue blood flow, blood volume, mean transit time, contrast arrival time, capillary permeability surface area product, and hepatic arterial fraction in the case of the liver.

CT imaging of angiogenesis. Lee TY, Purdie TG, Stewart E. Q J Nucl Med 2003 Sep; 47 (3), pp. 171–87.

Angiography
Gadopentetate Dimeglumine ([Gd]-DTPA)

Application: In subsets of cases with contraindications to iodinated contrast medium and for whom magnetic resonance angiography is inappropriate, [GD]-DTPA can function as an alternative contrast medium for computed tomography angiography (CTA).
Dosage: Gadopentetate dimeglumine, 0.5 mol/l (Berlex Laboratories, Wayne, NJ),
Implementation: Characterization of the cervical vessels for presurgical assessment of neck vascular anatomy (e.g., n = 3 cases) after 1st establishing proof of principle.
Animal Model: Canine (beagle 12.2 kg BW).
Human Model: Cases subjected to 3D CT scanning angiography (n = 3).
Interpretation and Analysis: Sufficient enhancement for CTA of the thoracic aorta, cervical vessels, and abdominal vessels can readily be achieved. Cervical vessels can be clearly defined in the vast majority of human cases (e.g., n = 3/3).
Instrumentation: Computed tomography: LightSpeed QX/i CT (General Electric Medical Systems, Milwaukee, WI), and an Ultra Sparc II (SUN Microsystems, Santa Clara, CA) running Advantage Windows 3.1 (General Electric Medical Systems).

Gadopentetate dimeglumine is potentially an alternative contrast agent for 3-dimensional computed tomography angiography with multidetector-row helical scanning. Gupta AK, Alberico RA, Litwin A, Kanter P, Grossman ZD. J Comput Assist Tomogr 2002 Nov–Dec; 26 (6), pp. 869–74.

Angiography/Abdominal Vasculature
Gadobutrol/Iopromide/Continuous Spiral Computed Tomography (CT)

Application: Angiography of abdominal vascular structures.
Route of Administration: Gadobutrol (intravenous); iopromide (intravenous).
Dosage: Gadobutrol (2 mmol/kg BW; 1 mol/L gadobutrol); iopromide (2 mmol/kg BW; 300 mg I/mL).
Implementation: Continuous spiral CT imaging of the chest and abdomen can be performed using either gadobutrol or iopromide (slice 5 mm, table feed 7.5 mm, reconstruction increment 5 mm). Comparisons can be made 1 week later, utilizing the same protocol and employing other contrast agents of interest. Serial CTs are performed at the same level using gadobutrol or iopromide on day 1 and the alternate agent on day 8 in order to determine contrast media kinetics, peak enhancement, and time-enhancement product in important vascular regions and parenchymal organs (abdominal aorta, inferior vena cava, liver, and renal parenchyma). Peak enhancement (net increase compared with non-enhanced baseline values) is measured in Hounsfield units (HU) in defined anatomical regions of interest.
Experimental Animal Model: Domestic pigs (n = 8; 35 ± 4 kg body weight).
Interpretation and Analysis: The mean *in vivo* peak enhancement at 5, 15, 30, and 120 seconds in the abdominal aorta after injection of 2 mL/kg BW gadobutrol and iopromide is 200 ± 11, 224 ± 10, 261 ± 13, and 95 ± 9 HU vs 232 ± 10, 298 ± 10, 152 ± 11, and 123 ± 10 HU, respectively. Differences in enhancement of vascular structures are statistically significant ($p < 0.05$) in carotid arteries (235 ± 20 HU for gadobutrol and 264 ± 19 HU for iopromide) and the aortic arch (261 ± 14 HU for gadobutrol and 279 HU ± 13 HU

for iopromide). No statistical significance can be appreciated in any other measured vascular structures and parenchymal organs. Contrast-enhanced CT with 1 mol/L gadobutrol at a dose of 2 mmol/kg body weight provides excellent enhancement of vascular structures and parenchymal tissues in most vascular regions and parenchymal organs similar to an equivalent volume of 300 mg/mL iodinated contrast media.

1-molar gadobutrol as a contrast agent for computed tomography: Results from a comparative porcine study. Kalinowski M, Kress O, Wels T, Alfke H, Klose KJ, Wagner HJ. Invest Radiol 2003 Apr; 38 (4), pp. 193–9.

Angiography: Arteriography/Abdominal Aorta and Renal Arteries/Digital Subtraction Arteriography (DSA)
Gadodiamide (Full-Strength)/Iodinated Contrast Imaging Agents (Half-Strength)

Cellular/Molecular Mechanism of Action: Iodinated compounds and gadodiamide possess radiopaque properties.
Application: Angiography procedures.
Route of Administration: Intra-arterial.
Dosage: Equal volumes (32 mL) of either half-strength (300 mg/mL iodine) iodinated contrast material or full-strength gadodiamide (0.11–0.25 mmol/kg).
Implementation: Conduction of DSA of the abdominal aorta and renal arteries utilizing gadodiamide for comparison with half-strength iodinated contrast media (equal volumes). The abdominal aorta, left and right renal main renal artery, and 1st and 2nd order segmental branches are graded separately as either diagnostic or non-diagnostic (e.g., blinded study, 4 angiographers).
Interpretation and Analysis: Images of the abdominal aorta are (i) diagnostic 100% of the time when using iodine and gadodiamide; (ii) 76% and 80% diagnostic for iodine and gadodiamide in the left main renal artery, respectively; and (iii) 100% and 80% diagnostic for iodine and gadodiamide in the right main renal artery, respectively. First-order segmental branches of the right and left renal arteries are graded diagnostic (i) 72% and 56% of the time, respectively, for dilute iodinated contrast material, and (ii) 40% and 28% of the time, respectively, for gadodiamide. Second-order segmental branches of the right and left kidney are graded diagnostic (i) 24% of the time for iodinated contrast, and (ii) 8% and 4% of the time, respectively, for gadodiamide. Full-strength, intra-arterial gadodiamide at doses smaller than 0.3 mmol/kg can produce diagnostic images of the abdominal aorta and main renal arteries. However, images of the intrarenal branches are usually not diagnostic.

Feasibility of gadodiamide compared with dilute iodinated contrast material for imaging of the abdominal aorta and renal arteries. Spinosa DJ, Angle JF, Hagspiel KD, Bissonette E, Conaway MR, Hartwell GD, Matsumoto AH. J Vasc Interv Radiol 2000 Jun; 11 (6), pp. 733–7.

Angiography: Arteriography/Selective Intra-Arterial Administration/X-Ray Digital Subtraction Angiography (DSA)
Gadobutrol/Gadolinium Diethylenetriamine Pentaacetic Acid ([Gd]-DTPA)

Cellular/Molecular Mechanism of Action: Gadolinium preparations and iodinated compounds possess radiopaque properties.
Application: Selective arterial angiography using x-ray DSA as an imaging modality.
Route of Administration: Gadolinium-DTPA (intra-arterial), gadobutrol (intra-arterial), iopromide (intra-arterial).
Dosage: Gadolinium-DTPA (0.5 mol/L), gadobutrol (1 mol/L), iopromide (300 mg I/mL).
Implementation: Flush arteriograms (ascending, descending, abdominal aorta, iliac, and femoral arteries) and selective angiograms (carotid, renal, and visceral arteries) are obtained utilizing bilateral femoral arterial access (5 F sheaths). Digital subtracted angiograms are obtained during injection of undiluted gadobutrol, iopromide, and gadopentetate with the injection parameters of volume and velocity maintained in a similar manner for each of the 3 contrast agents. In paired arteries, 2 different contrast media are used during the same angiographic run. Diagnostic quality and accuracy of the angiograms are evaluated on a 3-step scale.
Animal Model: Porcine (n = 10, 70 kg total BW).
Interpretation and Analysis: Sufficient non-selective angiographic images can be obtained in 90% of cases using iodinated contrast material (swine). Gadobutrol can achieve sufficient non-selective angiograms in 64% of acquired images Alternatively, selective angiograms are of sufficient diagnostic quality in 98% of the procedures using iodinated contrast material compared to 90% for 1 mol/L gadobutrol and 48% using 0.5 mol/L [Gd]-DTPA. Adverse reactions for any of the reagents is rarely appreciated. One mol/L gadobutrol solution allows x-ray DSA with a diagnostic accuracy equivalent to 300 mg/mL iodinated contrast media, if a selective injection technique is performed. Flush aortograms produce images of inferior quality compared to iodinated contrast material.

X-ray digital subtraction angiography with 1 mol/L gadobutrol: Results from a comparative porcine study With iodinated contrast agents. Kalinowski M, Kress O, Wels T, Alfke H, Klose KJ, Wagner HJ. Invest Radiol 2002 May; 37 (5), pp. 254–62.

Angiography: Arteriography/Catheter-Based Digital Subtraction Arteriography (DSA)
Gadolinium (Gadodiamide)

Cellular/Molecular Mechanism of Action: Gadolinium possesses radiopaque properties.
Application: Catheter-based DSA image-enhancing contrast agent.
Dosage: Gadolinium (gadodiamide, 287 mg/mL formulation).
Implementation: Catheter-based DSA studies performed in cases suffering from azotemia using gadolinium (gadodiamide, 287 mg/mL) as a contrast agent to evaluate renal arterial circulation characteristics (e.g., n = 25 images; n = 21 cases). Quantities of gadolinium administered range between 40 and 264 mL (mean = 124 ± 74 mL). Serial determinations of renal function are performed in cases as a comparative monitoring parameter. Example case population: Arteriography performed in cases prior renal revascularizations (e.g., n = 20); routine postoperative follow-up study (e.g., n = 7); increasing azotemia (e.g., n = 9); worsening hypertension (e.g., n = 3); evaluation of a known renal artery stenosis and abdominal aortic aneurysm (e.g., n = 1); evaluation of suspected renovascular hypertension (e.g., n = 3); and known aortic aneurysms in whom renal artery stenosis is suspected.
Human Model: Cases suffering from azotemia (n = 21 cases for n = 25 images).
Interpretation and Analysis: Adverse changes in renal function are infrequently appreciated following gadolinium arteriography. Prearteriography serum creatinine values can range between 1.6 to 9.1 mg/dL (3.0 ± 1.4 mg/dL), compared with postangiography values varying between 1.2 and 8.4 mg/dL (2.9 ± 1.3 mg/dL). Comparable blood urea nitrogen values range from 23 to 71 mg/dL (40.1 ± 13.5 mg/dL) before arteriography and from 21 to 68 mg/dL

(36.5 ± 13.3 mg/dL) after arteriography. Renal arteries can be successfully imaged in a high percentage of cases (e.g., n = 38/38). First-order and 2nd-order branches are well visualized on selective renal studies. Image evaluations reveal alterations, including renal artery stenosis, renal artery occlusion, renal artery thrombosis, renal artery dissecting pathology, and aortorenal bypass graft. Catheter-based arteriography in patients with azotemia with gadolinium as a contrast agent is a safe and effective means to evaluate the renal arterial circulation. Gadolinium should be considered as 1 of the preferred contrast agents for catheter-based arteriography procedures because it is a relatively safe alternative to nephrotoxic iodinated contrast agents in cases suffering from azotemia.

Gadolinium as a non-nephrotoxic contrast agent for catheter-based arteriographic evaluation of renal arteries in patients with azotemia. Ailawadi G, Stanley JC, Williams DM, Dimick JB, Henke PK, Upchurch GR Jr. J Vasc Surg 2003 Feb; 37 (2), pp. 346–52.

Angiography/Attenuation of Positive Contrast Imaging Enhancing Agents
Gadolinium (Gadobutrol)/Iopromide

Cellular/Molecular Mechanism of Action: Gadolinium and iodinated compounds possess radiopaque properties.
Application: Gadobutrol is a neutral lanthanide macrocyclic chelate containing gadolinium that is applied as a positive contrast agent for diagnostic imaging modalities, including computed tomography (CT). Iopromide is an iodinated compound that is utilized as a positive contrast agent.
Dosage: Gadolinium/gadobutrol (0.7, 1.0, and 1.5 mmol [Gd]/kg; rabbits). Iodine/iopromide (1.0 and 2.4 mmol I/kg; rabbits).
Implementation: Measurement of the degree of attenuation in Hounsfield units (HU) of increasing concentrations of aqueous gadolinium ([Gd] from gadobutrol) and iodine (I from iopromide) formulations by CT at 80 kV, 120 kV, and 137 kV. Peak enhancement (net increase in CT attenuation compared with baseline) and the time-enhancement product in the aorta and in the renal parenchyma of the outer and inner cortex are measured over a 5-minute period following the administration of a single intravenous injection of gadolinium or iopromide
Animal Model: Rabbit.
Interpretation and Analysis: The *in vitro* CT attenuation of gadolinium is approximately 40% higher than that of iodine at equivalent mass concentrations (120 kV). The mean peak enhancements in the aorta after injections of 0.7, 1.0, and 1.5 mmol [Gd]/kg and 1.0 and 2.4 mmol I/kg is 216, 313, 591, 224, and 498 Hounsfield units, respectively. In addition, a 30-second injection of high-dose gadobutrol creates an attenuation profile that is suitable for 3-dimensional reconstructions of the aorta and the renal vasculature. Due to the higher CT attenuation of gadolinium compared with that of iodine, gadobutrol is a more effective contrast agent than iopromide for CT at lower doses of the imaging atom.

Evaluation of gadobutrol in a rabbit model as a new lanthanide contrast agent for computed tomography. Schmitz SA, Wagner S, Schuhmann-Giampieri G, Wolf KJ. Invest Radiol 1995 Nov; 30 (11), pp. 644–9.

Angiography: Blood-Pool Imaging
Iopromide-Containing Liposomes/[Iopromide-Containing Soy Phosphatidyl Glycerol Liposomes]

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties. Liposomes protect agents from degradation or dilution and can under certain scenarios facilitate selective delivery.
Application: Soy phosphatidyl glycerol liposomes containing iopromide can be utilized as blood-pool imaging agents for computed tomography (CT) (non-human primates).
Route of Administration: Iopromide-containing soy phosphatidyl glycerol liposomes (intravenous).
Dosage: Iopromide-containing soy phosphatidyl glycerol liposomes (300 mg total iodine per kilogram).
Semi-Synthetic Methods: Incorporation of iopromide into soy phosphatidyl glycerol liposomes with a mean diameter of 195 nm (46% encapsulation).
Implementation: Evaluation of the diagnostic efficacy of intravenously injected iopromide contained within soy phosphatidyl glycerol liposomes.
Animal Model: Primate. Anesthetized adult baboons (15–21 kg).
Interpretation and Analysis: Injections of iopromide-containing soy phosphatidyl glycerol liposomes are well tolerated without any measurable changes in electrocardiographic parameters. Recoveries from anesthesia is uneventful. Sequential helical CT scans from the base of the skull to the symphysis pubis acquired up to 40 minutes after injection show persistent blood-pool enhancement. Maximum mean enhancement of major vascular structures is 106 Hounsfield units (HU) at 1 minute after contrast medium injection. Mean blood-pool enhancement is 76, 72, and 67 HU at 10, 20, and 40 minutes after injection, respectively. Liver and spleen can be enhanced by 40 and 41 HU, respectively, 40 minutes after injection. No significant enhancement can be measured in the brain and pancreas. Soy phosphatidyl glycerol liposomes containing iopromide therefore appear to have potential utility as blood-pool agents due to their ability to produce prolonged enhancement of CT generated images of vascular structures (non-human primates).

CT blood-pool enhancement in primates with lopromide-carrying liposomes containing soy phosphatidyl glycerol. Schmiedl UP, Krause W, Leike J, Sachse A. Acad Radiol 1999 Mar; 6 (3), pp. 164–9.

Angiography/Blood-Pool Imaging/Aorta/Liver/Renal Cortex
Liposomal Iohexol

Cellular/Molecular Mechanism of Action: Iohexol is an iodine-containing compound that possesses radiopaque properties. Liposomes protect encapsulated compounds from excessively rapid elimination (renal excretion), metabolic degradation, and sometimes faciltate selective delivery.
Background Information: Although soluble non-ionic iodine compounds with low systemic toxic effects have been developed for use in computed tomography (CT), they have short residence times of only a few minutes or mere seconds, and therefore last for an insufficient time period for blood-pool imaging, even with high-speed multidetector row spiral CT. Moreover, potential renal toxic effects preclude repeated administration of these contrast agents during imaging, as well as their use in patients with compromised renal function.
Application: Potential blood-pool agent that can be applied for cardiovascular imaging purposes.
Route of Administration: Liposomal iohexol (intravascular).
Dosage: Liposomal iohexol (475 mg of iodine per kilogram of body weight).
Implementation: Development and evaluation of a CT contrast agent for blood-pool imaging that remains in the blood for more than 3 hours and that is relatively non-toxic to the kidneys. One strategy for achieving this objective can entail liposomal encapsulation of iohexol formulations. Relevant properties and characteristics that need to be assessed for such preparations include (i) encapsulation efficiency in terms of milligrams of iodine per milliliter of lipid for-

mulation; (ii) stability *in vitro* in phosphate buffer solution and in human plasma; and (iii) *in vivo* stability, residence time, and enhancement of contrast on images of various organ systems.

Animal Model: Rabbit (*in vivo* investigations).

Interpretation and Analysis: Formulations that contained 34.8 mg of iodine per milliliter of liposomal iohexol solution, remain stable in blood plasma in both *in vitro* and *in vivo* environments, after intravascular injection (e.g., rabbit). An intravenous dose of 475 mg I/kg produces contrast enhancement of approximately 130 Hounsfield units (HU) in the aorta and liver cortex and approximately 100 HU in the renal cortex. Contrast enhancement is maintained for 3 hours after injection, and minimal clearance of the contrast agent via the kidneys is observed. Liposomal iohexol formulation may therefore have sufficient residence time for blood-pool imaging in a rabbit model. Future experiments with long-residence-time iohexol formulations may lead eventually to applications in cardiac imaging and in early tumor detection.

Long-residence-time nano-scale liposomal iohexol for x-ray-based blood-pool imaging. Kao CY, Hoffman EA, Beck KC, Bellamkonda RV, Annapragada AV. Acad Radiol 2003 May; 10 (5), pp. 475–83.

Angiography/Central Nervous System (CNS) Blood-Brain Barrier (BBB)/Monitoring BBB Disruption/ Monitoring CNS Chemotherapeutic Delivery
Meglumine Iothalamate/Metrizamide/Methotrexate

Background Information: Enhanced computed tomography (CT) is a non-invasive means of monitoring BBB disruption.

Application: Meglumine iothalamate and metrizamide can be applied as contrast-enhancing agents for the detection and characterization of vascular anomalies. Related examples include their utilization to monitor the degree, distribution, extent, and reversibility of osmotic BBB disruption.

Route of Administration: Meglumine iothalamate (intravascular), metrizamide (intravascular).

Dosage: Meglumine iothalamate.

Implementation: Intravenous and intracarotid injection of meglumine iothalamate and metrizamide for CT imaging of cranial vasculature and assessment of the integrity of the BBB (canine animal model).

Interpretation and Analysis: The timing of iodinated contrast agent administration is crucial to achieving optimal enhancement by CT of disruptions in the integrity of the BBB. Intravenous administration of meglumine iothalamate produces excellent levels of enhancement on CT scan images, while intracarotid infusions are less satisfactory. Under similar conditions, enhancement provided by metrizamide is less marked and more transient than that observed with meglumine iothalamate. Systemically administered methotrexate after osmotic BBB disruption results in increased brain levels of the agent in areas that closely correlated with CT scan enhancement. Based on these observations, CT can be utilized as an effective non-invasive approach to monitoring both BBB disruption and the delivery of chemotherapeutic agents to the brain.

Use of enhanced computerized tomography to evaluate osmotic blood-brain barrier disruption. Neuwelt EA, Maravilla KR, Frenkel EP, Barnett P, Hill S, Moore RJ. Neurosurgery 1980 Jan; 6 (1), pp. 49–56.

Angiography/Central Nervous System (CNS)/Mean Cerebral Blood Flow Volume/Regional Brain Perfusion
Iodinated Contrast Agents

Application: Synchrotron radiation computed tomography (CT) allows the *in vivo* measurement of absolute contrast-agent concentrations with high accuracy and precision, and absolute cerebral blood volume or flow can be derived from these measurements using tracer kinetic methods.

Implementation: Acquisition of CT images following intravenous bolus injection of an iodinated contrast agent in order to follow the temporal evolution of the contrast material within the intravascular compartment (blood circulation). The 1st images are acquired before iodine infusion and subtracted to obtain CT slices expressed in absolute iodine concentrations. Cerebral blood volume and cerebral blood flow maps are obtained after correction for partial volume effects.

Animal Model: Rat (e.g., $n = 7$ healthy animals).

Interpretation and Analysis: Mean cerebral blood volume and flow values are approximately 2.1 ± 0.38 mL/100 g and 129 ± 18 mL/(100 g/min) in the parietal cortex; and 1.92 ± 0.32 mL/100 g and 125 ± 17 mL/(100 g/min) in the caudate putamen, respectively. Synchrotron radiation CT has the potential to assess these 2 brain-perfusion parameters.

Absolute cerebral blood volume and blood flow measurements based on synchrotron radiation quantitative computed tomography. Adam JF, Elleaume H, Le Duc G, Corde S, Charvet AM, Tropres I, Le Bas JF, Esteve F. J Cereb Blood Flow Metab 2003 Apr; 23 (4), pp. 499–512.

Angiography/Central Nervous System (CNS) Neurovasculature/Anatomical Model of the Cerebrovascular System / Cranial Model for Instructional Imaging/Cranial Model of Anatomical Dissection
Tomographic Contrast Imaging Agent/Silicone

Application: Production of a physical anatomical model suitable for imaging and manual dissection of vascular structures of the cranium for instructional purposes.

Implementation: Volumetric ratios of computed tomographic contrast agent and a silicone preparation are formulated in a manner that creates a final mixture that is optimally suited as an imaging compound. The resultant mixture is injected into the cerebrovascular systems of 6 fresh human cadaver heads for the purpose of acquiring digital virtual exposures performed in parallel with laboratory dissection. The specimens are analyzed by computed tomography (CT) with static and dynamic 3-dimensional images of the cadaver vascular tree obtained as viewed through surgical corridors of various skull base approaches.

Interpretation and Analysis: A contrast agent to silicone rubber volumetric ratio of 1:8 creates a preparation that is most appropriate for both CT and subsequent laboratory dissection of specimens. The blood vessels in computed tomographic scans demonstrate a higher attenuation than surrounding soft tissues. The opacity consistency of injected vessels is a critical parameter for a clear 3-dimensional rendering of the vascular structures in the natural surroundings of the skull base. Preparations of cadaver skulls in the manner described are suitable models for both imaging and manual dissection. The model allows for static and dynamic 3-dimensional examination of the surgical anatomy from a neurosurgeon's perspective. Preparation of biological specimens in this manner can therefore facilitate the study of cerebrovascular system morphology/pathology in relation to the skull base as a tool for designing surgical approaches and methodologies.

Imaging the cerebrovascular tree in the cadaveric head for planning surgical strategy. Zhao JC, Chen C, Rosenblatt SS, Meyer JR, Edelman RR, Batjer HH, Ciric IS. Neurosurgery 2002 Nov; 51 (5), pp. 1222–7; discussion 1227–8.

Angiography/Contrast Agent Artifact/Computed Tomography (CT) Imaging/Positron Emission Tomography (PET)
Iodinated Contrast Imaging Agents

Application: Enhancement of PET/CT imaging.
Route of Administration: Intravenous (most frequently).
Implementation: Intravenous and oral iodinated contrast agent administered to oncology cases (e.g., n = 30) combined with whole-body PET/CT. Computed tomography images acquired in the presence of intravenous and oral iodinated contrast agent are used for PET attenuation correction. Positron emission tomography images are assessed for the presence of artifact, defined as a region of high count rate on attenuation-corrected images in accurate co-registration with a contrast-enhanced blood vessel. Intravascular enhancement of thoracic veins is quantified by application of regions of interest, and quantities in cases with artifact (Group 1) and without artifact (Group 2) correlated. Body surface area is calculated for all patients.
Interpretation and Analysis: Contrast-induced PET artifact can be appreciated in approximately 13% of cases (e.g., n = 4/30 patients). Mean density differences in intravascular enhancement are highly significant ($p < 0.001$) compared to case groups with artifact (2,262 ± 304 Hounsfield units [HU]) and without artifact (1,058 ± 209 HU). Body surface area is significantly lower ($p = 0.035$) in cases with artifact (1.67 ± 0.11 m^2) than in cases without artifact (2.01 ± 0.18 m^2). Contrast-enhanced dual-modality PET/CT examinations may result in a PET artifact that is due to the transient bolus passage of undiluted intravenous contrast agent.

Focal tracer uptake: A potential artifact in contrast-enhanced dual-modality PET/CT scans. Antoch G, Freudenberg LS, Egelhof T, Stattaus J, Jentzen W, Debatin JF, Bockisch A. J Nucl Med 2002 Oct; 43 (10), pp. 1339–42.

Angiography: Hepatic and Abdominal Vasculature
Iohexol/Non-Ionic Computed Tomography (CT) Contrast Agent

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Iohexol can be applied as a non-ionic contrast agent in spiral CT to enhance image analysis of hepatic and abdominal vascular structures.
Route of Administration: Iohexol (intravenous injection).
Dosage: Iohexol formulated at 240 mg I/ml and 350 mg I/ml injected at a rate of 2 ml/sec (New Zealand rabbits).
Implementation: Evaluation of the relative effectiveness of iohexol contrast agent (iodinated/non-ionic) formulated at 240 mg I/ml and 350 mg I/ml as contrast agents for enhancing the imaging of hepatic and abdominal vascular structures applying spiral CT. Each animal is evaluated using both concentrations of iohexol using 4 different contrast volumes: 2.3, 1.7, 1.1, and 0.57 ml/kg. Enhancement values of the aorta, hepatic veins, inferior vena cava, and hepatic parenchyma are measured using a region-of-interest cursor.
Animal Model: Rabbit (e.g., n = 10 New Zealand male rabbits).
Interpretation and Analysis: At all contrast doses, equal volumes of contrast iohexol-350 results in statistically higher hepatic ($p \leq 0.003$, paired Student's t test) as well as vascular ($p < 0.04$) enhancement compared with iohexol-240. The slopes and intercepts of the enhancement curves for iohexol-350 and iohexol-240 are not statistically different ($p > 0.05$) when enhancement is plotted as a function of total grams of iodine administered. In the application of spiral CT scanning, appropriate contrast doses and relative costs per dose of iohexol should be considered on the basis of total iodine load administered rather than total volume administered.

Effect of contrast concentration on abdominal enhancement in the rabbit: Spiral computed tomography evaluation. Bluemke DA, Fishman EK, Anderson JH. Acad Radiol 1995 Mar; 2 (3), pp. 226–31.

Angiography/Neoplasia/Perfusion Characteristics of Hepatic Metastatic Lesions
EOE-13/[^{99m}Technetium] ([^{99m}Tc]) Macroaggregated Albumin or [^{99m}Tc]-MAA

Cellular/Molecular Mechanism of Action: Biological preparation that is passively retained within the intravascular compartment for a prolonged period of time following intravenous injection due to its relatively large molecular weight.
Application: Assessment of vascular perfusion of organs and tissue structures in a manner that can detect hepatic metastatic lesions.
Route of Administration: Intravenous (catheter infusion at a rate of 0.66 ml/min.)
Implementation: Evaluation of hepatic perfusion patterns applying both nuclear scintigraphy and computed tomography imaging methodologies.
Interpretation and Analysis: Computed tomography with EOE-13 yields more precise anatomic detail and is unique in demonstrating the perfusion status of each hepatic metastasis. Further evaluation of EOE-13 for this purpose is warranted.
Instrumentation: Computer-integrated nuclear medicine scintigraphy camera imaging (CT with EOE-13 used as a validating procedure).

Assessment of perfusion patterns during hepatic artery infusion chemotherapy: EOE-13 CT and [^{99m}Tc]-MAA scintigraphy. Miller DL, Schneider PD, Gianola FJ, Willis M, Vermess M, Doppman JL. AJR Am J Roentgenol 1984 Oct; 143 (4), pp. 827–31.

Angiography/Pulmonary Artery Embolism/ Warning: Potential Risk of Cardiac Arrest
Carbon Dioxide (CO_2)/Multidetector Row Spiral Computed Tomography (CT) Image Analysis

Cellular/Molecular Mechanism of Action: Carbon dioxide possesses radioleucent properties.
Application: Carbon dioxide can be applied as a negative contrast agent in CT imaging procedures.
Route of Administration: Carbon dioxide (intravascular).
Dosage: Carbon dioxide (120 or 180 mL).
Implementation: Serial CT scanning is performed in the supine and prone positions utilizing different scan delays (10, 15, and 20 seconds) and different volumes of CO_2 (60, 120, 180, and 240 mL) that are hand-infused over a duration of 10 to 15 seconds (e.g., n = 4 swine). In pulmonary embolism models (e.g., n = 5 swine), scanning is performed with iodinated contrast medium and then rescanned utilizing CO_2 (120 or 180 mL) as a negative contrast agent. The supine and prone CT scans and the number and location of thrombi depicted in the CO_2- and contrast material-enhanced CT scans are then compared.
Animal Model: Creation of pulmonary emboli in swine (e.g., n = 7) followed by delineation of their anatomical characteristic using multidetector row spiral CT image analysis.
Interpretation and Analysis: Because the position of the pulmonary artery in swine, the prone position is more effective than the supine position with CO_2 enhancement. An infusion of 120 mL of CO_2 is sufficient to enhance the entire pulmonary artery, and scan-

ning can be timed to coincide with the completion of infusion, thereby creating the most effective results. Both the CO_2- and contrast-enhanced CT scans can aid in the detection of the vast majority of pulmonary thrombi (e.g., n = 7/7). Thrombi are more apparent using CO_2-enhanced CT scans compared to contrast-enhanced scans because of the high contrast interface between soft tissue and gas. However, a number of cases may not be able to be resuscitated after the occurrence of abrupt cardiac arrest following CO_2-enhanced scanning procedures (e.g., n = 2/7 swine). The CT depiction of pulmonary emboli is feasible with CO_2 gas as a negative contrast agent and may even be superior to that with iodinated contrast media. Further studies are required to evaluate the safety of this method and to develop an improved delivery of CO_2 gas for this application.

CO_2-enhanced CT imaging for the detection of pulmonary emboli: Feasibility study in a porcine model. Bae KT, Hong C, Becker CR, Prasad S, Nolte MA, Eisenbeis PE, Heiken JP. Acad Radiol 2003 Mar; 10 (3), pp. 313–20.

Angiography/Pulmonary Vasculature/ Pharmacokinetic Profile
Iodinated Macromolecule Contrast Agent Prototype (P743)/Iobitridol

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Imaging of vascular structures utilizing spiral computed tomography (CT) pulmonary angiography in rabbits.
Route of Administration: Iobitridol (intravascular) and P743 (intravascular).
Dosage: Iobitridol (iodine dosage = 250 mg I/kg), P743 (iodine dosage = 150 mg I/kg).
Implementation: Evaluation of the relative pharmacokinetic profiles for the iodinated macromolecular contrast medium P743 compared to the standard contrast agent iobitridol pertaining to their relative capacity to enhance imaging of vascular structures in both 1st-pass and delayed CT scans (manual and power injected delivery). Protocols can be applied that facilitate comparison of vessel enhancement in both 1st-pass and delayed scans for both P743 and iobitridol contrast agents with the help of a power injector.
Animal Model: Rabbit.
Interpretation and Analysis: In instances of manual-fast injection, the 1st pass of iobitridol can be observed only on proximal scans. Conversely, opacification of vessels is maintained during 3 spiral scans with P743 under the same injection conditions. Utilization of optimal bolus timing provides for higher vessel enhancement that can be appreciated during bolus 1st pass with iobitridol compared with P743 prototype. However, during the post-bolus phase, the decrease in attenuation values is markedly faster with iobitridol than with P743. Therefore, P743 remains within the intravascular compartment to a greater extent than does iobitridol, which may have clinical diagnostic implications (e.g., pulmonary embolism).

CT pulmonary angiography with a macromolecular contrast medium: A comparative study vs iobitridol in rabbits. Wiart M, Corot C, Berthezene Y, Violas X, Canet E. Invest Radiol 2001 Sep; 36 (9), pp. 547–53.

Angiography: Vena Cava (Inferior): Cavography/ Diagnostic Accuracy and Sensitivity
Iodinated Contrast Media/Gadolinium/Carbon Dioxide (CO_2)

Cellular/Molecular Mechanism of Action: Carbon dioxide has radioleucent properties, while gadolinum and iodinated compounds possess radiopaque properties.
Application: Carbon dioxide, gadolinium, and iodinated contrast medias can be applied as imaging enhancing contrast medias for the identification and characterization of vascular anomalies.
Route of Administration: Carbon dioxide, gadolinium, and iodinated contrast medias are utilized as intravascular contrast agents.
Implementation: Injection of CO_2, gadolinium, and iodinated contrast media into catheterized vascular structures for achieving enhanced imaging analysis of anatomical abnormalities within the inferior vena cava. Caval diameter is determined utilizing calibrating software applied in combination with visual evaluations based on measurements of the distance from the superior image border to the inferior margin of the renal veins and from the inferior image border to the iliac bifurcation. Measurements of anatomical structures using CO_2 and gadolinium can then be compared to those obtained with iodinated contrast materials in addition to establishing their relative sensitivity in detecting the presence or absence of caval thrombus formation and vascular anomalies.
Experimental Model: Cases suspected of suffering from various vascular anomalies (e.g., n = 40 injected with iodinated contrast material, CO_2, and gadolinium).
Interpretation and Analysis: Caval diameter measurements differ by 0.4 mm or less for all 3 agents. Measurements with all agents are within 2 mm of each other for all individual cases. Measurements acquired utilizing gadolinium and CO_2 are not significantly different from one another pertaining to estimates of caval diameter. At initial reading, compared with iodinated contrast material, gadolinium has greater mean inter-observer error in measuring the distance to the iliac bifurcation and both renal veins (range, 1.6–1.8 mm) than CO_2 (range, 0.2–1.4 mm). This finding, although statistically significant for gadolinium ($p < 0.05$), is doubtful to be of any clinical relevance. Inter-observer correlation is significantly worse for CO_2 at the levels of the iliac bifurcation ($p = 0.02$) and right renal vein ($p = 0.008$). Inter-observer correlation for gadolinium is similar to that for iodinated contrast material at all levels. At repeat reading, there are significantly inferior intra-observer correlations with use of CO_2 for both renal veins ($p < 0.05$) compared to iodinated contrast material and for the left renal vein ($p < 0.05$) compared to gadolinium. Renal vein anomalies can often be easier to detect with gadolinium and iodinated contrast media (e.g., n = 3/3) compared to negative CO_2 contrast enhancement (e.g., n = 1/3). Therefore, CO_2 and gadolinium appear to have limitations when compared with iodinated contrast material. Gadolinium provides superior consistency in identifying relevant landmarks for filter placement. Alternatively, CO_2 demonstrates significantly greater mean correlative error than gadolinium for initial and repeat readings.

Gadolinium, carbon dioxide, and iodinated contrast material for planning inferior vena cava filter placement: A prospective trial. Brown DB, Pappas JA, Vedantham S, Pilgram TK, Olsen RV, Duncan JR. J Vasc Interv Radiol 2003 Aug; 14 (8), pp. 1017–22.

Cardiovascular: Ventricular (Left) Heart Failure/ Atypical Hounsfield Attenuation in Cardiac Failure/ Unacceptable Screening Methods for Cardiopulmonary Function
Lopromid

Cellular/Molecular Mechanism of Action: Lopromid is a compound that possesses radiopaque properties.
Background Information: Bolus tracking systems have been developed that can improve the timing of intravenous contrast media application in helical computed tomography (CT).

Application: Contrast-enhancing agent for use in helical CT image analysis.

Dosage: Lopromid (75 ml; 300 mg/ml; 2 ml/sec).

Implementation: Evaluation of bolus tracking systems for improving the timing of intravenous contrast media delivery applied in CT imaging assessement of cardiac function. The displayed increase of vascular density over time after peripheral lopromid contrast media injection is classified according to 3 categories, including (Type A) rapid increase, (Type B) deceleration before a 100 HE threshold was reached, and (Type C) 1 or more peaks. The findings of the invasive investigation of the heart are correlated to findings of the bolus-tracking measurements.

Human Model: Cases subjected to bolus triggered contrast enhanced helical CT and invasive investigation of the heart within 1 week (n = 64).

Interpretation and Analysis: Examinations can have slightly different category distributions: Type A = 19; Type B = 34; Type C = 11 cases. A highly significant correlation exists between the type of the Hounsfield attenuation and systolic pressure in the left ventricle. There is no correlation between the type of the Hounsfield attenuation and the diastolic pressure in the left ventricle, pressures related to the right ventricle or the ejection fraction. The bolus-tacking system shows a sensitivity of 53%, a specificity of 82%, an accuracy of 70%, a positive predictive value of 70% and a negative predictive value of 70% in detection of left heart failure. The bolus tracking system C.A.R.E.-bolus often shows atypical Hounsfield attenuation in cases of cardiac failure but is not suitable as a screening method for cardiopulmonary function.

Instrumentation: CT scanner Somatom Plus 4 Volume Zoom (Siemens Corp., Forchheim, Germany) using the C.A.R.E. Bolus software that performs repetitive low-dose test scans (e.g., for the abdomen: 140 kV, 20 mA, T_1 0.5 sec) and measures the Hounsfield attenuation (increase over the baseline) in a preselected region of interest.

Observing the density increase curve after intravenous contrast medium administration using a bolus triggering system: A method for detection cardiovascular disorders. Stückle CA, Kickuth R, Kirchner EM, Liermann D, Kirchner J. Radiologe 2002 Jun; 42 (6), pp. 480–4.

CENTRAL NERVOUS SYSTEM (CNS)

Adenosine A2A Receptor Complexes (Central Nervous System [CNS])/A2A and A1 Selectivity *Styrylxanthine Derivatives/[7-Methyl-[^{11}C]-(E)-3,7-Dimethyl-8-(3-Iodostyryl)-1-Propargylxanthine or [^{11}C]-IS-DMPX/[7-Methyl-[^{11}C]-(E)-8-(3-Bromostyryl)-3,7-Dimethyl-1-Propargylxanthine or [^{11}C]-BS-DMPX/KF15372 A1 Antagonist/KF17837 A2A Antagonist*

Background Information: Previous investigations have evaluated the potential of iodinated and brominated styrylxanthine derivatives labeled with [^{11}C] as *in vivo* probes.

Application: Detection and characterization of the distribution patterns for adenosine A2A receptor complexes within the CNS (striatum) applying positron emission tomography (PET) or single photon emission computed tomography (SPECT) imaging modalities.

Semi-Synthesis: [7-methyl-[^{11}C]-(E)-3,7-dimethyl-8-(3-iodostyryl)-1-propargylxanthine ([^{11}C]-IS-DMPX) and [7-methyl-[^{11}C]-(E)-8-(3-bromostyryl)-3,7-dimethyl-1-propargylxanthine ([^{11}C]-BS-DMPX) can be prepared by the [^{11}C]-methylation of corresponding 7-demethyl derivatives.

Implementation: Evaluation of the diagnostic efficacy of [^{11}C]-BS-DMPX and [^{11}C]-IS-DMPX for delineating the distribution and relative binding avidity for A1 and A2A receptor complexes. Determination of the diagnostic efficacy of these agents can therefore be validated by performing *in vivo* imaging studies and *in vitro* membrane binding analyses.

Animal Model: Murine (CNS).

Interpretation and Analysis: Membrane binding studies performed *in vitro* show a high affinity (K_i values) of [^{11}C]-IS-DMPX and [^{11}C]-BS-DMPX ligands for A2A receptor: 8.9 nM for IS-DMPX and 7.7 nM for BS-DMPX, and a high A2A/A1 selectivity: $>$ 1,100 for IS-DMPX and 300 for BS-DMPX. In mice, [^{11}C]-IS-DMPX and [^{11}C]-BS-DMPX is taken up slightly more in the striatum than in the reference regions such as the cortex and cerebellum. The uptake ratios of striatum-to-cortex and striatum-to-cerebellum gradually increases but are very small: 1.6–1.7 for the striatum-to-cortex ratio and 1.2 for the striatum-to-cerebellum ratio at 60 minutes postinjection. The uptake by these 3 regions is reduced by co-injection of an excess amount of carrier or an A2A antagonist KF17837, but not by an A1 antagonist KF15372. The blocking effects in the 3 regions is greater for [^{11}C]-BS-DMPX (32–57%) than for [^{11}C]-IS-DMPX (6–29%). *Ex vivo* autoradiography analysis confirms that the 2 ligands are slightly concentrated in the striatum. [^{11}C]-BS-DMPX shows more selective affinity for adenosine A2A receptors than does [^{11}C]-IS-DMPX, but the 2 tracers are not suitable as *in vivo* ligands because of low selectivity for the striatal A2A receptors and a high level of non-specific binding.

Evaluation of iodinated and brominated [^{11}C]-styrylxanthine derivatives as *in vivo* radioligands mapping adenosine A2A receptor in the central nervous system. Ishiwata K, Shimada J, Wang WF, Harakawa H, Ishii S, Kiyosawa M, Suzuki F, Senda M. Ann Nucl Med 2000 Aug; 14 (4), pp. 247–53.

Alzheimer's Disease (AD): General Perspective *Amyloid Plaque Ligands/Computed Tomography (CT)/Positron Emission Tomography (PET)*

Alzheimer's disease, a progressive neurodegenerative disorder, is the most common cause of dementia in the elderly. Current consensus statements have emphasized the need for early recognition and the fact that a diagnosis of AD can be made with high accuracy by using clinical, neuropsychologic, and imaging assessments. Magnetic resonance (MR) or CT imaging is recommended for the routine evaluation of AD. Coronal MR images can be useful to document or quantify atrophy of the hippocampus and entorhinal cortex, both of which occur early during the course of the disease process. Both volumetric and subtraction MR techniques can be used to quantify and monitor dementia progression and rates of regional atrophy. MR measures are also increasingly being used to monitor treatment effects in clinical trials of cognitive enhancers and antidementia agents. Positron emission tomography and single photon emission CT (SPECT) offer value in the differential diagnosis of AD from other cortical and subcortical dementias and may also offer prognostic value. In addition, PET studies have demonstrated that subtle abnormalities may be apparent in the prodromal stages of AD and in subjects who carry susceptibility genes. Positron emission tomography ligands are in late-stage development for demonstration of amyloid plaques, and human studies have already begun. Functional MR-based memory challenge tests are in development as well.

Neuroimaging and early diagnosis of Alzheimer's disease: A look to the future. Petrella JR, Coleman RE, Doraiswamy PM. Radiology 2003 Feb; 226 (2), pp. 315–6.

Myelography: Image Quality/Adverse Reactions: Relative Frequency
Iomeprol/Iohexol/Iotrolan/Iopamidol

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Iomeprol is a non-ionic iodinated contrast medium that can be applied in myelogram procedures (conventional radiography and myelo-computed tomography [CT]). Other iodinated compounds that can be applied in a similar fashion include iohexol and iotrolan.
Route of Administration: Iomeprol (intrathecal).
Dosage: Iomeprol (3 g total iodine reference dose with doses as high as 4.5 g of total iodine).
Implementation: Evaluation of pharmacokinetics, tolerability, safety, and efficacy of iomeprol following intrathecal injection (e.g., n = 600 cases).
Interpretation and Analysis: Pharmacokinetics study results reveal that after intrathecal administration iomeprol is completely absorbed from the cerebrospinal fluid compartment (once absorbed into the systemic circulation, it is rapidly excreted, unmetabolized, by glomerular filtration). Dose-finding studies show that the opacification produced by iomeprol depends on the injected dose. Although a dose-dependent increase in efficacy is observed, no differences in neurotolerability and safety are detected between the doses tested. Doses of iomeprol greater than 3 g iodine do not cause a greater incidence of adverse events and produce significantly better contrast efficacy. Comparative clinical trials demonstrate that iomeprol and iopamidol, iohexol, and iotrolan always provided adequate opacification of the subarachnoid space, both in conventional myelography and myelo-CT, with adequate delineation of normal structures and pathologic processes. No serious adverse events occur up to a total dose of 4.5 g of iodine. Almost invariably, no differences between the agents with regard to tolerance, adverse events, and effects on vital signs, physical examination, and neurologic examination can be appreciated. Therefore, iomeprol is safe and effective and can be recommended for myelography and myelo-CT.

Clinical experience with iomeprol in myelography and myelo-CT: Clinical pharmacology and double blind comparisons with iopamidol, iohexol, and iotrolan. Katayama H, Heneine N, van Gessel R, Taroni P, Spinazzi A. Invest Radiol 2001 Jan; 36 (1), pp. 22–2.

GASTROINTESTINAL SYSTEM

Gastrointestinal/Abdominal and Pelvic Organs
Gastrografin (Oral)/Antiperistaltic Agents

Background Information: The oral administration of a dilute positive iodinated contrast agent such as gastrografin (2%) is usually necessary to achieve optimal delineation of abdominal and pelvic organs. The amount of contrast agent administered and the timing of its administration depends on the site of the suspected disease state. The simultaneous intravenous administration of antiperistaltic agents is generally unnecessary when utilizing modern fast computed tomography (CT) scanners. However, contrast agent-induced artifact may at times be troublesome, so it is therefore suggested by some radiologists that lesions in the left lobe of the liver be scanned without positive contrast in the stomach. Negative contrast may also be useful in delineating disease: gas (carbon dioxide) has been shown to be useful in evaluating bladder tumors. Air insufflation has also been utilized to facilitate the evaluation of small rectal tumors. Intravenous administration of contrast agent aids in the characterization of various disease entities and also facilitates the recognition of vascular structures (such as veins and arteries in the peripancreatic region). Both the infusion and bolus modes of contrast material administration have their place in the evaluation of abdominal and pelvic lesions evaluated by CT. Newer techniques such as dynamic CT scanning is a valuable diagnostic modality for both delineating and characterizing disease states.

Positive and negative contrast agents in CT evaluation of the abdomen and pelvis. Hamlin DJ, Burgener FA. J Comput Tomogr 1981 Jun; 5 (2), pp. 82–90.

Intestinal Opacification/Combined Abdominal/Pelvic Helical Computed Tomography (CT)
Oral Intravenous Contrast Media

Application: Enhancement of abdominal/gastrointestinal imaging studies.
Implementation: Dilution of intravenous contrast agents in water to a total volume of 400 ml and oral ingested 60 minutes prior to, in addition to 400 ml of water immediately before helical CT imaging (e.g., n = 19 cases).
Interpretation and Analysis: The distal small bowel is opacified by the positive contrast agent, and the stomach by the negative contrast agent in all patients. Gastric contents are homogenous, and the density is a mean ± standard deviation 2.5 ± 7.3 Hounsfield units (HU). The difference between the densities of the gastric contents and of the gastric wall (mean ± standard deviation 80.8 ± 20.9 HU) is statistically significant ($p < 0.05$). Applying the described protocol (diluted iodine solution 1st followed by water) can be used as a method for the oral administration of contrast agents for combined abdominal and pelvic helical CT with intravenous contrast media.

Positive and negative oral contrast agents for combined abdominal and pelvic helical CT: First iodinated agent and 2nd water. Matsuoka Y, Masumoto T, Koga H, Suzuki K, Ushimi T, Terada H, Tamura A, Yokoyama Y, Abe K, Kamata N. Radiat Med 2000 May–Jun; 18 (3), pp. 213–6.

HEPATIC SYSTEM

Hepatocyte Selective Contrast Agent/Arterial Portography/Carcinoma Lesions
Iodinated Tryglyceride/Iohexol

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties. Iodinated tryglycerides are rather selectively assimilated by hepatocytes.
Application: Iodinated tryglycerides can be injected as a radiodense contrast agent that is subsequently assimilated relatively selectively by hepatocytes in a manner that can provide enhance computed tomography (CT) images of the liver.
Route of Administration: Iodinated tryglycerides can be selectively injected directly into specific (catheterized) vascular structures (e.g., arterial portography).
Dosage (Rabbit): Iohexol-enhanced CT (600 mg iodine per kilogram of body weight [mg I/kg]); CT arterial portography (iohexol [600 mg I/kg]); triglyceride-enhanced computer tomograpy (126 mg I/kg); and dual-contrast-enhanced CT (triglyceride plus iohexol [425 mg I/kg]).
Implementation: Comparison of the relative effectiveness of iodinated tryglyceride (hepatocyte-selective contrast agent) and/or iohexol to function as CT image-enhancing agents during arterial portography procedures. Such an evaluation can be performed

utilizing livers (e.g., rabbit) directly inoculated with VX2 carcinoma. Results are compared for 5 helical CT examinations: (i) unenhanced CT; (ii) iohexol-enhanced CT; (iii) CT arterial portography; (iv) triglyceride-enhanced CT; (v) and dual-contrast-enhanced CT. Attenuation of normal liver and neoplastic lesions can be assessed using analysis of variance techniques and blinded-reader evaluation protocols.

Interpretation and Analysis: Normal liver attenuation is greatest at CT arterial portography (127.3 Hounsfield units [HU] ± 5.3 [mean ± standard error of the mean]), followed by dual-contrast-enhanced CT (112.4 HU ± 1.2), iohexol-enhanced CT (97.9 HU ± 2.2), triglyceride-enhanced CT (82.3 HU ± 1.1), and unenhanced CT (54.9 HU ± 1.8). Liver-to-lesion attenuation difference at triglyceride-enhanced CT is significantly greater than at iohexol-enhanced CT ($p < 0.05$), and attenuation differences at dual-contrast-enhanced CT are comparable to those at CTAP. Detection of tumors is not improved with triglyceride-enhanced CT, which increases conspicuity. Sensitivity values for lesion detection at dual-contrast-enhanced CT are greater than those with iohexol-enhanced CT or CT arterial portography ($p < .05$). Computed tomography imaging enhanced with triglyceride preparations (especially when combined with iohexol) produces sensitivity values and liver-to-lesion attenuation differences that are greater with lower iodine doses than with iohexol or with CT arterial portography methods.

CT depiction of experimental liver tumors: Contrast enhancement with hepatocyte-selective iodinated triglyceride vs conventional techniques. Lee FT Jr, Chosy SG, Naidu SG, Goldfarb S, Weichert JP, Bakan DA, Kuhlman JE, Tambeaux RH, Sproat IA. Radiology 1997 May; 203 (2), pp. 465–70.

Hepatic Biodistribution Profile/Emulsion Particle Diameter and Dose/Organ Radioactive Dosimetry
[^{125}I]-Radioiodinated Polyiodinated Triglyceride (ITG)-Lipid Emulsion (LE)/(Potential Computed Tomography [CT] Agent)

Physiological Mechanism of Action: Polyiodinated triglyceride lipid emulsions are rather selectively assimilated intracellularly by hepatocytes.

Application: Novel LE formulations can be applied as a delivery moiety for the development for hepatoselective contrast agents. Radioiodinated polyiodinated triglyceride can be used as an indicator/signal molecule. The production of a LE-ITG conjugated complex can therefore be utilized as a potential contrast image-enhancing agent for application in CT.

Route of Administration: [^{125}I]-ITG-LE (intravenous).

Semi-Synthetic Methods: The signal molecule polyiodinated triglyceride is covalently cross-linked to LE (carrier molecule) to create a biologically functional conjugate.

Implementation: Delineation of the *in vivo* biodistribution of radioactivity associated with intravenously administered [^{125}I]-ITG-LE as a function of (i) mean particle size; (ii) total administered dose; (iii) formulation composition; and (iv) time after injection. Computed tomography imaging studies of the abdomen can be applied to evaluate the extent of hepatic enhancement after ITG-LE administration.

Animal Model: Rat.

Interpretation and Analysis: Mean emulsion particle diameter and total administered dose exert the greatest effect on ITG-LE biodistribution profiles. In optimal delivery scenarios, > 70% of the administered dose localizes to the liver 30 minutes after injection. Liver enhancement profiles in CT imaging studies are consistent with biodistribution profiles. Therefore, an appropriately formulated and administered dose of ITG-LE provides tissue-selective localization of contrast material that can be effectively used in CT procedures.

Polyiodinated triglyceride lipid emulsions for use as hepatoselective contrast agents in CT: Effects of physicochemical properties on biodistribution and imaging profiles. Bakan DA, Weichert JP, Longino MA, Counsell RE. Invest Radiol 2000 Mar; 35 (3), pp. 158–69.

Hepatic Neoplastic Disease/Hepatocellular Carcinoma: Arteriography/Vascularity Pattern of Hepatocellular Carcinoma/Trans-Catheter Arterial Chemoembolization Response/Arteriography Prognostic Variables
Arterial Phase Computed Tomography (CT) Contrast Image-Enhancement Agents

Application: Hepatic arterial-phase helical CT can be utilized to assess tumor vascularity and predict the likelihood of response to trans-catheter arterial chemoembolization in patients with hepatocellular carcinoma.

Implementation: Classification of helical CT image results in conditions of hepatocellular carcinoma into 3 patterns of vascularity on the basis of the degree of tumor or liver enhancement during the hepatic arterial phase. Cases in which hypervascular lesions predominate are classified as a Type 1 pattern. In cases that primarily display hypovascular lesions are classified as a Type 2 patterns. Conditions can further be classified as responders or non-responders on the basis of changes in tumor size detected on CT images after 3 trans-catheter arterial chemoembolization treatments.

Human Model: Hepatocellular carcinoma (e.g., n = 57 cases).

Interpretation and Analysis: Approximately 65% of hepatocellular carcinoma cases subjected to 3 trans-catheter arterial embolization treatments can be classified as responders (e.g., n = 37/57), while 35% are classified as non-responders (e.g., n = 20/57). There is a statistically significant correlation between Type 1 hypervascular patterns and response to trans-catheter arterial chemoembolization. Conversely, Type 2 hypovascular patterns correlate with non-response to trans-catheter arterial chemoembolization ($chi^2 = 7.85$, $p = 0.02$). Patients classified as responders live significantly longer than those classified as non-responders with 12-, 24-, and 36-month survival rates of 90%, 67%, and 36%, respectively, for responders and 70%, 17%, and 10%, respectively, for non-responders. Therefore, patients that respond to trans-catheter arterial chemoembolization have a prolonged survival period ($p < 0.01$). Response correlates closely with tumor vascularity as shown on hepatic arterial phase helical CT images.

Prognostic significance of arterial phase CT for prediction of response to trans-catheter arterial chemoembolization in unresectable hepatocellular carcinoma: a retrospective analysis. Katyal S, Oliver JH, Peterson MS, Chang PJ, Baron RL, Carr BI. AJR Am J Roentgenol 2000 Dec; 175 (6), pp. 1665–72.

INFLAMMATION AND SEPSIS

Abscess: Focal Hepatic Lesions
Ethyl Ester of Diatrizoic Acid (EEDA)

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.

Background Information: Ethyl ester of diatrizoic acid is an iodinated (89 mg I/ml) nanoparticulate (200 nm) contrast agent that has undergone preclinical evaluation.

Application: Contrast agent for enhancement of computed tomography (CT) imaging of focal hepatic disease.

Route of Administration: Ethyl ester of diatrizoic acid (intravenous).

Dosage: Ethyl ester of diatrizoic acid is an iodinated (89 mg I/ml) nanoparticulate (200 nm).
Implementation: Evaluation of EEDA administered in conditions of focal liver abscesses (e.g., n = 11 New Zealand White rabbits). On different days EEDA and iohexol are individually administered. CT scanning is performed at intervals following contrast agent administration. Liver and abscess enhancement are measured and compared. Dynamic imaging experiments in normal animals are also performed using both agents.
Interpretation and Analysis: Preparations of EEDA result in a significantly greater enhancement of liver and liver-to-abscess contrast imaging compared to iohexol at all time points beyond 5 minutes at approximately 25% of the total iodine load. During dynamic imaging, liver and aortic enhancement is greater with EEDA than with iohexol, except during a 20- to 40-second period immediately following contrast agent administration. The contrast agent EEDA therefore is superior to iohexol for imaging liver abscesses. Liver-directed agents such as EEDA may prove to be more efficacious than currently available extracellular agents designed for liver CT scanning.

Hepatic imaging with iodinated nanoparticles: A comparison with iohexol in rabbits. Gazelle GS, Wolf GL, McIntire GL, Bacon ER, Na G, Halpern EF, Toner JL. Acad Radiol 1995 Aug; 2 (8), pp. 700–4.

LYMPH NODES AND LYMPHATICS

Lymphography/Percutaneous Computed Tomography (CT) Imaging
Iodinated Chylomicron Microemulsions

Application: Enhanced imaging of lymphoid structures.
Implementation: An iodinated chylomicron remnant-like microemulsion is formulated with a mean particle size of 91.3 nm and an iodine concentration of 91 mg I/mL. Contrast material (2 mL) can be injected into the subcutaneous tissues of the metatarsus and metacarpus (e.g., n = 6 normal dogs) to enhance visualization of popliteal and cervical lymph nodes, respectively. Computed tomography images are then acquired at 0, 15, 30, 45, 60, 240, 480, and 1,440 minutes.
Interpretation and Analysis: Significant lymph node enhancement occurs in as little as 15 minutes postinjection and persists for at least 8 hours. Node opacification is most pronounced at 1 to 4 hours postinjection and exceeds 200 HU in some nodes (precontrast attenuation = 45 HU). Marked enhancement of popliteal efferent lymphatics and of iliac and sacral node groups also occurs, indicating distribution to 2nd-order nodes. Attenuation of enhanced nodes reverts to precontrast levels by 24 hours. The new surface-modified, chylomicron remnant-like emulsion provides marked, selective enhancement of targeted lymph nodes after subcutaneous administration. Moreover, the formulation produces significant opacification of more distant node groups from a single injection.

A surface-modified chylomicron remnant-like emulsion for percutaneous computed tomography lymphography: synthesis and preliminary imaging findings. Wisner ER, Weichert JP, Longino MA, Counsell RE, Weisbrode SE. Invest Radiol 2002 Apr; 37 (4), pp. 232–9.

Lymphography: Regional Lymph Nodes
Iodinated Nanoparticle

Application: Computed tomography (CT) lymphography of the craniocervical and thoracic lymph nodes.
Route of Administration: Iodinated nanoparticulate suspension injected subcutaneously, submucosally, and intraperitoneally.
Dosage: Iodinated nanoparticulate suspension (2–4 ml of 15% wt/vol preparation).
Implementation: Evaluation of the imaging characteristics of iodinated particulate contrast agents administered interstitially or intraperitoneally for CT lymphography of the craniocervical and thoracic lymph nodes. Suspensions of iodinated nanoparticle are injected subcutaneously, submucosally, or intraperitoneally (e.g., n = 8 dogs). Computed tomography and plain radiographic images are obtained prior to contrast administration and 4 hours, 24 hours, and 7 days after injection. Correlations are then made by detailed postmortem assessment.
Animal Model: Canine (e.g., n = 8 dogs).
Interpretation and Analysis: Computed tomography images reveal enhancement of regional nodes draining injection sites. Mean attenuation of opacified nodes is approximately 313 ± 297 (mean ± standard deviation), 536 ± 453, and 492 ± 372 Hounsfield units at 4 hours, 24 hours, and 7 days postinjection, respectively. Lymph node opacification on CT images correlates well with node location found at postmortem. It is therefore feasible to effectively opacify craniocervical and thoracic lymph nodes with iodinated nanoparticulate contrast agent following interstitial or intraperitoneal delivery.

Iodinated nanoparticles for indirect computed tomography lymphography of the craniocervical and thoracic lymph nodes in normal dogs. Wisner ER, Katzberg RW, Koblik PD, Shelton DK, Fisher PE, Griffey SM, Drake C, Harnish PP, Vessey AR, Haley PJ, et al. Acad Radiol 1994 Dec; 1 (4), pp. 377–84.

Lymphography: Subdiaphragmatic Lymph Nodes
Iodinated Nanoparticles

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Computed tomography (CT) lymphography of lymph nodes (e.g., subdiaphragmatic).
Dosage: Iodinated nanoparticles (4 ml of a 15% [wt/vol]; n = 10 dogs [beagles]).
Implementation: Evaluation of the imaging characteristics of iodinated particulate contrast media for indirect CT lymphography of normal subdiaphragmatic lymph nodes (canine). Iodinated nanoparticle suspensions can be injected into the gastric, colonic, rectal, or cervical submucosa, loose paraprostatic fascia, or metatarsal subcutaneous tissues. Endoscopic, CT, or ultrasound guidance is used when necessary to facilitate contrast agent delivery. Computed tomography and radiographic images are obtained prior to contrast administration and at 4 hours, 24 hours, and 7 days postcontrast injection. Postmortem examinations are then performed as a validation procedure.
Interpretation and Analysis: Computed tomography images show enhancement of regional lymph nodes draining the various injection sites. The mean attenuation of opacified nodes is 678 ± 463 Hounsfield units 24 hours after injection, which remains elevated at 7 days postinjection. Lymph node opacification on CT images correlates well with node location observed on postmortem examinations. Subdiaphragmatic lymph nodes can be effectively opacified using an iodinated nanoparticle contrast agent for indirect CT lymphography.

Indirect computed tomography lymphography of subdiaphragmatic lymph nodes using iodinated nanoparticles in normal dogs. Wisner ER, Katzberg RW, Koblik PD, McGahan JP, Griffey SM, Drake CM, Harnish PP, Vessey AR, Haley PJ. Acad Radiol 1995 May; 2 (5), pp. 405–12.

NEOPLASIA

Neoplastic Disease/Hepatic Tumor Detection Enhancement
Vascular Endothelial Growth Factor (VEGF)/ Water-Soluble Contrast Imaging Agents

Application: Vascular endothelial growth factor may be a biopharmaceutical agent that can be applied to enhance the detection of hepatic neoplasia using water-soluble contrast agents and computed tomography (CT) imaging analyses.
Route of Administration: Vascular endothelial growth factor theoretically would most often be administered intravascularly in either a selective or non-selective manner.
Dosage: Vascular endothelial growth factor.
Implementation: Hepatic tumors (e.g., n = 11 hepatocellular carcinomas; 3 metastatic malignant lesions) are evaluated applying dual-phase dynamic helical computed tomography, or computed tomographic hepatic arteriography. The attenuation of each mass is classified as hyper-attenuation, isoattenuation or hypoattenuation with respect to the normal adjacent parenchyma. Gun-needle biopsy can be done for each tumor, and paraffin sections immunostained with anti-VEGF antibody by the avidin-biotin-peroxidase complex method. The pathologic grade is assigned according to intensity (1+, 2+, 3+) and area (±, 1+, 2+). Tumor size ranges are approximately 2.0–14.0 cm (mean, 5.8 cm).
Interpretation and Analysis: In the arterial phase, the intensity does not correlate with the degree of enhancement ($p = 0.086$). However, the correlation between the attenuation value of hepatic arterial phase and the area of positive tumor cells is statistically significant ($p = 0.002$). Vascular endothelial growth factor may be a biopharmaceutical agent that can be utilized to enhance hepatic mass with water-soluble iodinated contrast agent in computed tomography imaging methodologies.

Correlation of VEGF with contrast enhancement on dual-phase dynamic helical CT in liver tumors: Preliminary study. Kwak BK, Shim HJ, Park US, Lee TJ, Paeng SS, Lee CJ, Lim HK, Park CK. J Korean Med Sci 2001 Feb; 16 (1), pp. 83–7.

Neoplastic Disease: Hepatic VX2 Implants
Dy-EOB-DTPA/Iopromide

Application: Dy-EOB-DTPA is a liver-specific contrast imaging agents that can be applied for the detection of hepatic neoplastic cell implants using spiral computed tomography (CT) image analysis.
Route of Administration: Dy-EOB-DTPA (intravenous); iopromide (intravenous).
Dosage: Dy-EOB-DTPA (0.5 mmol/kg); iopromide (600 mg iodine/kg).
Implementation: The agent Dy-EOB-DTPA is injected intravenously as a hepatic image-enhancing agent. At 15, 30, 45, 60, and 90 minutes after administration spiral CT images are obtained and attenuation within the liver determined. Other studies involve the evaluation of neoplastic lesions (e.g., rabbits with VX2 tumor implants) receive in a random crossover design either Dy-EOB-DTPA, or 1 day later, iopromide contrast agent. At 60 minutes following injection of Dy-EOB-DTPA administration and both the arterial and portal-venous phases occur after iopromide injection, CT images are then acquired. Livers are subsequently harvested and histopathology examinations performed on hepatic neoplastic lesions. In tumor-bearing animals, 14 implanted tumors are 3–20 mm in diameter with 65% of the tumors were below 10 mm in diameter.
Animal Model: Rabbits (normal) and tumor-bearing rabbits (e.g., n = 14 VX2 tumor implants; 3–20 mm in diameter with 65% of the tumors below 10 mm in diameter). All rabbits are anesthetized during scanning procedures.
Interpretation and Analysis: In normal animals, 0.5 mmol/kg Dy-EOB-DTPA results in a liver enhancement of 30 HU during an entire 90-minute observation period. Dy-EOB-DTPA is able to detect 93% of the total number of neoplastic lesions present within the liver compared to 79% level of detection for iopromide both in the arterial and in the portal-venous phase (histopathology analysis serving as a comparative reference). Differences between the 2 agents is not statistically significant. Alternatively, plain CT image analysis detects approximately 50% of the total number of neoplastic lesions present ($p < 0.01$ vs iopromide and Dy-EOB-DTPA). Scar tissue and sites of necrosis are also detected by each of the methods. Dy-EOB-DTPA injection at a dose of 0.5 mmol/kg results in a long-lasting detectability of 93% of all implanted tumors vs 79% found with iopromide.

Efficacy of the iodine-free computed tomography liver contrast agent, Dy-EOB-DTPA, in comparison with a conventional iodinated agent in normal and in tumor-bearing rabbits. Krause W, Handreke K, Schuhmann-Giampieri G, Rupp K. Invest Radiol 2002 May; 37 (5), pp. 241–7.

Neoplastic Disease/Hepatic Metastasis Animal Model
Radioiodinated Polyiodinated Triglyceride (ITG)-Poly(Ethylene) Glycol (PEG): Blood-Pool Contrast Agent/ITG-Lipid Emulsion (LE): Hepatocyte-Selective Contrast Agent

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties.
Application: Combined formulation of ITG-PGE and ITG-LE for detection of hepatic metastatic neoplastic lesions (e.g., small [< 10 mm] VX2 tumors in rabbit liver).
Route of Administration: Iohexol (intravascular).
Dosage: Iohexol (600 mg I/kg iohexol); ITG-LE (200 mg I/kg).
Implementation: Evaluate the effectiveness of combining (i) lipid-based blood-pool contrast agent (ITG-PEG); with a (ii) hepatocyte-selective agent (ITG-LE) for accurate computed tomography (CT) detection of small neoplastic metastatic lesions within the liver parenchyma, in addition to determining the relative capacity of (iii) an iodinated lipid-based blood-pool contrast agent to remain within the intravascular compartment for extended periods of time compared to conventional water-soluble contrast agents.

One approach to achieving this investigation objective can involve conducting pharmacokinetic analyses utilizing injected preparations of either [^{125}I]-labeled ITG-PEG or [^{125}I]-labeled ITG-LE followed by subsequent blood collection and quantification of radioactivity. Computed tomography imaging is then performed in both normal reference control and tumor-bearing *in vivo* models. Scanning CT image analysis is then performed at 10 days with multiple imaging techniques, including non-contrast and helical intravenous enhanced (iohexol), and then 24 hours later using both ITG-PEG and ITG-LE. Tissue density measurements (Hounsfield units [HU]) of liver, tumor, and blood (descending aorta) are acquired in each case for comparison. Tumor morphology is verified by evaluation of gross pathological changes.

Animal Model: Rat (n = 12 SD rats) and rabbits (e.g., n = 3 normal New Zealand White rabbits and n = 2 New Zealand White rabbits with < 10 mm VX2 liver tumors). Tumor-bearing rabbits are laparotomized and VX2 cells injected directly into the hepatic parenchyma to produce a total of 8 focal lesions (2–10 mm diameter).

Interpretation and Analysis: Pharmacokinetic analysis in rats as well as CT studies in normal rabbits reveals that ITG-PEG remains in the blood-pool phase for more than 2 hours following intravenous administration. In fact, blood density in normal rabbits obtained with ITG-PEG is 95.1 HU ± 5.8 at 120 minutes compared to 90.7 HU ± 6.1 immediately after injection. Although liver enhancement is greater with iohexol (67 HU within 1 minute of injection), than for ITG-PEG/ITG-LE (32 HU, 60 minutes postinjection), liver-to-lesion ratios favor ITG-PEG/ITG-LE due to significant enhancement of tumors with iohexol (+40 HU). Tumor enhancement is minimal with ITG-PEG/ITG-LE. Lesions are subjectively much better defined with ITG-PEG/ITG-LE with sharper edge definition. The iodinated lipid-based contrast agent composed of both blood-pool and hepatocyte-selective components affords favorable CT imaging results compared to conventional urographic agent, albeit at one-third the total iodine dose.

Lipid-based blood-pool CT imaging of the liver. Weichert JP, Lee FT Jr, Longino MA, Chosy SG, Counsell RE. Acad Radiol 1998 Apr; 5 Suppl 1, pp. S16–9; discussion S28–30.

Neoplastic Disease/Hepatic Metastases: Carcinoma of the Colon/Improved Sensitivity of Detection: Computed Tomography (CT) Scan
Hepatic-Specific Lipid Emulsion (FP 736-03) Iohexol

Cellular/Molecular Mechanism of Action: Compound FP 736-03 is an iodinated lipid emulsion that has radiopaque properties.
Application: Hepatocyte-specific iodinated lipid emulsion applied to perform CT liver imaging (FP 736-03).
Implementation: Evaluation of multiple hepatic metastases of human colonic cancer (e.g., nude rat model). Animals are examined 3 weeks after intraportal tumor cell injection. Plain image examinations, and iohexol- and FP-736-03-enhanced CT are then performed. The rate of detection is determined on a lesion-by-lesion basis. Metastatic diameters measured on CT images are compared with corresponding macroscopic evaluation of serially collected liver specimens.
Animal Model: Rat (nude rat with human colon cancer transplants).
Interpretation and Analysis: In a lesion-by-lesion analysis (e.g., n = 140 metastases), the CT imaging detection rates when utilizing FP-736-03 are significantly greater than observed with plain images, or iohexol-enhanced imaging (e.g., 16.4% and 30.7% for native examination, 12.9% and 17.9% for iohexol-enhanced CT, and 53.6% and 72.1% for FP-736-03-enhanced CT, respectively [e.g., n = 2 diagnosticians]). Detection rates tend to increase with lesion diameter. The hepatocyte-specific contrast medium FP 736-03 improved the detection rate significantly in comparison with both plain non-contrast enhanced and iohexol-enhanced CT images.

An iodinated lipid emulsion for CT of the liver. Comparison with iohexol in the detection of experimental hepatic metastases. Bergman A, Sundin A, Magnusson A. Acta Radiol 1997 Jan; 38 (1), pp. 55–60.

RENAL SYSTEM

Urography: Image Enhancement Contrast Agents
Iopromide/Iotrolan/Urea/Iloprost/Nifedipine/ Papaverine/Sodium Nitroprusside

Cellular/Molecular Mechanism of Action: Iodinated compounds possess radiopaque properties. Certain molecular additives can be applied to influence the excretion rate of iodinated contrast agents during urography imaging procedures.
Application: Enhancement of the diagnostic quality of urography images produced with iodinated contrast-enhancing agents including iopromide and iotrolan (conventional radiography and computed tomography).
Route of Administration: Iopromide (300 mg/mL) and iotrolan preparations (280 mg/mL) are each administered intravenously.
Dosage: Iopromide (plain films = 300 and 600 mg iodine/kg) and iotrolan preparations (plain films = 300 and 600 mg/kg) in dogs and rabbit. Alternatively, iopromide (computed tomography [CT] scans = 50 mg iodine/kg) and iotrolan preparations (CT scans = 50 mg/kg) in rabbit.
Implementation: Conventional plain-film radiography urograms utilizing urea (52 mg/mL), iloprost (10, 20, 50, and 100 ng/mL), nifedipine (0.02 mg/mL), papaverine (0.625 mg/mL), and sodium nitroprusside (0.05 μg/mL) added to commercial iopromide (300 mg/mL) and iotrolan preparations (280 mg/mL). Images are obtained at different time points after contrast injection of iodine preparations (e.g., rabbits and dogs). Image analysis is then repeated using CT and iopromide or iotrolan plus urea (52 mg/mL) or iloprost (20 ng/mL) and iodine doses of 50 mg/kg body weight (rabbits).
Interpretation and Analysis: Urea and iloprost at low concentrations (10 and 20 ng/mL) increases image quality both in plain film and in CT urography. Iloprost at high concentrations (50 and 100 ng/mL), nifedipine, papaverine, and sodium nitroprusside decrease image quality. An increase in image quality is accompanied by faster renal elimination of the contrast agent. Therefore, image quality in plain film and CT urography can potentially be improved by the application of additives formulated with contrast medium preparations.

Use of additives to contrast media to improve imaging in plain film and computed tomography urography. Krause W, Schromm M. Invest Radiol 2003 Jan; 38 (1), pp. 17–26.$eref4

RESPIRATORY SYSTEM

Neoplasia/Lung Cancer Staging/Lymph Node Metastasis Detection
Iodinated Nanoparticles

Background Information: Lung cancer continues to be a leading cause of death around the world. Staging of this disease is critically dependent upon the involvement or non-involvement of the lymph nodes that drain the region of lung containing the lesion/tumor. Palpation, unenhanced computed tomography (CT), and lymph node excision (i.e., mediastinectomy) are currently used to ascertain the status of these regional draining lymph nodes.
Application: Detection and staging of lung cancer and metastatic lesions within regional lymph nodes.
Route of Administration: Iodinated nanoparticles (inhalation).
Implementation: Pulmonary instillation of iodinated nanoparticles for contrast-enhanced CT of lung draining lymph nodes.
Animal Model: Canine (beagles).
Interpretation and Analysis: The variables of dose, time post installation, and formulation type (surfactant) have an impact upon enhanced CT images of the tracheobronchial lymph nodes. Pulmonary instillation of small volumes of iodinated nanoparticles can be successfully used to aid in staging forms of lung cancer by CT imaging.

Pulmonary delivery of nanoparticles of insoluble, iodinated CT x-ray contrast agents to lung draining lymph nodes in dogs. McIntire GL, Bacon ER, Toner JL, Cornacoff JB, Losco PE, Illig KJ, Nikula KJ, Muggenburg BA, Ketai L. J Pharm Sci 1998 Nov; 87 (11), pp. 1466–70.

Pulmonary Arterial Embolism/Warning: Potential Risk of Cardiac Arrest
Carbon Dioxide (CO_2)/Multidetector Row Spiral Computed Tomography (CT) Image Analysis

Cellular/Molecular Mechanism of Action: Carbon dioxide possesses radioleucent properties.
Application: Carbon dioxide can be applied as a negative contrast agent in CT imaging procedures.
Route of Administration: Carbon dioxide (intravascular).
Dosage: Carbon dioxide (120 or 180 mL).
Implementation: Serial CT scanning can be performed in the supine and prone positions utilizing different scan delays (10, 15, and 20 seconds) and different volumes of CO_2 (60, 120, 180, and 240 mL) that are manually infused over a duration of 10 to 15 seconds (e.g., n = 4 swine). In pulmonary embolism models (e.g., n = 5 swine), scanning can be performed with iodinated contrast medium and then rescanned with CO_2 (120 or 180 mL). The supine and prone CT scans and the number and location of thrombi recognized in the CO_2- and contrast material-enhanced CT scans are then compared.

Experimental Model: Creation of pulmonary emboli in swine (e.g., n = 7) followed by delineation of their anatomical characteristics using multidetector row spiral CT image analysis.
Interpretation and Analysis: Because of the position of the pulmonary artery in swine, the prone position is more effective than the supine position with CO_2 enhancement. An infusion of 120 mL of CO_2 is sufficient to enhance the entire pulmonary artery, and scanning timed to coincide with the completion of infusion is the most effective. Both the CO_2- and contrast-enhanced CT scans can aid in the detection of the vast majority of pulmonary thrombi (e.g., n = 7/7). Thrombi are more apparent using CO_2-enhanced CT scans compared to contrast-enhanced scans because of the high contrast interface between soft tissue and gas. However, a number of cases cannot be resuscitated after the occurance of abrupt cardiac arrest following CO_2-enhanced scanning procedures (e.g., n = 2/7). The CT depiction of pulmonary emboli is feasible with CO_2 gas as a negative contrast agent and may even be superior to iodinated contrast media. Further studies are required to evaluate the safety of this method and to develop an improved delivery of CO_2 gas for this application.

CO_2-enhanced CT imaging for the detection of pulmonary emboli: Feasibility study in a porcine model. Bae KT, Hong C, Becker CR, Prasad S, Nolte MA, Eisenbeis PE, Heiken JP. Acad Radiol 2003 Mar; 10 (3), pp. 313–20.

TECHNIQUE

Computed Tomography: X-Ray Micro-Computed Tomography (CT)/Quantitative Imaging Enhancemet of Molecular Transport
Heavy Metal Chelates: Iodine Devoid/Radiopaque Indicators

Application: Utilized in x-ray micro-CT primarily for the purpose of (i)acquiring 3D microarchitecture images and their correlation with associated functional characteristics; and (ii) regional distribution of administered radiopaque indicators, within intact rodent organs or biopsies from large animals and humans.
Implementation: Current use of x-ray micro-CT can be extended in 3 ways to increase the quantitative imaging of molecular transport processes and accumulation within such tissue/organ specimens. Examples in this regard include: (i) Use of heavy elements, other than iodine, attached to molecules of interest or to surrogates for those molecules for detection of accumulation patterns of the indicator in physiological compartments, and their transport to and from such compartments, which can be quantitated from the imaged spatial distribution of the contrast agents. (ii) High spatial resolution of conventional x-ray attenuation-based CT images to improve the quantitative nature of radionuclide-based tomographic images (single photo emission computed tomography [SPECT] and positron emission tomography [PET]) by providing correction for the attenuation of emitted gamma rays and the accurate delineation of physiological spaces known to selectively accumulate those indicators. Similarly, other imaging modalities that also localize function in 2D images (such as histological sections subsequently obtained from the same specimen), can be used in synergistic combination with CT-based 3D microstructure. (iii) Increasing the sensitivity and specificity of x-ray CT image contrast by use of methods such as: K-edge subtraction imaging, x-ray fluorescence imaging, imaging of the various types of scattered x-ray and the consequences of the change in the speed of x-rays through different tissues, such as refraction and phase shift. These other methods of x-ray imaging can increase contrast by more than an order of magnitude over that from conventionally-used attenuation of x-ray. To fully exploit their potentials, much development of radiopaque indicators, scanner hardware and image reconstruction and analysis software are needed.

Molecular imaging in small animals—Roles for micro-CT. Ritman EL. J Cell Biochem Suppl 2002; 39, pp. 116–24.

FLUOROSCOPIC IMAGING

Angiography: Fluoroscopic Imaging/Dosimetry Performance: Comparisons
Iodine-Based Vascular Contrast Agents (Gadolinium Filter)/Gadolinium-Based Vascular Contrast Agents (Tungsten Filter)

Application: Fluoroscopic imaging.
Implementation: Evaluation of fluoroscopic imaging and dosimetric analyses utilizing iodine-based and gadolinium-based vascular contrast agents in combination with K-absorption edge filters with atomic numbers between 50 (tin) and 82 (lead). Such combinations are studied using a theoretical model for a range of diagnostic x-ray spectra (55–100 kVp) and for water phantoms representative of thin and thick anatomical structures. Performance can be characterized according to radiographic contrast patterns, a derived image quality index, patient integral and entrance skin doses, and x-ray tube load. For a given thickness of anatomical structures, an optimum combination of spectrum kVp, contrast agent, and supplemental filter is defined by maximum imaging performance for a minimum or tolerable x-ray tube load and patient dose.
Interpretation and Analysis: Improvement of both imaging performance and reduced dose is possible through the use of an appropriate combination of spectrum kVp and filter. For gadolinium-based contrast, performance is optimized with tungsten filtration at 90 kVp for both thin and thick anatomies. It is not possible, however, to optimize the iodinated contrast performance with a single combination of supplemental filter and spectrum kVp. The optimal performance for iodinated contrast is achieved with gadolinium filtration at 60 kVp for thin anatomical structures and with ytterbium filtration at 80 kVp for thick anatomical structures. The best performance for thin anatomies is observed with a combination of iodinated contrast/gadolinium filter at 60 kVp and the best performance for thick anatomy can be observed with a combination of gadolinium-based contrast/tungsten filter at 90 kVp.

X-ray image intensifier performance and patient doses for combinations of supplemental beam filters and vascular contrast agents. McParland BJ, Boyd MM. Phys Med Biol 2001 Jan; 46 (1), pp. 227–44.

21 Ultrasonography

GENERAL CONSIDERATIONS AND CONCEPTS

Acoustic Parameters
Optison/Definity/SonoVue/Sonazoid

Implementation: Evaluation of the acoustic properties of 4 ultrasonic contrast agents (Optison, Definity, SonoVue, and Sonazoid) at 30 MHz. A range of contrast agent concentrations can be studied using either saline or glucose as a diluent of choice. Mean backscatter power is measured over regions-of-interest (ROIs) at distances of 1, 1.5, 2, 3, 4, and 5 mm from the center of the intravascular probe and normalized to the ultrasound (US) data collected from a standard glass reflector.
Interpretation and Analysis: For all of the agents, the mean backscatter power at 30 MHz varies in a linear manner with concentration between 0.01 million microbubbles/mL and 1 million microbubbles/mL. Furthermore, for 2 of the agents, mean backscatter enhancement is detectable at concentrations as low as 2 microbubbles/sample volume.
Instrumentation: Boston Scientific ClearView Ultra intravascular US scanner modified to allow access to the unprocessed US data.

In vitro acoustic characterization of 4 intravenous ultrasonic contrast agents at 30 MHz. Moran CM, Watson RJ, Fox KA, McDicken WN. Ultrasound Med Biol 2002 Jun; 28 (6), pp. 785–91.

Contrast Image-Enhancing Agents

The concept of contrast imaging was introduced to ultrasound almost 30 years ago. The development of ultrasound contrast agents (USCAs), initially slowed by technical limitations, has become more dynamic during the past decade. The ideal USCA should be nontoxic, injectable intravenously, capable of crossing the pulmonary capillary bed after a peripheral injection, and stable enough to achieve enhancement for the duration of the examination. While satisfying cost-benefit requirements, it should provide not only Doppler but also gray-scale enhancement. Doppler examinations can be improved by using USCAs when studying deep and small vessels, vessels with low or slow flow, or vessels with a non-optimal insonation angle. Ultrasound contrast agents also enhance detection of flow within abnormal vessels, including tumor vascularization and stenotic vessels, and provide better delineation of ischemic areas. Research has focused on the development of specific contrast imaging sequences that allow detection of tissue enhancement similar to that obtained with computed tomography (CT) or magnetic resonance imaging (MRI). These sequences take advantage of the non-linear behavior of microbubbles within ultrasound fields, bringing real-time perfusion imaging for liver, kidney, and the myocardium into reach. New objectives include targeted agents that could further widen USCA applications to the specific delivery of active drugs such as anticoagulants or cytotoxic compounds. The combination of new generations of USCAs and new ultrasound image sequences appears to be very promising and currently represents a significant part of ultrasound research.

Ultrasound contrast agents: Properties, principles of action, tolerance, and artifacts. Correas JM, Bridal L, Lesavre A, Mejean A, Claudon M, Helenon O. Eur Radiol 2001; 11 (8), pp. 1316–28.

Contrast Image-Enhancing Agents
Microbubbles

Various applications of contrast-assisted ultrasound, including blood vessel detection, perfusion estimation, and drug delivery, require controlled destruction of contrast agent microbubbles. The lifespan of a bubble depends on the properties associated with the bubble shell, the gas core, and the acoustic waveform impinging on the bubble. At least 3 mechanisms of microbubble destruction can occur within a biological system, including fragmentation, acoustically driven diffusion, and static diffusion. Fragmentation is responsible for rapid destruction of contrast agents on a microsecond time scale. The primary characteristics of fragmentation are a very large expansion and subsequent contraction that results in bubble instability. Optical studies using a novel pulsed-laser optical system show the expansion and contraction of ultrasound microbubble contrast agents have a ratio of maximum diameter to minimum diameter greater than 10. Fragmentation is dependent on the transmission pressure, occurring in over 55% of bubbles insonified with a peak negative transmission pressure of 2.4 MPa and in less than 10% of bubbles insonified with a peak negative transmission pressure of 0.8 MPa. The echo received from a bubble decorrelates significantly within 2 pulses when the bubble becomes fragmented, creating an opportunity for rapid detection of bubbles via a decorrelation-based analysis. Preliminary findings observed in mouse tumor models have verified the occurrence of *in vivo* fragmentation. A much slower mechanism of bubble destruction is diffusion, which is driven by both a concentration gradient between the concentration of gas in the bubble compared with the concentration of gas within the liquid, as well as convective effects of motion of the gas-liquid interface. The rate of diffusion increases during insonation, because of acoustically driven diffusion, producing changes in diameter on the time scale of the acoustic pulse length, thus on the order of microseconds. Gas bubbles diffuse while they are not being insonified (referred to as static diffusion). An air bubble with initial diameter of 2 microns in water at 37° C is predicted to fully dissolve within a 25-ms timeframe. Clinical ultrasound contrast agents are often designed with a high molecular weight core in an attempt to decrease the diffusion rate. C3F8 and C4F10 gas bubbles of the same size are predicted to fully dissolve within 400 ms and 4,000 ms, respectively. Optical experiments involving gas diffusion of a contrast agent support the theoretical predictions; however, shelled agents diffuse at a much slower rate without insonation, on the order of minutes to hours. Shell properties play a significant role in the rate of static diffusion by blocking the gas-liquid interface and decreasing the transport of gas into the surrounding liquid. Static diffusion decreases the diameter of albumin-shelled agents to a greater extent than lipid-shelled agents after insonation.

Mechanisms of contrast agent destruction. Chomas JE, Dayton P, Allen J, Morgan K, Ferrara KW. IEEE Trans Ultrason Ferroelectr Freq Control 2001 Jan; 48 (1), pp. 232–48.

Contrast Image-Enhancing Agents/Adverse Effect of Soluble Radiographic Contrast Media (RCM)

The pulmonary adverse effects of intravascular administered water-soluble RCM include bronchospasm, pulmonary edema, and increases in pulmonary arterial blood pressure (Ppa). Symptomatic bronchospasm is rare but subclinical increase in airways resistance is common after intravascular injection of RCM. Experimental studies have demonstrated that the low osmolar ionic dimer ioxaglate can induce significant bronchospasm in comparison with other types of RCM. Histamine and endothelin, which are potent bronchoconstrictors and released in response to the administration of RCM, do not seem to mediate the bronchospastic effect of RCM. Pretreatment with corticosteroids or antihistamine does not appear to prevent RCM-induced bronchospasm, but the administration of β_2-adrenergic agonist can abolish this adverse effect. RCM-induced pulmonary edema can be secondary to endothelial injury causing an increase in the permeability of the microcirculation. It may also occur in cases with incipient cardiac failure, when large doses of RCM particularly of the high osmolar type are used. A rise in Ppa induced by RCM seems to be secondary to an increase in pulmonary vascular resistance through direct effects on the pulmonary circulation. Low osmolar non-ionic monomers induce the least noticeable changes in the pulmonary circulation and should be the contrast media of choice for intravascular use in cases with pulmonary hypertension. The mechanisms responsible for the effects of RCM on airway resistance and pulmonary circulation remain unclear. Intrabronchial administration of high osmolar water-soluble RCM is dangerous and can induce severe bronchial irritation and pulmonary edema. Low osmolar RCM are well tolerated by the lungs following aspiration with minimal histological reaction.

Effects of radiographic contrast media on the lung. Morcos SK. Br J Radiol 2003 May; 76 (905), pp. 290–5.

Contrast Image-Enhancing Agents/Adverse Effects to Endothelium Injury/Endothelial Cell Viability: *Ex Vivo*/[Local Delivery] *Microbubbles/Propidium Iodine*

Background Information: Microvascular endothelial cells (EC) may be subject to the bioeffects induced by contrast ultrasound (US) because of their proximity to circulating microbubbles.
Application: Potential modality for the selective delivery of diagnostic or therapeutic pharmaceutical agents contained with microbubble preparations and subsequently liberated by ultrasound-induced disruption (lysis).
Route of Administration: Intravascular.
Implementation: Evaluation of EC injury produced in mesentery microvessel sections (e.g., arteriole, capillary, or venule) by exposure of ultrasound microbubble contrast agents to ultrasound energy. Assessment of such phenomenon can involve injection of microbubble preparations followed by ultrasound exposure at a 1-Hz or 30-Hz frame rate. Propidium iodide (PI), a fluorescent indicator of cell injury, can then be employed to visualize impaired endothelial cell integrity. Note: PI-positive nuclei should be equally few among the 3 control preparations.

Animal Model: Rat (mesenteric vasculature) divided into 5 experimental groups including 3 controls (sham operation, microbubble injection alone, US exposure with saline injection); and 2 contrast ultrasound groups (1-Hz or 30-Hz frame rates).
Interpretation and Analysis: Contrast-US increases the number of PI-positive cells in capillaries (1-Hz frame rate, 2.4 ± 2.2 cells per 0.1-mm vessel length, $p = 0.09$; 30-Hz frame rate, 4.3 ± 1.8 cells, $p < 0.01$) and in venules (1-Hz frame rate, 4.1 ± 2.5 cells, $p < 0.05$; 30-Hz frame rate, 13.8 ± 3.6 cells, $p < 0.01$) compared with sham preparations (0.10 ± 0.22 cells). Such findings indicate that diagnostic contrast ultrasound potentially causes endothelial injury, particularly within venules and capillaries.

Endothelial cell injury in venule and capillary induced by contrast ultrasonography. Kobayashi N, Yasu T, Yamada S, Kudo N, Kuroki M, Kawakami M, Miyatake K, Saito M. Ultrasound Med Biol 2002 Jul; 28 (7), pp. 949–56.

Contrast Image-Enhancing Agents/Adverse Effects of Renal Hemorrhage: Transducer Power vs Frequency/Renal Hemorrhage: Intermittent vs Continuous Insonification *Microbubbles*

Background Information: The generation of ultrasound (US) bioeffects using a clinical imaging system is controversial.
Route of Administration: Intravascular.
Implementation: Evaluation of the hypothesis that the presence of microbubbles in the US field of a medical imager induces biologic effects. One approach to detecting such effects includes insonification of the kidneys for 5 minutes using a medical imaging system after the administration of microbubbles. One kidney is insonified using a continuous mode (30 Hz) while the opposite kidney is insonified using an intermittent (1 Hz) technique. The microbubbles are exposed to 3 different transducer frequencies and 4 transducer output powers. After insonification, the animals are euthanized, the kidneys removed, and their gross appearance scored under "blinded" conditions using a defined pathology scale.

Animal Model: Rat (anesthetized).
Interpretation and Analysis: Following the administration of microbubbles, ultrasound imaging of the kidney induces hemorrhage in the renal tissue. The severity and area of hemorrhage increases with increases in the transducer power and a decrease in the transducer frequency. Intermittent insonification in the presence of microbubbles produces a greater degree of renal hemorrhage than continuous imaging techniques.

Microbubbles induce renal hemorrhage when exposed to diagnostic ultrasound in anesthetized rats. Wible JH Jr, Galen KP, Wojdyla JK, Hughes MS, Klibanov AL, Brandenburger GH. Ultrasound Med Biol 2002 Nov–Dec; 28 (11–12), pp. 1535–46.

Constrast Image-Enhancing Agents/Adverse Effects/ Gastrointestinal Inflammation, Necrosis, Mucosal Ulceration *Gas-Carrying Ultrasound Contrast Agents/Sonazoid*

Cellular/Molecular Mechanism of Action: Gas-carrying agents interact with ultrasound energy in a manner that impedes their transmission and reflects a portion of the "wave energy" back toward the probe/detector.
Application: Enhanced detection and characterization of physiological conditions and pathological anomalies affecting tissues, organ systems, vascular structures, and body fluids utilizing ultrasound-based diagnostic modalities.
Route of Administration: Gas-carrying ultrasound contrast image-enhancing agents (usually intravenous but theoretically can be utilized in any body fluid compartment). However, please note findings described in the Interpretation and Analysis section.
Implementation: Evaluation of the potential for gas-carrying ultrasound contrast image-enhancing agents to create pathological changes within tissues and organs.

Animal Model: Dogs, guinea pigs, mice, rats injected intravenously with gas-carrying ultrasound contrast image-enhancing agents.

Interpretation and Analysis: Single intravenous administration of different gas-carrier contrast agents used in ultrasound imaging (e.g., n = 3 mice) cause inflammation, necrosis, and ulceration of cecum and proximal colon (cecocolonic area) and focal necrosis in the liver of mice. Similar intestinal lesions are also found in rats after treatment with a single intravenous administration of a gas-carrier contrast agent. Strain differences in the incidences of these lesions can be appreciated in both rats and mice. HsdHan:NMRI mice are among the most sensitive of the mouse strains evaluated to date. Even at the lowest dose of Sonazoid technically possible to inject in HsdHan:NMRI mice, lesions can be identified and a no-effect dose cannot be determined. In time-course experiments in HsdHan:NMRI mice, lesions begin to develop in the cecum and colon within 15 to 30 minutes after dosing. Lesions in the liver are 1st observed 120–240 minutes after dosing. Interestingly, diet plays a role in the etiology of the lesions, as HsdHan:NMRI mice given a diet with reduced amounts of cellulose and starch have reduced incidences of lesions, and when glucose is the only carbohydrate source, no lesions can be observed. No intestinal or hepatic lesions can be found in guinea pigs or rabbits after repeated intravenous administrations of Sonazoid. In dogs, minimal to mild granulocytic inflammation of the cecum and/or colon is found after daily repeated intravenous injections for 28 days, but not after daily repeated administration for 14 days nor after a single administration. Intestinal and hepatic lesions in rats and mice after a single intravenous injection of gas-carrier contrast agents have been proposed to be caused by a common mechanism: intravascular growth of gas-carrier agents in tissues with gas supersaturation, as occurs in the cecal wall of rats and mice. In this particular environment the growing gas bubbles cause ischemia and necrosis in the cecal and colonic wall and liver. Such a proposed mechanism of action is consistent with the absence of clinical reports indicative of intestinal and/or hepatic lesions in humans after administration of gas-carrier contrast agents.

Intestinal and hepatic lesions in mice, rats, and other laboratory animals after intravenous administration of gas-carrier contrast agents used in ultrasound imaging. Dirven HA, Rasmussen H, Johnsen H, Videm S, Walday P, Grant D. Toxicol Appl Pharmacol 2003 May 1; 188 (3), pp. 165–75.

Contrast Imaging Agent Stability/Microbubble Formulations/Saline in Plastic vs Glass Vials/Saline in Vacuum vs Non-Vacuum Vials/Bladder Contrast-Enhanced Voiding Urosonograpy

Microbubbles (Galactose-Based Formulations: Levovist)

Background Information: The sonographic diagnosis of vesicoureteral reflux with intravesical administration of ultrasound contrast media is increasingly more frequent.

Application: Lower urinary tract examination.

Route of Administration: Urethral catheter.

Considerations: Rapid dissolution of microbubbles in the bladder filled with normal saline can occasionally be appreciated during the course of ultrasound examinations.

Implementation: Evaluate the degree of interaction between normal saline and ultrasound contrast media in concert with elucidating any potential causes for such interactions. Such an objective can be addressed applying an *in vitro* experimental model to characterize normal saline solution and a galactose-based ultrasound contrast medium (Levovist) formulated at gradient concentrations and then scanned utilizing predefined parameters. As a point of reference, the oxygen concentration of the solutions can also be measured (pO_2 and O_2 mg/l).

Interpretation and Analysis: Ultrasound contrast medium has a significantly longer contrast duration when mixed with normal saline from plastic containers rather than glass containers. The contrast duration difference is even more marked between normal saline from containers sealed under vacuum and those not sealed under vacuum (e.g., 0.7 and 12 minutes, respectively). The oxygen concentration in normal saline from vacuum-sealed containers is one-third or less that from non-vacuum-sealed containers. The high concentration of dissolved oxygen in normal saline seems to prevent the diffusion of air from microbubbles into the solution and thus their collapse. Desaturated normal saline has a detrimental effect on microbubbles of galactose-based US contrast agents. For the purpose of filling the bladder during contrast-enhanced voiding urosonography only normal saline solution from non-vacuum-sealed containers should be used. For practical purposes, normal saline from plastic containers is safest.

Interaction of normal saline solution with ultrasound contrast medium: Significant implication for sonographic diagnosis of vesicoureteral reflux. Darge K, Bruchelt W, Roessling G, Troeger J. Eur Radiol 2003 Jan; 13 (1), pp. 213–8.

Echocardiography/Myocardial Contrast Imaging

Microbubbles

Myocardial contrast echocardiography (MCE) has undergone many advances in the past several years through remarkable developments in contrast agent and ultrasound equipment technology. Microbubble ultrasound contrast agents can now safely transit the pulmonary circulation to provide opacification of the left ventricular cavity and improved endocardial border definition and to detect myocardial perfusion. The role of contrast echocardiography in enhancing technically difficult images is now well established in clinical practice and has proven especially useful in the stress and intensive care unit settings. Major progress has been made in the application of MCE for myocardial perfusion assessment in acute and chronic ischemic heart disease syndromes. Advances in novel applications of contrast echocardiography, including targeted delivery of genetic and pharmaceutical materials, have also occurred but remain in a preclinical phase. In summary, the combination of recent innovations in ultrasound equipment, and microbubble acoustics, allows for exciting exploration of the expanding role of contrast echocardiography in clinical practice.

Recent advances in myocardial contrast echocardiography. Gunda M, Mulvagh SL. Curr Opin Cardiol 2001 Jul; 16 (4), pp. 231–9.

Oxygen-Carrying Compounds/Vasculature/Liver or Spleen and Associated Neoplasia

Perflurochemicals (PFCs)

Cellular/Molecular Mechanism of Action: Perfluorochemicals possess oxygen-carrying properties. The characteristics that make them unique diagnostic agents pertain to their lack of hydrogen atoms, immiscibility with water, low surface tension, compressibility, and long intravascular persistence when emulsified and given intravenously.

Application: Perfluorochemicals can function as versatile contrast agents with important applications related to radiology (x-ray), ultrasound, and magnetic resonance imaging (MRI) modalities. When made radiopaque, they are visible with x-ray computed tomography (CT) and standard radiography.

Interpretation and Analysis: Because the neat liquid is inert it can be ingested, instilled in the lung, or introduced into any hollow organ to image the lumen without untoward effects. The long intravascular persistence allows imaging of blood vessels and vascularized

tissues. Small or deep vessels become visible with color Doppler imaging, and on angiographic images of any vascular tree including the coronary arteries, which can be rendered from serial CT images. As PFCs accumulate within reticuloendothelial system (RE) cells, specific liver and spleen enhancement can be achieved allowing the detection of small tumors within these organs. When injected interstitially, the particles find their way to draining (regional) lymph nodes providing detail of the internal architecture to detect the presence or absence of tumor involvement using both CT and sonography. Applying 19F MR, tissue perfusion and tissue pO_2 measurements can be obtained. The applications of PFC for diagnostic purposes are vast, unique, and important. These new capabilities will advance radiological tools to new horizons.

Intravascular behavior of a perfluorochemical emulsion. Tsuda Y, Yamanouchi K, Okamoto H, Yokoyama K, Heldebrant C. J Pharmacobiodyn 1990 Mar; 13 (3), pp. 165–71.

Synthetic carriers of oxygen. Dellacherie E, Labrude P, Vigneron C, Riess JG. Crit Rev Ther Drug Carrier Syst 1987; 3 (1), pp. 41–94.

Pharmaceutical Delivery/Ultrasound-Triggered Focal Delivery/Acoustic Power: Optimization/Signal Intensity Maximization/Bulk Modulus/Influence of Difference in Shell Characteristics
Microbubbles/Acoustic Lability

Background Information: Low mechanical index perfusion imaging relies on the detection of signals produced by microbubble oscillation at low acoustic powers that results in minimal microbubble destruction.
Route of Administration: Intravascular.
Consideration: Localized ultrasound-mediated disruption of microbubble preparations containing encapsulated pharmaceutical agents has been employed as a modality for selective delivery.
Implementation: Evaluation of the hypothesis that the optimal acoustic power for real-time imaging differs for microbubbles with different shell characteristics. One approach for pursuing this hypothesis can involve the evaluation of 3 microbubble agents with varying shell elastic properties according to their polymer composition. Differences in the elastic properties of these microbubbles can be demonstrated by (i) measurement of their bulk modulus, and (ii) evaluation of their acoustic lability by microscopic visualization of microbubble destruction during insonification at incremental acoustic powers. The ultrasound signal generated by these microbubbles at various mechanical indexes and the degree of microbubble destruction during continuous imaging is then determined both in an *in vitro* flow system and during *in vivo* open-thorax imaging procedures.
Animal Model: Canine (open-thorax preparations).
Interpretation and Analysis: Optimal power for achieving maximal signal intensity with minimal microbubble destruction is influenced by the shell elastic properties. Acoustic power for maximizing acoustic signal without destroying microbubbles during low mechanical index imaging varies according to specific shell characteristics.

Influence of microbubble shell properties on ultrasound signal: Implications for low-power perfusion imaging. Leong-Poi H, Song J, Rim SJ, Christiansen J, Kaul S, Lindner JR. J Am Soc Echocardiogr 2002 Oct; 15 (10 Pt 2), pp. 1269–76.

Phase Aberration Correction: Acoustic Imaging
Droplet Emulsion

Application: Potentially useful for phase aberration correction in acoustic imaging.
Route of Administration: Intravascular.
Considerations: Acoustic pressure thresholds exist, above which droplets vaporize into bubbles approximately 25 times the original diameter. For frequencies between 1.5 and 8 MHz, the threshold decreases from 4.5 to 0.75 MPa peak rarefactional pressure.
Semi-Synthetic Methods: Droplet emulsion (90% < 6 microm diameter) are produced by mixing saline, bovine albumin, and dodecafluoropentane.
Implementation: Evaluation of droplet preparations pertaining to their evaporation as a function of applied acoustic pressure and frequency, as well as simulations of gas bubble lifetime based on gas diffusion. Simulation of *in vivo* environments can be effectively achieved by the evaporation of droplets in blood flowing under attenuating material.
Interpretation and Analysis: The droplet emulsion agent may be potentially useful for tissue occlusion in cancer treatment, as well as for phase aberration corrections in acoustic imaging.

Acoustic droplet vaporization for therapeutic and diagnostic applications. Kripfgans OD, Fowlkes JB, Miller DL, Eldevik OP, Carson PL. Ultrasound Med Biol 2000 Sep; 26 (7), pp. 1177–89.

Stability of Contrast Preparations/Concentration and Preparation Time
Galactose-Based Ultrasound Contrast Agents (USCA)

Application: Galactose-based USCA (Levovist) can be applied as an ultrasound contrast agent for the detection of physiological alterations and pathological anomalies affecting tissues, organ systems, and vascular structures.
Route of Administration: Galactose-based USCA (intravascular).
Dosage: Galactose-based USCA (volumetric doses = 0.1 ml, 0.5 ml, and 1 mL; formulated at concentrations of 200, 300, and 400 mg/mL administered at various times since reconstitution = 2 minutes, 12 minutes, 22 minutes, and 32 minutes).
Implementation: Characterization (*in vitro*) of changes in microbubble population formulated as a galactose-based USCA (Levovist) based on, or a function of attenuation measurements. Additional parameters evaluated include dose-effect, formulation concentrations, and time, because reconstitution is evaluated using 2 broadband pulses at different peak negative pressures (0.39 and 0.49 MPa; n = 72 injections).
Analysis and Interpretation: Two minutes after reconstitution, a linear relationship can be appreciated between attenuation measurements and the amount of USCA (slope 0.92 dB × mg/cm/ml, r = 0.86). For a given dose and concentration, microbubble stability is significantly reduced with an increase in time between reconstitution and administration, particularly at lower concentrations. The persistence (image duration) and contrast effect of Levovist can be improved by administering the agent within the recommended time period following reconstitution, and by the injection of maximum suggested concentrations.

Optimizing an ultrasound contrast agent's stability using *in vitro* attenuation measurements. Correas JM, Kurtisovski E, Bridal SL, Amararene A, Helenon O, Berger G. Invest Radiol 2002 Dec; 37 (12), pp. 672–9.

CARDIOVASCULAR SYSTEM

Anatomical and Physiological Anomalies/Solid Organ Lesions
Fluorocarbon Microbubble/C_3F_8 Microbubbles/ SF_6 Microbubbles

Cellular/Molecular Mechanism of Action: Gas microbubbles resonate when impacted by ultrasound frequencies and there-

fore reflect a fraction of the ultrasound energy they come in contact with.

Background Information: Ultrasonography has, until recently, lacked effective contrast-enhancing agents. Micrometer-sized gas bubbles that resonate at a diagnostic frequency are ideal reflectors for ultrasound. However, simple air bubbles, when injected into the blood stream, disappear within seconds through the combined effects of Laplace pressure, blood pressure, and exposure to ultrasound energy.

Semi-Synthetic Methods: Production of microbubbles containing fluorocarbon vapor (C_3F_8 or SF_6).

Implementation: Positive contrast media for performing ultrasonographic imaging of fluid-filled body compartments.

Analysis and Interpretation: Fluorocarbon vapor extends the *in vivo* persistence of microbubbles from seconds to minutes in a manner that substantially improves their level of clinical utility. Imaging techniques that selectively suppress tissue, but not microbubble signals, further increase image contrast. Approved products consist of C_3F_8 or SF_6 microbubbles and N_2 microbubbles osmotically stabilized with C_6F_{14}. These agents allow the detection and characterization of cardiovascular abnormalities and solid organ lesions, such as tumors. By providing higher quality images, they improve the accuracy and confidence of disease diagnosis and can play a decisive role in clinical decision making. New objectives include agents that target specific cells for the molecular imaging of disease, and drug and gene delivery, including ultrasound-triggered delivery.

Injectable microbubbles as contrast agents for diagnostic ultrasound imaging: The key role of perfluorochemicals. Schutt EG, Klein DH, Mattrey RM, Riess JG. Angew Chem Int Ed Engl 2003 Jul 21; 42 (28), pp. 3218–35.

Angina/Typical Stress-Induced Angina
Microbubbles/Levovist and Optison

Cellular/Molecular Mechanism of Action: Microbubbles variably impede, absorb, and reflect the unidirectional flow of ultrasound energy, causing a portion of the "waves" to be directed back toward the transducer sensor (detector).

Background Information: Power Doppler harmonic imaging (PDHI) is normally performed in a triggered mode.

Application: Potential detection of hypoperfusion events utilizing myocardial contrast echocardiography (MCE) performed with PDHI modalities.

Route of Administration: Intravascular.

Considerations: Microbubbles are destroyed by ultrasound energy in the myocardium, requiring refilling of the myocardium with microbubbles within the time interval between the ultrasound pulses to obtain repetitive information about perfusion. Using the contrast agent Levovist, however, real-time PDHI also results in myocardial opacification presumably due to perfusion signals of the arteriolar microbubble passage.

Implementation: Evaluation of coronary artery integrity and patency.

Human Model: Typical stress-induced angina (e.g., female 45 years old).

Interpretation and Analysis: Prior to angiography a conventional echocardiogram may show normal left ventricular function. By contrast, however, tissue Doppler may demonstrate postsystolic longitudinal shortening of the septal, anterior, and lateral wall regions. Myocardial contrast echocardiography with triggered PDHI can reveal complete opacification of the myocardium at rest. Using real-time PDHI with Levovist, the septum can lack opacification. It is possible with consecutive angiography studies to document the presence of pathological conditions, including severe unprotected main coronary artery stenosis. After angioplasty and stent implantation, MCE measurements can be repeated. Repetitive intravenous bolus injections of Optison during triggered PDHI may reveal no difference relative to images acquired prior to angioplasty. Using real-time PDHI with Levovist, however, there may be a marked difference in comparisons to the preinterventional analyses. Complete opacification of the apical septum may be observed. Evaluation of some cases may suggest that different MCE techniques can analyze different compartments of the myocardial vasculature in clinical practice. Such methodological comparisons between triggered and real-time PDHIs show obvious differences in the Doppler intensity (DI) signal detection due to different microbubble behaviors. Clinicians should be aware of the potentials of MCE to improve non-invasive diagnostic procedures in patients with ischemic heart disease.

Instrumentation: Echocardiographic instrumentation.

Myocardial contrast echocardiography for assessment of myocardial perfusion at rest in a patient with left main coronary artery stenosis. Hagendorff A, Pfeiffer D, Rother T, Becher H. Z Kardiol 2003 Oct; 92 (10), pp. 876–83.

Angiography/Blood Flow and Vascular Patency/ Ultrasound Contrast-Enhancing Agents
C_3F_8 Microbubbles/N_2 Microbubbles/SF_6 Microbubbles

Cellular/Molecular Mechanism of Action: Use of fluorocarbon vapor extends the persistence of microbubbles *in vivo* from seconds to minutes and has greatly facilitated the application of contrast ultrasonography into clinical practice.

Application: Imaging techniques and reagents that selectively suppress the intensity of signal from tissue structures, but not injected microbubbles, serve to further enhance image contrast. Approved products consist of C_3F_8 or SF_6 microbubbles, and N_2 microbubbles osmotically stabilized with C_6F_{14}. These agents allow the detection and characterization of cardiovascular abnormalities and solid organ lesions, such as tumors. By providing higher quality images, they improve the accuracy and confidence of disease diagnosis and can play a decisive role in clinical decision making. New objectives include the design of agents that target specific cells for the molecular imaging of disease, and drug and gene delivery, including ultrasound-triggered delivery.

Route of Administration: Preparations of C_3F_8 microbubbles, SF_6 microbubbles, and N_2 microbubbles osmotically stabilized with C_6F_{14} are administered intravenously.

Dosage: C_3F_8 microbubbles, SF_6 microbubbles, and N_2 microbubbles osmotically stabilized with C_6F_{14} are administered at dosages commensurate with the diagnostic procedure being performed.

Considerations: Ultrasonography has, until recently, lacked effective contrast-enhancing agents. Micrometer-sized gas bubbles that resonate at a diagnostic frequency are ideal reflectors for ultrasound. However, simple air bubbles, when injected into the blood stream, disappear within seconds through the combined effects of Laplace pressure, blood pressure, and exposure to ultrasound energy.

Analysis and Interpretation: These agents allow the detection and characterization of cardiovascular abnormalities and solid organ lesions, such as tumors. By providing higher quality images, they improve the accuracy and confidence of disease diagnosis and can play a decisive role in clinical decision making. New design objectives include development agents that target specific cells for the molecular imaging of disease, and drug and gene delivery, including ultrasound-triggered delivery.

Injectable microbubbles as contrast agents for diagnostic ultrasound imaging: The key role of perfluorochemicals. Schutt EG, Klein DH, Mattrey RM, Riess JG. Angew Chem Int Ed Engl 2003 Jul 21; 42 (28), pp. 3218–35.

Angiography/Blood Flow Quantification
Microbubble Contrast Image-Enhancing Agent

Application: Enhanced detection and characterization of physiological conditions and pathological anomalies affecting tissues, organ systems, vascular structures, and body fluids utilizing ultrasound-based diagnostic modalities.
Route of Administration: Microbubble contrast image-enhancing agents (intravascular).
Implementation: Evaluate changes in destruction-replenishment curves (*in vitro* and *in vivo*) that result from microbubble destruction in feeding vessels that pass through the imaging plane before microbubbles enter the region-of-interest (ROI). Establishing the nature of such parameters can be applicable to the assessment of renal perfusion applying a microbubble contrast media. Such investigations can entail performing continuous injection of an ultrasonographic contrast agent, in concert with non-linear gray-scale image analysis obtained *in vitro* in the longitudinal plane of a renal dialysis cartridge flow phantom (flow rates of 100, 200, and 400 mL/min) and *in vivo* in the coronal plane of the left kidneys (n = 2 left kidneys). Destruction-replenishment curves are obtained for the dialysis cartridge in the form of ROIs located immediately after the entrance of microbubbles into the image plane and further from the entrance, after microbubbles have traveled across the complete length of the imaging plane. Replenishment curves are also obtained from ROIs corresponding to the kidneys at the level of segmental arteries, distal interlobar arteries, and the cortex.

Animal Model: Rabbit renal preparations (n = 2 left kidneys, n = 2 rabbits).
Analysis and Interpretation: The ROIs immediately after the entrance of the microbubbles in the image plane of the dialysis cartridge and in the segmental artery of the kidney follow a typical exponential function, represented as $A^{1-e\text{-}alphat}$. Early portions of curves for ROIs representing large number of microbubbles that have already passed through the image plane of the dialysis cartridge or reside within the renal cortex are not well described by such a function. The shape of the curve and the variations as a function of flow rate can be explained by means of a mathematical model based on indicator-dilution theory. Therefore, when the "feeding" vessels of an ROI travel across the ultrasound field before they reach the measurement region, the typical shape of the replenishment curve is modified (reduced velocity parameter and plateau).

Blood flow quantification with contrast-enhanced US: "Entrance in the section" phenomenon-phantom and rabbit study. Lucidarme O, Franchi-Abella S, Correas JM, Bridal SL, Kurtisovski E, Berger G. Radiology 2003 Aug; 228 (2), pp. 473–9. Epub 2003 Jun 11.

Angiography/Carotid Artery (Internal): Occlusion vs Pseudoocclusion/High-Grade Stenosis: Maximal Narrowing/Extracranial Vertebral Artery/Trans-Cranial Arteriovenous Malformation/Trans-Cranial Thrombosis of Cerebral Veins/Sinuses/Trans-Cranial Intracranial Aneurysms
Echo-Contrast Agents

Application: Enhanced detection and characterization of physiological conditions and pathological anomalies affecting tissues, organ systems, vascular structures, and body fluids utilizing ultrasound-based diagnostic modalities.
Route of Administration: Echo-contrast agents (intravascular).
Implementation: Utilization of echo-contrast agents to differentiate internal carotid artery occlusion and pseudoocclusion; better delineate the maximal narrowing in high-grade stenoses; and better visualize the extracranial vertebral artery and its collaterals. Trans-cranial applications include assessment of insufficient foraminal or temporal window, assessment of arteriovenous malformations, thrombosis of cerebral veins and sinuses, and intracranial aneurysms.
Analysis and Interpretation: The use of echo-contrast can have direct diagnostic and therapeutic consequences. Harmonic imaging, perfusion imaging, stimulated acoustic emission, and drug delivery are possible future domains of the technique. Besides the support of conventional neurovascular ultrasound in poor examination conditions due to variations in anatomy or pathology, echo-contrast agents may allow for novel applications in the diagnosis and treatment of cerebrovascular disease states.

Ultrasound contrast-enhancing agents in neurosonology: Principles, methods, future possibilities. Droste DW, Kaps M, Navabi DG, Ringelstein EB. Acta Neurol Scand 2000 Jul; 102 (1), pp. 1–10.

Angiography: Central Nervous System/Stimulated Acoustic Emission/High-Acoustic Disintegration of Microbubbles/Insonation of Echo-Contrast Agents
Microbubbles/Non-Moving Microbubbles (SHU 563 A)/Levovist (SHU 508 A)

Background Information: Experimental and clinical data suggest that insonation of echo-contrast agents with high acoustical power produces disintegration of microbubbles, resulting in a pseudo-Doppler phenomenon called stimulated acoustic emission (SAE).
Application: Potential measurement of cerebral vascular perfusion applying trans-cranial Doppler image detection of SAE signals.
Implementation: Evaluation of non-moving microbubbles (SHU 563 A) insonated *in vitro* through the temporal parts of a human cadaver skull, and contrast signals detected by velocity-coded color Doppler and power Doppler recordings. Complementary investigations can include the trans-cranial color Doppler and power Doppler imaging (e.g., n = 10 normal controls) utilizing the echo-contrast agent Levovist (SHU 508 A).
Analysis and Interpretation: Color Doppler signals reveal that SAE can be observed *in vitro* and in trans-cranial human investigations. These signals are characterized as a mosaic of color Doppler pixels ranging over a full color scale. Apparent velocity information and spatial distribution of SAE signals changes from image frame to image frame. In experimental models, the intensity of SAE signals decreases exponentially over time. With an increase of acoustic power, there is a significant increase in the maximum signal intensity ($p < 0.01$) and they are significantly shorter in signal duration ($p < 0.01$), consistent with stronger and more rapid disintegration. In humans, SAE signals can be clearly detected in cerebral tissue regions. The intensity of SAE signals in these regions (e.g., temporal cortex, 3.7 ± 1.2 dB) is approximately 8 times lower than the signal enhancement in the major cerebral arteries (e.g., 29.5 ± 5.6).

Stimulated acoustic emission detected by trans-cranial color Doppler ultrasound: A contrast-specific phenomenon useful for the detection of cerebral tissue perfusion. Pohl C, Tiemann K, Schlosser T, Becher H. Stroke. 2000 Jul; 31 (7), pp. 1661–6.

Angiography/Endothelium: ICAM-1 Expression/Optimization of Binding/Antibody-Density/Wall Shear Rate
Anti-ICAM 1 Microbubbles/Lipid-Based Perfluorobutane-Filled Microbubble

Background Information: The early stages of atherosclerosis are characterized by increased endothelial cell (EC) surface expression of leukocyte adhesion molecules (LAMs). Ultrasound detection

of acoustically active LAM-targeted microbubbles might provide a means to non-invasively assess the functional status of the endothelium.

Application: Non-invasive ultrasound assessment of the functional status of the endothelium.

Route of Administration: Intravascular.

Semi-Synthetic Methods: Production of a lipid-based perfluorobutane-filled microbubble synthesized with various densities of anti-ICAM 1 monoclonal antibodies conjugated to the bubble shell (1%, 5%, 10%, 50%, 75%, and 100% of maximum coverage) and possessing various wall shear rates (100, 175, 250, 350, and 500/s).

Implementation: Evaluation of the hypothesis that modulating the surface antibody density can permit regulation of the endothelial adhesion characteristics of microbubbles, and that microbubble adhesion is dependent on local wall shear rate. Such a hypothesis can be addressed by exposing coronary artery ECs to microbubbles produced with different surface antibody densities and various wall shear rates in a parallel plate perfusion chamber.

***Ex Vivo* Model:** Human coronary artery ECs cultured on coverslips. Endothelial cells are either normal or activated by interleukin-1 beta (IL-1β) in a manner that induces ICAM-1 overexpression.

Interpretation and Analysis: Immunomicrobubble adhesion is greater in the presence of activated vs normal endothelial cells ($p < 0.001$); increases with increasing surface antibody density ($p < 0.01$); and decreases with increasing wall shear rate ($p = 0.02$). In conclusion, shell antibody density and wall shear rate are critical parameters controlling differential microbubble adhesion. This phenomenon might ultimately permit imaging of clinically relevant (*in vivo*) LAM expression.

Modulating targeted adhesion of an ultrasound contrast agent to dysfunctional endothelium. Weller GE, Villanueva FS, Klibanov AL, Wagner WR. Ann Biomed Eng 2002 Sep; 30 (8), pp. 1012–9.

Angiography: Pulmonary Veins (Right and Left)
Optison (FS069)

Background Information: Pulmonary vein spectral Doppler signals to characterize ventricular diastolic and systolic function, though often difficult to obtain, can be enhanced using contrast imaging agents.

Application: Optison can be used as a positive contrast image-enhancing agent for application in diagnostic ultrasound procedures where it has the ability to improve Doppler-generated images in scenarios of poor signal transmission during 2-dimensional echocardiography procedures.

Dosage: Gradient injection volumes of 0.2, 0.5, 3.0, and 5.0 ml.

Implementation: Evaluation of the relative enhancement of pulmonary venous flow applying various injection volumes (e.g., $n = 191$ cases).

Analysis and Interpretation: Greatest contrast enhancement for right and left pulmonary veins is observed at the highest doses. At 0.5 ml, conversion from inadequate to adequate can be observed in right and left pulmonary veins in 48.0% and 79.3% of injection studies, respectively while the degree of improvement is 54.4% and 65.8%, respectively. The adverse event rate (6.5%) is similar to a 1st-generation agent. Optison is a safe and effective contrast agent for improving the visualization of pulmonary Doppler signals, especially the left pulmonary vein.

Intravenous Optison (FS069) enhances pulmonary vein flow velocity signals: A multicenter study. Ellahham S, Hausnerova E, Gottdiener J. Clin Cardiol 2000 Feb; 23 (2), pp. 91–5.

Angiography: Trans-Cranial Color-Coded Sonography (TCCS)/Duration of Contrast Enhancement
Levovist

Background Information: Since the introduction of contrast media, TCCS has become increasingly important for the examination of intracranial vessels. However, the widely practiced bolus injection of these agents leads to the initial creation of blooming artifacts and thereafter the level of contrast enhancement decreases rapidly, reducing the effective time of enhancement.

Application: Trans-cranial color-coded sonography examination of intracranial vessel structure, integrity, and function.

Route of Administration: Levovist (antecubital vein.)

Dosage: Levovist (300 mg/ml; 4 g at a constant infusion rate of 60 ml/hr).

Implementation: Trans-cranial color-coded sonography examination of intracranial vessels using a 2.25-MHz phased-array transducer during administration of Levovist contrast agent. The degree of enhancement is graded chronologically over time.

Human Model: Normal subjects ($n = 26$).

Analysis and Interpretation: During continuous administration, a constant level of contrast enhancement is achieved after 60.1 ± 26.2 seconds and lasts approximately 663.4 ± 55.8 seconds (evaluator 1 = 66.6 ± 26.2 seconds; evaluator 2 = 664.3 ± 55.9 seconds). The limits of interobserver agreement ranges from -10.1% to 9.9%. No major blooming effect is seen in the initial phases of the examinations.

Continuous administration of contrast medium for trans-cranial color-coded sonography. Schminke U, Motsch L, Bleiss A, von Smekal U, Griewing B, Kessler C. Neuroradiology 2001 Jan; 43 (1), pp. 24–8.

Angiography: Vascular Blood Flow Characterization/Aorta/Renal Artery and Vein/Portal Vein/Vena Cava
SHU 508 Microbubbles (Levovist)/Indocyanine Green (ICG)

Cellular/Molecular Mechanism of Action: Indocyanine green and microbubbles can be infused into the intravascular compartment, where they disperse throughout the blood volume.

Application: Indocyanine green and microbubbles can be applied to characterize blood flow through vascular structures.

Route of Administration: Indocyanine green and microbubbles are both administered intravascularly.

Dosage: Indocyanine green (1.25 mg/mL at 0.1 mL/kg); SHU 508 A (300 mg/mL at 0.1 mL/kg).

Considerations: SHU 508 A microbubbles (Levovist) can be detected by Doppler ultrasound.

Implementation: Evaluation of blood flow in the vena cava, aorta, renal artery, renal vein, and portal vein. The kinetics of ICG can be evaluated by measuring absorbance values using a photodiode, while SHU 508 A microbubbles (Levovist) can be detected using Doppler ultrasound.

Animal Model: Rabbit ($n = 5$).

Interpretation and Analysis: Peak signal intensity is observed immediately after injection of SHU 508 A, followed by a biphasic decay pattern. The rates of biphasic decay are similar in all vessels. A 2nd peak of the signal that represents microbubble recirculation can be observed within the vena cava. The circulation and recirculation times of the microbubbles after injection of SHU 508 A are similar to that of ICG. These findings suggest that majority of SHU 508 A microbubbles circulate through the body similarly to blood flow, without retention in microvasculature structures.

In vivo kinetics of microbubbles of SHU 508 A (Levovist): Comparison with indocyanine green in rabbits. Uchimoto R, Niwa K, Eguchi H, Kamiyama N, Mine Y, Miyazawa T, Brautigam M. Ultrasound Med Biol 1999 Nov; 25 (9), pp. 1365–70.

Angiography/Thrombosis
[Lys-Gla-Ala-Gly-Asp-Val]-Aerosomes-Entrapped Microbubbles

Cellular/Molecular Mechanism of Action: Microbubbles can function as a means for delaying excretion, suppressing biological degradation, and enhancing site-specific accumulation of diagnostic and therapeutic pharmaceutical agents.
Application: Ultrasound can be used as a primary diagnostic technique for the detection of deep venous thrombosis. Microbubble preparations can potentially enhance the detection and characterization of intravascular thrombi.
Route of Administration: Intravascular.
Semi-Synthetic Methods: In the 1st phase of production, synthetic linear hexapeptide (lysine-glutamine-alanine-glycine-aspartate-valine) can be coupled to a lipid moiety. The targeted lipid moiety is then incorporated into a lipid blend, followed by the entrapment of Aerosomes contrast agent (ImaRx) yielding perfluorobutane-entrapped microbubbles. Prior to administration microbubble preparations are then sized for uniformity.
Implementation: Evaluation of the *in vitro* capacity of sized perfluorobutane-entrapped microbubbles to (i) retain high levels of acoustic stability; (ii) recognize (bind) to blood clots; and (iii) enhance ultrasound image analysis of intravascular thromboses (blood clots).
Interpretation and Analysis: Experimental results reveal that the mean size of the specific ultrasound contrast agent (MRX-408) is about 2.0 microm. Microbubbles appear as smooth spherical structures. Microscopic analysis reveals that targeted bubbles bind to blood clots, whereas control, non-targeted bubbles do not bind to blood clots. *In vitro* acoustic studies reveal similar levels of stability for modified microbubbles compared with control microbubbles. Targeted microbubbles enhance *in vitro* blood clot visualization, whereas non-targeted microbubbles do not increase clot detection. Therefore, thrombus-specific ultrasound contrast agents could potentially improve the detection of thrombosis by ultrasound and may be useful for distinguishing between new and old thrombosis. Investigations performed *in vivo* have been initiated to further characterize the applicability of these preparations.

In vitro studies of a new thrombus-specific ultrasound contrast agent. Unger EC, McCreery TP, Sweitzer RH, Shen D, Wu G. Am J Cardiol 1998 Jun 18; 81 (12A), pp. 58G–61G.

Arrythmias
Microbubble Echosonography Contrast Media/ Contrast-Enhanced Pulsed Ultrasound

Dosage: Microbubble preparations (0.25 ml in rats).
Implementation: Conduction of contrast-enhanced pulsed ultrasound imaging to acquire conduction complex and heart lesion recording data. The volume of the contrast agent administered is based on body weight and the mechanical index (ultrasonic pressure) exceeding those used in echocardiography by 14 to 345 and 3 to 29 times, respectively.
Animal Model: Development of an experimental protocol suitable for characterizing electrocardiographic arrhythmias in rat hearts exposed to ultrasound after injection of a microbubble contrast agent.
Analysis and Interpretation: Premature atrial complexes, premature ventricular complexes, or polymorphic ventricular tachycardia can be detected in about 50% of rats (e.g., n = 10/20). Cessation of ultrasound image analyses results in a termination of arrhythmia patterns; however, such alterations can reoccur (e.g., n = 4/20) when ultrasound exposure is resumed. Myocardial degeneration identified by histochemical staining (hematoxylin-basic fuchsinpicric acid) can be appreciated *in vivo* in a large percentage of test model subjects (e.g., n = 16/20) with a smaller percentage of these actually displaying arrhythmias (e.g., n = 10/20). There is no significant difference in the amount of histochemical staining in hearts from *in vivo* preparations with arrhythmias when compared to scenarios when arrhythmias are not detected.

Because arrhythmias are induced principally when the contrast agent interacts with ultrasound energy during exposure, the presence of myocardial degeneration alone is not a sufficient explanation for ectopic electrical activity. Under these extreme exposure conditions, the data suggest that pulsed ultrasound through its biomechanical interactions with contrast agents has the potential to induce arrhythmias.
Instrumentation: Pulsed ultrasound examination (frequency, 3.1 MHz; pulse duration, 1.3 microseconds; pulse repetition frequency, 1,700 Hz; and *in situ* peak rarefactional pressure, 15.9 MPa).

Arrhythmias in rat hearts exposed to pulsed ultrasound after intravenous injection of a contrast agent. Zachary JF, Hartleben SA, Frizzell LA, O'Brien WD Jr. J Ultrasound Med 2002 Dec; 21 (12), pp. 1347–56.

Arteriography: Aortic Stenosis/Aortic Valve Replacement (AVR)/Coronary Flow Reserve (CFR)
Dipyridamole

Physiological Mechanism of Action: Dipyridamole induces vasodilatory responses.
Background Information: In patients with aortic stenosis and a normal coronary angiogram, CFR is impaired.
Route of Administration: Dipyridamole (intravascular).
Dosage: Dipyridamole (0.56 mg/kg for 4 minutes).
Implementation: Evaluate the effect of AVR on the CFR after a long-term follow-up. In part, this can be assessed by measuring CFR 123 ± 137 days before and 497 ± 167 days after aortic valve replacement. Coronary flow reserve measurements are performed according to a standardized protocol, with a vasodilator stimulus (e.g., dipyridamole) and peak diastolic velocity measurements recorded at 6 minutes.
Human Model: Cases of aortic stenosis with normal coronary angiogram images (e.g., n = 30). Cases with AVR (e.g., n = 21).
Interpretation and Analysis: The initial average peak gradient of aortic stenosis is 89.5 ± 22.4 mm Hg. After AVR, it decreases to 26.2 ± 9 mm Hg. Left ventricular mass is significantly lower after AVR: 354.9 ± 107.9 g vs 223.8 ± 73.6 g ($p < 0.001$). The average baseline diastolic velocity measured by pulsed Doppler in the left anterior descending coronary artery amounts to 62.2 ± 25.5 cm/s before and 40.1 ± 13.6 cm/s after AVR. The difference is statistically significant ($p < 0.01$). The average diastolic velocity at maximum stress equals 117 ± 42.8 cm/s pre- and 91.5 ± 34 cm/s postoperatively ($p < 0.005$). Calculated CFR before AVR amounts to 1.96 ± 0.5 and increases to 2.37 ± 0.8 postoperatively. The difference is statistically significant ($p < 0.05$). Prosthetic AVR is of considerable benefit concerning the CFR in cases with a normal coronary angiogram after a long-term follow-up.

The effect of aortic valve replacement on coronary flow reserve in patients with a normal coronary angiogram. Nemes A, Forster T, Kovács Z, Thury A, Ungi I, Csanády M. Herz 2002 Dec; 27 (8), pp. 780–4.

Arteriography: Aortic Stenoses and Atherosclerotic Plaques
Microbubbles (AF0150)

Cellular/Molecular Mechanism of Action: The microbubble agent AF0150 absorbs and reflects ultrasound energy.
Application: Detection and characterization of vascular anamalies, including atherosclerotic plaque deposits in the coronary arteries.
Route of Administration: AF0150 (intravenous).
Dosage: AF0150 (0.3 ml in rabbits and 1.0/L porcine blood for *ex vivo* vascular preparations).
Implementation: Evaluation of contrast media pertaining to (i) testing the hypothesis that filling the arterial lumen with echoes at B-mode ultrasound (US) enables the assessment of wall and luminal abnormalities, and (ii) comparing contrast material-enhanced B-mode US with color and power Doppler US angiography. Such diagnostic procedures can be performed to delineate the presence of aortic atherosclerotic lesions utilizing AF0150 as an ultrasound contrast image-enhancing agent applied in combination with color Doppler and B-mode ultrasound. Radiopaque plastic casts of the rabbit aortas and contact radiographs of the plastic replicas can serve as standard reference controls.

Animal Model: Rabbit (created atherosclerotic aorta lesions).

Human *Ex Vivo* Preparation: Replicas of diseased human carotid arteries immersed in a tissue-mimicking phantom (e.g., $n = 4$).
Interpretation and Analysis: Although color and power Doppler US allow immediate localization of the lumen, precise estimation of stenoses and reliable visualization of surface irregularities is not possible. After AF0150 administration, angiogram-like images of the lumen can be created with B-mode US, allowing rapid assessment of the entire vessel lumen and wall. Consequently, luminal stenoses can be more accurately measured than with unenhanced B-mode US ($r^2 = 0.94$, $p < 0.0001$ vs $r^2 = 0.21$, $p = 0.25$) or Doppler ($r^2 = 0.42$, $p < 0.03$). In addition, plaques and ulcerations are visible only with contrast-enhanced B-mode US. Microbubbles fill the arterial lumen with echoes at B-mode US, creating an angiogram-like image. The ability to visualize the inner and outer surfaces of the vascular wall improves the evaluation of luminal and wall abnormalities

Contrast-enhanced B-mode US angiography in the assessment of experimental *in vivo* and *in vitro* atherosclerotic disease. Sirlin CB, Lee YZ, Girard MS, Peterson TM, Steinbach GC, Baker KG, Mattrey RF. Acad Radiol 2001 Feb; 8 (2), pp. 162–72.

Arteriography: Aortic Stent Graft Monitoring/ Cost-Effectiveness: Relative Expense
Contrast Duplex Ultrasound/Computed Tomography (CT) Angiography

Background Information: The use of endovascular stent graft repair for aortic aneurysmal disease has become increasingly common, with the added requirement for close postoperative surveillance to detect the presence of endoleaks or graft migration. The most commonly used technique for surveillance is CT angiography, with the need for intravenous contrast posing one limitation in those cases with renal dysfunction and the cost of such testing posing an economic limitation.
Analysis and Interpretation: Early results of duplex imaging using an intravenous ultrasound contrast agent have shown sensitivity and specificity equivalent to CT angiography, with no evidence of any related morbidity. The difference in cost-effectiveness for using duplex ultrasound imaging as the primary surveillance technique for postoperative follow-up in cases with aortic stent grafts is substantial. Surveillance protocols now require performing 8 follow-up examinations during the 1st 3 years after stent graft placement. The charges for CT angiography in some institutions average $2,779 per study, for a 3-year total of $22,232 per patient. The charges for aortic duplex ultrasound average $525 per study, with a 3-year total of $4,200 per patient. Adding the cost of routine abdominal radiographs to confirm stent graft position ($147 per study) would bring this 3-year total to $5,376, a savings of $16,856 per case. For every 100 patients that are monitored after stent graft placement, this represents a 3-year savings of more than $1.6M. Promising early results of duplex ultrasound imaging with an intravenous contrast agent show sensitivity and specificity equivalent to those of CT angiography in detecting aneurysm size and graft endoleaks or other hemodynamic abnormalities. If these results can be demonstrated in larger patient series, this technique should become the method of choice for stent graft surveillance, for it offers very significant economic advantages and avoids the complications of intravenous contrast-induced renal dysfunction.

Duplex ultrasound imaging with an ultrasound contrast agent: The economic alternative to CT angiography for aortic stent graft surveillance. Bendick PJ, Zelenock GB, Bove PG, Long GW, Shanley CJ, Brown OW. Vasc Endovascular Surg 2003 May–Jun; 37 (3), pp. 165–70.

Arteriography: Arterial Disease (Peripheral)/ Relative Sensitivity and Specificity
Ultrasound Contrast-Enhancing Agents/Digital Subtraction Arteriography (DSA)

Application: Ultrasound contrast-enhancing agents can be utilized to improve the diagnostic ability of duplex-ultrasound and increase agreement with DSA when examining difficult arterial segments in conditions of peripheral arterial disease.
Route of Administration: Ultrasound contrast agent (intra-arterial).
Implementation: Conduction of a duplex-ultrasound scan and DSA for the evaluation of vascular trifurcation structures. In situations when duplex-ultrasound scans are inconclusive, the procedure can be repeated during continuous ultrasound contrast agent infusion. Digital subtraction arteriography findings serve as a master reference control.

Human Model: Cases suffering from peripheral arterial disease (PAD: $n = 60$; $n = 15$ inconclusive duplex-ultrasound scan of the trifurcation; $n = 8/15$ male; $n = 15$; DSA exam $n = 15$ candidates for vascular surgery (20% or $n = 3/15$ due to claudication; 33% or $n = 5/15$ due to rest pain; and 47% or $n = 7/15$ due to tissue loss).
Analysis and Interpretation: Following contrast agent administration, the number of inconclusively diagnosed segments is significantly reduced by 26 (70%), from 37 to 11 ($p < 0.001$). In 19 segments (73%), contrast agent infusion changes the diagnosis in accordance with the DSA ($p < 0.05$). Values of sensitivity and positive predictive value are improved from 0.20 (0.04–0.62) to 0.47 (0.26–0.69) and 0.50 (0.10–0.91) to 0.80 (0.49–0.93), respectively. Specificity and negative predictive value are unchanged. Agreement between duplex-ultrasound and DSA are improved from poor (kappa = 0.18 [95% CI: 0–0.82]) to moderate (kappa = 0.45 [0.17–0.74]) ($p = 0.44$). Ultrasound contrast agents improve the diagnostic ability of duplex-ultrasound when scanning difficult arterial segments in patients suffering from PAD.

Ultrasound contrast agent improves imaging of lower limb occlusive disease. Eiberg JP, Hansen MA, Jensen F, Rasmussen JB, Schroeder TV. Eur J Vasc Endovasc Surg 2003 Jan; 25 (1), pp. 23–8.

Arteriography: Arterial Function (Peripheral: Brachial Artery Vasodilation)
Nitroglycerin

Cellular/Molecular Mechanism of Action: Nitroglycerin activates guanylate cyclase, thereby increasing the synthesis of guanosine 3′5′ monophosphate (cGMP) within smooth muscle tissue. In part this is mediated by increasing nitric oxide production that in turn increases cGMP levels. Ultimately light chain myosin is dephosphorylated, which functions in the contractile process. Analogs of cGMP also relax vascular and bronchial smooth muscle.
Physiological Mechanism of Action: Nitroglycerin promotes primarily venodilation properties at lower dosages compared to its effect on arterioles.
Application: Echocardiography/pharmaceutical provocative test procedures designed to detect and characterize physiological and pathological anomalies affecting vascular structures.
Route of Administration: Nitroglycerin (multiple).
Dosage: Nitroglycerin (variable).
Implementation: Investigate the effects of non-ECG-triggered imaging during ultrasound studies of endothelial function. Brachial artery diameters can be measured throughout the cardiac cycle at rest, during reactive hyperemia, and after nitroglycerin administration. Cardiac cycle-related variations are computed as the maximum percent change from the end-diastolic diameter. The range of possible errors in flow-mediated dilation (FMD) and nitroglycerin-mediated vasodilation that may result from ignoring cyclic variations in diameter are determined for each condition.

Human Model: n = 24 subjects.
Interpretation and Analysis: True FMD, true nitroglycerin-mediated vasodilation, and the maximum and minimum values that can be erroneously calculated for FMD if timing is ignored all differ dramatically ($p < 0.05$). The range of apparent FMD values that can be measured is nearly 3 times the true FMD value. Ignoring temporal position within the cardiac cycle artifactually increases calculated FMD into the normal range, despite truly impaired FMD. Peak arterial dilation occurs before end-systole and greater baseline vessel compliance is associated with greater FMD. Brachial arterial diameters vary significantly throughout the cardiac cycle. The magnitude of this variation is similar to the arterial dilation induced by reactive hyperemia and nitroglycerin, making ECG-triggered imaging mandatory for accurate and reproducible clinical and research measurements of artery diameters and FMD. Measurement of diameters at end-diastole may be preferred to other time points in the cardiac cycle.
Instrumentation: 7.5-MHz ultrasound transducer (R-wave-triggered imaging with acquisition every 41.7–66.7 ms).

Effect of cardiac cycle on ultrasound assessment of endothelial function. Chuang ML, Douglas PS, Bisinov EA, Stein JH. Vasc Med 2002 May; 7 (2), pp. 103–8.

Arteriography: Athrosclerosis Imaging by Ultrasonography/Atheroma: Regions of Fibrous Content/Atheroma: Regions of Early Phase Deposition
Anti-Fibrinogen-Liposomes/Anti-ICAM-Liposomes

Background Information: Previous reports have demonstrated the development of acoustically reflective liposomes that can be conjugated for site-specific acoustic enhancement.
Application: Detect and characterize (*in vivo*) atherosclerotic plaque deposits affecting vascular structures, including the coronary artery.
Semi-Synthetic Methods: Liposomes can be prepared with phospholipids and cholesterol using a dehydration/rehydration method. Antibodies are thiolated for liposome conjugation with *N*-succinimidyl 3-(2-pyridyldithio) propionate resulting in the generation of a thioether linkage between the protein molecule and the phospholipid. As a synthesis test model, liposomes can be conjugated to antifibrinogen or anti-intercellular adhesion molecule 1 (anti-ICAM 1).
Implementation: Evaluate the ability of acoustically reflective liposomes coupled to antibodies specific for different components of atherosclerotic plaques and thrombi to target and enhance *in vivo* ultrasonic image analysis. One approach to achieving this objective can involve assessing arterial plaques imaged (20-MHz intravascular ultrasound catheter and 7.5-MHz trans-vascular linear probe) after injection of saline, unconjugated liposomes, and antibody conjugated liposomes.

Animal Model: Swine (Yucatan "mini-hogs" for atherosclerosis developed by crush injury of 1 carotid and 1 femoral artery in concert with the ingestion of a hypercholesterolemic diet).
Interpretation and Analysis: Conjugated liposomes retain their acoustically reflective properties and provide ultrasonic image enhancement of their targeted structures. Liposomes conjugated to antifibrinogen attach to thrombi and fibrous portions of atheromas, whereas liposomes conjugated to anti-ICAM 1 attach to early atheroma. Such conjugated acoustic agents can therefore provide varying targeting with different antibodies with retention of intravascular and trans-vascular acoustic properties.

In vivo targeting of acoustically reflective liposomes for intravascular and trans-vascular ultrasonic enhancement. Demos SM, Alkan-Onyuksel H, Kane BJ, Ramani K, Nagaraj A, Greene R, Klegerman M, McPherson DD. J Am Coll Cardiol 1999 Mar; 33 (3), pp. 867–75.

Arteriography/Atherosclerotic Plaque Deformation
Acetylcholine/Isosorbide Dinitrate (Vasodilation)

Cellular/Molecular Mechanism of Action: Acetylcholine functions as a parasympathetic/cholinergic neurotransmitter by physically binding to and activating its corresponding receptor complex.
Physiological Mechanism of Action: Isosorbide dinitrate promotes vasodilation, while acetylcholine promotes vasoconstriction.
Application: Visualize ultrasonic deformations of atherosclerotic plaques in human coronary arteries during vasoconstriction and vasodilatation.
Route of Administration: Acetylcholine (selective intravascular); isosorbide dinitrate (intracoronary injection).
Considerations: The cross-sectional area of the atherosclerotic plaque appears to be constant during vasomotion of human coronary arteries.
Interpretation and Analysis: Intravascular ultrasound can detect occult atherosclerosis in angiographically normal coronary arteries in instances of chest pain at rest (n = 8). During the acetylcholine provocative tests, intravascular ultrasound can be used to monitor deformations of the atherosclerotic plaques. During the last phase of evaluations, vasodilation can be promoted with the intracoronary injection of isosorbide dinitrate. Under control, acetylcholine-treated, and isosorbide dinitrate-treated conditions, the cross-sectional areas of sonolucent circle and vessel lumen can be measured to establish the area of atherosclerotic plaques. In the process of vasoconstriction and vasodilation, the plaque area does not change significantly.

Intravascular ultrasonic evidence by constant cross-sectional area of atherosclerotic plaques during coronary vasomotion in humans. Matsu-ura Y, Kijima Y, Hashimura K, Kato Y, Ueda T, Yasuda T, Mori I, Hoshida S, Hori M. Eur Heart J 1997 Jun; 18 (6), pp. 949–53.

Arteriography/Coronary Artery Blood Flow in Athletes
Microbubbles (Levovist)

Application: Quantitative myocardial contrast echocardiography imaging.
Route of Administration: Intravascular (selective intra-arterial delivery).
Implementation: Characterization of coronary artery blood flow in atheletes.
Interpretation and Analysis: Coronary microcirculation patterns in athletes are similar, but at higher levels compared to "control" cases. Training influences the development of physiologic left ventricular hypertrophy that counterbalances the dilatation in the left ventricular chambers because of the higher blood volume in athletes compared with control participants. Angiogenesis and several functional adaptations (relaxation of small coronary arteries, increased production of nitric oxide by the coronary endothelium, or both) represent the potential mechanisms that allow an optimal distribution of oxygen and of nutrient substrates to the hypertrophied myocardium of athletes.
Instrumentation: Harmonic power Doppler analysis using background subtraction. Quantitative analysis of echo-contrast can be performed off-line with PC software, obtaining the transit curves of microbubbles through the coronary capillary system.

The role of quantitative myocardial contrast echocardiography in the study of coronary microcirculation in athlete's heart. Di Bello V, Giorgi D, Bertini A, Talini E, Mengozzi G, Palagi C, Nardi C, Paterni M, Mariani M. J Am Soc Echocardiogr 2002 Jul; 15 (7), pp. 678–85.

Arteriography/Endothelium: ICAM-1 Expression/ Endothelial Dysfunction: Coronary Ischemia
Microbubbles

Background Information: Clinical signs and symptoms of coronary artery disease are predated in decades by endothelial dysfunction, an aberration in the vascular lining that permits the development and propagation of atherosclerotic lesions and vasomotor dysfunction in the arterial circulation. These ultimately lead to acute and chronic coronary ischemic syndromes. Other pathophysiologic scenarios encountered in clinical cardiology practice, such as cardiac transplant rejection and the period following coronary angioplasty or cardiac surgery, also are associated with endothelial dysfunction. Endothelial dysfunction parallels coronary risk factors and is potentially reversible, rendering early identification of the phenomenon a clinically important end point. Current methods for detecting endothelial dysfunction are limited.
Application: Characterization of vascular endothelial integrity based on the detection of alterations in the expression of surface membrane complexes, including intracellular adhesion molecule 1 (ICAM-1) using targeted ultrasound imaging techniques.
Route of Administration: Intravascular.
Considerations: Implications pertain to other disease processes beside coronary ischemia and encompass both diagnostic and therapeutic delivery strategies.
Implementation: Evaluation of endothelial cell viability and biology as a function of unique surface marker expression (e.g., ICAM-1).
Interpretation and Analysis: Myocardial contrast echocardiography using microbubbles targeted to bind to cell surface markers uniquely expressed by dysfunctional endothelial cells may offer an approach for the non-invasive detection of endothelial disease using clinical ultrasound imaging techniques.

Microbubble-endothelial cell interactions as a basis for assessing endothelial function. Villanueva FS, Klibanov A, Wagner WR. Echocardiography 2002 Jul; 19 (5), pp. 427–38.

Arteriography: Coronary Artery Microcirculation Regulation/Vasodilative and Perfusion Reserve
Colored Microspheres/Adenosine Phosphate

Cellular/Molecular Mechanism of Action: Adenosine phosphate promotes relaxation of vascular smooth muscle. Colored microspheres impede the transmission and reflect a fraction of ultrasound wave energy back in the direction of the detector/probe.
Application: Evaluate and characterize microvascular perfusion parameters.
Route of Administration: Adenosine phosphate (intravenous/rat), colored microspheres (intravenous).
Dosage: Adenosine phosphate (3 mg/kg/min).
Implementation: Measurement of perfusion parameters based on perfusion-sensitive T1 relaxation after magnetic spin labeling of water protons. Regional blood volume (RBV) is determined from steady state measurements of T1 before and after administration of an intravascular contrast agent. A colored microsphere technique can be used as a reference method for perfusion measurements. Regional blood volume and perfusion maps are obtained at rest and during administration of adenosine phosphate (3 mg/kg of body weight per minute).
Animal Model: Rat.
Interpretation and Analysis: At magnetic resonance imaging (MRI), perfusion during resting conditions is 3.5 mL/g/min $\pm$ 0.1 (standard error of measurement [SEM]), while RBV is 11.6% $\pm$ 0.6 (SEM). Adenosine phosphate significantly increases perfusion to 4.5 mL/g/min $\pm$ 0.3 (SEM) and decreases mean arterial pressure from 120 mm Hg to 65 mm Hg, which implies a reduction of coronary resistance of 40% relative to baseline values. Regional blood volume increases consistently to 23.8% $\pm$ 0.6 (SEM). Quantitative mapping of perfusion and regional blood volume can therefore be performed non-invasively *in vivo* by means of MRI. Such methods allow determination of vasodilative and perfusion reserve, which reflects the *in vivo* regulation of coronary microcirculation in response to a given stimulus.

Myocardial perfusion and intracapillary blood volume in rats at rest and with coronary dilatation: MR imaging *in vivo* with use of a spin-labeling technique. Waller C, Kahler E, Hiller KH, Hu K, Nahrendorf M, Voll S, Haase A, Ertl G, Bauer WR. Radiology 2000 Apr; 215 (1), pp. 189–97.

Arteriography: Coronary Artery/Myocardial Contrast Echocardiography (MCE)
SonoVue

Background Information: Myocardial contrast echocardiography has become a major field of clinical research in echocardiography in the last decade. The introduction of new contrast agents has been accompanied by a large variety of new contrast specific imaging techniques.
Route of Administration: SonoVue (intravascular).
Implementation: Intravascular injection of ultrasound contrast media (e.g., SonoVue) for visualization of vascular anatomical abnormalities affecting vessel structures, including disease affecting the coronary artery.
Analysis and Interpretation: Myocardial echocardiography imaging can be performed utilizing SonoVue as an enhancing agent for improved characterization of coronary artery disease (CAD) in clinical environments.

Clinical value of myocardial contrast echocardiography in chronic coronary artery disease. Tiemann K. Echocardiography 2002 Oct; 19 (7 Pt 2), pp. 635–9.

Arteriography: Coronary Artery/Myocardial Perfusion/Contrast Media Formulation for Echocardiography
Albumin/Dextrose/Deca-Fluorobutane

Route of Administration: Albumin/dextrose/deca-fluorobutane gas contrast media (PESDA: continuous intravascular infusion at 1 to 2 ml/min and titrated for best myocardial contrast); adenosine (intravenous bolus).
Dosage: PESDA contrast media (dependent upon diagnostic procedure of interest); adenosine (6–18 mg).
Semi-Synthetic Methods: Ultrasound contrast media formulation (PESDA: sonocated solution composed of albumin 20% [1 ml]; dextrose 5% [1 ml]; deca-fluorobutane gas [8 ml]).
Implementation: Utilization of PESDA as a positive contrast agent in (i) echocardiography for the evaluation of myocardial perfusion at rest and after bolus injection of adenosine; and (ii) coronary angiography (1 month lag phase between procedures; n = 81 cases; n = 64 males). Diagnostically, the left ventricular perfusion beds can be evaluated; they are usually of prime clinical interest in conditions of cardiac disease (e.g., n = 208 territories analyzed). Acquisition of images is established with triggered (1:1) 2nd harmonic.
Analysis and Interpretation: Coronary angiography is capable of detetecting flow limiting (> 75%; e.g., n = 70); and no-flow limiting lesions (e.g., n = 138). At rest, obvious myocardium contrast enhancement can be appreciated in at least 1 segment of a territory in all cases. After adenosine injection an unquestionable further increase in myocardial contrast can be observed in territories related to no-flow limiting lesions lasting < 10 seconds (e.g., 99% or n = 138 territories); and myocardial perfusion defects detected in territories related to flow limiting lesions (e.g., 97% or n = 68 territories). Usually false results are very low, as is the incidence of serious complications. Myocardial perfusion study with PESDA and adenosine protocol is a practical, safe, and accurate method for analyzing coronary flow reserve.

Study of coronary flow reserve with intravenous use of microbubbles (contrast echocardiography) and adenosine: Protocol for clinical application in patients suspected of having coronary heart disease. Morcerf F, Moraes A, Carrinho M, Dohmann HJ. Arq Bras Cardiol 2002 Mar; 78 (3), pp. 281–98.

Arteriography: Coronary Artery Stenosis/Myocardial Ischemia/Wall Motion
Levovist (Ultrasound Contrast Media)/Dipyridamole Stress Test/[^{99m}Tc]-SestaMIBI

Background Information: Power Doppler imaging is a method that can be used to perform myocardial contrast echocardiography (MCE) analysis. It can selectively evaluate signals coming from an ultrasound contrast agent, allowing myocardial perfusion studies.
Application: Detection and characterization of coronary artery stenosis and myocardial ischemia/perfusion.
Implementation: Evaluation of the relative accuracy of the noninvasive imaging modalities of power Doppler MCE, stress-echo wall motion, and nuclear scan imaging (single photon emission computed tomography [SPECT]) employed to assess (i) coronary artery stenosis; (ii) myocardial ischemia/perfusion; and (iii) responses during pharmacological stress, using coronary angiography as a reference standard. Power Doppler MCE images are acquired using continuous intravenous infusion of Levovist contrast agent. Echo wall motion images are acquired. Complementary diagnostic procedures can involve the injection of [^{99m}Tc]-sestaMIBI at peak stress in concert with the acquisition of stress SPECT images 30 minutes after diagnostic agent administration.
Human Model: Dipyridamole stress test (e.g., n = 25 subjects).
Analysis and Interpretation: Power Doppler MCE and SPECT show an 84% concordance (e.g., n = 21/25 cases; kappa = 0.67) for the detection of ischemia. Concordance based on coronary artery territories for normal perfusion vs fixed defects vs reversible defects is 92% (69% of 75; kappa = 0.81), with 100% for left anterior descending, 92% for right coronary artery, and 84% for circumflex. Power Doppler MCE has a lower sensitivity than SPECT (89% vs 100%) but higher specificity (100% vs 88%) for identifying stenotic (≥ 70%) coronary arteries as assessed by angiography. Echo wall motion analysis displays the lowest sensitivity (68%) with 100% specificity. Accuracy is 94% for both power Doppler MCE and SPECT, and 83% for wall motion analysis. Based on the nature of such findings, power Doppler MCE can function as a sensitive and specific method for identifying myocardial perfusion during pharmacological stress. Accuracy of power Doppler MCE for stenotic coronary arteries appears to be slightly higher than stress-echo wall motion and similar to SPECT.

Non-invasive detection of coronary artery stenosis: A comparison among power Doppler contrast echo, [^{99m}Tc]-sestaMIBI SPECT and echo wall motion analysis. Rocchi G, Fallani F, Bracchetti G, Rapezzi C, Ferlito M, Levorato M, Reggiani LB, Branzi A. Coron Artery Dis 2003 May; 14 (3), pp. 239–45.

Arteriography: Neoplasia/Hepatocellular Carcinoma: Response to Percutaneous Radio Frequency Abalation/Relative Effectiveness: 3-Phase Dynamic Computed Tomography (CT) vs Coded Harmonic Angiography
Levovist Microbubbles

Cellular/Molecular Mechanism of Action: Microbubbles have a different level of acoustic impedance compared to homogenous biological fluids. Upon contact, transmission of ultrasound energy is slowed, absorbed, and/or reflected away from the microbubbles, a fraction of which is detected by the probe/detector.
Application: Microbubbles can be utilized in contrast-enhanced coded phase-inversion harmonic sonography to assess the therapeutic response of percutaneous radio frequency ablation in conditions of hepatocellular carcinoma.
Route of Administration: Microbubbles (e.g., Levovist given by intravenous injection).
Implementation: Evaluation of hepatocellular carcinoma nodules applying coded harmonic angio involving coded phase-inversion harmonic sonography facilitated by the application of microbubble contrast image-enhancing agents before and after percutaneous radio frequency ablation. The intratumor blood vessels and tumor parenchymal can be detected in the early arterial phase and the late vascular phase, respectively. The results of contrast-enhanced imaging with coded harmonic angio can be compared with those of 3-phase dynamic CT.
Human Model: Cases of hepatocellular carcinoma nodules (e.g., n = 67 cases, n = 107 exams; n = 91 nodular lesions).
Analysis and Interpretation: A vast majority of hepatocellular carcinoma nodules are found to be hypervascular on contrast-enhanced imaging with coded harmonic angiography (e.g., n = 107/107). Following radio frequency ablation, contrast-enhanced coded harmonic angiography detects persistent signal enhancement of nodules (e.g., n = 41/107 or 38.3%), whereas the technique shows

no intratumoral enhancement in greater than half of examined nodules (e.g., n = 66/107 or 61.7%). Compared with dynamic CT, the sensitivity, specificity, and diagnostic accuracy of contrast-enhanced coded harmonic angio is approximately 95.3%, 100%, and 98.1%, respectively. With contrast-enhanced coded harmonic angio, it is difficult to identify the safety margin that can be detected on dynamic CT. Contrast-enhanced imaging with coded harmonic angio may provide an alternative approach that has high diagnostic agreement with dynamic CT in assessing the therapeutic effect of radio frequency ablation in hypervascular hepatocellular carcinomas, in spite of having limitations in identifying the safety margin.

Radio frequency ablation of hepatocellular carcinoma: Therapeutic response using contrast-enhanced coded phase-inversion harmonic sonography. Wen YL, Kudo M, Zheng RQ, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T, Maekawa K. AJR Am J Roentgenol 2003 Jul; 181 (1), pp. 57–63.

Arteriography: Neoplasia/Prostatic Carcinoma (PC-3m)/Vascular Structures: Vascular Density
Contrast Agent/[Protamine]

Cellular/Molecular Mechanism of Action: Influences the transmission of ultrasound energy by creating changes in impedance, absorption, or reflectance in a manner that is detectably different from most biological fluids (echogenic properties).
Application: Contrast media can be utilized to enhance Doppler ultrasound image quality and sensitivity for the purpose of detecting and characterizing tumor vascularity patterns.
Route of Administration: Intravenous.
Implementation: Evaluation of tissue density and vascular structures perfusing neoplastic lesions applying color flow signal-pixel rate (SPR) of images calculated using the number of pixels showing color Doppler signals as a ratio of total pixel number associated with the entire lesion.

Animal Model: Mice (BALB/c nude mice; n = 48) receiving subcutaneous transplants of human prostate carcinoma PC-3m tumor followed by protamine injections (SQ) as an angiogenesis inhibitor. Animals are randomly divided into 3 groups according to protamine dose.
Analysis and Interpretation: The SPR of the high- and low-dose groups is significantly lower than that of control groups ($p < 0.01$). Mean SPR ± standard deviation (SD) is 0.09 ± 0.05, 0.11 ± 0.05, and 0.22 ± 0.10 for different treatment groups, respectively. The SPR parameters correlate significantly with the density of microvessel structure ($r = 0.86–0.94$, $p < 0.01$). Contrast agent-enhanced Doppler ultrasound imaging can effectively reveal changes in vascularity in neoplastic lesions (e.g., ± protamine). In addition to microvessel density, contrast agent-enhanced Doppler ultrasound imaging may become 1 of several independent prognostic indexes of angiogenesis inhibitor therapy.

Evaluation of the effect of protamine on human prostate carcinoma PC-3m using contrast-enhanced Doppler ultrasound. Tang J, Li S, Li J, Zhang Y, Li X, Dong B, Shi H, Zhang G. J Urol 2003 Aug; 170 (2 Pt 1), pp. 611–4.

Arteriography: Splanchnic Hemodynamics
Levovist

Application: Enhancement of Doppler ultrasound acquired images of splanchnic hemodynamic properties.
Route of Administration: Levovist infused as an intra-arterial bolus.
Dosage: Levovist at 2.5 g/dose.
Implementation: Doppler ultrasound examination of portal vein and branches of the hepatic, splenic, and superior mesenteric arteries before and 5–8 and 12–15 minutes after the initiation of an 8-minute long infusion of image-enhancing contrast media (e.g., Levovist). Mean velocity and mean diameter are calculated for the portal vein. Resistance indexes are determined for individual arteries.

Human Model: Cases suffering from hepatic cirrhosis (e.g., n = 12) and healthy normal reference controls (e.g., n = 12).
Analysis and Interpretation: A significant increase in resistance index can be observed in the hepatic (0.80 ± 0.07 vs 0.71 ± 0.06; $p < 0.01$) and splenic arteries (0.72 ± 0.06 vs 0.64 ± 0.06; $p < 0.01$) 5–8 minutes after contrast agent injection in conditions of cirrhosis but not in controls. Neither portal vein diameter nor portal flow mean velocity change during test challenges in both controls and cirrhosis cases. This effect may be related to a selective trapping of microbubbles in the altered hepatic and splenic microvasculature in cirrhosis conditions rather than being an imaging artifact. Such phenomena may have implications regarding harmonic ultrasound protocols designed to image cirrhotic liver tissue in the early arterial phase.

Effect of Levovist on splanchnic hemodynamics in cirrhotic patients. Gaiani S, Serra C, Piscaglia F, Celli N, Rasciti L, Miglioli M, Bolondi L. Ultrasound Med Biol 2003 May; 29 (5), pp. 643–8.

Capillary Retention/Myocardial and Pulmonary Retention
Neutral vs Anionic Microbubbles (± Poly(Ethylene Glycol) [PEG])

Cellular/Molecular Mechanism of Action: Microbubbles do not transmit ultrasound energy to the same extent (degree) as do "homogeneous" biological fluids due to partial impedance, absorption, and reflectance phenomena ultimately detected by the ultrasound sensor/probe/detector.
Background Information: Interactions between serum proteins and lipid membranes are greater with anionic compared to neutral membranes. Such interactions may influence the microvascular behavior of anionic lipid microbubbles. Lipid microbubbles with a net negative charge can be retained within capillaries via complement-mediated attachment to the external membrane surface of vascular endothelium. This property may be useful for the development of ultrasound contrast agents that can be imaged late after venous injection.
Application: Enhanced detection and characterization of physiological conditions and pathological anomalies affecting tissues, organ systems, vascular structures, and body fluids utilizing ultrasound-based diagnostic modalities.
Route of Administration: Lipid-shelled microbubbles (bolus intravenous injection).
Semi-Synthetic Methods: Lipid-shelled microbubbles with either a neutral or a negative charge can be prepared either with or without a protective surface layer of PEG. Complement attachment to microbubbles can be assessed by flow cytometry with flourescein isothiocyanate-conjugated anti-C3b monoclonal antibody.
Implementation: Intravascular administration to delineate the influence of microbubble charge on microvascular retention of microbubble contrast agents.

Intravital microscopy can be performed on the cremaster muscle wild-type and C3-deficient mice during the intravenous injection of either anionic/negative or neutral-charged PEG lipid-shelled microbubbles. Pulmonary and myocardial retention of lipid-shelled microbubbles can be established by myocardial contrast echocardiography.

Analysis and Interpretation: Size-independent capillary retention of microbubbles, occurring for a few seconds to > 10 minutes, is frequently observed with anionic, but rarely with neutral, microbubbles (4.3 ± 0.3 mm^{-3} vs 0.4 ± 0.1 mm^{-3}, $p < 0.01$). Anionic microbubble retention is reduced by 70% by surface PEG and also markedly reduced in C3-deficient mice (1.4 ± 0.1 mm^{-3}, $p < 0.05$ vs wild-type). Flow cytometry demonstrates that complement binds only to anionic microbubbles. Contrast echocardiography reveals both pulmonary and myocardial retention of only anionic microbubbles, the latter appreciated as a function of persistent opacification > 10 minutes after bolus intravenous injection. Lipid microbubbles with a net negative charge can be retained within capillaries due to complement-mediated attachment to endothelial cell populations. Such properties may be useful for the development of ultrasound contrast agents that can be imaged late after venous injection.

Influence of microbubble surface charge on capillary transit and myocardial contrast enhancement. Fisher NG, Christiansen JP, Klibanov A, Taylor RP, Kaul S, Lindner JR. J Am Coll Cardiol 2002 Aug 21; 40 (4), pp. 811–9.

Cardiac Failure: Chronic/Inotropic Activity/Cardiac Output/Pressor Effect/Inotropic Agent Selection
Dobutamine/Carvedilol/Metoprolol

Cellular/Molecular Mechanism of Action: Dobutamine functions as a positive adrenergic (sympathomimetic) agent. Metoprolol is a selective β_1-adrenoreceptor antagonist (blocker), while carvedilol is a non-selective β-adrenoreceptor blocker.
Physiological Mechanism of Action: Dobutamine promotes positive cardiac inotropic responses while also promoting increases in cardiac output and increases in systemic blood pressure.
Application: Enhance the accuracy and sensitivity of stress echocardiography procedures and serve as a guide during the process of selecting (therapeutic) inotropic agents.
Route of Administration: Dobutamine (intravenous).
Dosage: Carvedilol and metoprolol CR/XL (target dose 50 mg twice daily and 200 mg once daily, respectively) for 8 weeks. Dobutamine (5 μg/kg/min and high-dose 15 μg/kg/min).
Implementation: Evaluation of carvedilol and metoprolol CR/XL as potential adjunct forms of intervention during dobutamine-stress echocardiography procedures performed at the end of each maintenance period. Parameters evaluated include determining if conditions of congestive heart failure display different hemodynamic responses during dobutamine challenge in the presence of β-adrenoreceptor blocking agents.

Human Model: Cases with stable chronic congestive heart failure (ejection fraction < 40%) on chronic treatment with metoprolol CR/XL (n = 10 subjects).

Study Design: Single center, single blind, randomized, 2-period crossover study comparing carvedilol with metoprolol CR/XL.
Interpretation and Analysis: No significant hemodynamic differences can be seen at rest after 2 treatments. There is a more pronounced increase in heart rate and cardiac output that occurs during dobutamine infusion when cases are given metoprolol compared to carvedilol. Mean arterial pressure increases significantly with carvedilol Cardiac output increases during low-dose dobutamine, without further changes being appreciated with high-dose dobutamine administration. During infusions of dobutamine, there is no significant difference in ejection fraction between carvedilol and metoprolol treatment. Therefore, during conditions of congestive heart failure on a non-selective β-adrenoreceptor blocker or β_1-selective blocker respond differently to the inotropic drug dobutamine. Selective β_1 blockade with metoprolol can be counteracted by dobutamine, whereas with carvedilol a low dose of dobutamine increases cardiac output, and a higher dose of dobutamine initiates a pressor effect. These findings may be clinically relevant when choosing an inotropic pharmaceutical agent.

Different responses to dobutamine in the presence of carvedilol or metoprolol in patients with chronic heart failure. Bollano E, Täng MS, Hjalmarson A, Waagstein F, Andersson B. Heart 2003 Jun; 89 (6), pp. 621–4.

Cardiac Shunts: (Right to Left)
Echovist 200/Echovist 300

Cellular/Molecular Mechanism of Action: Echovist 200 and Echovist 300 are agents that impede/absorb/reflect ultrasound energy.
Background Information: Cardiac right-to-left shunts (RLS) can be identified by trans-esophageal echocardiography (TEE) as well as by trans-cranial Doppler ultrasound (TCD) using contrast agents, such as Echovist 200 or Echovist 300, in conjunction with a Valsalva maneuver (VM) as a provocation procedure. Both Echovist preparations are in clinical use. Currently, the appropriate timing of the VM is controversial.
Application: Characterization of cardiac RLS.
Route of Administration: Intravascular.
Implementation: Evaluation of TEE and bilateral TCD as modalities for characterizing the middle cerebral arteries. One approach for objective assessment can entail the application of randomized protocols including (i) no utilization of VM; (ii) VM for 5 seconds starting with the beginning of Echovist 300 injection; (iii) VM for 5 seconds starting 5 seconds after the beginning of Echovist 300 injection; (iv) VM for 5 seconds starting 10 seconds after the beginning of Echovist 300 injection; and (v) VM for 5 seconds starting 5 seconds after the beginning of Echovist 200 injection.

Human Model: Cases with right-to-left shunts (n = 64).
Interpretation and Analysis: Cardiac RLS can be demonstrated by both TEE and contrast TCD in a relatively large percentage of cases (n = 27/64 shunt-positive cases).

Conversely, a relatively large number of cases are negative for both TEE and TCD analyses (e.g., n = 22/64). A very small percentage of cases are TEE positive, but TCD negative (e.g., n = 0/64). Alternatively, a considerable number of cases will be only positive on at least 1 TCD investigation but negative on TEE (e.g., n = 15/64). Evaluation of methods employing VM for 5 seconds starting 5 seconds after the beginning of Echovist 300 injection, or VM for 5 seconds starting 5 seconds after the beginning of Echovist 200 injection, are diagnostically the most appropriate with the former procedures being slightly superior. Trans-cranial Doppler ultrasound using Echovist 300 or Echovist 200 is a sensitive method to identify TEE proven cardiac RLS. To achieve the best diagnostic accuracy, the VM should be performed for a duration of 5 seconds starting at 5 seconds following the beginning of contrast injection.

Contrast trans-cranial Doppler ultrasound in the detection of right-to-left shunts: Comparison of Echovist 200 and Echovist 300, timing of the Valsalva maneuver, and general recommendations for the performance of the test. Droste DW, Jekentaite R, Stypmann J, Grude M, Hansberg T, Ritter M, Nabavi D, Nam EM, Dittrich R, Wichter T, Ringelstein EB. Cerebrovasc Dis 2002; 13 (4), pp. 235–41.

Cardiomyopathy: Idiopathic Dilated (IDCM)/ Prognostic Parameters
Dobutamine/Low-Dose Dobutamine Echocardiography

Physiological Mechanism of Action: Dobutamine exerts adrenergic agonist properties.

Background Information: Dobutamine echocardiography is widely used for the evaluation of myocardial contractile reserve.
Application: The response of the left ventricle to low-dose dobutamine infusion adds clinically valuable prognostic information for the evaluation of IDCM.
Dosage: Dobutamine (10 μg/kg/min for 5 minutes).
Implementation: Evaluate the prognostic value of low-dose dobutamine echocardiography in conditions of IDCM. Image analysis can entail measurement of 2-dimensional and Doppler echocardiographic variables before and after the infusion of dobutamine.

Human Model: Cardiovascular disease (n = 77 consecutively diagnosed cases of IDCM; left ventricular (LV) ejection fractions of < 40%; age range = 49 ± 9 years mean ± standard deviation [SD]; men = 82%; n = 30/77 died; n = 5/77 successful heart transplant over a 63 ± 7-month follow-up period within a 49- to 75-month range).
Interpretation and Analysis: Using multivariate regression analysis, the only significant factors related to fatal outcome or the need for cardiac transplantation include the following: (i) LV end-systolic volume of > 150 mL after low-dose dobutamine infusion (odds ratio [OR], 2.2; confidence interval [CI], 1.2 to 4.1; p = 0.011); (ii) no decrease of LV end-diastolic volume after dobutamine infusion (OR, 1.9; CI, 1.1–3.4; p = 0.031); (iii) atrial fibrillation (OR, 2.7; CI, 1.4–5.3; p = 0.003); and (iv) male gender (OR, 2.6; CI, 1.2–5.5; p = 0.017). A scoring system has been proposed with 1 point assigned for each of the above-mentioned factors. The mortality rates for total scores of 0, 1, 2, 3, and 4 are approximately 0%, 19%, 48%, 83%, and 100%, respectively. The response of the LV to low-dose dobutamine infusion adds clinically valuable prognostic information to the evaluation of IDCM conditions.

Prognostic value of low-dose dobutamine echocardiography in patients with idiopathic dilated cardiomyopathy. Drozdz J, Krzemińska-Pakula M, Plewka M, Ciesielczyk M, Kasprzak JD. Chest 2002 Apr; 121 (4), pp. 1216–22.

Cardiomyopathy: Ischemic/Low-Dose Wall Motion Scores: Risk Stratification/Prediction of Benefit from Revascularization
Dobutamine

Physiological Mechanism of Action: Dobutamine exerts adrenergic/sympathetic agonist properties.
Application: Evaluation of dobutamine low-dose wall motion scores for estimating risk stratification of ischemic cardiomyopathy conditions and identification of those who do and do not benefit from revascularization.
Route of Administration: Dobutamine (intravascular).
Dosage: Dobutamine (low-dose = 10 μg/kg/min and high-dose/maximal dose 50 μg/kg/min) infusion.
Implementation: Delineation of the value of wall motion scores at rest and with low- and high-dose dobutamine infusion for predicting the outcome and benefit associated with revascularization in conditions with ischemic cardiomyopathy. Complementary follow-up evaluation entails assessment of ischemic cardiomyopathy cases utilizing echocardiography analyses acquired at rest and during low-dose and high-dose/maximum dobutamine infusion.

Human Model: Cases of ischemic cardiomyopathy (n = 139).
Interpretation and Analysis: Both rest and low-dose wall motion scores are multivariate predictors of cardiac death, but ischemia and peak dose scores are not appropriate predictors. Cases can be classified into resting condition scores that have been risk stratified into 3 groups, including (i) score (1.00–1.99) with 11% cardiac death; (ii) score (2.00–2.49) with 30% death; and (iii) score ≥ 2.50 with 47% death. One-third of cases with rest scores ≥ 2.50 have improvement in scores to < 2.50 with low-dose dobutamine. Frequency of cardiac death is reduced to 23% compared with 60% (p = 0.04) in those that remain with low-dose scores ≥ 2.50. Low-dose scores also can identify those cases who benefited from revascularization. In casess with low-dose scores (1.00–1.99), the frequency of cardiac death is marginally lower in revascularized than in non-revascularized conditions (10% vs 21%, p = 0.28). In cases with scores (2.00 to 2.49), revascularized conditions have a significantly lower frequency of cardiac death than non-revascularized cases (15% vs 41%, p < 0.05). The frequency of death in those with low-dose scores ≥ 2.50 is very high in both revascularized (75%) and non-revascularized (56%, p = 0.42) conditions.

Usefulness of rest and low-dose dobutamine wall motion scores in predicting survival and benefit from revascularization in patients with ischemic cardiomyopathy. Sawada SG, Lewis SJ, Foltz J, Ando A, Khouri S, Kaser S, Gradus-Pizlo I, Gill W, Fineberg N, Segar D, Feigenbaum H. Am J Cardiol 2002 Apr 1; 89 (7), pp. 811–6.

Cardiovascular Functional Reserve/Global Ventricular Function/Tei Index
Dobutamine-Stress Echocardiography

Cellular/Molecular Mechanism of Action: Dobutamine exerts adrenergic/sympathomimetic properties.
Background Information: Dobutamine stress echocardiography has become an accepted approach for the assessment of cardiac functional reserve. Although the Doppler-derived index of combined systolic/diastolic myocardial performance (Tei index) has been described as being easily obtainable and useful for predicting left ventricular performance, the effect of dobutamine on the Tei index has not been thoroughly established in children.
Application: Dobutamine can be applied in combination with diagnostic ultrasound to characterize various aspects of cardiac physiology (e.g., ventricular function).
Route of Administration: Dobutamine (infusion).
Dosage: Dobutamine (5 μg/kg per minute).
Implementation: Assess the effect of dobutamine on the Tei index in cases of postoperative ventricular septal defect and Kawasaki disease. Echocardiographic recordings are obtained before and after dobutamine infusion. Variables measured include trans-mitral flow velocities (E, A, E/A), rate-corrected mean velocity of circumferential fiber shortening (rate-corrected Vcf), and idiopathic mitral prolapse (IMP). Isovolumic contraction time (ICT), isovolumic relaxation time (IRT), and ejection time (ET) are measured and then applied to calculate the Tei index using the following formula: Tei index = (ICT + IRT)/ET.

Human Model: Postoperative ventricular septal defect (n = 8 cases); and cases of Kawasaki disease (n = 7).
Interpretation and Analysis: Dobutamine infusion increases rate-corrected Vcf (29%, p < 0.01), peak E (7%, p < 0.05), and peak A (13%, p < 0.05). E/A ratios do not change during dobutamine infusion. ET, ICT, and IRT decrease during dobutamine infusion. The magnitude of the change in ICT (−21%, p < 0.01) is greater than that in IRT (−12%, p < 0.01) and ET (−8%, p < 0.05). The decrease in Tei index (−16%, p < 0.01) results in a decrease in ICT/ET (−21%, p < 0.01) and a slight decrease in IRT/ET (−12%, p < 0.05). The Tei index has potential as a sensitive indicator of the effects of inotropic stimulation on global left ventricular function.

Effect of dobutamine on a Doppler echocardiographic index of combined systolic and diastolic performance. Harada K, Tamura M, Toyono M, Yasuoka K. Pediatr Cardiol 2002 Nov–Dec; 23 (6), pp. 613–7.

Cardiovascular Thrombi
Ligand-Targeted Lipid-Encapsulated Perfluorodichlorooctane/Perfluorohexane (PFH)/ Perfluorooctane (PFO)/Perfluorooctyl Bromide (PFOB)/ Perfluorodichlorooctane (PFDCO)/Perfluorodecalin (PFD)

Background Information: Targeted acoustic contrast agents offer the potential for sensitive ultrasonic detection of pathologic tissues. Previous reports have described the development of a ligand-targeted, lipid-encapsulated, liquid PFDCO ultrasonic contrast system with a small nominal particle size (approximately 250-nm diameter). Perfluorocarbon nanoparticles substantially increase reflectivity when bound to targeted surfaces, and it has been proposed that such a system can be approximated physically as a simple, thin layer, acoustic transmission line.
Application: Ultrasound detection of intravascular thrombi (reference model).
Route of Administration: Intravascular.
Implementation: Evaluate the ultrasonic reflectivity of different perfluorocarbon formulations with widely varying acoustic impedances that have been "targeted" to either nitrocellulose membranes or *in vitro* plasma thrombi. Examples of 5 perfluorocarbons include PFH, PFO, PFOB, PFDCO, while PFD. Ultrasonic reflection can be measured by acoustic microscopy (17 MHz to 35 MHz).
Interpretation and Analysis: Acoustic reflectivity is increased ($p < 0.05$) by all targeted perfluorocarbon formulations, and the magnitude of the contrast effect is inversely correlated with the perfluorocarbon acoustic impedance. Perfluorohexane nanoparticles exhibit the greatest enhancement, while PFD nanoparticles show the least. The acoustic transmission line model effectively predicts the relative differences in acoustic reflectivity and frequency dependence among different perfluorocarbon formulations. For future clinical applications, PFO nanoparticles may provide the best combination of acoustic enhancement, *in vivo* physical stability, and safety.

Improvements in the ultrasonic contrast of targeted perfluorocarbon nanoparticles using an acoustic transmission line model. Marsh JN, Hall CS, Scott MJ, Fuhrhop RW, Gaffney PJ, Wickline SA, Lanza GM. IEEE Trans Ultrason Ferroelectr Freq Control 2002 Jan; 49 (1), pp. 29–38.

Catheter Placement Validation
Levovist Microbubble Contrast Agent

Background Information: Intraperitoneal port catheter systems for local delivery of cytotoxic drugs require imaging prior to chemotherapy to confirm homogenous distribution of an injected fluid in the entire peritoneal cavity.
Application: Image analysis to validate correct placement of intraperitoneal port catheter systems such as those applied in the delivery of cytotoxic pharmaceuticals.
Route of Administration: Levovist (infusion as a bolus).
Implementation: Ultrasound examinations are performed after bolus injection of microbubble contrast medium (e.g., Levovist). Distribution of the contrast medium in the peritoneal cavity is imaged using B-mode ultrasound (US) and color-stimulated acoustic emission imaging (SAE). Contrast-enhanced computed tomography (CT) imaging can be used as a validation reference control for evaluating the accuracy of ultrasound images.

Human Model: Peritoneal carcinoma ($n = 12$ cases) implanted with intraperitoneal port catheter system prior to the initiation of chemotherapy administration.
Analysis and Interpretation: Distribution of microbubbles in the peritoneal cavity can be easily detected by both US methods. Free distribution in all abdominal quadrants can be visualized with both US techniques in most cases (e.g., $n = 10/12$). In a small number of cases, CT and US reveal contrast medium limited to the perihepatic area (e.g., $n = 2/12$, requiring surgical catheter repositioning). Ultrasound after intraperitoneal injection of a microbubble contrast agent provides reliable information about the distribution of intraperitoneally injected fluid in the peritoneal cavity. This method is therefore well suited for imaging port catheter systems prior to chemotherapy.

Intraperitoneal distribution of ultrasound contrast medium imaged with B-mode ultrasound and color-stimulated acoustic emission imaging. Puls R, Gebauer B, Hildebrandt B, Riess H, Herrmann M, Hosten N, Albrecht T. Eur Radiol 2003 Apr; 13 (4), pp. 695–9. Epub 2002 Sep 07.

Myocardial Akinesis Postinfarction
Perflutren Microbubbles: Myocardial Contrast Echocardiography (MCE)/Dobutamine Echocardiography (DE)

Background Information: Akinesia after acute myocardial infarction (AMI) may be reversible or irreversible. Distinguishing these 2 entities early after AMI is difficult, but clinically important. Previous studies have shown that MCE and low-dose DE may both be useful in this setting. However, there are few data regarding the relative and combined value of these techniques.
Implementation: Evaluate and compare the utility of real-time intravenous MCE and low-dose DE in the early prediction of akinetic myocardial functional recovery following an AMI episode. Real-time MCE can be performed using perflutren microbubbles followed immediately by performing low-dose DE analysis. Contrast opacification and wall motion are determined by experienced observers blinded to clinical data. Repeat echocardiograms are obtained 51 ± 19 days later and wall motion at rest is scored by an observer blinded to clinical data.

Human Model: Cases of AMI ($n = 37$ at 3 ± 2 days after AMI).
Interpretation and Analysis: Normal contrast opacification can predict functional recovery with a positive predictive value of 63%, a negative predictive value of 73%, and an accuracy of 66%. Residual contractility during low-dose DE can have a positive predictive value of 82%, a negative predictive value of 72%, and a predictive accuracy of 76%. When the 2 tests are concordant (64%), they have a positive predictive value of 81%, a negative predictive value of 85%, and a predictive accuracy of 83%. Low-dose DE is superior to intravenous MCE in the prediction of functional recovery of akinetic myocardium after AMI, but the combination of both maximizes prediction accuracy.

Comparison of intravenous myocardial contrast echocardiography and low-dose dobutamine echocardiography for predicting left ventricular functional recovery following acute myocardial infarction. Hillis GS, Mulvagh SL, Pellikka PA, Hagen ME, Gunda M, Wright RS, Oh JK. Am J Cardiol 2003 Sep 1; 92 (5), pp. 504–8.

Myocardial Akinesis (Post–Acute Infarction)/ Independent Predictor of Functional Recovery/ Left Anterior Descending Coronary Artery Segment
Octafluoropropane Microbubbles/Myocardial Contrast Echocardiography (MCE)

Background Information: Akinesia after acute myocardial infarction (MI) may be reversible, secondary to stunning, or irreversible, as a result of extensive myocyte necrosis. Distinguishing these 2 entities soon after MI is difficult but has important clinical implications.

Application: Myocardial contrast echocardiography.
Route of Administration: Intravenous infusion.
Implementation: Evaluation of the effectiveness of MCE (real-time mode) in combination with the intravenous infusion of octafluoropropane microbubbles to assess myocardial perfusion. Repeated echocardiograms can be reevaluated 56 (± 29) days following initial MI episodes.

Human Model: Cases of myocardial infarction (n = 35 at 2 ± 1 days after an acute episode [e.g., n = 31/35 or 91% underwent myocardial revascularization]).
Interpretation and Analysis: Development of normal perfusion profiles predicts functional recovery with a positive predictive value of 66% and a negative predictive value of 81%. The accuracy of the technique is superior in myocardial segments supplied by the left anterior descending coronary artery (positive and negative predictive value: 70% and 90%, respectively). In multivariable analysis, the mean MCE perfusion score in akinetic segments is the most powerful independent predictor of functional recovery (odds ratio 8.6, p = 0.02). Such findings suggest that real-time intravenous MCE is a useful predictor of functional recovery of akinetic myocardium after acute MI.

Contrast echocardiography using intravenous octafluoropropane and real-time perfusion imaging predicts functional recovery after acute myocardial infarction. Hillis GS, Mulvagh SL, Gunda M, Hagen ME, Reeder GS, Oh JK. J Am Soc Echocardiogr 2003 Jun; 16 (6), pp. 638–45.

Myocardial/Border Detection of Endocardium Postischemia/Fundamental and Harmonic Imaging/ Stress Echocardiography
Sulfur Hexafluoride Microbubbles: (Stabilized)/ Dobutamine Stress

Background Information: Although stress echocardiography has been established as a diagnostic technique for the detection and assessment of ischemia, there are still a number of limitations to the application of the technique. These are related to suboptimal image quality with poor visualization of endocardial borders. Because assessment of wall motion is fundamental to the diagnostic value of stress echocardiography (both pharmacologic and exercise), endocardial border visualization is of the utmost clinical importance. Furthermore, interinstitutional observer agreement for image interpretation can approach 100% in cases that have high-quality images, compared to a cumbersome 43% level of agreement for cases that have images of a lower quality.
Application: Decreasing the number of redundant diagnostic tests used in the evaluation of myocardial function (e.g., postischemic insult).
Route of Administration: Intravascular.
Considerations: Harmonic imaging improves visualization of endocardial borders at rest and during dobutamine stress tests. However, there is room for improvement. Since the introduction of ultrasound contrast agents, contrast has been increasingly used for better endocardial border visualization. Data from centers that conduct a large number of stress echocardiography tests have shown that the addition of contrast agents decreases redundant diagnostic testing.
Implementation: Evaluation of myocardial dynamic function.
Interpretation and Analysis: Data obtained by some centers from subsets of clinical cases administered SonoVue (stabilized sulfur hexafluoride microbubble contrast agent; SF_6 = inert gas) show an improvement in the number of evaluatable segments with fundamental and harmonic imaging, and in endocardial border detection during dobutamine stress echocardiography. Contrast also enables future quantitative analysis using acoustic quantification (AQ) and color kinesis. These studies should be carried out now since the contrast agent has been approved for clinical application.

Usefulness of ultrasound contrast for image enhancement during stress echocardiography. Ten Cate FJ. Echocardiography 2002 Oct; 19 (7 Pt 2), pp. 621–5.

Myocardial Contrast Echocardiography (MCE)

Myocardial contrast echocardiography has undergone many advances in the past several years through remarkable developments in contrast agent and ultrasound instrumentation/technology. Microbubble ultrasound contrast agents can now safely transit the pulmonary circulation to provide opacification of the left ventricular cavity, improve endocardial border definition, and detect myocardial perfusion. The role of contrast echocardiography in enhancing technically difficult images is now well established in clinical practice and has proven especially useful in the stress and intensive care unit settings. Major progress has been made in the application of MCE for myocardial perfusion assessment in acute and chronic ischemic heart disease syndromes. Advances in novel applications of contrast echocardiography, including targeted delivery of genetic and pharmaceutical materials, have also occurred, but remain in a preclinical phase. The combination of recent innovations in ultrasound equipment and microbubble acoustics allows for exciting exploration of the expanding role of contrast echocardiography in clinical practice.

Recent advances in myocardial contrast echocardiography. Gunda M, Mulvagh SL. Curr Opin Cardiol 2001 Jul; 16 (4), pp. 231–9.

Myocardial Function/Ventricular Physiology
Dobutamine/Nitroglycerin

Cellular/Molecular Mechanism of Action: Dobutamine is an adrenergic agonist/sympathomimetic agent.
Physiological Mechanism of Action: Nitroglycerin exerts vasodilatory properties.
Background Information: Dobutamine-inducible left ventricular obstruction lacks a sound clinical meaning. This phenomenon may be related to an abnormal response of the heart to the sympathetic stimulation, and head-up tilt test would elicit intraventricular obstruction in cases known to develop it during dobutamine administration, through the synergistic effects of reduced preload, hypercontractility, and reflex increase in the cardiac sympathetic tone.
Application: Dobutamine echocardiography analyses for the evaluation of ventricular function.
Route of Administration: Dobutamine (infusion) and nitroglycerin (infusion).
Dosage: Dobutamine and nitroglycerin (0.3 mcg/kg/min).
Implementation: Evaluation of dobutamine stress-echocardiography and 60° head-up tilt on echocardiographic and electrocardiographic monitoring in drug-free and nitroglycerin infusion (0.3 mcg/kg/min) protocols. Doppler echocardiographic measurements of left ventricular diameters, function, and outflow tract flow velocity can be acquired in addition to assessment of power heart rate variability analyzed on short electrocardiographic recordings (low/high frequency ratio). In addition, plasma catecholamines can be determined at baseline and during tilt maneuvers.

Human Model: Normal coronaries (n = 21; of which n = 12 cases with left ventricular obstruction during dobutamine stress-echocardiography [n = 7/12 males, aged 61 ± 7 years]; n = 9 failed to develop complications [n = 3 males, aged 60 ± 9 years]).

Interpretation and Analysis: Intraventricular obstruction does not develop in cases with complications during dobutamine stress electrocardiography. Baseline differences can be appreciated in ventricular dimensions and mitral early-to-late velocity ratio between cases that have or have not developed complications during dobutamine stress electrocardiography procedures. However, hemodynamic behavior during either tilt maneuver is similar. The low/high frequency ratio, that is similar at baseline and similarly increased on head-up tilt, increases significantly less on nitroglycerin-tilt in cases that develop complications during dobutamine electrocardiography procedures. Differences in plasma catecholamines can usually not be appreciated.

Dobutamine-inducible left ventricular obstruction: Head-up tilt and autonomic balance. Barletta G. Int J Cardiol 2003 Feb; 87 (2–3), pp. 245–52.

Myocardial Function/Ventricular (Left) Dysfunction: Chronic/Ventricular Wall Motion Score Index
Glucose-Insulin-Potassium (GIK)/Dobutamine

Background Information: Glucose-insulin-potassium infusion improves cardiac function and outcome during acute ischemia.
Application: Characterization of chronic left ventricular dysfunction as a function of ventricular wall motion score index parameters.
Route of Administration: Dobutamine (intravenous) and GIK (4-hour intravenous infusion).
Implementation: Evaluation of the capacity for GIK infusion to benefit conditions of chronic ischemic left ventricular dysfunction and determination of whether there is a relationship to the presence and integrity of viable myocardium. In this manner dobutamine echocardiography can be combined with a 4-hour infusion of GIK. Segmental responses are quantified by improvement in wall motion score index (WMSI) and peak systolic velocity using tissue Doppler. Global responses are assessed by left ventricular volume and ejection fraction, measured using 3-dimensional reconstruction methods. Myocardial perfusion is also determined using contrast echocardiography.

Human Model: Chronic ischemic left ventricular dysfunction ($n = 30$ cases).
Interpretation and Analysis: Ventricular WMSI (mean $\pm$ standard deviation [SD]) improves with dobutamine (from 1.8 ± 0.4) to 1.6 ± 0.4), $p < 0.001$) and with GIK (from 1.8 ± 0.4 to 1.7 ± 0.4, $p < 0.001$); there is a similar increment for both. Improvement in wall motion score with GIK may be observed in 55% of the 62 segments classed as viable by dobutamine echocardiography, and in 5% of 162 classed as non-viable. There can be an increment in peak systolic velocity after both dobutamine echocardiography (from 2.5 ± 1.8 to 3.2 ± 2.2 cm/s, $p < 0.01$) and GIK (from 3.0 ± 1.6 to 3.5 ± 1.7 cm/s, $p < 0.001$). The GIK effects are not mediated by changes in pulse, mean arterial pressure, lactate, or catecholamines, nor do they correlate with myocardial perfusion. End systolic volume improves after GIK ($p = 0.03$), but only in cases that have a viable myocardium detected by dobutamine echocardiography. Therefore, in cases with a viable myocardium and chronic left ventricular dysfunction, GIK improves wall motion score, myocardial velocity, and end systolic volume, independent of effects on hemodynamics or catecholamines. Such a response to GIK is observed in areas of normal and abnormal perfusion assessed by contrast echocardiography.

Effects of glucose-insulin-potassium infusion on chronic ischaemic left ventricular dysfunction. Khoury VK, Haluska B, Prins J, Marwick TH. Heart 2003 Jan; 89 (1), pp. 61–5.

Myocardial Function/Ventricular (Left) Dysfunction: Halothane-Induced/Acute Coronary Occlusion/ Pericardial Effusion/Echocardiography-Calculated Ejection Fractions
Optison/Phased-Array Intracardiac Echocardiography/ Ultrasound (ICE) Catheter

Application: Phased-array ICE catheter may be a useful clinical tool for the diagnosis of heart failure, ischemia, tamponade, and pulmonary embolism.
Route of Administration: Optison (intracardiac/intravascular applying an ICE catheter system).
Implementation: ICE catheter-facilitated images are obtained from the right atrium during (i) acute left ventricular dysfunction; (ii) acute coronary occlusion; (iii) pericardial effusion and tamponade; and (iv) pulmonary embolism.

Experimental Model: Halothane-induced left ventricular dysfunction and pericardial effusion induced by direct injection/ infusion of saline into the pericardium.
Analysis and Interpretation: In experimental halothane-induced left ventricular dysfunction, echocardiography-calculated ejection fractions reveal declines from $47\% \pm 11\%$ to $25\% \pm 10\%$ ($p < 0.01$). Regional contraction abnormalities after acute coronary occlusion are identified without and with ultrasound contrast agent (e.g., Optison). Pericardial effusion produced by saline infusion into the pericardium can be detected in amounts as small as 15 mL. Right ventricular and atrial compression and respiratory variation in right ventricular inflow during tamponade can be demonstrated on images. After injection of intravenous thrombin to create venous thromboembolism, right ventricular dilatation/dysfunction and thrombi attached to the tricuspid and pulmonary valves and within the pulmonary artery can be identified.

Phased-array intracardiac echocardiographic imaging of acute cardiovascular emergencies: Experimental studies in dogs. Yamada E, Zhang Y, Davies R, Coddington W, Kerber RE. J Am Soc Echocardiogr 2002 Oct; 15 (10 Pt 2), pp. 1309–14.

Myocardial Function/Ventricular (Left) Function/ Poor Thoracic Window Visualization: Chronic Obstructive Pulmonary Disease (COPD)/ Mechanical Ventilation/Positioning
Ultrasound Contrast Media

Application: Enhancement of echocardiographic imaging in cases with a poor thoracic window to cardiovascular structures due to COPD, mechanical ventilation, or suboptimal positioning.
Dosage: Contrast media (1–2 mls).
Implementation: Echocardiographic evaluation of left ventricular function. Outcome measure: The total scores for the left ventricle (LV) in the 2-chamber and 4-chamber views are calculated at baseline and following injection of 1 to 2 mL of a contrast agent. The mean numbers of segments visualized in all cases at baseline and after injection of contrast agent are compared to assess any improvements in anatomical visualization of the heart (e.g., $n = 40$ cases).
Analysis and Interpretation: The endocardium can be adequately visualized to assess LV function in a relatively small percentage of cases admitted to an intensive care unit (ICU) service (e.g., 37% or $n = 15/40$ cases). In population subgroups where the endocardium is only poorly visualized, the simultaneous administation of contrast media is necessary in efforts to acquire suitable diagnostic images (e.g., 63% or $n = 25/40$). Such cases display a rela-

tively low average baseline segmental score of approximately 4.5 compared to a value of 11.6 in cases receiving the benefit of contrast image-enhancement media. Of the population of cases with poor image acquisitions, a relatively large percentage have average baseline segmental scores of 3.9 (e.g., n = 19/25) and display non-diagnostic images. However, the vast majority of these cases following the administration of IV contrast media will convert and display diagnostic images with an average of 11.6 segments visualized. Use of echocardiographic contrast agents in selected patients with poor baseline echocardiographic windows in the ICU setting significantly enhances segmental LV visualization and yields 100% conversion from non-diagnostic to diagnostic studies.

Contrast microbubbles improve diagnostic yield in ICU patients with poor echocardiographic windows. Nguyen TT, Dhond MR, Sabapathy R, Bommer WJ. Chest 2001 Oct; 120 (4), pp. 1287–92.

Myocardial Function/Left Ventricular (LV) Opacification
Octafluoropropane-Filled Human Albumin Microbubbles/Air-Filled Human Albumin Microbubbles

Cellular/Molecular Mechanism of Action: Microbubbles variably impede, absorb, and reflect ultrasound energy in a manner that is different from surrounding tissues or biological fluids.

Background Information: Echocardiographic contrast enhancement of the LV has diagnostic value in the assessment of regional and global LV function. The efficacy of both octafluoropropane-filled human albumin microbubbles (OCTA) and of air-filled human albumin microbubbles (AIR) for LV endocardial delineation and qualitative LV opacification has been reported. However, pulmonary disease, obesity, impaired LV function, and decreased echogenicity may diminish the efficacy of contrast agents for LV opacification.

Application: Octafluoropropane-filled human albumin microbubbles (OCTA: 0.5 mL) and air-filled human albumin microbubbles can be applied as positive contrast image-enhancing agents for ultrasonography procedures.

Dosage: Octafluoropropane-filled human albumin microbubbles (OCTA: 0.5 mL); air-filled human albumin microbubbles (AIR: 0.22 mL/kg).

Implementation: Compare quantitative LV opacification provided by octafluoropropane-filled human albumin microbubbles (OCTA: 0.2, 0.5, 3.0, 5.0 mL) vs air-filled human albumin microbubbles (AIR: 0.08 mL/kg, 0.22 mL/kg) during videodensitometry procedures. Changes in videodensity from non-contrast to contrast agent with the same gain settings can be determined at end diastole and end systole (gray scale 0–255 U) for 2 regions of interest: LV apex-to-mid-cavity; and mid-cavity-to-base. The relative influence of clinically evident pulmonary disease, impaired LV function on echocardiography, and echogenicity on LV opacification produced by both contrast agents can be determined by multivariate analysis.

Human Model: (i) n = 199 cases of suspected cardiac dysfunction; 59.2 ± 13.3 years, n = 157/199 or 79% males; (ii) n = 74 with impaired LV function, pulmonary disease, or both; (iii) n = 70 obese with a body mass index > 30 kg/m^2; (iv) n = 45 non-echogenic (≥ 4 of 6 endocardial segments not seen in the apical 4-chamber view).

Analysis and Interpretation: Significant videodensity increases ranging from 67% to 143% are observed with both agents. At the recommended initial doses (0.5 mL for OCTA, 0.22 mL/kg for AIR), OCTA produces greater opacification than AIR in both regions of interest and at both phases of the cardiac cycle. Poor LV function is associated with decreases in LV opacification for AIR but not for OCTA. Diminished echogenicity is more strongly associated with impaired opacification for AIR than for OCTA. Obesity and clinically evident pulmonary disease are associated with diminished chamber opacification with both OCTA and AIR. In addition to the superiority of octafluoropropane-filled microspheres to air-filled microspheres for LV opacification, the efficacy of OCTA is relatively unaffected by impaired LV function and is less susceptible to the effects of poor echogenicity than AIR.

Differing susceptibility of echocardiographic contrast agents to adverse effects of biologic factors: Multicenter, videodensitometric comparison of octafluoropropane-filled microspheres with air-filled microspheres for left ventricular opacification. Hausnerova E, Gottdiener JS, Gould JJ, Pezzullo JC, Callahan JD, Cohen JL, Bruns DE, Kuvelas MT, West HE, Dittrich HC. J Am Soc Echocardiogr 1999 Nov; 12 (11), pp. 957–66.

Myocardial Function/Ventricular Wall Motion: Post–Heart Transplant/Myocardial Contractile Response
Dipyridamole

Background Information: Coronary allograft vasculopathy (CAV) remains a major factor limiting long-term survival after heart transplantation (HTX). The diagnosis of CAV is still based on serial coronary angiography.

Application: Evaluation of coronary allograft vasculopathy applying combined dipyridamole administration with echocardiography image analysis. Dipyridamole echocardiography is a simple, non-invasive test that after heart transplantation may identify cases with altered wall motion that will subsequently require stricter surveillance.

Dosage: Dipyridamole (high dose).

Implementation: Evaluation of the prognostic value of high-dose dipyridamole echocardiography in heart transplant cases. Coronary allograft vasculopathy is defined as CAV 1 (focal or diffuse stenosis < 50%) or CAV 2 (focal or diffuse stenosis ≥ 50%). The wall motion score index (WMSI) is measured at rest and after dipyridamole administration.

Human Model: Heart transplant cases (n = 68) 48 hours prior to scheduled annual coronary angiography.

Interpretation and Analysis: Results of coronary angiography imaging are normal in a large number of cases (e.g., n = 43/68 or 63%); approximately 16% show CAV 1 (e.g., n = 11/68) and CAV 2 in about 21% of cases (e.g., n = 14/68). Similarly, rest wall motion is normal in a large percentage of cases (e.g., n = 39/68 normal; n = 29/68 abnormal). Following dipyridamole administration, wall motion remains unchanged in normal cases (e.g., n = 39/39; Group 1, no CAV in n = 34 and CAV 1 in n = 5). In cases with rest wall motion abnormalities (e.g., n = 29), dipyridamole promotes no change or worsening in most instances (e.g., n = 21/29; Group 3, CAV 2 in n = 14 and no CAV or CAV 1 in n = 7) but can reverse to normal in a modest number of cases (e.g., n = 8/29; Group 2, no CAV in n = 7, and CAV 1 in n = 1). During a 6-year (± 3 years) monitoring period, a large number of cases may experience a major cardiac event (e.g., n = 15; n = 11 Group 3, n = 4 Groups 1 and 2). Wall motion at rest and after dipyridamole administration and CAV is an independent predictor for cardiac events; only dipyridamole WMSI > 1 remains significant ($p < 0.0001$) when assessed by multivariate analysis.

Prognostic value of contractile response during high-dose dipyridamole echocardiography test in heart transplant recipients. Ciliberto GR, Parodi O, Cataldo G, Mangiavacchi M, Alberti A, Parolini M, Frigerio M. J Heart Lung Transplant 2003 May; 22 (5), pp. 526–32.

Myocardial Function/Ventricular (Left) Wall Segment
Optison Contrast Agent/Dobutamine Stress Echocardiography

Application: A 2nd-generation echocardiogaphic contrast agent (e.g., Optison).
Route of Administration: Optison (intravascular).
Implementation: Determine the effectiveness and quality of left ventricular wall segment images, image quality, and confidence of interpretation with and without the use of Optison as an intravenous contrast agent during rest and peak dobutamine stress echocardiography tests (e.g., n = 300 cases).
Analysis and Interpretation: At rest and at peak stress, the percentage of wall segments visualized, image quality, and confidence of interpretation is superior with the use of contrast agent compared with non-contrast imaging. No significant decrease can be seen in wall segment visualization, image quality, or confidence of interpretation from rest to peak stress in images obtained with contrast, unlike the images obtained without contrast from rest to peak stress. The use of intravenous echocardiographic contrast agents like Optison during dobutamine stress echocardiography significantly improves wall segment visualization and image quality at rest and at peak stress, resulting in improved confidence of interpretation.

Contrast dobutamine stress echocardiography: Clinical practice assessment in 300 consecutive patients. Rainbird AJ, Mulvagh SL, Oh JK, McCully RB, Klarich KW, Shub C, Mahoney DW, Pellikka PA. J Am Soc Echocardiogr 2001 May; 14 (5), pp. 378–85.

Myocardial Infarction: Akinesia of the Myocardial Wall
Perflutren Microbubbles/Dobutamine Echocardiography (DE)

Background Information: Akinesia after acute myocardial infarction (AMI) may be reversible or irreversible. Distinguishing these 2 entities early after AMI is difficult but clinically important. Previous studies have shown that myocardial contrast echocardiography (MCE) and low-dose DE may both be useful in this setting. However, minimal data exists regarding the relative and combined value of these techniques.
Route of Administration: Intravascular.
Implementation: Evaluate and compare the utility of real-time intravenous MCE and low-dose DE in the early prediction of functional recovery of akinetic myocardium after AMI. Real-time MCE is performed using perflutren microbubbles. Subsequently, low-dose DE is immediately performed. Contrast opacification and wall motion are determined by experienced observers blinded to clinical data. Repeat echocardiograms are obtained 51 ± 19 days later and wall motion at rest is scored blinded to clinical data.
Human Model: Cases of AMI (n = 37 at 3 ± 2 days after AMI).
Interpretation and Analysis: Normal contrast opacification predicts functional recovery with a positive predictive value of 63%, a negative predictive value of 73%, and an accuracy of 66%. Residual contractility during low-dose DE has a positive predictive value of 82%, a negative predictive value of 72%, and a predictive accuracy of 76%. When the 2 tests are concordant (64%), they have a positive predictive value of 81%, a negative predictive value of 85%, and a predictive accuracy of 83%. Low-dose DE is superior to intravenous MCE for the prediction of functional recovery of akinetic myocardium after AMI, but the combination of both maximizes predictive accuracy.

Comparison of intravenous myocardial contrast echocardiography and low-dose dobutamine echocardiography for predicting left ventricular functional recovery following acute myocardial infarction. Hillis GS, Mulvagh SL, Pellikka PA, Hagen ME, Gunda M, Wright RS, Oh JK. Am J Cardiol 2003 Sep 1; 92 (5), pp. 504–8.

Myocardial Infarction/Coronary Artery Disease
Contrast Echocardiography

The application of non-invasive imaging techniques to assess myocardial viability has become an important part of routine management of acute myocardial infarction and chronic coronary artery disease. Information regarding the presence and extent of viability may help identify patients likely to benefit from revascularization or therapy directed at attenuating left ventricular remodeling. Myocardial contrast echocardiography (MCE) is capable of defining the presence and extent of viability by providing an accurate assessment of microvascular integrity needed to maintain myocellular viability. It is especially suited for the spatial assessment of perfusion, even when myocardial blood flow is reduced substantially in the presence of severe epicardial stenoses or in a vascular bed dependent on collateral perfusion. The routine use of MCE to evaluate viability in conditions of acute and chronic coronary artery disease is now feasible with the advent of new imaging technologies and microbubble agents capable of myocardial opacification from venous injections. The utility of this technique for determining treatment strategies has not been established but is forthcoming.

Assessment of myocardial viability with myocardial contrast echocardiography. Lindner JR. Echocardiography 2002 Jul; 19 (5), pp. 417–25.

Myocardial Infarction: Uncomplicated/Prediction of Favorable Outcome/High Negative Predictive Value Assessment
Dobutamine/Electropharmacology

Cellular/Molecular Mechanism of Action: Dobutamine is an adrenergic agonist/sympathomimetic.
Background Information: Risk stratification after uncomplicated myocardial infarction is a major clinical challenge. In particular, the prognostic value of residual inducible ischemia is still controversial.
Application: Electrocardiogram (ECG) and dobutamine stress echocardiography evaluation of cases with uncomplicated myocardial infarction.
Route of Administration: Intravenous.
Implementation: Evaluation of the relative prognostic value of exercise ECG and dobutamine stress echocardiography performed in the early postinfarction period. One approach to achieving this objective can entail assessment of maximal exercise ECG and dobutamine stress echocardiography within 10 days of an uncomplicated myocardial infarction off therapy and then prospectively monitored for 8.8 months. The variables of age, sex, diabetes, smoking habit, hypertension, dyslipidemia, infarct location, thrombolysis, and resting wall motion score index are taken into account as clinical variables. Prognostic correlations are then made vs spontaneous events (cardiac death, non-fatal reinfarction, and unstable angina requiring hospitalization) while cases undergo revascularization (by means of percutaneous trans-luminal coronary angioplasty or coronary artery bypass surgery).
Human Model: Cases of an uncomplicated myocardial infarction off therapy and prospectively monitored for 8.8 months (n = 406 with n = 53 females; age = 57 ± 9 years).

Interpretation and Analysis: A relatively high number of "events" can be anticipated to occur during the monitoring period (e.g., n = 127; n = 41 or 10% spontaneous [5 deaths; 12 reinfarctions; 24 unstable angina], n = 86 procedural related [27 angioplasty; 59 bypass surgery]). Spontaneous events cannot be predicted by any clinical, exercise ECG, or dobutamine stress echocardiography variable. However, the negative predictive value of both tests is excellent (91% and 90%, respectively). With a multivariate Cox analysis, male gender, positive low-workload (< 100 W) exercise ECG ($p < 0.0001$), positive low-dose dobutamine stress echocardiography ($p < 0.0001$), and rest-stress wall motion score index variation ($p < 0.001$) are capable of predicting cumulative cardiac events with an independent and additive value.

Dobutamine stress echocardiography is significantly more sensitive ($p < 0.05$) and less specific ($p < 0.01$) in predicting the outcome of anterior infarction conditions, while exercise ECG is significantly more sensitive ($p < 0.05$) in cases with non-Q wave infarction. The most valuable concepts related to these observations include (i) spontaneous events are poorly predicted by provocative tests in low-risk patients after uncomplicated myocardial infarction; (ii) however, both exercise ECG and dobutamine stress echocardiography can predict a favorable outcome with a very high negative predictive value; (iii) dobutamine stress echocardiography should be considered a secondary option in cases where the exercise ECG is equivocal or when the location of ischemia is a relevant issue; (iv) the possibility that the 2 tests have a differential utility depending on the infarct location and type (Q wave vs non-Q wave) may be clinically relevant and deserves further evaluation.

Prognostic value of residual ischemia assessed by exercise electrocardiography and dobutamine stress echocardiography in low-risk patients following acute myocardial infarction. Bigi R, Galati A, Curti G, Coletta C, Barlera S, Partesana N, Bordi L, Ceci V, Occhi G, Fiorentini C. Eur Heart J 1997 Dec; 18 (12), pp. 1873–81.

Myocardial Perfusion/Blood Flow and Chamber Volume
Microbubbles

Cellular/Molecular Mechanism of Action: Microbubbles do not propagate ultrasound energy as effectively as do homogeneous (biological) fluids or other types of liquid media.
Application: Microbubbles are excellent tracers for assessment of red blood cell kinetics.

In this fashion, microbubble contrast agents and ultrasound imaging modalities can facilitate the evaluation of various dynamic vascular processes, including the assessment of myocardial perfusion by echocardiography. Apart from providing a spatial assessment of myocardial perfusion, myocardial contrast echocardiography (MCE) can also be used to quantify the 2 specific components of myocardial blood flow-flow velocity and myocardial blood volume.
Implementation: One method that can be applied to quantify myocardial blood flow velocity is based on rapid destruction of microbubbles by ultrasound, and the subsequent assessment of the rate of replenishment of microbubbles into the myocardial microcirculation within the ultrasound beam elevation. Assessment of steady state myocardial video intensity (VI) provides a measure of myocardial or capillary blood volume.
Analysis and Interpretation: Perfusion defects that develop distal to a stenosis during hyperemia are due to capillary derecruitment. The degree of derecruitment (and therefore the severity of a perfusion defect) is proportional to stenosis severity. Because the capillary bed also provides the greatest resistance to hyperemic flow, decreases in capillary blood volume distal to a stenosis during hyperemia result in increases in microvascular resistance, which is the mechanism underlying the progressive decrease in flow reserve in the presence of a stenosis. Consequently, both the severity of a perfusion defect and quantification of abnormal myocardial blood flow reserve on MCE can be used to determine stenosis severity. As imaging methods with MCE continue to be refined, the optimal imaging algorithms for clinical practice still need to be determined. Myocardial contrast echocardiography, however, holds promise as a non-invasive, instantaneous, on-line method for the detection and quantification of coronary artery disease.

Detection and quantification of coronary stenosis severity with myocardial contrast echocardiography. Wei K. Prog Cardiovasc Dis 2001 Sep–Oct; 44 (2), pp. 81–100.

Myocardial Perfusion/Blood Flow Mismatch (Ischemic vs Normal)/Blood Flow Mismatch: Ischemic vs Normal Myocardium
NC 100100/Fluorescent Microspheres

Application: The ultrasound contrast agent NC 100100 can be applied to perform myocardial contrast echocardiography (MCE) imaging. Fluorescent microspheres can be utilized to determine regional myocardial blood flow parameters. The degree of blood flow mismatch between ischemic and normal myocardial regions during graded coronary stenoses can be estimated by quantitative assessment of myocardial perfusion produced by IV MCE (e.g., canine models).
Route of Administration: NC 100100 (intravenous).
Implementation: Coronary artery flow is measured with a transit time flowmeter during baseline, pharmacological-induced vasodilation, a non-flow-limiting stenosis at rest in conjunction with vasodilation, a flow-limiting stenosis, and total occlusion. Myocardial contrast echocardiography is then performed, facilitated by the injection of NC 100100 contrast agent. Time-intensity curves are obtained off-line for the left anterior descending (LAD) coronary artery risk area and the adjacent left circumflex (LCx) territory, and peak background-subtracted video intensity determined. Fluorescent microspheres are injected at each intervention for determination of regional myocardial blood flow.

Animal Model: Variable obstruction of the LAD coronary artery in dogs produced by a screw occluder.
Analysis and Interpretation: During non-flow-limiting stenosis, flow-limiting stenosis, and total occlusion, LAD/LCx ratios of peak myocardial video intensity and blood flow decrease proportionately. Both LAD/LCx ratios of video intensity and blood flow identify the non-flow-limiting and flow-limiting stenoses as well as total occlusion of the LAD artery. A significant correlation exists between LAD/LCx video intensity and blood flow ratios ($r = 0.83$, $p < 0.0001$).

Quantitative assessment of myocardial perfusion during graded coronary artery stenoses by intravenous myocardial contrast echocardiography. Leistad E, Ohmori K, Peterson TA, Christensen G, DeMaria AN. J Am Coll Cardiol 2001 Feb; 37 (2), pp. 624–31.

Myocardial Perfusion/Blood Flow with Vasculature/Red Blood Cell Velocity
Microbubbles/Microbubble "Replenishment"

Background Information: The development of new microbubble agents and ultrasound imaging modalities now allow the assessment of myocardial perfusion with echocardiography.
Application: Evaluation of vascular blood flow.

Route of Administration: Intravascular (selective delivery).
Implementation: Microbubbles can be administered intravenously as constant infusions, thereby allowing their concentration in blood to reach a steady state. If the relation between microbubble concentration and video intensity is within the linear range, then myocardial video intensity will reflect the concentration of microbubbles in that region, which at steady state represents the myocardial blood volume.
Interpretation and Analysis: The ability to destroy microbubbles and measure their replenishment within the ultrasound beam provides an opportunity to evaluate microbubble (or red blood cell) velocity. The product of myocardial blood volume and red blood cell velocity reflects properties of myocardial blood flow.

Assessment of myocardial blood flow and volume using myocardial contrast echocardiography. Wei K. Echocardiography 2002 Jul; 19 (5), pp. 409–16.

Myocardial Perfusion/Coronary Flow Quantification/Myocardioviability/Bedside Diagnostic Capabilities
Echocardiography Contrast Media

Background Information: Contrast echocardiography can delineate myocardial perfusion and has the potential for quantitating coronary flow and assessing myocardial viability.
Application: Assessment of myocardial perfusion, quantifying coronary artery flow, and determination of myocardial viability.
Analysis and Interpretation: Utilization of contrast echocardiography for quantifying coronary artery blood flow and assessment of myocardial viability provides important physiologic information that complements the characterization of vascular anatomical anomalies. Because it can be rapidly performed at the bedside, contrast echocardiography may be a valuable tool for use in acute myocardial ischemia. When contrast echocardiography has been used after recanalization of occluded coronary arteries, the assessment of myocardial salvage conveys information concerning reflow, stunning, and prognosis, and in the case of angioplasty it provides immediate information regarding the success of the procedure. Contrast echocardiography can also assess myocardial areas at risk of irreversible damage and the presence or absence of collateral flow. Myocardial contrast echocardiography is a rapidly changing field and with the continued development of newer contrast agents and refinement of ultrasound imaging equipment, the applications of contrast echocardiography will continue to grow.

Assessment of myocardial perfusion by contrast echocardiography. Perez JE. Rev Port Cardiol 2001 Jan; 20 Suppl 1, pp. I49–56.

Myocardial Perfusion/Ischemia/Occulsion and Reperfusion Injury/Myeloperoxidase Score
Microsphere Contrast Image-Enhancing Agents/BR14

Cellular/Molecular Mechanism of Action: Microspheres when applied as an ultrasound contrast image-enhancing agent variably absorb, impede, and reflect ultrasound energy in a manner that is different than patterns observed for biological fluids.
Background Information: Ultrasound exposure may destroy the ultrasound imaging agent BR-14 and eliminate visualization of sustained myocardial opacification from retained microbubbles.
Application: Microsphere contrast image-enhancing agents can be applied to evaluate alteration in anatomical integrity and physiological function of vascular structures, including the assessement of myocardial perfusion parameters.
Route of Administration: Microsphere contrast image-enhancing agent (intra-arterial).
Implementation: Determine if delays in postinjection imaging with the ultrasound contrast agent BR-14 produce prolonged opacification and hyperenhancement of myocardium subjected to coronary occlusion/reperfusion.
Animal Model: Canine cardiac preparations (n = 8 open-thorax; 3 hours left anterior descending coronary artery occlusion followed by 3 hours of reperfusion). Myocardial contrast echocardiography (MCE) is performed before occlusion and 120 minutes after the onset of both occlusion and reperfusion events. Ultrasound imaging is initiated 15 minutes after injection of microspheres to assess myocardial blood flow (MBF).
Analysis and Interpretation: Preocclusion images reveal a uniform opacification of the left ventricular myocardium that is greater than that of the ventricular cavity, with a mean intensity of the left anterior descending (LAD) coronary artery bed of 8.66 $\pm$ 1.38 dB. During occlusion, MCE results in the appearance of a perfusion defect in the LAD risk area (intensity 2.08 $\pm$ 1.10 dB). After 120 minutes of reperfusion, the LAD risk-area myocardium develops dense opacification of a higher intensity ("hot spot") than baseline (13.7 vs 8.7 dB), but with reduced MBF consistent with accumulation of a high concentration of microbubbles. Increased MCE intensity is associated with a greater myeloperoxidase score. Contrast opacification by BR-14 may be selectively retained within the perfusion bed of a coronary artery subjected to occlusion/reperfusion. Such opacification exhibits defects with occlusion, manifests hyperenhanced intensity (hot spot) with reperfusion, is associated (correlates) with levels of myeloperoxidase activity, and conforms to the area of myocardium subjected to altered flow.

Visualization of risk-area myocardium as a high-intensity, hyperenhanced "hot spot" by myocardial contrast echocardiography following coronary reperfusion: Quantitative analysis. Kunichika H, Peters B, Cotter B, Masugata H, Kunichika N, Wolf PL, DeMaria AN. J Am Coll Cardiol 2003 Aug 6; 42 (3), pp. 552–7.

Myocardial Perfusion Post–Myocardial Infarction/ Reflow Patterns/Stress Echocardiography
Perfluorocarbon-Encapsulated Microbubbles/ [Adenosine Stress Echocardiography]/[Dipyridamole Stress Echocardiography]/[Dobutamine Stress Echocardiography]

Background Information: One of the major challenges facing the field of ultrasonography is the accurate interpretation of images.
Application: Enhancement of the diagnostic accuracy of adenosine, dypyridamole, and dobutamine stress echocardiography procedures.
Route of Administration: Intravascular.
Implementation: Assessment of myocardial contrast patterns (e.g., perfusion abnormalities) during dipyridamole, adenosine, and dobutamine stress echocardiography procedures.
Interpretation and Analysis: Myocardial contrast patterns can detect myocardial perfusion abnormalities during dipyridamole, adenosine, and dobutamine stress echocardiography. Validity of such findings remains to be established in larger multicenter clinical studies.

Clinical experience in the detection of coronary artery disease with myocardial contrast echocardiography. Porter TR, Xie F. Echocardiography 2002 Jul; 19 (5), pp. 399–407.

Myocardial Perfusion/Myocardial Contrast Echocardiography (MCE) Flow or No-Flow: Correlation with Cardiac Physiology Parameters (Pressure-Derived Collateral Flow Index [CFIp]) *Microbubbles*

Cellular/Molecular Mechanism of Action: Microbubble preparations transmit ultrasound energy to a much lesser extent than do relatively "homogenous" biological fluid mediums due to variable increased impedance, absorbance, and reflectance, which can be detected by ultrasound instrumentation.

Background Information: Higher CFIp values are associated with less severe myocardial ischemia during angioplasty in the noninfarcted heart. It remains unknown whether CFIp also identifies collateral function in acute myocardial infarction cases with and without no-reflow phenomenon.

Application: Acquisition of MCE images for the assessment of myocardial perfusion.

Route of Administration: Microbubble myocardial perfusion imaging (intracoronary artery).

Implementation: Following successful percutaneous transluminal coronary angioplasty (PTCA) stent, measurements are acquired for mean aortic pressure (Pa), central venous pressure (Pv), and coronary wedge pressure (Pcw) of the infarct-related artery to calculate: CFIp = (Pcw − Pv)/(Pa − Pv). Myocardial contrast echocardiography is performed with an intracoronary injection of microbubbles to assess myocardial perfusion. Left ventriculograms on day 1 and day 28 are evaluated for the measurement of regional wall motion (RWM, SD/chord).

Human Model: Acute myocardial infarction (e.g., n = 48 cases).

Interpretation and Analysis: There is no difference in CFIp among subsets based on angiographic collateral grades (grade 0, 1, 2, 3; 0.28 ± 0.07, 0.27 ± 0.09, 0.27 ± 0.08, 0.23 ± 0.08, p = NS). The CFIp is significantly higher in cases with MCE no-reflow (n = 16) than in those with MCE reflow (n = 32, respectively 0.34 ± 0.07 vs 0.23 ± 0.06, $p < 0.01$). There is a significant inverse correlation between the extent of functional improvement (DeltaRWM [28 d-1 d]) and CFIp ($r = 0.56$, $p < 0.01$), implying that higher CFIp is associated with worse functional improvement.

In AMI, CFIp is unlikely to reflect collateral function but seems to increase with the severity of microvascular dysfunction. Because higher CFIp is generally associated with a poorer functional recovery, it provides a simple and useful estimate of clinical outcomes in AMI.

Pressure-derived collateral flow index as a parameter of microvascular dysfunction in acute myocardial infarction. Yamamoto K, Ito H, Iwakura K, Shintani Y, Masuyama T, Hori M, Kawano S, Higashino Y, Fujii K. J Am Coll Cardiol 2001 Nov 1; 38 (5), pp. 1383–9.

Myocardial Perfusion Postinfarction *Microbubbles*

Background Information: Intense research efforts have been made to bring forth the use of myocardial contrast echocardiography (MCE) as a viable clinical procedure for the diagnosis and evaluation of coronary artery disease.

Implementation: Evaluation of myocardial perfusion parameters and characterize myocardial infarction and ischemia.

Interpretation and Analysis: A number of ultrasound contrast agents have been developed that act as red blood cell tracers and display myocardial perfusion profiles when imaged by dedicated ultrasound imaging modalities. A considerable amount of experimental and clinical research has shown that MCE can aid in the recognition of acute and chronic myocardial infarction, viable myocardium, and functionally significant coronary stenoses. Comparison of this diagnostic technique to nuclear imaging and coronary arteriography has demonstrated that an excellent level of diagnostic accuracy can be achieved in evaluating various coronary syndromes. Optimal practice of perfusion imaging requires a thorough knowledge of microbubble characteristics and imaging modalities, as well as experience with specific methodologies. In this context, techniques continue to evolve from intermittent gated examination to real-time perfusion imaging in a manner that allows evaluation of both perfusion and functional parameters. The opportunity to target sites of pathology with specially engineered microbubbles could also aid in many therapeutic applications besides diagnostic imaging.

Myocardial perfusion imaging using contrast echocardiography. Nesser HJ, Morcerf F, Teupe C, Pandian NG. Herz 2002 May; 27 (3), pp. 217–26.

Thrombosis: Intravascular *Perfluorooctylbromide/Perfluorooctane/Nanoparticles*

Cellular/Molecular Mechanism of Action: Perfluorooctylbromide and perfluorooctane impede/absorb ultrasound emissions.

Background Information: Complex molecular signaling heralds the early stages of pathologies such as angiogenesis, inflammation, unstable atherosclerotic plaques, and areas of remote thrombi. Acoustic enhancement of blood clot morphology has been demonstrated to be feasible with the use of a non-gaseous, fibrin-targeted acoustic nanoparticle emulsion delivered *in vitro* and *in vivo* to areas of thrombosis.

Application: Perfluorooctylbromide and perfluorooctane can be applied as acoustic contrast image-enhancing agents for the detection of intravascular thrombosis.

Route of Administration: Perfluorooctylbromide and perfluorooctane (intravenous).

Implementation: Design and construct a system that allows visualization of the evolution of acoustic contrast enhancement. To evaluate the system 2 targeting strategies can be utilized, including (i) avidin-complexed nitrocellulose membrane; and (ii) human plasma clots. The time evolution of enhancement can be visualized in 10-minute increments over a 1-hour time period.

Interpretation and Analysis: A monotonic increase can be observed in ultrasonic reflection enhancement from specially treated nitrocellulose membranes for targeted emulsions containing perfluorooctylbromide (1.30 ± 0.3 dB) and for perfluorooctane (2.64 ± 0.5 dB) within the 1st 60 minutes of imaging. In comparison, inherently non-echogenic plasma clots show a substantial increase of 12.0 ± 0.9 dB when targeted with a perfluorooctane emulsion and effectively demonstrate the concept of molecular imaging. The methodologies can quantify the time evolution of the binding of a site-targeted ultrasonic contrast agent. Moreover, with the incorporation of specific drug treatments into the nanoparticulate contrast agent, ultrasonic molecular imaging may yield reliable strategies for detecting and quantifying nascent pathologies and facilitate targeted drug therapy.

Time evolution of enhanced ultrasonic reflection using a fibrin-targeted nanoparticulate contrast agent. Hall CS, Marsh JN, Scott MJ, Gaffney PJ, Wickline SA, Lanza GM. J Acoust Soc Am 2000 Dec; 108 (6), pp. 3049–57.

Valve Structures: Mitral Regurgitation (MR): Quantification of Severity
Microbubbles/(Power Injected)

Cellular/Molecular Mechanism of Action: Microbubbles variably absorb, impede, and reflect ultrasound energy relative to normal tissues and biological fluids.
Background Information: There is no method of quantifying the severity of MR from the injection of tracer directly into the left ventricular (LV) cavity (common method used in the cardiac catheterization laboratory).
Application: Measurement of the severity of cardiac chamber mitral regurgitation. Apart from being quantitative, the methodology can be safely used during cardiac catheterization in cases where iodinated contrast agents may be potentially harmful.
Route of Administration: Microbubble formulations (power injected into catheterized cardiac chamber).
Dosage: Microbubble formulation (5 ml/LV chamber). Five milliliters of a microbubble mixture is power injected into the LV during simultaneously performed contrast echocardiography.
Implementation: Characterization of relative tracer washout from the left atrial (LA) and LV chambers facilitated by the use of a mathematical model that derives regurgitant fraction (RF) parameters (e.g., n = 39 cases). Relative changes in background-subtracted video intensity are measured within the LV and LA, and the resultant model-derived RF is correlated with the severity of mitral regurgitation on cineangiography.
Human Model: Diagnostic cardiac catheterization in conditions of possible mitral regurgitation.
Analysis and Interpretation: The severity of MR ranges between 0 and 4+ on cineangiography with a corresponding model-derived RF of 0 to 0.69 on contrast echocardiography. A close linear relation is noted between angiographic severity of MR and model-derived RF on contrast echocardiography ($y = 0.1x + 0.03$, $r = 0.89$, $p < 0.001$). Contrast echocardiography is more sensitive than cineangiography for detecting mild MR.

Quantification of mitral regurgitation in the cardiac catheterization laboratory with contrast echocardiography. Buckley RS, Kaul S, Jayaweera AR, Gimple LW, Powers ER, Dent JM. Am Heart J 2000 Jun; 139 (6), pp. 1109–13.

Valve Structures: Tricuspid Regurgitation (TR)/ Right Heart Function
Agitated Colloid/Levovist (Lev)/Saline (Sal)

Background Information: Doppler imaging spectrum of TR can be used to non-invasively assess right ventricular (RV) pressure. With mild TR, the native (Nat) TR envelope may not allow accurate pressure evaluation. Proprietary contrast agents such as Lev can be used to augment TR Doppler and opacify right-sided heart chambers, but they are expensive and their efficacy has not been objectively evaluated in cases with difficult baseline studies or compared with less expensive Sal or colloid solutions, such as Gelofusine (Gel).
Application: Agitated colloid, Lev, and Sal can be used to assess pulmonary systolic pressure and right-sided heart opacification. Note: Cost $0.10, $2.50, and $75.00 per study for Sal, Gel, and Lev contrast agents, respectively.
Route of Administration: Cardiac catheterization.
Implementation: Reexamination of poor quality Nat TR envelopes detected on trans-thoracic echocardiogram with serial intravenous injection of 3 contrast agents (Sal, Gel, and Lev). Doppler signals for each agent are recorded on video and digitally on optical disk to facilitate assessment of signal quality, estimate RV pressure, and approximate signal longevity. Quality of RV-right atrial (RV-RA) opacification is also determined for Sal and Gel, in addition to the measurement of pressures obtained from cardiac catheterization in order to independently evaluate pressure estimates from echocardiography.
Human Model: Trans-thoracic contrast echocardiogram (n = 25 cases); percutaneous right-sided heart cathetrization (e.g., n = 9/25). Pressures obtained from the catheterization are used to independently evaluate the pressure estimates from echocardiography.
Analysis and Interpretation: The 3 contrast agents Sal, Gel, and Lev all significantly improve the mean quality grade (grades 0–5) of TR envelopes (Nat 1.12, Sal 1.97, Gel 2.56, Lev 2.41, $p < 0.001$), decrease the number of uninterpretable envelopes (grade 0) (Nat 49%, Sal 12%, Gel 4%, Lev 12%, $p < 0.0001$ for comparison of each agent relative to Nat), and improve the correlation between echocardiographic and catheter-derived RV-RA pressure measurements (Nat $r = 0.65$, Sal $r = 0.75$, Gel $r = 0.90$, Lev $r = 0.88$). The persistence of enhanced Doppler signals of interpretable quality (> grade 1) is greater for Lev (15.8 seconds) and Gel (15 seconds) than for Sal (7.6 seconds) ($p = 0.002$). Opacification of RV and RA, measured as mean luminosity score during 2-dimensional harmonic imaging, is significantly higher for Gel than Sal (92.84 ± 31.2 vs 56.06 ± 25.6, respectively; $p = 0.0003$).

Agitated colloid is superior to saline and equivalent to levovist in enhancing tricuspid regurgitation Doppler envelope and in the opacification of right heart chambers: A quantitative, qualitative, and cost-effectiveness study. Tan HC, Fung KC, Kritharides L. J Am Soc Echocardiogr 2002 Apr; 15 (4), pp. 309–15.

CENTRAL NERVOUS SYSTEM

Angiography/Stimulated Acoustic Emission (SAE)/ High-Acoustic Disintegration of Microbubbles/ Insonation of Echo-Contrast Agents
Microbubbles/Non-Moving Microbubbles (SHU 563 A)/ Levovist (SHU 508 A)

Background Information: Experimental and clinical data suggest that insonation of echo-contrast agents with high acoustical power induces the disintegration of microbubbles, resulting in pseudo-Doppler phenomenon SAE.
Application: Potential measurement of cerebral vascular perfusion applying trans-cranial Doppler image detection of SAE signals.
Implementation: Evaluation of the feasibility for performing trans-cranial imaging analyses. One investigative approach for achieving this objective can involve the evaluation of non-moving microbubbles (SHU 563 A) insonated *in vitro* through the temporal parts of a human cadaver skull, and contrast signals detected by velocity-coded color Doppler and power Doppler recordings. Complementary investigations can include trans-cranial color Doppler and power Doppler imaging (e.g., n = 10 normal controls) utilizing the echo-contrast agent SHU 508 A (e.g., Levovist).
Analysis and Interpretation: Color Doppler signals reveal that SAE can be observed *in vitro* and in trans-cranial human investigations. Such signals are composed of a mosaic of color Doppler pixels ranging over the full color scale. Apparent velocity information and spatial distribution of SAE signals ultimately changes from image frame to image frame. In experimental models, the intensity of SAE signals decreases exponentially over time. With an increase of acoustic power, there is a significant increase of the maximum signal intensity ($p < 0.01$) and a significantly shorter signal duration ($p < 0.01$), consistent with stronger and more rapid disintegration. In humans, SAE signals can be clearly detected in cerebral tissue regions. The intensity of SAE signals in these regions (e.g., temporal

cortex, 3.7 ± 1.2 dB) is approximately 8 times lower than the signal enhancement in the major cerebral arteries (e.g., 29.5 ± 5.6).

Stimulated acoustic emission detected by trans-cranial color Doppler ultrasound: A contrast-specific phenomenon useful for the detection of cerebral tissue perfusion. Pohl C, Tiemann K, Schlosser T, Becher H. Stroke 2000 Jul; 31 (7), pp. 1661–6.

Angiography: Trans-Cranial Color-Coded Sonography/Duration of Contrast Enhancement
Levovist

Background Information: Since the introduction of contrast media, trans-cranial color-coded sonography (TCCS) has become increasingly important for the examination of intracranial vessels. However, the widely practiced bolus injection of these agents leads to initial blooming artifacts, and thereafter the level of contrast enhancement decreases rapidly, reducing the effective time of imaging enhancement.
Application: Trans-cranial color-coded sonography examination of intracranial vessel structure, integrity, and function.
Route of Administration: Levovist (antecubital vein).
Dosage: Levovist (300 mg/ml; 4 g at a constant infusion rate of 60 ml/h).
Implementation: Trans-cranial color-coded sonography examination of intracranial vessels using a 2.25-MHz phased-array transducer during administration of Levovist contrast agent. The degree of enhancement can be graded chronologically over time.
Human Model: Normal subjects (n = 26).
Analysis and Interpretation: During continuous administration, a constant level of contrast enhancement is achieved after 60.1 ± 26.2 seconds and lasts approximately 663.4 ± 55.8 seconds (evaluator 1 = 66.6 ± 26.2 seconds; evaluator 2 = 664.3 ± 55.9 seconds). The limits of interobserver agreement can range from −10.1% to 9.9%. No major blooming effect is seen in the initial phases of examination.

Continuous administration of contrast medium for trans-cranial color-coded sonography. Schminke U, Motsch L, Bleiss A, von Smekal U, Griewing B, Kessler C. Neuroradiology 2001 Jan; 43 (1), pp. 24–8.

Arteriography: Intracranial Arteries
Galactose-Based Microbubble Suspension (Levovist)

Cellular/Molecular Mechanism of Action: Microbubbles variably impede, absorb, and reflect ultrasound energy in a manner that is different from surrounding tissues or biological fluids.
Application: Contrast-enhanced trans-cranial ultrasound (TU) demonstrates a high sensitivity and specificity for detecting and characterizing intracranial stenosis.
Implementation: Evaluate the frequency at which the ultrasound contrast agent (UCA) Levovist is used in routine TU.

In this context, it has been utilized as a secondary imaging strategy for identifying intracranial arteries insufficiently characterized with combined trans-cranial color-coded duplex and trans-cranial Doppler examination. Trans-cranial ultrasound images can be compared with 3-dimensional time-of-flight magnetic resonance (MR) angiography as a validation procedure.
Human Model: Cases with intracranial arteries that could not be adequately visualized with combined trans-cranial color-coded duplex and trans-cranial Doppler examination (e.g., n = 61/687).
Analysis and Interpretation: A diagnostic result with ultrasound contrast agent can be achieved in approximately 75% of cases during trans-temporal and in 81% during trans-foraminal insonation. The sensitivity and specificity of TU in the diagnosis of intracranial stenosis is approximately 83% and 82%, respectively. Use of UCA is necessary in approximately 8.8% of cases. A diagnostic benefit of between 75% and 80% can be achieved in relevant case populations.

Echo-contrast-enhanced trans-cranial ultrasound: Frequency of use, diagnostic benefit, and validity of results compared with MRA. Zunker P, Wilms H, Brossmann J, Georgiadis D, Weber S, Deuschl G. Stroke 2002 Nov; 33 (11), pp. 2600–3.

Brain Blood Flow Measurements/Induction of Neurologic Pathology by Ultrasound (US) Contrast Agents
US Contrast Agent/Burst US

Cellular/Molecular Mechanism of Action: Microbubbles variably impede, absorb, and reflect US energy in a manner that is different from surrounding tissues or biological fluids.
Application: Burst US and US contrast agents have been used to acquire brain blood flow measurements.
Implementation: Evaluation of the potential for burst US in the presence of a US contrast agent to cause injury to central nervous system (CNS) tissues using parameters similar to those during the acquisition of brain blood flow measurements. One approach to achieving this objective involves the sonication of brains in 3 to 8 locations with 1.5-MHz, 10-microsecond bursts repeated at a frequency of 1 kHz at temporal peak acoustic pressure amplitudes ranging from 2 to 12.7 MPa. The total sonication time for each location is 20 seconds. Before each sonication, a bolus of US contrast agent is injected intravenously. Contrast-enhanced magnetic resonance (MR) images are then obtained after sonications to detect local enhancement in the brain. Whole brain histological evaluation is performed, and sections are stained with H & E, TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling), and vanadium acid fuchsin (VAF) to evaluate tissue effects, including apoptosis and ischemia.
Animal Model: Intact rabbit brains (e.g., n = 10).
Analysis and Interpretation: Both the magnetic resonance imaging (MRI) contrast enhancement and histology findings indicate that brain tissue damage is induced at a pressure amplitude level of 6.3 MPa. Induced pathological injury that can be detected includes vascular wall damage, hemorrhage, and, eventually, necrosis. Mild vascular damage can be observed localized in a few microscopic tissue volumes in about half of the sonicated locations at all pressure values tested (down to 2 MPa). However, these sonications do not induce any detectable tissue effects such as ischemia or apoptosis. Therefore, ultrasound exposure levels that are currently used for blood flow measurements within the brain are below the threshold of blood-brain barrier opening or brain tissue damage. However, it is important to note that brain damage can be induced if exposure levels are increased.
Instrumentation: Ultrasound instrumentation (1.5 MHz).

The threshold for brain damage in rabbits induced by bursts of ultrasound in the presence of an ultrasound contrast agent (Optison). Hynynen K, McDannold N, Martin H, Jolesz FA, Vykhodtseva N. Ultrasound Med Biol 2003 Mar; 29 (3), pp. 473–81.

Brain Parenchyma Acoustic Density/Influence of Dose
BR14 [Perfluorobutane-Based]/Trans-Cranial Gray-Scale Harmonic Imaging

Cellular/Molecular Mechanism of Action: The agent BR14 is a perfluorobutane-based compound that has an increased level of acoustic density compared to homogeneous biological fluids.

Application: BR14 is a perfluorobutane-based ultrasound contrast agent that can be used to image the brain parenchyma by transcranial gray-scale harmonic imaging (SONOS 5500, 1.8/3.6 MHz).
Route of Administration: BR14.
Dosage: BR14 (dose 1 = 0.05 ml/kg BW; dose 2 = 0.2 ml/kg BW).
Implementation: Trans-cranial gray-scale harmonic imaging of brain parenchyma facilitated by the administration of BR14 (perfluorobutane-based ultrasound contrast agent). Following bolus injection of 2 different doses of BR14, acoustic densitometry is performed to quantify changes in regional contrast intensity.

Animal Model: Canine (n = 6 beagles; sedated).
Analysis and Interpretation: In brain parenchyma (canine), the mean relative peak increase in acoustic intensity is +61% and +24% following the administration of high- and low-dose BR14, respectively. In the masticatory muscle, application of the higher dose results in a stronger increase in contrast intensity compared to lower dosage levels. Evaluation of the contralateral base of the skull shows a dose-dependent decrease in acoustic intensity. Bolus injection of BR14 produces an increase in acoustic intensity that can be used for visualization of contrast agent within the brain parenchyma. Using high dosages, a strong signal-enhancing effect in the regions near the ultrasound probe leads to a consecutive attenuation of signals from structures being located beyond ("shadowing effect"). This is the explanation for the paradoxical result that higher dosage levels lead to a lower peak signal increase within brain parenchyma.
Instrumentation: SONOS 5500, 1.8/3.6 MHz.

Harmonic imaging of the brain parenchyma using a perfluorobutane-containing ultrasound contrast agent. Seidel G, Meyer K, Algermissen C, Broillet A. Ultrasound Med Biol 2001 Jul; 27 (7), pp. 915–8.

Brain Perfusion/Neural Parenchyma
Contrast Echo-Enhanced Ultrasound

Advances in neurosonology have generated several techniques of ultrasonic perfusion imaging employing ultrasound echo-contrast agents (ECAs). Doppler imaging techniques cannot measure the low flow velocities that are associated with parenchymal perfusion. Ultrasonic perfusion imaging, therefore, is a combination of a contrast agent-specific ultrasound imaging technique (CAI) mode and a data acquisition and processing (DAP) technique that is suited to observe and evaluate perfusion kinetics. The intensity in CAI images is a measure of ECA concentration but also depends on various other parameters, (e.g., depth of examination). Moreover, ECAs can be destroyed by ultrasound, which is an artifact but can also be a desirable feature. Thus, many different DAPs have been developed for certain CAI techniques, ECAs and target organs. Although substantial progress in ECA and CAI technology can be foreseen, ultrasound contrast imaging has yet to reliably differentiate between normal and conditions associated with pathological levels of perfusion. Destructive imaging techniques, such as contrast burst imaging (CBI) or time variance imaging (TVI), in combination with new DAP techniques provide sufficient signal-to-noise ratios (SNR) for trans-cranial applications, and the potential to provide information regarding contrast agent kinetics and sufficient destruction to eliminate depth dependency and facilitate the calculation of semi-quantitative parameters. Because ultrasound machines are widely accessible and cost-effective, ultrasonic perfusion imaging techniques should become supplementary standard perfusion imaging techniques in acute stroke diagnosis and monitoring.

Brain perfusion and ultrasonic imaging techniques. Eyding J, Wilkening W, Postert T. Eur J Ultrasound 2002 Nov; 16, pp. 91–104.

Brain Perfusion: Cerebral/Physiological and Pathological Perfusion Differentiation in Brain Parenchyma Regions
Galactose-Based Microbubble Suspension (Levovist)

Cellular/Molecular Mechanism of Action: Microbubbles variably impede, absorb, and reflect ultrasound energy in a manner that is different from surrounding tissues or biological fluids.
Background Information: Contrast burst imaging (CBI) and time variance imaging (TVI) are new ultrasonic imaging modes that enable visualization of intravenously injected echo-contrast agents in brain parenchyma.
Application: Enhanced visual analysis of brain tissues utilizing echo-contrast agent-specific imaging modalities. Evaluation of cerebral perfusion in a capacity that may serve to differentiate physiological and pathological perfusion in different parenchymal regions of the brain.
Route of Administration: Galactose-based microbubble suspension (Levovist: intravenous).
Dosage: Galactose-based microbubble suspension (Levovist: 4 g dose; 400 mg/mg formulation).
Implementation: Evaluation of cerebral perfusion in a manner that may facilitate differentiation between physiological and pathological perfusion in different parenchymal regions of the brain. In this context, normal vascular structures can be characterized applying CBI and TVI ultrasound methodologies in concert with a galactose-based microbubble suspension (Levovist). Complementary evaluations include performing a dynamic susceptibility contrast magnetic resonance imaging (MRI) (e.g., p-MRI obtained for each case). In both ultrasound (US) methods and p-MRI, the time-intensity curves are calculated off-line. Absolute time to peak intensities (TPI), peak intensities (PI), and peak width (PW) of US investigations and TPI, relative cerebral blood flow (CBF), and relative cerebral blood volume (CBV) of p-MRI examinations are determined in the following regions-of-interest (ROIs): lentiform nucleus (LN), white matter (WM), posterior (PT), and anterior thalamus (AT). In addition, the M_2 segment of the middle cerebral artery (MCA) is evaluated by US, and the precentral gyrus (PG) can be examined in p-MRI examinations. In relation to a reference parenchymal ROI (AT), relative TPIs are compared between the US and p-MRI methods and relative PI of US investigations with the ratio of CBF (rCBF) of p-MRI examinations in identical ROIs.

Human Model: Cases with no vascular abnormalities examined applying CBI and TVI ultrasound imaging methodologies (e.g., n = 12).
Analysis and Interpretation: Mean TPI vary from 18.3 ± 5.0 (AT) to 20.1 ± 5.8 (WM) to 17.2 ± 4.9 (MCA) seconds in CBI examinations and from 19.4 ± 5.3 (AT) to 20.4 ± 4.3 (WM) to 17.3 ± 4.0 (MCA) seconds in TVI examinations. Mean PIs vary from 581.9 ± 342.4 (WM) to 1522.9 ± 574.2 (LN) to 3400.9 ± 621.7 arbitrary units (MCA) in CBI mode and from 7.5 ± 4.6 (WM) to 17.5 ± 4.9 (LN) to 46.3 ± 7.1 (MCA) arbitrary units in TVI mode. PW range from 7.3 ± 4.5 (AT) to 9.1 ± 4.0 (LN) to 24.3 ± 12.8 (MCA) seconds in CBI examinations and from 7.1 ± 3.9 (AT) to 8.7 ± 3.5 (LN) to 26.7 ± 18.2 (MCA) seconds in TVI examinations. Mean TPI is significantly shorter and mean PI and mean PW are significantly higher in the MCA compared with all other ROIs ($p < 0.05$). Mean TPI of the p-MRI examinations range from 22.0 ± 6.9 (LN) to 23.0 ± 6.8 (WM) seconds; mean CBF range from 0.0093 ± 0.0041 (LN) to 0.0043 ± 0.0021 (WM). There is no significant difference in rTPI in any ROI between US and p-MRI measurements ($p > 0.2$), whereas relative PIs are significantly higher in areas with lower insonation depth such as the LN compared with rCBF. Com-

pared to PI, TPI and rTPI in US techniques are robust parameters for the evaluation of cerebral perfusion and may help to differentiate between physiological and pathological perfusion patterns in different parenchymal regions of the brain.

Comparison between echo-contrast agent-specific imaging modes and perfusion-weighted magnetic resonance imaging for the assessment of brain perfusion. Meves SH, Wilkening W, Thies T, Eyding J, Holscher T, Finger M, Schmid G, Ermert H, Postert T; Ruhr Center of Competence for Medical Engineering. Stroke 2002 Oct; 33 (10), pp. 2433–7.

Brain Perfusion/Lentiform Nucleus/Thalamus/ Cerebral White Matter/Trans-Cranial Sonography *Optison*

Background Information: Perfusion trans-cranial sonography is a technique that can detect pathological alterations in brain tissue perfusion in conventional hospital environments (e.g., bedside).
Application: Imaging of cranial vascular structures applying methodologies such as perfusion trans-cranial sonography.
Implementation: Perfusion trans-cranial sonography (P-TCS) is performed in an axial insonation plane through the thalamus and 3rd ventricle. Signal increases induced by a bolus of an echo-contrast agent (Optison) are quantified within regions-of-interest (ROIs) corresponding with the thalamus (TH), lentiform nucleus (LN), and the cerebral white matter (WM).

Human Model: Cases suffering from Moyamoya disease and bilateral thalamic edema due to thrombosis of the internal cerebral veins, in addition to healthy negative reference controls (e.g., n = 10).
Analysis and Interpretation: In Moyamoya disease P-TCS is capable of demonstrating decreases in perfusion in the WM and LN as a result of bilateral, high-grade stenoses of the intracranial part of the internal carotid artery. Peak intensity can be increased in the TH due to collateral crossover flow from the vertebrobasilar system. Thalamus perfusion can be decreased in thrombosis of the internal cerebral vein thrombosis with normal LN perfusion. The extention of the thalamic edema into the white matter can also be demonstrated by decreased perfusion in the adjacent WM. Regions-of-interest ratios of different tissues can prove helpful in quantifying perfusion parameters.

Sonographic harmonic grey scale imaging of brain perfusion: Scope of a new method demonstrated in selected cases. Stolz E, Allendorfer J, Jauss M, Traupe H, Kaps M. Ultraschall Med 2002 Oct; 23 (5), pp. 320–4.

Cardiovascular/Artifact Reduction: Minimization (Color Blooming)/Echoscintigraphy *Microbubbles/SHU 563A*

Background Information: Echoscintigraphy is based on the recording and summation of low-intensity signals that are emitted during ultrasound (US)-induced destruction of microbubbles.
Application: Reduction of color blooming and acoustic shadowing in contrast sonography imaging analyses through the use of an alternative imaging modality (echoscintigraphy).
Route of Administration: Intravascular.
Implementation: Evaluation of a new imaging modality (echoscintigraphy) to reduce color blooming and acoustic shadowing in contrast sonography. One strategy that has been implemented to achieve this objective has involved the use of the prototype echo-contrast agent SHU 563A to enhance imaging using a flow phantom (artificial vessels) as a reference. Following the injection of various amounts of SHU 563A (700– 40,000 bubbles/mL), preparations are insonated in the intermittent harmonic-power Doppler imaging (H-PDI) mode. The amount of receive gain is varied from 50% to 75%. The cross-sectional area (CSA) of the tube can be assessed using a summation algorithm (echoscintigraphy) and a conventional single-frame analysis (S-FA) of the H-PDI signals.
Interpretation and Analysis: Application of the summation algorithm at low-contrast concentration allows a gain-independent automatic calculation of the CSA at medium and high gain settings. Using the S-FA method, assessment of vessel diameter and the CSA is gain dependent and allows correct measurements only from 60% to 65% gain. At a high receive gain and high contrast concentration, S-FA results in an overestimation of the CSA up to 35.5%. Echoscintigraphy allows correct display of contrast-filled vessels over a wide range of gain settings at low-contrast concentrations, where S-FA does not adequately display echo contrast. Thus, echoscintigraphy minimizes artifacts resulting from color blooming and acoustic shadowing.

Echoscintigraphy: A new imaging modality for the reduction of color blooming and acoustic shadowing in contrast sonography. Schlosser T, Pohl C, Kuntz-Hehner S, Omran H, Becher H, Tiemann K. Ultrasound Med Biol 2003 Jul; 29 (7), pp. 985–91.

Neurosonology: Trans-Cranial Doppler Sonography

Background Information: Temporal hyperostosis is the major limitation of trans-temporal insonation of the basal cerebral arteries in trans-cranial Doppler sonography. Alternative contrast agents capable of traversing the pulmonary bed offer new prospects for overcoming this limitation.
Application: Echo-contrast agents improve the diagnostic potentiality of ultrasound techniques, increasing the diagnostic accuracy of these methods in cerebrovascular diseases, vascular malformations, venous pathologies, and tumors, and may contribute to reducing the need for more invasive and expensive examinations.

Echo-contrast agents in neurosonology. Totaro R, Del Sette M, Marini C. Funct Neurol 1999 Oct–Dec; 14 (4), pp. 235–9.

GASTROINTESTINAL SYSTEM

Abdominal Mass Detection (Small)/Phlegmon and Abscess Differentiation/Crohn's Disease *Levovist*

Application: Contrast-enhanced power Doppler ultrasound performed with contrast media (e.g., Levovist) can be used to detect small inflammatory masses in Crohn's disease in addition to facilitating differentiation between phlegmon and abscess formation.
Route of Administration: Levovist (intravascular).
Implementation: Evaluation of abdominal inflammatory masses utilizing power Doppler ultrasound before and after intravenous administration of ultrasound contrast agent (e.g., Levovist). Particular focus is directed at characterizing the vascularity within and/or around inflammatory masses. Color Doppler flow images are categorized as clearly visible, minimally visible, or absent. Computed tomography images can serve as validation reference controls.

Human Model: Abdominal inflammatory masses (e.g., n = 28 cases).
Analysis and Interpretation: Power Doppler ultrasound performed after the administration of contrast medium can detect small inflammatory masses in Crohn's disease, particularly those under 2 cm in diameter (validated by computed tomography). Contrast-enhanced power Doppler ultrasound is a sensitive method for detecting small abdominal inflammatory masses and for differentiation between conditions of phlegmon and abscess formation.

Contrast-enhanced power Doppler ultrasound in the diagnosis and follow-up of inflammatory abdominal masses in Crohn's disease. Esteban JM, Aleixandre A, Hurtado MJ, Maldonado L, Mora FJ, Nogues E. Eur J Gastroenterol Hepatol 2003 Mar; 15 (3), pp. 253–9.

Microstructures of the Gastrointestinal System/ Enhanced Optical Coherence Tomography
Propylene Glycol

Cellular/Molecular Mechanism of Action: The contact of certain cell types and tissues with propylene glycol subtly modifies some of their individual physical properties.
Background Information: Optical coherence tomography (OCT) is a recently developed imaging technique that has the potential to advance the early diagnosis of diseases in the human gastrointestinal (GI) tract. However, the high scattering nature of GI tissue limits its imaging depth and contrast. For more effective diagnosis using OCT, a concurrent improvement of imaging depth and contrast is, therefore, needed.
Application: Enhancement of non-invasive imaging of the GI tract applying OCT.
Implementation: Evaluate the administration of chemical agents as a means of improving the capability of OCT imaging of clinically relevant microstructures of the GI tract. Tissue preparations are imaged with OCT within 0.5 to 2 hours of harvest. Immediately after the 1st OCT imaging of tissue specimens, about 0.5 ml of 80% propylene glycol solution is applied onto tissue surfaces and the tissue allowed to absorb the compounds for 20 minutes. Another image is then taken at the same position. The specimens are then embedded and stained in preparation for histologic evaluation. Co-registration of the images obtained using OCT before and after topical application of propylene glycol formulations and standard histopathologic processing provide a basis for comparison.

***Ex Vivo* Tissue Preparations:** Normal human GI tissues, including stomach and esophagus, were obtained from hospitalized patients.
Interpretation and Analysis: More detailed microstructures, including the basal layer position and the cellular composition of the mucosal layer of GI tract tissues, can be observed after the topical application of propylene glycol solution. These same structures are not resolvable with conventional OCT imaging methods. Propylene glycol could be used as a contrasting agent for OCT imaging of human GI tract tissues, allowing an increased capability of OCT for rapid *in vivo* clinical diagnoses.

Propylene glycol as a contrasting agent for optical coherence tomography to image gastrointestinal tissues. Wang RK, Elder JB. Lasers Surg Med 2002; 30 (3), pp. 201–8.

HEPATIC SYSTEM

Angiography/Liver Transplant: Postoperative Assessment/Angiography of Portal and Hepatic Venous Structures/Arteriography of Hepatic Arteries/Budd-Chiari Syndrome: Hepatic Vein Occlusion/Pretransplant: Portal Vein Patency ± Thrombus/Flow Direction/Cavernous Transformation/Posttransplantation: Hepatic Artery Thrombosis/Hepatic Artery Stenosis ± Pseudoaneurysm
Levovist Microbubbles

Background Information: Color Doppler ultrasound (US) is a well established technique for imaging of hepatic vessels in the assessment of pre- and post–liver transplant conditions. Unfortunately, a full-color Doppler US examination of the portal or hepatic venous and hepatic arterial systems is frequently precluded by technical factors.
Application: Ultrasound contrast agents are useful in enhancing vascular Doppler signal images and play an important role in liver transplantation assessment.
Route of Administration: Levovist microbubbles (intravascular).
Implementation: Evaluation of vascular abnormalities in the pretransplant candidate with specific emphasis on establishing aspects of (i) portal vein patency and direction of flow; (ii) presence of portal vein thrombus; and (iii) demonstration of cavernous transformations.
Analysis and Interpretation: Occlusion of hepatic veins in Budd-Chiari syndrome can be confidently confirmed. Following liver transplantation, contrast ultrasonography allows for comprehensive assessment of hepatic artery thrombosis, hepatic artery stenosis, and pseudoaneurysm formation. The need for further imaging is reduced or confidently deferred in many instances. Ultrasound contrast agents play an important role in liver transplant candidates.

Clinical use of Levovist, an ultrasound contrast agent, in the imaging of liver transplantation: Assessment of the pre- and posttransplant patient. Sidhu PS, Marshall MM, Ryan SM, Ellis SM. Eur Radiol 2000; 10 (7), pp. 1114–26.

Biliary Colic/(Sphincter of Oddi Spasm)
Codeine/(Ultrasound)

Cellular/Molecular Mechanism of Action: Codeine.
Application: Codeine can be a used as a provocative agent to improve ultrasound assessment of changes in common bile duct diameter and provides an effective approach in the diagnostic detection of sphincter of Oddi spasms.
Considerations: Biliary colic from spasm of the sphincter of Oddi is a condition that is relatively difficult to detect and characterize.
Analysis and Interpretation: Diagnosis can be established by (i) using a pharmacological provocative tests employing codeine to reproduce pain; (ii) detection of changes in liver and pancreatic serum enzymes; and (iii) ultrasound imaging to demonstrate a transient increase in common bile duct diameter. Implementation of such procedures prior to surgical sphincteroplasty reproduces symptoms and brings about marked elevations in pancreatic and liver enzyme fractions and increases in common bile duct diameter that can be measured by ultrasound examination (e.g., 0.4–1.0 cm). Such events often cannot be appreciated after sphincteroplasty.

Ultrasound in the diagnosis of sphincter of Oddi spasm. Berezny GM, Beck IT, DaCosta LR, O'Brien PG, Paloschi GB. J Clin Gastroenterol 1985 Dec; 7 (6), pp. 528–32.

Cirrhosis with Focal Lesions/Hepatocellular Carcinoma: Tumor Vessels/Hyperechoic and Hypoechoic Image Patterns
Levovist Microbubbles/Pulse Inversion Harmonic Imaging (PIHI)/Color Doppler and Power Doppler

Application: Detection and characterization of focal hepatic lesions in conditions of cirrhosis applying microbubbles (e.g., Levovist) as a hepatospecific contrast image-enhancing agent in combination with PIHI.
Route of Administration: Levovist (intravenous).
Implementation: Examination of cirrhosis cases with focal hepatic lesions initially utilizing conventional ultrasound (US), followed by evaluation using color Doppler (CD), power Doppler (PD)

with spectral analysis of tumoral vessels, and PIHI. The latter modality, PIHI, is performed 30 seconds (vascular phase) and 3–5 minutes (late phase) after Levovist injection.

Human Model: Cirrhosis cases with focal hepatic lesions (e.g., n = 25). To definitely characterize and evaluate focal hepatic lesions, helical-CT (HCT) enhancement patterns (e.g., n = 15 cases) and/or surgical/bioptic histologic findings (e.g., n = 10 cases) are utilized as reference procedures (e.g., n = 30 focal hepatic lesions classified as hepatocellular carcinoma [HCC]).

Analysis and Interpretation: In focal hepatic lesions classified as HCC by reference procedures, approximately 63% appear hypoechoic (e.g., n = 19/30); 17% appear isoechoic (e.g., n = 5/30); or 20% hyperechoic (e.g., n = 6/30) on conventional US and are accompanied by basket arterial patterns (e.g., n = 10/30), vessels within the tumor (e.g., n = 6/30), peripheral arterial patterns (e.g., n = 4/30), or no vascular pattern (e.g., n = 10/30) on CD and PD. On PIHI, acquired images appear hyperechoic (e.g., n = 26/30) or isoechoic (e.g., n = 4/30) in the vascular phase, if compared to the surrounding liver parenchyma, and hypoechoic (e.g., n = 23/30) or isoechoic (e.g., n = 7/30) in the late phase. Some lesions classified as regenerative nodules (RNs) by reference procedures appear hypoechoic on conventional US, with peripheral venous/arterial pattern (e.g., n = 1/4) or no vascular pattern (e.g., n = 3/4) on CD and PD. On PIHI, lesions appear hypoechoic (n = 3) or isoechoic (n = 1) in the vascular phase, and remain prevalently hypoechoic (n = 3) or isoechoic (n = 1) in the late phase. Hepatic lesions classified as hemangioma by reference procedures appear hyperechoic (e.g., n = 4/5) or hypoechoic (e.g., n = 1/5) on conventional US with few peripheral venous vessels on CD and PD. On PIHI, a progressive fill-in from the periphery toward the center during the vascular and late phase can be appreciated following microbubble (Levovist) injection. Pulse Inversion Harmonic Imaging seems to be a reliable technique to characterize focal lesions in cirrhotic patients.

Characterization of focal hepatic lesions in cirrhotic patients by Pulse Inversion Harmonic Imaging US contrast specific technique with Levovist. Quaia E, Forgacs B, Calderan L, Bertolotto M, Pozzi Mucelli R. Radiol Med (Torino) 2002 Oct; 104 (4), pp. 285–94.

Hepatic Lesion Characterization/Neoplasia: Hepatic Nodules (Malignant vs Benign)/Hepatic Cirrhosis vs Diffuse Parenchymal Liver Disease/ Backscatter Ultrasound
Microbubbles/Contrast Medium Transit Time

Cellular/Molecular Mechanism of Action: Modern ultrasound contrast media are gas-containing stabilized microbubble preparations that remain intact in the circulating blood for several minutes after intravenous injection and increase the intensity of backscattered ultrasound.

Background Information: When the microbubbles disappear from the blood, they can be detected in the parenchyma of the liver and the spleen for about 30 more minutes (late liver- and spleen-specific phase). The insonated microbubbles produce 2nd-harmonic ultrasound frequencies, whose detection requires non-conventional ultrasound modalities such as pulsed inversion imaging. Non-conventional ultrasound techniques can also be used without microbubbles because 2nd harmonics can be generated by ultrasound in tissues as well.

Application: Circulating microbubbles can be used not only to enhance weak Doppler signals but also to perform dynamic contrast studies. Contrast-enhanced dynamic ultrasound studies—similar to contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) examinations—have been used in humans to non-invasively characterize lesions (i.e., without biopsies) found during conventional ultrasound examinations. To map the distribution of contrast medium in a nodule or in an organ, specific scanning techniques such as stimulated acoustic emission have been developed. Stimulated acoustic emission occurs when high acoustic pressure ultrasonic waves disrupt the stationary or slowly moving microbubbles. This results in the release of a large amount of harmonic ultrasound frequencies. When the stimulated acoustic emission technique is used for dynamic studies, scanning must be interrupted several times to allow the microvasculature of the lesion to refill with microbubbles (interval delay imaging). The contrast patterns of malignant and benign hepatic nodules in humans have been the most intensively studied. Another type of dynamic study in humans measures the transit time of the contrast medium; that is, how fast the peripherally injected microbubbles reach the hepatic veins. Hepatic cirrhosis can be differentiated from other diffuse parenchymal liver diseases by a shorter transit time. Introducing non-conventional ultrasound techniques and ultrasound contrast media in veterinary diagnostic imaging may have potential value; however, intensive research should be carried out before ultrasound contrast agents can routinely be used in clinical practice.

A review of non-conventional ultrasound techniques and contrast-enhanced ultrasonography of non-cardiac canine disorders. Szatmári V, Harkányi Z, Vörös K. Vet Radiol Ultrasound 2003 Jul–Aug; 44 (4), pp. 380–91.

Kupffer Cell Phagocytic Function
Perfluorobutane Microbubbles: Sonazoid/ Radioiodinated Albumin

Cellular/Molecular Mechanism of Action: Perfluorobutane microbubbles (Sonazoid) are eliminated from the intravascular compartment by hepatic tissues (mononuclear phagocytic system or reticuloendothelial system).

Application: Detection and characterization of hepatic mononuclear phagocytic system integrity (sinusoidal macrophages).

Route of Administration: Perfluorobutane microbubbles (Sonazoid) can be infused intravascularly. In a research environment, they can also be infused *ex vivo* into fresh whole- liver preparations (e.g., rat).

Dosage: Perfluorobutane microbubbles (Sonazoid ≤ 20 times the anticipated clinical dose for hepatic imaging).

Implementation: Delineation of the cell type within the liver that is responsible for removing perfluorobutane microbubbles (Sonazoid) from the intravascular compartment and determination of their potential to disrupt the normal function of hepatic cell populations. Following intravascular injection of perfluorobutane microbubbles, hepatic tissue are harvested and analyzed by electron microscopy.

Animal Model: Administration of perfluorobutane microbubbles (Sonazoid) to rats intravascularly, and to perfused liver preparations.

Analysis and Interpretation: Electron microscopy reveals that perfluorobutane microbubbles (Sonazoid) are exclusively internalized within Kupffer cells (hepatic macrophages of the mononuclear phagocytic system) that line the liver sinusoids. The hepatic parenchymal, stellate, or endothelial cell populations do not appear to internalize the contrast agent. Uptake of perfluorobutane microbubbles (Sonazoid) by the Kupffer cells following injection of a dose corresponding to 20 times the anticipated clinical dose for liver imaging does not result in measurable changes in the uptake and degradation of radioactively labelled albumin microspheres previously shown to be a useful indicator marker for Kupffer cell phagocytosis.

Hepatic clearance of Sonazoid perfluorobutane microbubbles by Kupffer cells does not reduce the ability of liver to phagocytose or degrade albumin

microspheres. Kindberg GM, Tolleshaug H, Roos N, Skotland T. Cell Tissue Res 2003 Apr; 312 (1), pp. 49–54. Epub 2003 Feb 27.

Neoplasia: Hepatic Carcinoma Vascularity
Levovist

Cellular/Molecular Mechanism of Action: Microbubble preparations variably impede, absorb, and reflect ultrasound energy (acoustical impedance) in a manner that is different from surrounding tissues/organs and relatively homogenous biological fluids.
Application: Characterization of alterations is tissues, organ systems, vascular structures, and biological fluids.
Route of Administration: Microbubble preparations (e.g., Levovist) are most frequently administered intravascularly.
Implementation: Evaluate the usefulness of contrast-enhanced harmonic wideband gray-scale sonography to assess the therapeutic effects of trans-catheter arterial chemoembolization in conditions of hepatocellular carcinoma for comparison with findings from histopathological analysis. In this context, hepatocellular carcinoma lesion vascularity can be characterized before and 7 days after trans-catheter arterial chemoembolization. Imaging analysis is performed by contrast-enhanced harmonic wideband gray-scale sonography combined with the injection of an ultrasound contrast image-enhancing agent (Levovist). Biopsy specimens are obtained from the lesions evaluated, and the histologic findings compared with contrast-enhanced sonography images.

Human Model: Cases of hepatocellular carcinoma lesions (e.g., n = 29).
Analysis and Interpretation: Contrast-enhanced harmonic wideband gray-scale sonography performed after trans-catheter arterial chemoembolization can detect tumor vascularity in approximately half of hepatocellular carcinoma lesions evaluated (e.g., n = 16/29). Histopathology evaluation detects residual tumor in most lesions determined to have positive vascularity patterns (e.g., n = 13/16 lesions). In contrast, essentially all lesions without detectable tumor vascularity have no histologically evident tumor residue (e.g., n = 13/13). Some of these lesions may display tumor progression that can be detected by computed tomography (CT) imaging during 9 to 12-month follow-up periods (e.g., n = 3/13). Sensitivity and specificity of images for discerning viable and non-viable hepatocellular carcinoma after trans-catheter arterial chemoembolization is approximately 100% and 81%, respectively, based on comparisons between contrast-enhanced harmonic wideband gray-scale sonographic images compared with histopathology findings. Contrast-enhanced harmonic wideband gray-scale sonography is potentially useful for evaluating the therapeutic effects of trans-catheter arterial chemoembolization on hepatocellular carcinoma.

Contrast-enhanced harmonic gray-scale sonographic-histologic correlation of the therapeutic effects of trans-catheter arterial chemoembolization in patients with hepatocellular carcinoma. Morimoto M, Shirato K, Sugimori K, Kokawa A, Tomita N, Saito T, Imada T, Tanaka N, Nozawa A, Numata K, Tanaka K. AJR Am J Roentgenol 2003 Jul; 181 (1), pp. 65–9.

Neoplasia: Hepatic VX2 Tumor Carrier Model
Bubble Contrast Image-Enhancing Agent (Non-Destructive)/B-Mode Ultrasound/2nd-Harmonic Imaging (SHI)

Application: Enhanced detection and characterization of physiological conditions and pathological anomalies affecting tissues, organ systems, and vascular structures utilizing ultrasound-based diagnostic modalities.
Route of Administration: Ultrasound contrast image-enhancement agent (intravenous/rabbit ear vein).
Dosage: Ultrasound contrast image-enhancement agent (0.01 mL/kg for B-mode and 0.05 ml/kg for SHI).
Implementation: Hepatic VX2 tumors can be imaged with B-mode ultrasonography and by SHI (high mechanical index [MI] = 1.6 and low MI = 0.1). Echo agents can be intravenously injected through an ear vein (B-mode = 0.01 mL/kg; 0.05 ml/kg = high MI SHI). Venous channels are then evacuated with sterilized saline. Images are recorded by magnetic optics (MO) and analyzed further by direct visual inspection.

Animal Model: New Zealand rabbits (e.g., n = 6; mean = 2.7 ± 0.4 kg) developed as a construct hepatic VX2 tumor carrier model.
Analysis and Interpretation: Ultrasound (B-mode) is capable of detecting both hypoechoic and hyperechoic lesions with a mean size of about 2.1 ± 0.4 and are oval or round in shape. Such lesions appear to have a clear outline or a hypoechoic halo at their margin (e.g., n = 6 rabbits; n = 6 hypoechoic; n = 3 hyperechoic lesions). Ultrasound contrast agent does not change the echogenicity of the lesions under B-mode ultrasound and SHI under high acoustic pressure. However, contrast media greatly increases real-time visualization sensitivity of lesions with SHI under low acoustic pressure. Contrast-enhanced SHI with low MI and a bubble non-destructive method is a much more effective methodology for detecting and characterizing hepatic VX2 tumors than is conventional SHI for clinical applications.
Instrumentation: B-mode ultrasonography; SHI and MO.

Assessment of hepatic VX2 tumors of rabbits with 2nd-harmonic imaging under high and low acoustic pressures. Du WH, Yang WX, Wang X, Xiong XQ, Zhou Y, Li T. World J Gastroenterol 2003 Aug; 9 (8), pp. 1679–82.

Neoplasia: Hepatocellular Carcinoma (Angiography)/Response to Percutaneous Radio Frequency Abalation/Relative Effectiveness: 3-Phase Dynamic Computed Tomography (CT) vs Coded Harmonic Angiography
Levovist Microbubbles

Cellular/Molecular Mechanism of Action: Microbubbles variably impede, absorb, and reflect ultrasound energy in a manner that is different than surrounding tissues or biological fluids, which in turn are detected by imaging probes (detectors).
Application: Characterization of pathological conditions affecting organ systems (e.g., hepatic carcinoma) and vascular structures.
Route of Administration: Levovist microbubbles (intravenous).
Implementation: Evaluation of the capacity for microbubbles in contrast-enhanced coded phase-inversion harmonic sonography angiography to assess therapeutic responses to percutaneous radio frequency ablation of hepatocellular carcinoma lesions. The intratumoral blood vessels and tumor parenchymal stain are detected in the early arterial phase and the late vascular phase, respectively. The results of contrast-enhanced imaging with coded harmonic angiography are compared with those of 3-phase dynamic CT.

Human Model: Cases of hepatocellular carcinoma (e.g., n = 67 cases, n = 107 exams; n = 91 nodular lesions).
Analysis and Interpretation: The vast majority of hepatocellular carcinoma nodules are hypervascular on contrast-enhanced imaging with coded harmonic angiography (e.g., n = 107/107). Following radio frequency ablation, contrast-enhanced coded harmonic angiography detects persistent signal enhancement of nodules (e.g., n = 41/107 or 38.3%), whereas the technique shows no intratumoral enhancement in greater than half of examined nodules (e.g.,

n = 66/107 or 61.7%). Compared with dynamic CT, the sensitivity, specificity, and diagnostic accuracy of contrast-enhanced coded harmonic angio is approximately 95.3%, 100%, and 98.1%, respectively. With contrast-enhanced coded harmonic angiography, it is difficult to identify the safety margin that can be detected on dynamic CT. Contrast-enhanced imaging with coded harmonic angiography may provide an alternative diagnostic approach that has a high level of agreement with dynamic CT in assessing the therapeutic effect of radio frequency ablation in hypervascular hepatocellular carcinomas, in spite of having limitations in safety margin identification.

Radio frequency ablation of hepatocellular carcinoma: Therapeutic response using contrast-enhanced coded phase-inversion harmonic sonography. Wen YL, Kudo M, Zheng RQ, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T, Maekawa K. AJR Am J Roentgenol 2003 Jul; 181 (1), pp. 57–63.

Neoplasia: Hepatocellular Carcinoma/ Hemangiosarcoma/Nodular Hyperplasia/Hepatic Metastases
Sulfur Hexafluoride Vapor Microbubbles

Cellular/Molecular Mechanism of Action: Microbubbles impede the unidirectional progression of ultrasound (US) energy through tissues, and therefore variably impede, absorb, and reflect a portion of the waves generated by the transducer.
Application: Enhancement of focal hepatic lesions.
Route of Administration: Intravascular.
Implementation: Determine if US performed with a microbubble contrast imaging agent (e.g., SonoVue), or a contrast agent containing microbubbles filled with sulfur hexafluoride (SF_6) vapor, produces differential patterns of contrast enhancement of focal hepatic lesions. Such an objective can be addressed by assessing hepatic lesions utilizing ultrasound, color Doppler ultrasound, and contrast-enhanced ultrasound performed in intermittent high-acoustic-power mode. Reference control procedures can consist of contrast-enhanced helical computed tomography (e.g., n = 11 cases) and US-guided fine needle aspiration (e.g., n = 28 cases). Contrast enhancement patterns are defined by means of both subjective and objective analysis. Baseline and contrast-enhanced ultrasound scans can be reviewed off-line.

Human Model: Focal hepatic lesions associated with various clinical conditions (n = 39 cases; n = 40 focal hepatic lesions; n = 15 hepatocellular carcinomas [HCCs]; n = 10 metastases; n = 11 hemangiomas; n = 4 focal nodular hyperplasias).
Interpretation and Analysis: Injection of SonoVue can correctly characterize a large number of conditions affecting the liver parenchyma (e.g., n = 13/15 HCCs; n = 8/10 metastases; and n = 4/4 hemangiomas) based on the appreciation of an atypical pattern (baseline ultrasound images). A low percentage of HCCs and metastases may remain "indeterminate" with no identification of characteristic baseline or contrast-enhanced patterns (e.g., n = 2/15 HCCs; n = 2/10 metastases).

Baseline ultrasound imaging analysis appears to be essential in characterizing all hemangiomas with a typical pattern (n = 7), and color Doppler ultrasound with spectral analysis of tumoral vessels is essential in characterizing focal nodular hyperplasia. The percent diagnostic agreement with reference procedures is significantly higher ($p < 0.001$) for contrast-enhanced US compared with baseline ultrasound. Therefore, characteristic patterns of ultrasound contrast enhancement with SonoVue help in characterizing and differentiating focal hepatic lesions.

US characterization of focal hepatic lesions with intermittent high-acoustic-power mode and contrast material. Quaia E, Bertolotto M, Calderan L, Mosconi E, Mucelli RP. Acad Radiol 2003 Jul; 10 (7), pp. 739–50.

Neoplasia: Malignant and Benign Mass Lesions/ Nodular Hyperplasia: Central Starlike Fill-In Patterns/Hemangioma
Levovist/Iminodiacetic Acid-Enhanced Scintigraphy

Cellular/Molecular Mechanism of Action: Microbubbles (e.g., Levovist) variably impede and absorb ultrasound energy. Because their acoustical impedance differs from surrounding tissues or more "homogenous" biological fluids, the properties can be detected by imaging probes.
Application: Detection and characterization of benign and malignant mass lesions affecting the liver, including conditions of nodular hyperplasia and hemangioma.
Route of Administration: Microbubbles (e.g., Levovist) are usually administered intravascularly.
Dosage: Levovist (2 g).
Implementation: Histopathology analysis can initially be performed on biopsy specimens collected from hepatic mass lesions. Complementary forms of diagnostic evaluation can entail the application of computed tomography or hepatic iminodiacetic acid-enhanced scintigraphy. Ultrasound images are then acquired after injection of Levovist injection and analysis performed in order to characterize the arrival of contrast media utilizing the interval delay imaging technique for 60 seconds.

Human Model: Cases with focal heptic lesions (e.g., n = 72; n = 42/72 malignant, n = 30 benign).
Analysis and Interpretation: The early arrival of Levovist at less than 30 seconds after injection can be used as an indicator for malignancy and has a specificity level of 67% and sensitivity of 60% ($p < 0.05$). The central starlike "fill-in" pattern as a sign for focal nodular hyperplasia has a level of specificity of 100% and sensitivity of 67% ($p < 0.001$). The rimlike pattern followed by centripetal fill-in as a sign for hemangioma has a specificity level of 100% and sensitivity of 18% ($p < 0.01$). Alternatively, the early diffuse stippled arrival pattern can be found in 60% of malignant lesions and also in 33% of cases of focal nodular hyperplasia and in some cases of adenoma (e.g., n = 1 case). Analysis of Levovist arrival time cannot distinguish between a malignant or benign lesion in individual cases. However, the central starlike arrival pattern is characteristic of focal nodular hyperplasia.

Pulse inversion sonography in the early phase of the sonographic contrast agent Levovist: Differentiation between benign and malignant focal liver lesions. von Herbay A, Vogt C, Haussinger D. J Ultrasound Med 2002 Nov; 21 (11), pp. 1191–200.

Neoplasia: Metastatic Cancer/Colorectal Carcinoma
Styrene Maleic Acid Neocarzinostatin [SMANCS/ Lipiodol (S/L)]

Background Information: Liver metastasis is the major cause of death in conditions of colorectal cancer. Surgical resection is at present the only curative option. Styrene maleic acid neocarzinostatin targets the unique vascular architecture of tumor blood vessels, which are hyperpermeable and lack a well-developed lymphatic system.
Implementation: Evaluation of changes in the tumor microvasculature associated with hepatic metastases detected by the application of scanning electron microscopy (SEM) of corrosion casts following S/L administation.

Animal Model: Murine (mice with hepatic metastatic lesions induced by intrasplenic injection of dimethylhydrazine-induced colon cancer cells). In this model tumor angiogenesis occurs at day 10, while exponential tumor growth occurs at day 16.
Analysis and Interpretation: Tumors treated with S/L at day 10 appear similar to day 10 controls. Tumor vessels, 50 ± 18 microm

in diameter, are easily identified from hepatic vessels. Within the hepatic sinusoids are avascular spaces, 144 ± 60 μm in diameter, that correspond to tumor cell aggregates at the initial stages of growth. Similarly, day 16 treated tumors appear comparable to day 16 controls. These vessels are narrower (84 ± 32 μm vs 150 ± 70 μm) than their control counterparts. This is in contrast to vessels (216 ± 36 μm in diameter) of a complex nature appreciated at 3 weeks.

S/L exerts a marked and immediate effect on the tumor microvessels at both the angiogenic and the exponential phases of tumor growth. This agent is effective at the microvascular level during inhibition of metastatic growth.

Changes in the microvascular architecture of colorectal liver metastases following the administration of SMANCS/lipiodol. Kuruppu D, Christophi C, Maeda H, O'Brien PE. J Surg Res. 2002 Mar; 103 (1), pp. 47–54.

Neoplasia: Metastatic Cancer/Detection Limits and Resolution/Comparisons with Contrast Helical Computed Tomography (CT) and B-Mode Ultrasound (US)
Levovist/Wideband Harmonic Imaging (WHI)

Application: Contrast-enhanced WHI can be applied to detect and characterize various types of hepatic metastases (nodules < 1 cm diameter).
Route of Administration: Microbubbles (Levovist) are most commonly given by intervascular injection depending on the diagnostic procedure of interest.
Dosage: Levovist at 2.5 g/dose (300 mg/ml).
Implementation: Evaluation of the capability of contrast-enhanced WHI to detect liver metastases compared to fundamental B-mode US and postcontrast CT. Conventional B-mode sonography can be applied to characterize hepatic metastatic lesions. Wideband harmonic imaging is performed 3 minutes after injection of Levovist contrast agent and postcontrast helical CT (HCT). The number and location of the lesions detected and the smallest lesion for individual cases are then recorded for comparison analysis.

Human Model: Cases suffering from hepatic metastases originating from different types of primary malignant lesions (e.g., n = 27).
Analysis and Interpretation: Comparisons reveal an increase in the number of lesions in harmonic mode compared with conventional B-mode images in cases with hepatic metastasis (e.g., n = 27/27) with a mean increase from 9.3 lesions with B-mode to 18.8 lesions with WHI. The smallest lesions are detected with WHI when compared with conventional US and helical CT (HCT) (2 mm with WHI, 5 mm with B-mode, and 5 mm with CT). Wideband harmonic imaging detects more lesions than conventional ultrasound or contrast HCT. Contrast-enhanced WHI appears to be superior to conventional US and HCT for the detection of hepatic metastases, especially for those nodules under 1 cm of diameter.

Improved detection of liver metastases with contrast-enhanced wideband harmonic imaging: Comparison with CT findings. Esteban JM, Molla MA, Tomas C, Maldonado L. Eur J Ultrasound 2002 Oct; 15 (3), pp. 119–26.

Neoplasia: Metastatic Carcinoma/Ring Enhancement Pattern (Early Phase)/Metastatic Carcinoma: Clear Contrast Defect (Delayed Phase)/ Gray-Scale Conventional vs Harmonic Imaging/ Lesion Neovascularization
Sonazoid

Cellular/Molecular Mechanism of Action: Sonazoid possesses a different level of acoustical impedance compared to tissues, organ systems, and "homogenous" biological fluids.
Application: Detection and characterization of hepatic neoplastic lesions (rabbit tumor model).
Route of Administration: Sonazoid echo-enhancing agent (intravenous).
Implementation: Examination of normal livers with Sonazoid echo-enhancing contrast imaging agent utilized in concert with the ultrasonographic imaging modes (i) gray-scale conventional; and (ii) harmonic performed as a time-related analysis. Data is evaluated quantitatively and visually with tumor-model rabbits in order to estimate the diagnostic potential of each modality.

Animal Model: Rabbit liver (*in vivo* normal vs tumor model).
Interpretation and Analysis: Peak enhancement of vessels and parenchyma is observed 1 minute after injection of Sonazid echo-enhancing contrast agent when evaluated with both modes. Signal enhancement in the parenchyma lasts for 120 minutes compared with rapid decay (5–10 minutes) within vessels. When Sonazoid is intravenously injected into metastatic carcinoma-model (VX-2) rabbits, all hepatic tumors show ring enhancement in the early phase, followed by clear contrast defects in the delayed phase because signal enhancement remains only in normal parenchyma. Visual analysis scores for the diagnosis of tumors are improved by Sonazoid injection, and the videodensitometric differences between tumor and normal tissues are significantly greater after injection. Although the harmonic mode tends to show better contrast effects, the conventional mode provides significant contrast enhancement in this hepatic tumor model. Sonazoid might be useful for the detection of undifferentiated tumors in the liver by making it possible to visualize neovascularity in the early phase and clear contrast defects in the delayed phase, not only in the harmonic but also in the conventional mode.

Gray-scale liver enhancement with Sonazoid (NC100100), a novel ultrasound contrast agent: Detection of hepatic tumors in a rabbit model. Watanabe R, Matsumura M, Chen CJ, Kaneda Y, Ishihara M, Fujimaki M. Biol Pharm Bull 2003 Sep; 26 (9), pp. 1272–7.

Neoplasia: Metastatic and Primary/Hepatocellular Carcinoma (HCC)/Hemangioma/Nodular Hyperplasia/Metastases
Microbubble Contrast Agent Prototype (SHU 508 A)

Application: Detection and characterization of physiological conditions and pathological anomalies affecting tissues, organ systems, and vascular structures when used in combination with ultrasound-based imaging modalities.
Route of Administration: Prototype microbubble contrast agent (SHU 508 A) injected intravenously.
Implementation: Evaluate the degree that the microbubble contrast agent SHU 508 A enhances lesion characterization capabilities (imaging profiles) of various focal hepatic lesions utilizing contrast-enhanced gray-scale harmonic ultrasound (US) in combination with coded harmonic angio (CHA) techniques to enhance lesion characterization capabilities. Procedures are performed with a combination of continuous scanning to assess the intratumoral vasculature (vascular imaging) and interval-delay scanning to determine the sequential enhancement pattern (acoustic emission imaging).

Human Model: Hepatic lesions (e.g., n = 95 cases; n = 105 focal lesions; n = 51/105 hepatocellular carcinoma, n = 22/105 metastases, n = 22/105 hemangiomas, n = 4/105 nodular hyperplasia, n = 6/105 non-tumerous nodules).
Analysis and Interpretation: Of the total number of vascular images acquired, approximately 69% of hepatocellular carcinomas show irregular branching vessels (e.g., n = 35/51 HCCs), while 91%

of metastases (e.g., n = 20/22) display a peripherally stippled pattern. Intratumoral vessels are absent in about 95% of hemangiomas (e.g., n = 21/22) and all non-tumorous lesions (e.g., n = 6/6), while approximately 75% of focal nodular hyperplasias display a spoke-wheel pattern (e.g., n = 3/4). In acoustic emission imaging, 71% of HCCs (e.g., n = 36/51) show heterogeneous enhancement and 86% (e.g., n = 19/22) of metastases showed rim- or flamelike peripheral enhancement during the early phase, with washout occurring in essentially all hepatocellular carcinomas and metastases during the late phase (e.g., n = 73/73 or 100%). In hemangiomas, enhancement is either peripheral and nodular (e.g., n = 19/22 or 86%) or persistent and homogeneous (e.g., n = 3/22 or 14%), and 75% of focal nodular hyperplasias (e.g., n = 3/4) become isoechoic during the late phase. Utilizing contrast-enhanced gray-scale US and the CHA technique, a period of continuous scanning reveals the intratumoral vasculature pattern, and interval-delay scanning demonstrates the sequential enhancement pattern. The characteristic findings of various focal hepatic lesions can then be determined.

Focal hepatic lesions: Evaluation with contrast-enhanced gray-scale harmonic US. Jang HJ, Lim HK, Lee WJ, Kim SH, Kim MJ, Choi D, Lee SJ, Lim JH. Korean J Radiol 2003 Apr–Jun; 4 (2), pp. 91–100.

Neoplasia: Metastatic and Primary/Hemangioma/Hepatocellular Carcinoma/Cholangiocellular Carcinoma/Hepatic Metastases/Sensitivity and Specificity

Sonocontrast Agent/Contrast-Enhanced Power Doppler Sonography

Application: Detection and characterization of liver hemangiomas and various forms of hepatic neoplastic disease applying contrast-enhanced power Doppler sonography.
Route of Administration: Sonocontrast agent (intravascular bolus).
Implementation: Classification of the distribution (peripheral, central, mosaic) and extent (none, minimal, moderate, and strong) of intratumoral flow pattern in sonographic images acquired in conditions of hepatic neoplasia.

Human Model: Cases with hepatic neoplasia (e.g., n = 45 cases; n = 57 tumors; n = 22 hemangiomas; n = 24 metastases; n = 10 hepatocellular carcinoma (HCC); n = 1 cholandiocellular carcinoma).
Analysis and Interpretation: The administration of the sonocontrast agent by bolus injection causes enhancement to gradually increase up to 2 minutes and lasts for 4 to 5 minutes. After injection of contrast agent, flow signals appear or increase in 59% of tumors (e.g., n = 34/57). No signal enhancement is observed in most hemangiomas (e.g., n = 18/22), 15% of metastases (e.g., n = 4/22), and 10% of hepatocellular carcinoma (e.g., n = 1/10). The sensitivity and specificity of intratumoral vascularity for the detection of malignant liver tumors is 37.1% and 90.9% for unenhanced power Doppler sonography, and 85.7% and 81.8% for contrast-enhanced power Doppler sonography, respectively. Contrast-enhanced power Doppler sonography is superior to unenhanced power Doppler sonography in the demonstration of malignant tumor vascularity, and is helpful in differentiating between hemangiomas and malignant liver tumors. A specific flow pattern within the tumor is not established in primary and metastatic malignant tumors with contrast-enhanced power Doppler sonography.

Contrast media-enhanced power Doppler sonography for evaluation of hemangiomas and malignant tumors in the liver. Karabacakoglu A, Karakose S, Cil AS, Kaya A. J Gastroenterol Hepatol 2003 Jan; 18 (1), pp. 92–8.

Neoplasia: Vascularity Patterns (Hepatic)/Differential Diagnoses

Levovist Microbubbles

Cellular/Molecular Mechanism of Action: Microbubble preparations possess a different level of acoustic impedance compared to homogenous biological fluids.
Application: Enhance the detection and characterization of physiological processes and anomalies of tissues (e.g., hepatic neoplasias) and vascular structures visualized with the aid of color Doppler imaging modalities.
Route of Administration: Levovist microbubbles (intravenous).
Implementation: Determine the efficacy of using an ultrasound contrast agent (Levovist) to enhance the color Doppler ultrasound imaging of liver neoplasms. One aspect of such analyses can involve delineating the profiles of vascular structures within normal compared to neoplastic hepatic lesions.

Human Model: Cases with hepatic neoplasia (e.g., n = 39).
Analysis and Interpretation: Contrast image-enhanced color Doppler is capable of detecting various hepatic tumors that possess different patterns of lesion vascularity.

In conditions of hepatocarcinoma, a rather unique image signal can be observed in the central region of tumors (e.g., n = 11/16). Conversely, metastatic liver lesions generate enhanced color Doppler signals that appear only at the tumor periphery (e.g., n = 3/16). A rimlike color enhancement can be seen in many of these lesions (e.g., n = 2/3). In cases with hepatic hemangiomas, contrast-enhanced color Doppler imaging demonstrates blood vessels at the margin of neoplasms (e.g., n = 6). In this context, contrast-enhanced color Doppler imaging improves the visualization of hepatic neoplasm vascularity. Such techniques can potentially detect small liver tumors and serve as an approach for differentiating various types of hepatic neoplasms.

Feasibility study of an ultrasound contrast agent (Levovist) in color Doppler imaging of liver neoplasms. Jiang Y, Dai Q, Liu J, Zhang J, Chang X, Cai S, Tan L. Chin Med Sci J 1999 Sep; 14 (3), pp. 167–9.

Neoplasia: Vascularity and Architecture (Hepatic)

Optison/Contrast-Enhanced Power Doppler Sonography (CEPD)/Phase Inversion Harmonic Imaging (PIHI)

Application: Optison is an echo-enhancing agent that can be utilized for the detection and characterization of alterations in vascular structures associated with liver pathology, including neoplastic disease.
Route of Administration: Optison (intravascular).
Implementation: Assessment of liver lesion vascularity and characterization of focal liver lesions applying CEPD and PIHI in combination with Optison as an echo-enhancing agent. Extent and architecture of lesion vascularity, in addition to the kinetics of contrast enhancement within lesions, can also be evaluated.

Human Model: Focal liver lesions (e.g., n = 101).
Analysis and Interpretation: Analysis of tumor vascularity may not be possible in approximately 30% of cases (e.g., n = 30/101) due to motion or blooming artifacts produced by CEPD. Vascularity can be detected in 61% of liver lesions by CEPD and in 95% by PIHI. Phase inversion harmonic imaging identifies significantly more tumor vascularity pattern (93%) than CEPD (57%). Specific lesion diagnosis based on PIHI can result in correct results in 92% compared to 59% of cases utilizing CEPD. Phase inversion harmonic imaging is highly efficient in detecting tumor vascularity and is superior to CEPD in characterizing focal liver lesions.

Phase inversion harmonic imaging vs contrast-enhanced power Doppler sonography for the characterization of focal liver lesions. Strobel D, Raeker S, Martus P, Hahn EG, Becker D. Int J Colorectal Dis 2003 Jan; 18 (1), pp. 63–72. Epub 2002 May 04.

IMMUNE SYSTEM

Lymph Nodes: Regional
Poly(Ethylene Glycol) (PEG)-Coated Magnetite Nanospheres

Semi-Synthetic Methods: Magnetite nanospheres can be prepared with a grafted PEG layer using various PEG lengths from 350 to 1,000 Da. Thermogravimetric analysis is utilized to measure the adsorbed amount of PEG. Colloid stability is confirmed by measurement of particle size and electrophoretic mobility.
Implementation: Evaluation of injection site drainage kinetics and lymph node retention 2 hours after subcutaneous administration of nanospheres coated with PEG lengths of 350, 550, 750, and 1,000 Da. For 750 PEG-coated nanospheres, the kinetics of distribution can be determined over a 48-hour time course.
Interpretation and Analysis: The distribution of the nanospheres is modified and the lymph node localization is enhanced by altering the surface coverage of PEG on the magnetic surface. PEG-coated magnetite nanospheres with different surface characteristics can be utilized to target a diagnostic agent for regional lymph nodes.

Development of systems for targeting the regional lymph nodes for diagnostic imaging: *In vivo* behavior of colloidal PEG-coated magnetite nanospheres in the rat following interstitial administration. Illum L, Church AE, Butterworth MD, Arien A, Whetstone J, Davis SS. Pharm Res 2001 May; 18 (5), pp. 640–5.

Lymph Nodes: Sentinel Nodes for Neoplasia/ Normal vs Metastatic
Carbon Dioxide Microbubbles

Background Information: Although endoscopic ultrasonography is considered the most useful diagnostic modality for regional staging, the capability of diagnosing lymph node metastasis based on endoscopic ultrasonography images alone is not sufficient. To improve the effectiveness of differential diagnosis of lymph node enlargement, contrast-enhanced echolymphography can be performed using endoscopic ultrasonography-guided puncture.
Implementation: Evaluation of the ability of contrast-enhanced echolymphography to differentiate between normal and metastatic lymph nodes. Lymph nodes are punctured under real-time endoscopic ultrasonography guidance, and carbon dioxide microbubbles can be injected to evaluate echo profiles and features before and after microbubbles injection.

***Ex Vivo* Model:** Metastatic lymph nodes (n = 8) surgically resected from cases with gastrointestinal cancers (*in vitro* study).

Human Model: Cases with abdominal lymph node swelling detected by endoscopic ultrasonography (n = 55).
Interpretation and Analysis: Contrast-enhanced echolymphography of freshly resected metastatic lymph nodes show nonhomogeneous echo patterns. In regions demonstrating filling defects detected by contrast-enhanced echolymphography, neoplastic infiltration pathology can be observed. In almost all of the malignant lymph nodes studied *in vivo*, filling defects and heterogeneous enhancements can be observed by contrast-enhanced echolymphography. However, contrast-enhanced echolymphography demonstrates uniform patterns in most of the benign group. Sensitivity, specificity, positive and negative predictive value, and accuracy of differential diagnosis by contrast-enhanced echolymphography are 95.8%, 90.3%, 88.5%, 96.6%, and 92.7%, respectively. Contrast-enhanced echolymphography is a useful method that aids in differentiating between reactive responses and malignant alterations affecting lymph nodes.

Differentiation of benign and malignant lymph nodes with contrast-enhanced echolymphography using endoscopic ultrasound-guided puncture. Kojima S, Goto H, Hirooka Y, Itoh A, Ishiguro Y, Hashimoto S, Hirai T, Hayakawa T. Hepatogastroenterology 2003 Sep–Oct; 50 (53), pp. 1285–91.

MUSCULOSKELETAL SYSTEM

Articular Cartilage/Cartilage Volume and Thickness Estimation/Ultrasonography Compared to Computed Tomography (CT)
Ultrasound Contrast Image-Enhancing Agent

Background Information: A non-invasive imaging technique that can quantify articular cartilage is needed for diagnosis, monitoring, and therapeutic control of osteoarthritis.
Route of Administration: Ultrasound contrast image-enhancing agent (intra-articular).
Implementation: Evaluate the accuracy of 3-dimensional cartilage volume and thickness measurements in the knee with magnetic resonance imaging (MRI) analysis. Such investigations can involve the use of of cadaveric specimens evaluated by sagittal imaging with a fat-suppressed gradient echo sequence. Following contrast agent injection, 2 sagittal computed tomography data sets are obtained, with knee joints repositioned between the examinations. Cartilage thickness is determined, after 3-dimensional reconstruction, using a minimal distance algorithm.

Human Model: Cadaveric specimens (e.g., n = 8).
Analysis and Interpretation: The mean absolute volume deviation between MRI and CT arthrography is 3.3% and that between the 2 computed tomography data sets is 3.6%. The absolute error in determining the maximal cartilage thickness with MRI is on average 0.6 intervals (of 0.5-mm thickness) and that between the CT examinations is 0.5 intervals. In some cases with anterior knee pain, a focal cartilage defect can be seen with MRI, and this can be verified by arthroscopic examination. Using 3-dimensional image processing, MRI can provide accurate data on cartilage volume and thickness in the human knee joint surfaces. This imaging technique potentially may be valuable in the treatment of patients with joint disease.

Accuracy of cartilage volume and thickness measurements with magnetic resonance imaging. Eckstein F, Schnier M, Haubner M, Priebsch J, Glaser C, Englmeier KH, Reiser M. Clin Orthop 1998 Jul (352), pp. 137–48.

Rheumatoid Arthritis/Intra-Articular Vascularization Patterns
Levovist Microbubbles

Application: Microbubbles (Levovist) can be applied to detect and characterize various forms of joint pathology. In cases of rheumatoid arthritis, this contrast agent can improve the detection of increased patterns of intra-articular vascularization between individual joints.
Route of Administration: Levovist microbubbles (intravenous).
Implementation: Assess the usefulness of contrast-enhanced color Doppler ultrasound (CDUS) in the evaluation of intra-articular vascularization pathology of finger joints in patients with rheumatoid arthritis (RA). Prior to image analysis of individual joints, varying levels of inflammation can be classified as active, moderately

active, or inactive. Contrast-enhanced CDUS is performed with a high-frequency multi-D linear array transducer. A microbubble-based US contrast agent (Levovist) is intravenously infused for image enhancement. Doppler findings are rated on the basis of both unenhanced and CDUS images.

Human Model: Rheumatoid arthritis (e.g., n = 198 fingers; n = 46 patients; n = 10 controls; n = 80 control fingers).

Analysis and Interpretation: Healthy joints show no intra-articular vascularization on either unenhanced or contrast-enhanced CDUS. Unenhanced CDUS detects intra-articular vascularization in approximately 8% of inactive joints (e.g., n = 7/83); in 52% of moderately active joints (e.g., n = 31/60); and in 58% of active joints (e.g., n = 32/55). Contrast-enhanced CDUS can detect intra-articular vascularization in 49% of inactive rheumatoid arthritis joints (e.g., n = 41/83); 98% of moderately active rheumatoid arthritis joints (e.g., n = 59/60); and essentially all active rheumatoid arthritis joints (e.g., n = 55/55). Detection of intra-articular vascularization is improved by the administration of a microbubble-based US contrast agent ($p < 0.001$). Contrast-enhanced CDUS can detect differences in intra-articular vascularization between joints with inactive rheumatoid arthritis and those with active rheumatoid arthritis ($p < 0.001$), between joints with inactive rheumatoid arthritis and those with moderately active rheumatoid arthritis ($p < 0.001$), and between joints with moderately active rheumatoid arthritis and those with active rheumatoid arthritis ($p < 0.001$).

The use of a microbubble-based US contrast agent significantly improves the detection of intra-articular vascularization in rheumatoid arthritis–affected finger joints. Such imaging techniques seem to be a useful adjunct in the assessment of disease activity.

Instrumentation: Color Doppler ultrasound equipped with a high-frequency multi-D linear array transducer.

The value of contrast-enhanced color Doppler ultrasound in the detection of vascularization of finger joints in patients with rheumatoid arthritis. Klauser A, Frauscher F, Schirmer M, Halpern E, Pallwein L, Herold M, Helweg G, ZurNedden D. Arthritis Rheum 2002 Mar; 46 (3), pp. 647–53.

Rheumatoid Arthritis/Knee: Increased Intra-Articular Vascular Perfusion/Inflammatory vs Non-Inflammatory Pannus
Levovist Microbubbles

Application: Detection and characterization of physiological alterations and pathological anomalies affecting tissues, organ systems, and vascular structures using ultrasound diagnostic modalities.

Route of Administration: Levovist ultrasound contrast image-enhancing agent (intravenous).

Implementation: Evaluation of the ability for Levovist microbubble preparations to detect, characterize, and distinguish between conditions of rheumatoid arthritis (inflammatory pannus) and non-inflammatory pannus affecting the knee joint. In this context, knee joints can be examined utilizing conventional ultrasonography and power Doppler ultrasonography before and after injection of intravenous ultrasound contrast agent (Levovist). The quantitative estimation of the vascularization of the synovial membrane is performed with time-intensity curves and calculation of the area-under-the-curve (AUC).

Human Model: Cases of rheumatoid arthritis of the knee (e.g., n = 42; n = 31 women) with the index of synovitis activity classified as active, moderately active, or inactive). Clinical and functional assessment (number of swollen joints, intensity of pain, general health-visual analogue scale, disability index-Health Assessment Questionnaire, Ritchie articular index) and laboratory evaluations are performed in addition to establishing the level of disease activity according to the calculation of a disease activity score (DAS) and the chronic arthritis systemic index (CASI) for each individual case.

Analysis and Interpretation: The mean (SD) value of the area underlying time-intensity curves is 216.2 (33.4) in conditions of active synovitis, 186.8 (25.8) with moderately active synovitis, and 169.6 (20.6) in conditions of inactive synovitis. The mean value of the areas differs significantly between cases with active compared to inactive synovitis ($p < 0.01$). The mean value of the AUC of the entire group is weakly correlated with the number of swollen joints ($p = 0.038$), but a strong correlation is found with composite indexes of disease activity such as the DAS ($p = 0.006$) and CASI ($p = 0.01$). No correlation can be appreciated with age, disease duration, and other laboratory and clinical variables.

Power Doppler ultrasonography may be a valuable tool to detect fractional vascular volume and to assist clinicians in distinguishing between inflammatory and non-inflammatory pannus. The transit of microbubbles of ultrasound contrast across a tissue can be used to estimate hemodynamic alterations and may have a role in assessing synovial activity and the therapeutic response to treatment of synovitis of the knee joint.

Power Doppler sonography in the assessment of synovial tissue of the knee joint in rheumatoid arthritis: A preliminary experience. Carotti M, Salaffi F, Manganelli P, Salera D, Simonetti B, Grassi W. Ann Rheum Dis 2002 Oct; 61 (10), pp. 877–82.

Rheumatoid Arthritis/Shoulder Joint: Poor Synovitis Detection and Differentiation of Synovial Inflammation
Levovist Microbubble Contrast Agent

Application: Using magnetic resonance imaging (MRI) as the "gold standard," power Doppler ultrasonography with and without echo-enhancing contrast agent cannot reliably identify synovitis or distinguish synovial inflammation from effusion in the shoulder joint.

Dosage: Levovist microbubble echo-enhancing contrast agent (2.5 g, 300 mg/ml).

Implementation: Evaluation of 1 symptomatic shoulder affected by rheumatoid arthritis utilizing conventional gray-scale sonography and power Doppler ultrasonography before and after intravenous administration of Levovist microbubble echo-enhancing contrast agent.

The degree and extent of altered echo patterns in the subacromial bursa, axillary recess, and glenohumeral joint seen by conventional gray-scale sonography and the intensity of vascular signals of power Doppler ultrasonography are compared with findings from MRI results obtained with T_2-weighted turbo spin-echo sequences and contrast-enhanced T_1-weighted fat-saturated spin-echo sequences. To increase the objectivity of the analysis, MRI findings can be evaluated by 2 readers without knowledge of the sonographic findings.

Human Model: Cases of rheumatoid arthritis affecting the shoulder (e.g., n = 24; mean age = 64).

Analysis and Interpretation: Reference standard MRI examinations can detect joint effusion in 71% (e.g., n = 17/24) and synovitis in 92% (e.g., n = 22/24) of affected cases. Conventional sonography reveals an abnormal articular echo pattern in 96% (e.g., n = 23/24) of cases, particularly in the axillary recess and subacromial bursa. Unfortunately, conventional sonography fails to attribute altered echo patterns to either fluid or specific synovitis. Power Doppler ultrasonography can facilitate establishing a specific diagnosis of synovitis in 33% of affected cases (e.g., n = 8/24), but the simultaneous administration of echo-enchaning contrast agent

increases this parameter to 50% (e.g., n = 12/24). In 42% of affected cases (e.g., n = 10/24), the findings of synovitis demonstrated by MRI correspond to an altered echo pattern by conventional sonography, but vascular signals are absent with power Doppler ultrasonography either with or without echo-enhancing contrast agent. Using MRI as the "gold standard," power Doppler sonography with and without echo-enhancing contrast agent cannot reliably identify synovitis or distinguish synovial inflammation from effusion in the shoulder joint.

Instrumentation: power Doppler ultrasonography and MRI instrumentation with T_2-weighted turbo spin-echo and contrast-enhanced T_1-weighted fat-saturated spin-echo capabilities.

Power Doppler sonography with and without echo-enhancing contrast agent and contrast-enhanced MRI for the evaluation of rheumatoid arthritis of the shoulder joint: Differentiation between synovitis and joint effusion. Wamser G, Bohndorf K, Vollert K, Bücklein W, Schalm J. Skeletal Radiol 2003 Jun; 32 (6), pp. 351–9.

NEOPLASIA

Arteriography of Neoplastic Lesions/Hepatocellular Carcinoma: Response to Percutaneous Radio Frequency Ablation/Relative Effectiveness: 3-Phase Dynamic Computed Tomography (CT) vs Coded Harmonic Angiography
Levovist Microbubbles

Cellular/Molecular Mechanism of Action: Microbubbles variably impede, absorb, and reflect ultrasound energy in a manner different from surrounding tissues, organ systems, or biological fluids.

Application: Microbubbles can be utilized in concert with contrast-enhanced coded phase-inversion harmonic sonography to assess the therapeutic response of hepatocellular carcinoma to percutaneous radio frequency ablation.

Route of Administration: Microbubbles (Levovist) can be infused into the intravascular compartment and into certain other body compartments.

Implementation: Evaluation of hepatocellular carcinoma nodules applying coded harmonic angiography (e.g., coded phase-inversion harmonic sonography) facilitated by the application of microbubble contrast image-enhancing agents before and after percutaneous radio frequency ablation. Intratumoral blood vessels and tumor parenchymal stain can be detected in the early arterial phase and late vascular phase, respectively. The results of contrast-enhanced imaging with coded harmonic angiography can subsequently be compared with those of 3-phase dynamic CT.

Human Model: Cases of hepatocellular carcinoma nodules (e.g., n = 67 cases, n = 107 exams; n = 91 nodular lesions).

Analysis and Interpretation: The vast majority of hepatocellular carcinoma nodules display hypervascular patterns on contrast-enhanced imaging with coded harmonic angiography (e.g., n = 107/107). Following radio frequency ablation, contrast-enhanced coded harmonic angiography detects a persistent signal enhancement of nodules (e.g., n = 41/107 or 38.3%), but the methodology shows no intratumoral enhancement in more than half of examined nodules (e.g., n = 66/107 or 61.7%). Compared with dynamic CT, the sensitivity, specificity, and diagnostic accuracy of contrast-enhanced coded harmonic angiography is approximately 95.3%, 100%, and 98.1%, respectively. With contrast-enhanced coded harmonic angiography, it is difficult to identify the safety margin that can be detected on dynamic CT. Contrast-enhanced imaging with coded harmonic angiography may provide an alternative approach that provides high diagnostic agreement with dynamic CT in assessing the therapeutic effect of radio frequency ablation in hypervascular hepatocellular carcinomas. However, it also has limitations in identifying safety margin parameters.

Radio frequency ablation of hepatocellular carcinoma: Therapeutic response using contrast-enhanced coded phase-inversion harmonic sonography. Wen YL, Kudo M, Zheng RQ, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T, Maekawa K. AJR Am J Roentgenol 2003 Jul; 181 (1), pp. 57–63.

Assimilation Enhancement of Neoplasia/Enhanced Selective Systemic/Local-Regional Uptake/Slowing of Local-Regional Blood Flow
Starch Microspheres/Small Unilamellar Vesicles (SUV)-Polyethylene Glycol (PEG) Liposomes

Physiological Mechanism of Action: Starch microspheres can be used to slow local-regional blood flow in a manner that can maximize assimilation of encapsulated liposomes into neoplastic lesions.

Background Information: Systemic adjuvant chemotherapy achieves unsatisfactory results for inoperable liver tumors and metastases. Various clinical studies have shown that loco-regional chemotherapy increases survival rate by several months.

Application: Enhancement of systemic and local-regional uptake of diagnostic and therapeutic pharmaceutical agents.

Route of Administration: Systemic and direct local-regional injection.

Implementation: Evaluate the capacity of liposome encapsulation to selectively facilitate local-regional/systemic targeting of neoplastic cell populations with cytostatic 5-fluouracil (5-FU).

Interpretation and Analysis: Tumor concentrations of 5-FU encapsulated in SUV-PEG liposomes increases by a factor of 27 in systemic therapy and a factor of 90 in loco-regional therapy. The tumor concentration of 5-FU increases by a factor of 8,000 if the blood flow is additionally slowed by starch microspheres (Spherex) during loco-regional therapy with 5-FU encapsulated within liposome preparations.

Liposome encapsulation of cystostatic drugs and starch microspheres improve tumor targeting in loco-regional therapy. An animal experiment study of CC 531 liver tumor. Berger G, Reszka R, Pohlen U, Jung M, Lucas S, Buhr HJ. Langenbecks Arch Chir Suppl Kongressbd 1998; 115 (Suppl I), pp. 693–7.

Blood Flow and Vascularity Patterns of Neoplasia/Mammary Carcinoma/Transplated Tumor Model
Definity Contrast Media

Background Information: Angiogenesis, the development of new blood vessels, is necessary for tumor growth. Antiangiogenic therapies have recently received attention as a possible cancer treatment.

Application: Sonographic imaging and definity contrast agent can be utilized to monitor the vascularity of induced tumors and detect their response to antiangiogenic therapy.

Route of Administration: Definity (intravenous).

Implementation: Evaluation of blood flow patterns to mammary carcinoma transplants applying sonographic imaging (i) prior to drug administration, (ii) during the 1st 51 hours following initial drug administration, and (iii) on days 8 and 15 after initiation of therapy. Tumor volumes can be estimated at each time point using a prolate ellipsoid method from linear dimensions measured on the B-mode ultrasound image in the 3 major axes. A destruction-replenishment technique is used for tumor blood flow evaluation using a con-

stant rate infusion of intravenously delivered ultrasound contrast media (Definity). A destructive pulse is fired 1st, followed by a chain of non-destructive pulses that allow for visualization of vascular contrast agent replenishment. Parametric maps of the time required for contrast agent replenishment and the time-integrated intensity are generated for both the tumor and kidney. Following ultrasound examination, contrast-enhanced computed tomography (CT) of each tumor is performed in the same imaging plane as data used to acquire ultrasound images. Fifteen days after the start of treatment, tumors are excised, preserved in 10% formalin, and sectioned in a plane approximating the ultrasound and CT imaging planes. Sections are prepared for light microscopy with H & E, CD31, and factor VIII immunostain to evaluate overall morphology and vessel distribution.

Animal Model: Rats subcutaneously implanted with R3230 murine mammary adenocarcinomas (e.g., n = 6). Animals are treated orally with an antiangiogenic agent (SU11657) beginning 28 days after tumor implantation (20 mg/kg BW once daily). Additional tumor-bearing control rats can be treated with an equivalent volume of vehicle alone (e.g., n = 3).

Analysis and Interpretation: Ultrasound measurements of tumor volume, the spatial extent of contrast enhancement, and the time required for contrast replenishment within control tumors are significantly different from those of treated tumors. The time-integrated ultrasound contrast enhancement decreases and the time required for replenishment of the contrast agent within the tumor volume increases over the course of antiangiogenic therapy. Parametric maps of integrated intensity correlate with the regions of viable tumor demonstrated on H & E and regions of elevated contrast intensity found on CT images. Contrast-enhanced ultrasound imaging of implanted tumors provides a tool to assess differences in the microcirculation of treated and control tumors in studies of antiangiogenic agents.

Instrumentation: Siemens Sonoline Elegra equipped with a 5.0-MHz linear transducer.

Contrast-assisted destruction-replenishment ultrasound for the assessment of tumor microvasculature in a rat model. Pollard RE, Sadlowski AR, Bloch SH, Murray L, Wisner ER, Griffey S, Ferrara KW. Technol Cancer Res Treat 2002 Dec; 1 (6), pp. 459–70.

Detection Limits and Resolution/Contrast Helical Computed Tomography (CT) and B-Mode Ultrasound (US)
Levovist/Wideband Harmonic Imaging (WHI)

Application: Microbubbles (e.g., Levovist) can be utilized in concert with contrast-enhanced WHI to detect and characterize various types of hepatic metastases (nodules < 1 cm diameter).

Route of Administration: Microbubbles (e.g., Levovist) can be given intravascularly.

Dosage: Levovist at 2.5 g/dose (300 mg/ml).

Implementation: Evaluate the relative capacity for contrast-enhanced WHI to detect liver metastases compared to conventional B-mode US and postcontrast CT. Wideband harmonic imaging can be performed 3 minutes after injection of Levovist contrast agent and postcontrast helical CT (HCT). The number and location of the lesions and the smallest lesion detectable for each case image are recorded.

Human Model: Cases suffering from hepatic metastases originating from different types of primary malignant lesions (e.g., n = 27).

Analysis and Interpretation: Comparisons reveal an increase in the number of lesions in harmonic mode compared with conventional B-mode images in cases with hepatic metastasis (e.g., n = 27/27) with a mean increase from 9.3 lesions with B-mode to 18.8 lesions with WHI. The smallest lesions can be detected with WHI (2 mm), compared with conventional US and HCT (B-mode = 5 mm, CT = 5 mm). Wideband harmonic imaging detects more lesions than conventional ultrasound or contrast HCT. Contrast-enhanced WHI appears to be superior to conventional US and HCT for the detection of hepatic metastases, especially for those nodules under 1 cm of diameter.

Improved detection of liver metastases with contrast-enhanced wideband harmonic imaging: Comparison with CT findings. Esteban JM, Molla MA, Tomas C, Maldonado L. Eur J Ultrasound 2002 Oct; 15 (3), pp. 119–26.

Gene Delivery/Selective Tumor DNA Delivery via Vasculature
Plasmid-Microspheres/Plasmid-Complexed Liposomes

Application: Selective targeting of plasmid-incorporated genetic DNA sequences for neoplastic cell types.

Route of Administration: Arterial catheterization.

Implementation: Evaluation of plasmid DNA delivery into to a renal solid tumor involving the utilization of plasmid bound to microspheres and plasmids complexed with liposomes to evaluate their potential to function as a selective delivery modality to tumors. Selective delivery is in part facilitated by selective catheterization of the major arterial network supplying individual neoplastic tissue masses.

***Ex Vivo* Model:** Rat (established renal solid tumor model).

Interpretation and Analysis: Forty-eight hours postdelivery, tumor-to-normal kidney tissue chloramphenicol acetyltransferase expression ratios are as follows: free (1.8:1), microspherical (3.9:1), and liposomal (1.2:1). Microspheres are able to selectively deliver plasmids to tumors, whereas cationic liposomes distribute plasmids to the parenchymal of both kidneys and tumor cells. The tumor model has the potential of screening delivery vehicles as well as therapeutic agents for the capacity of selective delivery to tumors via vasculature structures.

A model for evaluating selective delivery of plasmid DNA to tumors via the vasculature. Dass CR, Burton MA. Cancer Biother Radiopharm 2002 Oct; 17 (5), pp. 501–5.

Glioma: Malignant/$\alpha_V\beta_3$ Complexes
Echistatin Microbubbles

Background Information: Angiogenesis is a critical determinant that influences tumor growth and metastasis. It has been hypothesized that contrast-enhanced ultrasound (CEU) with microbubbles targeted to α_V-integrins expressed on the neovascular endothelium could be used to image angiogenesis phenomenon.

Implementation: Evaluation of malignant glioma CEU utilizing contrast-enhanced ultrasound in combination with microbubbles targeted to $\alpha_V\beta_3$ by surface conjugation with echistatin. Contrast-enhanced ultrasound perfusion imaging with non-targeted microbubbles can be used to derive tumor microvascular blood volume and blood velocity. Vascular α_V-integrin expression can be assessed by immunohistochemistry, and microbubble adhesion characterized by confocal microscopy.

Animal Model: Rat (athymic; n = 14). Malignant gliomas are produced by intracerebral implantation of U87MG human glioma cell populations. On day 14 or day 28 after implantation, CEU is performed.

Interpretation and Analysis: Mean tumor size increases markedly from 14 to 28 days (2 ± 1 vs 35 ± 14 mm^2, $p < 0.001$).

Tumor blood volume increases by approximately 35% from day 14 to day 28, whereas microvascular blood velocity decreases, especially at the central portions of the tumors. On confocal microscopy, $\alpha_V\beta_3$-targeted but not control microbubbles are retained preferentially within tumor microcirculation structures. Contrast-enhanced ultrasound signals produced by $\alpha_V\beta_3$-targeted microbubbles in tumors increase significantly from 14 to 28 days (1.7 ± 0.4 vs 3.3 ± 1.0 relative units, $p < 0.05$). Contrast-enhanced ultrasound signals from $\alpha_V\beta_3$-targeted microbubbles is greatest at the periphery of tumors, where α_V-integrin expression is the most prominent, and correlates well with tumor microvascular blood volume ($r = 0.86$). Contrast-enhanced ultrasound with $\alpha_V\beta_3$-targeted microbubbles can non-invasively detect early tumor angiogenesis. Such a technique, when coupled with changes in blood volume and velocity, may provide insights into the biology of tumor angiogenesis and be used for various diagnostic applications.

Imaging tumor angiogenesis with contrast ultrasound and microbubbles targeted to $\alpha_V\beta_3$. Ellegala DB, Leong-Poi H, Carpenter JE, Klibanov AL, Kaul S, Shaffrey ME, Sklenar J, Lindner JR. Circulation 2003 Jul 22; 108 (3), pp. 336–41.

Mammary Carcinoma
Levovist

Application: Detection and characterization of vascular anomalies, including microcirculation features of breast lesions, utilizing contrast-enhanced ultrasound (US).
Route of Administration: Microbubbles (e.g., Levovist) bolus injection via a 20-gauge catheter probe.
Dosage: Microbubbles (Levovist) at a rate of 2 ml/s.
Implementation: Evaluation of breast lesions using contrast-enhanced US; these lesions have previously been identified on mammography examination. Ultrasound examinations are performed before and after administration of US contrast medium (e.g., Levovist microbubbles) delivered as a bolus injection (2 ml/s) via a 20-gauge catheter probe. The mechanical index is set to high to obtain instant rupture of the microbubbles under insonation. Intensity/time curves of the transient scattering in the region-of-interest (ROI) after contrast administration is extrapolated. The areas under the ROI curves can also be evaluated to quantitatively assess scattering phenomenon.

Human Model: Cases of female breast cancer ($n = 36$; age = 42–63 years; mean age 55 years) with a single breast lesion previously detected by standard mammography procedures.
Interpretation and Analysis: Ultrasound and color Doppler US imaging can identify benign lesions ($n = 19$; $n = 15$ histologically proven) and malignant lesions ($n = 17$; $n = 16$ histologically proven). Administration of contrast medium does not improve the specificity of the examination. Only by plotting the intensity/time curves of the ROIs and evaluation of the areas under the ROI curves can a significant correlation be detected between US and histopathologic diagnosis. Although further evaluation of a larger case population is indicated, the analysis of breast lesions by contrast-enhanced perfusion US seems to be a promising technique. Perfusion US can effectively complement high-frequency US, especially when mammography procedures are inconclusive.
Instrumentation: ATL HDI 5000 ultrasound instrument (a 5- to 10-MHz linear-array transducer).

Breast lesion characterization with contrast-enhanced US. Work in progress. Caruso G, Ienzi R, Cirino A, Salvaggio G, Campione M, Lagalla R, Cardinale AE. Radiol Med (Torino) 2002 Nov–Dec; 104 (5–6), pp. 443–5.

Mammary Solid Masses/Benign vs Malignant Image Patterns
Microbubbles/Ultrasound Image-Enhancing Contrast Agent

Cellular/Molecular Mechanism of Action: Microbubbles variably impede, absorb, and reflect ultrasound energy in a manner that differs from surrounding tissue, organ systems, vascular structures, and body fluids.
Application: Detection and characterization of physiological alterations and pathological anomalies affecting vascular structures.
Implementation: Comparative evaluation of solid mammary mass lesions utilizing (i) gray-scale sonographic imaging following initial identification by (ii) diagnostic sonography or screening mammography and subsequent (iii) image analysis with contrast-enhanced power Doppler sonography before and after the injection of a microbubble echo-enhancing contrast agent. Such imaging modalities can be employed to determine their relative capacity to differentiate between malignant and benign mass lesions of the breast that are of relatively small size (measuring < 2 cm). Sonographic findings for all 3 techniques, as well as the morphologic features of the Doppler signals for each case before and after injection of the contrast agent on contrast-enhanced power Doppler sonography, are then collectively evaluated. Each lesion is classified as "benign" or "malignant" on the basis of specific criteria for sonographic interpretation. A hemodynamic study is then performed in which time-transit profiles of the Doppler signals on contrast-enhanced power Doppler sonography are generated using a computer-assisted program, and the results for each case compared with the findings from the histopathologic examination of surgical specimens.

Human Model: Females with mammary solid masses (e.g., $n = 36$; $n = 35/36$ women; mean age = 43.5 years; range = 18–69). The tumors ranged from 4 to 19 mm in the largest dimension.
Analysis and Interpretation: Histopathologic examination reveals that approximately 53% of mammary solid masses are benign (e.g., $n = 19/36$), while about 47% (e.g., $n = 17/36$) are malignant. For the morphologic diagnosis of malignant lesions, the sensitivity of gray-scale sonography is 100%, compared with 29% for power Doppler sonography without contrast enhancement. The specificity of gray-scale sonography is 47%, compared with 74% power Doppler sonography without contrast enhancement. Contrast-enhanced power Doppler sonography has a sensitivity of 71% and a specificity of 58%. The diagnostic accuracy is 72% for gray-scale sonography, 53% for power Doppler sonography without contrast enhancement, and 64% for contrast-enhanced power Doppler sonography. The time-transit profiles of the hemodynamic study do not reveal a statistically significant difference in the accuracy rates of contrast-enhanced power Doppler sonography between benign and malignant breast lesions. Compared with power Doppler sonography without contrast enhancement, contrast-enhanced power Doppler sonography provides better visualization of the morphology of vascular Doppler signals that are characteristic of malignancy, and therefore has a higher sensitivity and diagnostic accuracy, albeit a lower specificity. In differentiating between benign and malignant small breast lesions, contrast-enhanced power Doppler sonography can be helpful when used with gray-scale sonography and power Doppler sonography without contrast enhancement.

Value of contrast-enhanced power Doppler sonography using a microbubble echo-enhancing agent in evaluation of small breast lesions. Kook SH, Kwag HJ. J Clin Ultrasound 2003 Jun; 31 (5), pp. 227–38.

Mammary Tumors/Benign vs Malignant Features/ Tumor Vascularization
Ultrasound Contrast Agent: SHU 508

Background Information: An ultrasound contrast agent that survives transit through the pulmonary circulation after injection into a peripheral vein can also be used for the demonstration of flow characteristics within peripheral organs.
Implementation: Examination of breast tumors applying enhancing color-coded Doppler ultrasonography in combination with a prototype ultrasound contrast image-enhancing agent (SHU 508). Observations and interpretations made are then compared with histopathology findings (e.g., confirmed diagnoses).

Human Model: Cases with evidence of mammary tumors (e.g., n = 10).
Analysis and Interpretation: The very distinct enhancement of color-coded Doppler signals by use of the contrast agent allows reliable demonstration of the vascularization characteristics, not only for malignant lesions but also of essentially all benign solid tumors. Color signals are even recorded from normal mammary tissue, which indicates that the demonstration of perfusion no longer has to be used on its own as a diagnostic criterion. Alternatively, the observation of blood supply characteristics for a given tumor can be advantageous, not only with respect to tumor typing but also as it pertains to tumor prognosis. Given this perception, the registration of the arrival, retention, and washout phases specific to a contrast agent appears to furnish particularly interesting information about tumor growth dynamics.

Echo-contrast agent-enhanced color flow imaging of the breast. Duda VF, Rode G, Schlief R. Ultrasound Obstet Gynecol 1993 May; 3 (3), pp. 191–4.

Ovarian Tumors: Malignant vs Benign Lesions/ Relative Degree/Onset/Duration of Doppler Ultrasound Enhancement
Microbubbles

Application: Differentiation of malignant from benign ovarian lesions based on degree, onset, and duration of Doppler ultrasound enhancement facilitated by the infusion of microbubble ultrasound image-enhancing agents.
Route of Administration: Microbubbles (intravascular).
Implementation: Preoperative examination of suspected cases of ovarian neoplasia applying contrast agent-enhanced ultrasound. Images obtained during a 5-minute examination are stored digitally, and the behavior of the contrast agent evaluated objectively with measurements of the time-dependent image intensity within the region of interest acquired with a computer-integrated software program. A time-intensity curve is derived and analyzed for each individual image. The Mann-Whitney U test can then used to compare intensity changes and tumor parameters in benign and malignant adnexal tumors.

Human Model: Suspected cases of ovarian carcinoma.
Analysis and Interpretation: Both the baseline and maximum power Doppler intensities, as well as the absolute and relative (percent) rise in intensity, are significantly higher ($p < 0.001$) in malignant compared to benign tumors. The arrival time is shorter (17.5 vs 22.5 seconds; $p = 0.005$) and the duration of contrast agent effect longer (190.4 vs 103.6 seconds; $p < 0.001$) in malignant tumors in contrast to benign tumors. The area under the time-intensity curve is significantly greater in malignant tumors compared with that in benign tumors ($p < 0.001$). In summary, following microbubble contrast agent injection, malignant and benign adnexal lesions behave differently in degree, onset, and duration with contrast-enhanced Doppler ultrasound image analysis.

Kinetics of a US contrast agent in benign and malignant adnexal tumors. Orden MR, Jurvelin JS, Kirkinen PP. Radiology 2003 Feb; 226 (2), pp. 405–10.

Vascularity of Tumors/Mammary Carcinoma Transplants
Gadolinium: [Gd]-Diethylenetriamine Pentaacetic Acid (DTPA) for MRI/Galactose-Based Ultrasound (US) Contrast Agent

Cellular/Molecular Mechanism of Action: Gadolinium is classified as a paramagnetic metallic ion and its spacial orientation becomes altered when placed within a magnetic field.
Background Information: Angiogenesis is essential for spread and growth of malignant tumors. Because non-invasive methods for observing tumor vascularization are limited, most previous forms of diagnostic analysis have been based on histologic findings alone.
Application: Detection and characterization of physiological alterations and pathological anomalies affecting tissues, organ systems, and vascular structures.
Route of Administration: [Gd]-DTPA (intravenous for MRI); galactose-based contrast image-enhancing agent (intravenous for Doppler US).
Implementation: Evaluation of microvessel density within neoplastic lesions utilizing dynamic parameters obtained using intermittent contrast-enhanced Doppler sonography for comparison with results observed with dynamic magnetic resonance imaging (MRI) modalities. In this context, transplanted tumors are examined with dynamic T_1-weighted sequences using [Gd]-DTPA and images obtained by magnetic resonance (MR) for comparison with images obtained with intermittent power Doppler sonography after a single bolus of galactose-based contrast agent. Following examination, tumors are harvested for examination applying immunofluorescence microscopy (e.g., n = 6 CD31 stained lesions). Using a 2-compartment model, the MR parameters amplitude (reflecting plasma volume) and k_{ep} (influenced by the vessel permeability) are calculated and compared with maximal enhancement (max) and perfusion measured by ultrasound.

Animal Model: Tumor-bearing nude mice (e.g., n = 11).
Analysis and Interpretation: The MR amplitude correlates with the ultrasound parameter max significantly ($r = 0.61$; $p = 0.01$). Max ($r = 0.67$; $p = 0.01$), amplitude ($r = 0.72$; $p = 0.01$), and perfusion ($r = 0.62$; $p = 0.05$) correlate with the microvessel density. Values for k_{ep} moderately correlate with max, but not with perfusion and microvessel density. Dynamic MRI and contrast-enhanced ultrasound are complementary methods for examining perfusion and vascularity of experimental tumors.
Instrumentation: Magnetic resonance scanner (1.5 tesla).

Comparing dynamic parameters of tumor vascularization in nude mice revealed by magnetic resonance imaging and contrast-enhanced intermittent power Doppler sonography. Kiessling F, Krix M, Heilmann M, Vosseler S, Lichy M, Fink C, Farhan N, Kleinschmidt K, Schad L, Fusenig NE, Delorme S. Invest Radiol 2003 Aug; 38 (8), pp. 516–24.

Vascularity of Tumors/Mammary Carcinoma Microcirculation
Levovist

Application: Detection and characterization of vascular anomalies, including microcirculation features of breast lesions, utilizing contrast-enhanced ultrasound (US).

Route of Administration: Microbubbles (e.g., Levovist) bolus injection via a 20-gauge catheter probe.
Dosage: Microbubbles (Levovist) at a rate of 2 ml/s.
Implementation: Evaluation of breast lesions using contrast-enhanced US that have previously been identified on mammography examination. Ultrasound examinations are performed before and after administration of US contrast medium (e.g., Levovist microbubbles) delivered as a bolus injection (2 ml/s) via a 20-gauge catheter probe. The mechanical index is set to high to obtain instant rupture of the microbubbles under insonation. Intensity/time curves of the transient scattering in the region-of-interest (ROI) after contrast administration is extrapolated. The areas under the ROI curves can also be evaluated to quantitatively assess scattering phenomenon.

Human Model: Cases of female breast cancer (n = 36; age = 42–63 years; mean age 55 years) with a single breast lesion previously detected by standard mammography procedures.
Interpretation and Analysis: Ultrasound and color Doppler US imaging can identify benign lesions (n = 19; n = 15 histologically proven) and malignant lesions (n = 17; n = 16 histologically proven). Administration of contrast medium does not improve the specificity of the examination. Only by plotting the intensity/time curves of the ROIs and evaluation of the areas under the ROI curves can a significant correlation be detected between US and histopathologic diagnosis. Although further evaluation of a larger case population is indicated, the analysis of breast lesions by contrast-enhanced perfusion US seems to be a promising technique. Perfusion US can effectively complement high-frequency US, especially when mammography procedures are inconclusive.
Instrumentation: ATL HDI 5000 ultrasound instrument (a 5- to 10-MHz linear-array transducer).

Breast lesion characterization with contrast-enhanced US. Work in progress. Caruso G, Ienzi R, Cirino A, Salvaggio G, Campione M, Lagalla R, Cardinale AE. Radiol Med (Torino) 2002 Nov–Dec; 104 (5–6), pp. 443–5.

PANCREAS

Pancreatic Masses: Characterization
Microbubble Enhancement Media

Cellular/Molecular Mechanism of Action: Microbubbles variably impede, absorb, and reflect ultrasound energy in a manner that differs from surrounding tissues, organ systems, vascular structures, and body fluids.
Application: Characterize and detect alterations in physiological processes and pathological anomalies affecting tissues, organ systems, and vascular structures.
Implementation: Evaluation of the capacity for microbubble contrast-enhanced ultrasonograpy to characterize the vascularity of pancreatic ductal carcinomas. In this context, possible pathological conditions affecting the pancreas include pancreatic masses (e.g., n = 106) consisting of ductal carcinomas (e.g., n = 83/106); endocrine carcinomas (e.g., n = 7/106); intraductal papillary mucinous tumors (e.g., n = 5/106); autoimmune-related pancreatitis (e.g., n = 3/106); solid pseudopapillary tumors (e.g., n = 3/106); chronic pancreatitis (e.g., n = 2/106); serous cystadenoma (e.g., n = 1/106); osteoclastoid giant cell tumor (e.g., n = 1/106); and follicular lymphoma (e.g., n = 1/106). Such pancreatic lesions can be evaluated utilizing contrast-enhanced sonography with coded harmonic imaging utilizing a phase inversion harmonic technique. Contrast enhancement can be achieved through the application of microbubble enhancement media.
Analysis and Interpretation: Internal tumoral vascularity can be detected in a little over half of all ductal carcinomas (e.g., n = 47/83 or 56.6%). Vascular image spreading and homogeneous staining throughout the tumors can be observed in essentially all endocrine carcinomas. Enhancement effects may be appreciated in some cases of intraductal papillary mucinous tumors (e.g., n = 2/5) and in most cases of autoimmune-related pancreatitis (e.g., n = 3/3), but the degree varies. A significant correlation exists between the intensity of enhancement effects and the ratio of patent vessels within tumors ($p < 0.05$). Vascularity can be detected by contrast-enhanced sonography in only about half of all ductal carcinomas, confirming the difficulty in distinguishing those tumors from other pancreatic tumors. A degree of correlation exists between the patency of the vessels in the tumors and their vascularity.

Evaluation of contrast enhancement patterns in pancreatic tumors by coded harmonic sonographic imaging with a microbubble contrast agent. Nagase M, Furuse J, Ishii H, Yoshino M. J Ultrasound Med 2003 Aug; 22 (8), pp. 789–95.

Pancreatic Neoplasia
Contrast-Enhanced Power Doppler Sonography

Background Information: Echo-enhanced power Doppler sonography (power Doppler sonography after injection of a contrast agent) is a non-invasive and increasingly used procedure for differentiating between pancreatic tumors. However, the diagnostic accuracy of this method compared to conventional ultrasound or unenhanced power Doppler sonography has never been investigated in a large prospective controlled study.
Application: Echo-enhanced power Doppler sonography has a high sensitivity and specificity in the differential diagnosis of pancreatic tumors. However, histology serves as a valuable standard reference control to establish diagnostic accuracy (e.g., diagnostic confirmation).
Implementation: Sonography evaluation of suspected conditions of pancreatic cancer. The exact diagnosis is ultimately based on histological evidence from biopsy examination (surgical and fine needle biopsy) or on follow-up exams performed at least 18 months later.

Human Model: Cases suspected of pancreatic neoplasia (e.g., n = 137; mean age = 60 years; range 16–85).
Analysis and Interpretation: Sonographic imaging can detect pancreatic cancer in approximately 35% of case populations suspected of having pancreatic cancer (e.g., n = 47/137). In the residual cases, 30% may have pancreatitis (e.g., n = 41/137); 12% suffer from neuroendocrine tumors (e.g., n = 17/137); 9% have cystic lesions (e.g., n = 12/137); and 7% have other forms of pancreatic disease (e.g., n = 10/137). A normal pancreas can be appreciated in a relatively small percentage of suspected cases (e.g., n = 10/137). The sensitivity of echo-enhanced power Doppler sonography with respect to diagnosing pancreatic carcinoma is approximately 87% and its specificity 94%. The corresponding values for chronic pancreatitis are approximately 85% and 99%, respectively.

Differentiation of pancreatic tumors by conventional ultrasound, unenhanced and echo-enhanced power Doppler sonography. Rickes S, Unkrodt K, Neye H, Ocran KW, Wermke W. Scand J Gastroenterol 2002 Nov; 37 (11), pp. 1313–20.

PROSTATE

Prostatic Carcinoma/Malignant vs. Benign
Galactose-Based Air Microbubbles

Implementation: Evaluate and compare gray-scale and color Doppler before and during intravenous injection of ultrasound contrast agent made of galactose-based air microbubbles. Biopsy sam-

ples can be harvested during contrast injection or directed cores can be collected based on contrast-enhanced imaging (e.g., n = 18). Diagnostic efficiency with and without contrast medium injection for detecting prostate neoplasia is then compared and validated based on biopsy results.

Human Model: Cases evaluated by biopsy of the prostate (n = 85 suspects).

Implications and Analysis: Cancer can be identified in a large number of suspected cases (e.g., n = 58 biopsies/54 cases). Gray-scale imaging can detect a large number of abnormal hypoechoic nodules or irregular zones inside the outer gland (e.g., n = 96/54 cases), of which approximately 50% are found to be malignant on pathological evaluation (e.g., n = 48/96). Contrast-enhanced color Doppler has a higher sensitivity (93%) than unenhanced color Doppler (54%), while specificity increases only 79% to 87% for enhanced imaging. Approximately 90% (e.g., n = 9/10) of isoechoic suspicious zones can be depicted with enhancement, while unenhanced Doppler detects approximately 70% (e.g., n = 7/10). There is no significant difference between the intensity of enhancement and tumor Gleason scores. Contrast-enhanced color Doppler endorectal sonography increases the detection of prostate cancer. Improvement in sensitivity is high, while the difference in specificity is not as pertinent. It is accurate when using a common and routine application ultrasound unit. Such a technique is easy to perform and not time-consuming. Obtaining additional biopsy cores of suspicious enhancing foci significantly improves the detection rate of cancer.

Contrast-enhanced color Doppler endorectal sonography of prostate: Efficiency for detecting peripheral zone tumors and role for biopsy procedure. Roy C, Buy X, Lang H, Saussine C, Jacqmin D. J Urol 2003 Jul; 170 (1), pp. 69–72.

Prostatic Neoplastic Disease/Accuracy of Detection: Left-Right vs Dorsal-Ventral/Time-to-Start Detection/Maximum Enhancement Detection/Rise Time of Enhancement Detection
Ultrasound Contrast-Enhancing Agent

Application: Detect and characterize physiological alterations and pathological anomalies of tissues, organ systems, vascular structures, and body fluids.

Route of Administration: Contrast agent (intravenous bolus).

Implementation: Define enhancement characteristics that correlate to the presence of prostate cancer and to evaluate the value of these characteristics in the localization of prostate cancer applying trans-rectal contrast-enhanced power Doppler ultrasonography.

The biodistribution of contrast-enhanced blood to the prostate is monitored using trans-rectal contrast-enhanced power Doppler ultrasonography. The percentage of a selected area that shows enhancement is then observed over time. The resulting enhancement curves are described using the parameters (i) time to start; (ii) time to maximum point of enhancement; (iii) maximum value of enhancement; and (iv) rise time of the enhancement. Three evaluation protocols divide the prostate into a number of areas of interest, including (i) 2 areas using the left-right and dorsal-ventral protocols; and into (ii) 4 areas using the quadrant-protocol. The enhancement parameters of the areas of interest are compared to identify the most affected area. The results are compared to the histopathological findings.

Human Model: Confirmed cases of intact primary malignant neoplasia of the prostate scheduled for radical prostatectomy (e.g., n = 29).

Analysis and Interpretation: For the left-right protocol, the minimal time to peak can prove to be the most predictive parameter for selecting the major regions of malignancy. Accuracy of establishing a correct diagnosis is approximately 78% (e.g., n = 23/29). Accurate localization of the major malignancy in either the ventral or dorsal side of the prostate, however, is not feasible utilizing the methodology employed. Malignancies can be accurately localized in either the left or the right side of the prostate based on the time to the maximum moment of enhancement. An accurate discrimination between malignancies in either the dorsal or ventral side of the prostate cannot be performed. This is most likely due to anatomical differences between the dorsal and ventral area.

The value of dynamic contrast-enhanced power Doppler ultrasound imaging in the localization of prostate cancer. Goossen TE, de la Rosette JJ, Hulsbergen-van de Kaa CA, van Leenders GJ, Wijkstra H. Eur Urol 2003 Feb; 43 (2), pp. 124–31.

Prostatic Carcinoma (PC-3m)/Vascular Structures and Perfusion Density
Contrast Agent/[Protamine]

Cellular/Molecular Mechanism of Action: Contrast media is utilized to enhance Doppler ultrasound image quality and sensitivity based on the interaction of ultrasound energy with the physical properties of media preparation (echogenic properties/acoustic impedance).

Application: Detection of alterations in tumor vascularity patterns.

Route of Administration: Intravenous.

Implementation: Evaluation of neoplastic lesion vascular structures and tissue density applying color flow signal-pixel rate (SPR) analysis of images aided by calculations established using the number of pixels showing color Doppler signals as a ratio of total pixel number residing within the region-of-interest (ROI) for the suspected lesion of interest.

Animal Model: Mice (BALB/c nude mice; n = 48) with subcutaneous transplants of human prostate carcinoma PC-3m tumor followed by protamine injections (SQ) as an angiogenesis inhibitor. Animals are randomly divided into 3 groups according to protamine dose.

Analysis and Interpretation: The SPR of the high- and low-dose groups is significantly lower than that of control groups ($p < 0.01$). Mean SPR $\pm$ standard deviation is 0.09 ± 0.05, 0.11 ± 0.05, and 0.22 ± 0.10 for different treatment groups, respectively. The SPR parameters correlate significantly with the density of microvessel structures ($r = 0.86$ to 0.94, $p < 0.01$). Contrast agent-enhanced Doppler ultrasound imaging can effectively reveal change in vascularity within neoplastic lesions (e.g., $\pm$ protamine). In addition to microvessel density, contrast agent-enhanced Doppler ultrasound imaging may become 1 of several independent prognostic indexes for initiating angiogenesis inhibitor therapy.

Evaluation of the effect of protamine on human prostate carcinoma PC-3m using contrast-enhanced Doppler ultrasound.Tang J, Li S, Li J, Zhang Y, Li X, Dong B, Shi H, Zhang G. J Urol 2003 Aug; 170 (2 Pt 1), pp. 611–4.

Prostatic Neoplastic Disease/Accuracy of Detection: Left-Right vs Dorsal-Ventral/Time-to-Start Detection/Maximum Enhancement Detection/Rise Time of Enhancement Detection
Ultrasound Contrast-Enhancing Agent

Application: Detect and characterize physiological alterations and pathological anomalies of tissues, organ systems, vascular structures, and body fluids.

Route of Administration: Contrast agent (intravenous bolus).

Implementation: Define enhancement characteristics that correlate to the presence of prostate cancer and to evaluate the value of these characteristics in the localization of prostate cancer applying trans-rectal contrast-enhanced power Doppler ultrasonography.

The biodistribution of contrast-enhanced blood to the prostate is monitored using trans-rectal contrast-enhanced power Doppler ultrasonography. The percentage of a selected area that shows enhancement is then observed over time. The resulting enhancement curves are described using the parameters (i) time to start; (ii) time to maximum point of enhancement; (iii) maximum value of enhancement; and (iv) rise time of the enhancement. Three evaluation protocols divide the prostate into a number of areas of interest, including (i) 2 areas using the left-right and dorsal-ventral protocols; and into (ii) 4 areas using the quadrant-protocol. The enhancement parameters of the areas of interest are compared to identify the most affected area. The results are compared to histopathological findings.

Human Model: Confirmed cases of intact primary malignant neoplasia of the prostate scheduled for radical prostatectomy (e.g., n = 29).

Analysis and Interpretation: For the left-right protocol, the minimal time to peak can prove to be the most predictive parameter for selecting the major regions of malignancy. Accuracy of establishing a correct diagnosis is approximately 78% (e.g., n = 23/29). Accurate localization of the major malignancy in either the ventral or dorsal side of the prostate, however, is not feasible utilizing the methodology employed. Malignancies can be accurately localized in either the left or the right side of the prostate based on the time to the maximum moment of enhancement. An accurate discrimination between malignancies in either the dorsal or ventral side of the prostate cannot be performed. This is most likely due to anatomical differences between the dorsal and ventral area.

The value of dynamic contrast-enhanced power Doppler ultrasound imaging in the localization of prostate cancer. Goossen TE, de la Rosette JJ, Hulsbergen-van de Kaa CA, van Leenders GJ, Wijkstra H. Eur Urol 2003 Feb; 43 (2), pp. 124–31.

RENAL SYSTEM

Perfusion and Contrast Distribution/Spatial Temporal Resolution
Levovist

Background Information: Knowledge of the relative tissue perfusion and distribution of tissue perfusion patterns is a valuable parameter that can be beneficial in the diagnosis of numerous disease states.

Application: Detection and characterization of physiological alterations and pathological anomalies within tissues, organ systems, and vascular structures. Microbubbles (e.g., Levovist) are a type of contrast agent that can be applied for the calculation of functional images with high spatial resolution applying ultrasound (UA) techniques.

Route of Administration: Levovist (IA).

Implementation: Evaluation of techniques for assessing the relative vascular perfusion and distribution of tissue perfusion patterns based on images generated utilizing US contrast agents. In this context, functional imaging can be performed to characterize the transit of contrast agent bolus for delineation of distribution, peak time, peak value, transit time, peak area, wash-in rate, and washout decay constant.

Animal Model: Canine preparations performed under optimized conditions where motion artifacts can be minimized.

Analysis and Interpretation: Processing of radio frequency signals obtained with a 3.5-MHz echo system can provide functional images with a high spatial resolution of 2 mm in axial resolution and 2–5 mm in lateral resolution and a slice thickness of 2 mm. The functional images expose several known aspects of kidney perfusion, like perfusion heterogeneity of the kidney cortex and a different peripheral cortical perfusion compared to the inner cortex. Results suggest that it is likely that the functional images reflect the relative perfusion distribution of the kidney.

Contrast-enhanced ultrasonography provides several advantages compared to established nuclear techniques. Examples include, among others, a better spatial and temporal resolution, the lack of exposure of the patient to ionizing radiation, and the relatively low cost.

High-resolution functional imaging with ultrasound contrast agents based on RF processing in an *in vivo* kidney experiment. Verbeek XA, Willigers JM, Prinzen FW, Peschar M, Ledoux LA, Hoeks AP. Ultrasound Med Biol 2001 Feb; 27 (2), pp. 223–33.

Urinary/Vesicoureteral Reflux Grading (VUR)
Levovist

Background Information: The sonographic diagnosis of VUR with contrast-enhanced voiding urosonography (VUS) is gradually increasing in application. Introduction of VUS as part of the routine diagnostic imaging strategy for detecting reflux significantly reduces the number of voiding cystourethrographies (VCUG) that need to be performed. Like in VCUG grading of reflux, VUS is becoming more and more relevant.

Implementation: Evaluation of any sonomorphologic and sonomorphometric parameters that can correlate with reflux grading in VCUG. In this context, a reflux grading system for VUS can be applied for correlation with VCUG.

The VUS is conducted with intravesical administration of ultrasound (US) contrast medium (Levovist). Ureteral and pelvicalyceal dilatations before administration of US contrast medium and during reflux are then documented and the renal pelvic diameter measured. The density of microbubbles in the renal pelves is scored on a scale of 1 to 3 (low to high). A grading system for reflux in VUS is set up similar to the international reflux grading system for VCUG with the addition of 1 more differentiation parameter, namely whether the reflux is primarily in a dilated or non-dilated urinary tract. Reflux grades in VUS are compared with those in VCUG.

Human Model: C186 children (cases undergoing both VUS and VCUG; n = 186 children; n = 89/186 with VUR in at least 1 and the same kidney-ureter-unit [KUU] in both diagnostic imagings).

Interpretation and Analysis: None of the sonomorphologic and sonomorphometric parameters demonstrate any clear-cut finding that would simplify reflux grading in VUS. In approximately 63% (e.g., n = 59/95) KUUs the reflux grades are the same in both examinations. In approximately 11% (e.g., n = 10/95) and 27% (e.g., n = 26/95) KUUs, the reflux is graded lower or higher, respectively, in VUS than in VCUG. Approximately 57% are in a primarily dilated system and the remaining 43% in a non-dilated one. Approximately 70% of KUUs diagnosed as having grade 1 reflux in VCUG appear as grade 2 on VUS. A reflux grading system similar to the 1 used in VCUG can be applied in VUS. Adding the parameter of reflux into a primarily dilated or non-dilated ureter and/or pelvicalyceal system may function as a valuable dimension to reflux grading in VUS. Most of the refluxes labeled as grade 1 in VCUG are actually grade 2 or higher.

Vesicoureteral reflux grading in contrast-enhanced voiding urosonography. Darge K, Troeger J. Eur J Radiol 2002 Aug; 43 (2), pp. 122–8.

Urinary Bladder Rupture: Ultrasonography
Microbubbles

Background Information: Because urinary bladder rupture can be life threatening, a simple, safe technique for evaluating patients is desirable. Current diagnostic protocols involve radiographic imaging, but ultrasound-based contrast techniques have not been methodically evaluated in veterinary patients with urologic trauma.
Application: Detection of anatomical defects affecting the urinary bladder.
Route of Administration: Urinary catheter.
Implementation: Ultrasound contrast cystography (contrast cystosonography) involves infusion of microbubbled saline solution through a urinary catheter.

Animal (Practice) Model: Canine (naturally occurring urinary bladder rupture).
Interpretation and Analysis: A positive result consists of visualizing microbubbles sonographically in fluid surrounding the bladder immediately after infusion of contrast into the urinary catheter. A positive result can be easily appreciated in both an *in vitro* and *in vivo* (n = 2 dogs) model. Confirmation can be established with radiographic and surgical confirmation of naturally occurring intraperitoneal urinary bladder rupture. Based on results from such studies, ultrasound contrast cystography appears to be more sensitive than 2-dimensional abdominal sonography for detecting naturally occurring urinary bladder rupture in dogs.

Diagnosis of urinary bladder rupture using ultrasound contrast cystography: *In vitro* model and 2 case-history reports. Ccoté E, Carroll MC, Beck KA, Good L, Gannon K. Vet Radiol Ultrasound 2002 May–Jun; 43 (3), pp. 281–6.

Vesiculoureteral Reflux (VUR): Neonatal/Renal Ultrasonography: Contrast-Enhanced/Fluoroscopic Micturating Cystourethrography (MCU)
Stabilized Microbubbles

Background Information: Fluoroscopic MCU can be used for screening and grading conditions of VUR but involves the application of ionizing radiation. The role of sonography is somewhat limited in practice as a screening examination for neonatal populations.
Application: Contrast-enhanced sonography can be applied in predicting the presence or absence of VUR. Its ability to detect cases of high-grade reflux may make it an attractive alternative in case follow-ups of known conditions of VUR, and may help to reduce neonatal radiation exposure.
Implementation: Evaluation of renal ultrasonography imaging to determine the feasibility of grading conditions of VUR. Echo-enhanced sonography in concert with stabilized microbubbles can be utilized to evaluate function. Validation of findings can subsequently be achieved by performing fluoroscopic MCU. Criteria for the diagnosis of VUR is based upon the detection of transient hyperechogenicity appearing within the pelvicalyceal system or ureter.

Applying such a diagnostic strategy, it is possible to compare ultrasound contrast agents for the detection of VUR in at-risk infants and to compare these findings with fluoroscopic MCU with the aim of determining the relative effectiveness and ability to substitute echo-enhanced sonography for fluoroscopic MCU in order to identify neonates who do not have VUR, thus avoiding the use of radiation in this group.

Human Model: Neonates (n = 97/100; n = 69 males, n = 31 females; age = 28–90 days; mean micturations = 2.7 [1–6 range]).
Analysis and Interpretation: Reflux can be detected in 14% of suspect neonatal cases (e.g., n = 14/95 babies, n = 19 kidneys/14 babies) by 1 or the other diagnostic technique. The findings are concordant in approximately 93.2% of kidneys (e.g., n = 181/194 kidneys). Echo-enhanced sonography has a sensitivity of 64% (95% confidence interval 35–87%), a specificity of 100% (95–100%), a positive predictive value of 100% (66–100%), and a negative predictive value of 94% (87–98%).

Comparison of echo-enhanced ultrasound with fluoroscopic MCU for the detection of vesicoureteral reflux in neonates. McEwing RL, Anderson NG, Hellewell S, Mitchell J. Pediatr Radiol 2002 Dec; 32 (12), pp. 853–8. Epub 2002 Sep 25.

REPRODUCTIVE SYSTEM

Infertility (Female): Hysterosalpingography (Tubal Patency)
Levovist

Background Information: Previous experimental and clinical data suggest that insonation of echo-enhancing contrast agents with high acoustic power produces disintegration of microbubbles, resulting in a phenomenon called simulated acoustic emission (SAE).
Implementation: Evaluation of female infertility using x-ray hysterosalpingography (HSG) and SAE-HyCoSy. The order of the 2 procedures can be established randomly for each case after placement of a trans-cervical balloon catheter. For SAE-HyCoSy, the ultrasound contrast medium Levovist is injected, with the acoustic power set at the maximum level permitted on ultrasound machines employing dedicated algorithms. Conventional HSG is performed for comparison.

Human Model: Cases of female infertility (n = 41 and n = 77 fallopian tubes). X-ray HSG for infertility evaluation also receiving SAE-HyCoSy.
Interpretation and Analysis: In the majority of the cases examined, it is possible to detect the creation of SAE phenomenon. In 13% of tubes (e.g., n = 10), proximal filling may not be observed by either SAE-HyCoSy or HSG. In the remaining 87% of tubes (e.g., n = 67), free spill from the distal end of the lumen can be demonstrated in 96% of cases (e.g., n = 64/67) with SAE-HyCoSy and in 97% of cases (e.g., n = 65/67) with HSG. Disagreement between the 2 techniques may be observed in only a few instances (e.g., n = 5/77) with a Cohen's kappa coefficient of 0.76 (95% confidence interval, 0.56–0.96). Therefore, SAE techniques can be successfully applied to HyCoSy and allow visualization of the free spill of contrast agent into the peritoneal cavity in a majority of cases examined. In previous investigations, SAE-HyCoSy has shown good agreement with HSG diagnostic methods.

The sonographic evaluation of tubal patency with stimulated acoustic emission imaging. Prefumo F, Serafini G, Martinoli C, Gandolfo N, Gandolfo NG, Derchi LE. Ultrasound Obstet Gynecol 2002 Oct; 20 (4), pp. 386–9.

Mammary Gland Disease Differentiation/Optical Imaging
Gadolinium/Indocyanine Green (ICG)/Near-Infrared Diffuse Optical Tomography (DOT)

Cellular/Molecular Mechanism of Action: Indocyanine green is an absorbing dye that is bound to albumin within the intravascular compartment.
Application: Indocyanine green is an absorbing dye commonly used as a marker system within the plasma compartment. Under certain conditions, ICG possesses the potential to differentiate disease based on the quantified enhancement of suspicious lesions using magnetic resonance imaging (MRI) concurrently with DOT (contrast enhancement properties).

Route of Administration: Intravascular.

Implementation: Near-infrared diffuse optical tomography analysis of the mammary gland applied in combination/concurrently with MRI. Results are then correlated with histopathological evaluation of suspicious lesions.

Human Model: Cases scheduled for excisional biopsy or surgery.

Interpretation and Analysis: The ICG-enhanced optical images co-register accurately with gadolinium-enhanced magnetic resonance images, thereby validating the ability of DOT to image breast tissue. In contrast to simple transillumination, DOT appears to provide for localization and quantification of exogenous tissue chromophore concentrations. Additionally, ICG has demonstrated a potential to differentiate disease based on the quantified enhancement of suspicious lesions.

Concurrent MRI and diffuse optical tomography of breast after indocyanine green enhancement. Ntziachristos V, Yodh AG, Schnall M, Chance B. Proc Natl Acad Sci U S A 2000 Mar 14; 97 (6), pp. 2767–72.

4 DELIVERY MODALITIES

22 Biological and Synthetic Delivery Modalities

MACRO-BIOLOGICAL PLATFORMS

CELLULAR-BASED DELIVERY SYSTEMS

Lymphocytes/Selective Peripheral Lymphocyte-Mediated Delivery/Hunter's Syndrome: Mucopolysaccharidosis Type II
Iduronate-2-Sulfatase (IDS)/L2sN and L2/Avian β-Actin Promoter in LB2/CMV Early Promoter in LNC2

Cellular/Molecular Mechanism of Action: Utilization of the natural (native) homing properties of peripheral blood lymphocytes (PBL) to selectively deliver diagnostic agents.
Route of Adminstration: Intravenous.
Considerations: Measurement of trans-gene lysosomal enzyme soon after exposure of target cells to vector supernatant may yield a gross overestimate of long-term trans-gene expression by transduced cells.
Implementation: Characterization of IDS expression from 3 different promoters in 4 retroviral vectors performed in peripheral blood lymphocytes (PBL) from cases with Hunter's syndrome (PBL[MPS]). Relevant vectors for LTR include L2SN and L2, avian β-actin promoter in LB2, and the CMV early promoter in LNC2. The production of PBL(MPS) in this manner involves their exposure to packaging cell supernatant.
Interpretation and Analysis: Surprisingly, IDS activities are equally high in all transduced lymphocyte populations: 515 U/mg/h in PBL(MPS)-L2SN, 734 in PBL(MPS)-LB2, 352 in PBL(MPS)-L2, and 389 in PBL(MPS)-LNC2 compared to controls (< 10 in PBL[MPS]-LXSN or PBL[MPS]). The half-life of endocytosed IDS in PBL(MPS) is approximately 1.9 days. However, the level of lymphocyte IDS activity from proviral expression is only a fraction of the total, a large portion being derived from reuptake to enzyme from murine packaging cells, that is, a "2nd source" of enzyme.

Cells can be charged with a lysosomal enzyme to levels much higher than those found in nature. By "supercharging" cells with a lysosomal protein (or other molecule bearing the mannose-6-phosphate ligand), such cells may be exploited as vehicles for systemic delivery of therapeutic or diagnostic agents.

"Supercharged cells" for delivery of recombinant human iduronate-2-sulfatase. Pan D, Jonsson JJ, Braun SE, McIvor RS, Whitley CB. Mol Genet Metab 2000 Jul: 70 (3), pp. 170–8.

Lymphocytes/Selective Peripheral Lymphocyte-Mediated Delivery/Hunter's Syndrome: Mucopolysaccharidosis Type II
Retroviral Vectors (L2SN) /Iduronate-2-Sulfatase (IDS) /Neomycin Resistance Genes

Cellular/Molecular Mechanism of Action: Delivery of genetic sequences.
Background Information: The process of growing and transducing large quantities of human primary peripheral blood lymphocytes (PBLs) with high gene transfer efficiency continues to be one of the major challenges for clinical and experimental therapy.
Implementation: Development of a clinical trial of lymphocyte gene therapy for mucopolysaccharidosis type II (i.e., Hunter's syndrome). One approach to such an objective can entail investigating methods that exploited the innate capability of a hollow-fiber bioreactor system to filter large quantities of vector supernatant and facilitate transduction.
Interpretation and Analysis: The total number of PBLs could be expanded up to 187-fold, ielding up to 10^{10} cells at the end of a 7-day culture period. The multiplicity of infection can be increased (up to 20-fold) by ultrafiltrating a large volume of vector supernatant through a semipermeable membrane of this system. A high level of transduction efficiency (up to 57%) can be achieved, resulting in IDS enzyme activity as high as 1250 U/mg/h in transduced PBL(MPS) 15 days after transduction. This level is markedly increased from that of non-transduced cells (< 3 U/mg/h) and is even greater than that of normal PBLs (mean, 809; n = 10). After 12 days of G418 selection, PBL(MPS) transductants exhibits a proviral IDS enzyme level approximately 3-fold higher than that in normal PBLs. These results indicated t hat a hollow-fiber bioreactor could be used to culture and transduce human primary PBLs in clinically useful quantities with relatively high gene transfer efficiency and transgene expression.

Combined ultrafiltration-transduction in a hollow-fiber bioreactor facilitates retrovirus-mediated gene transfer into peripheral blood lymphocytes from patients with mucopolysaccharidosis type II. Pan D, Shankar R, Stroncek DF, Whitley CB, Hum Gene Ther 1999 Nov 20; 10 (17), pp.2799–810.

Lymphocytes: Natural Killer Cells: Interleukin-2 (IL-2)-Activated Natural Killer Cells/Pulmonary Metastases/Zyn-205 Linker/Doxorubicin
Melanoma

Cellular/Molecular Mechanism of Action: Natural killer cells have an innate ability to localize within and resolve neoplastic lesions perceived as foreign. Exposure of NK cells to IL-2 enhances their cytotoxic potential. Zyn-Linkers are membrane-binding lipophilic dyes.
Application: Adoptively transferred IL-2-activated NK (A-NK) cells selectively accumulate within tumor metastases and can therefore be applied as a biological vehicle for loco-regional drug delivery. Previous investigations have demonstrated that A-NK cells labeled with PKH26 are able to accumulate within established B16 melanoma pulmonary metastases by 16 hours at a concentration of over 600 cells/mm^2 of tumor tissue. Zyn-Linkers are membrane-binding lipophilic dyes that can be coupled by a variety of conjugation chemistries to various pharmaceutical agents.
Route of Administration: Intravascular.

Semi-Synthetic Methods: Zyn-205 is a prodrug in which doxorubicin is attached to a similar Zyn-Linker through an acid-sensitive bond.
Implementation: Optimization of *ex vivo* labeling conditions applying a 10-minute incubation period with 25 μM Zyn-205.
Interpretation and Analysis: Zyn-205 results in uptake of over 10^8 drug molecules per cell and creates no effect on either cell viability or cytolytic activity up to 24 hours postlabeling. Given these parameters, the amount of drug that may be carried to and locally concentrated within metastatic lesions can approach approximately 15 μM. In addition, A-NK cells carrying Zyn-linked doxorubicin at an equivalent dose of 25 μg/kg is therapeutically comparable to a systemic dose of 8 mg/kg (320x more) in the 3LL model of experimental metastasis. These data indicate that A-NK cells bearing Zyn-linked chemotherapeutic agents represent a unique and feasible method to target diagnostic and therapeutic pharmaceutical agents to cancer metastases and that therapeutic doses can be attained without unwanted systemic exposure.

A novel drug delivery system using IL-2 activated NK cells and Zyn-linked doxorubicin. Goldfarb RH, Koelemij R, Muirhead KA, Ohlsson-Wilhelm BM, Gray BD, Kuppen PJ, Basse PH, al-Atrash G, Kitson RP. In Vivo 2000 Jan–Feb; 14 (1), pp. 101–4.

Macrophages: Hepatic Kupffer Cell (KC) Selective Delivery/Antigen Processing Inhibition: Enhanced Adenoviral Vector Delivery
Clodronate Liposomes

Background Information: Tissue macrophages, in particular hepatic KCs, contribute to early inflammatory responses following adenoviral vector administration.
Application: Characterization of hepatic KCs and enhancement of adenoviral vector delivery.
Route of Administration: Intravascular.
Implementation: Evaluate the effect of selective and transient (3 days) depletion of hepatic KCs by a single injection of clodronate liposomes on the *in vivo* activity of high-capacity adenoviral (HC-Ad) vectors that express the human alpha-1 anti-trypsin (hAAT) gene.
Animal Model: Murine (C57BL/6 and C3H strain mice).
Interpretation and Analysis: In mice, KC depletion increases and stabilizes hAAT production levels following intravenous injection of HC-Ad vectors expressing hAAT either from the hAAT promoter or from the human cytomegalovirus promoter. Comparable increases in hAAT levels can be obtained by preinjection with a transcriptionally silent HC-Ad vector. Interestingly, in most instances, depletion of KCs is sufficient to prevent the generation of anti-hAAT antibodies, resulting in prolonged trans-genic expression. Thus, short-term and selective depletion of hepatic macrophages at the same time significantly increases hepatic trans-gene expression and reduces humoral immune response to trans-genic proteins.

Selective depletion or blockade of Kupffer cells leads to enhanced and prolonged hepatic trans-genic expression using high-capacity adenoviral vectors. Schiedner G, Hertel S, Johnston M, Dries V, van Rooijen N, Kochanek S. Mol Ther 2003 Jan; 7 (1), pp. 35–43.

Neoplasia/Cardiovascular Endothelium Adhesion of Neoplastic Cells/Intravital Observation of Cancer Cell/Microcirculation Interactions
CalceinAM Labeled Neoplastic Cells

Background Information: Organ-specific tumor cell adhesion within the microcirculation of host organs is an important step in the metastatic cascade. Circulating tumor cells adhere to the luminal surface of microcirculatory vessels, then quickly stabilize these adhesion interactions. Ultimately, they leave the circulation to avoid the toxic effects of hydrodynamic shear forces exerted by circulating blood.
Application: Intravital observation of colon carcinoma cell adhesion within the hepatic microcirculation.
Route of Administration: Intravascular.
Implementation: CalceinAM can be used to label colon carcinoma cell populations (e.g., HT-29 [human] and CC531 [rat]) in order to track their biodistribution following intra-arterial injection as a single-cell suspension. Intravital fluorescence microscopy can then be applied to detect the development of adhesive interactions between circulating tumor cells and vascular endothelial surfaces within the hepatic microcirculation. These interactions are then analyzed regarding their time course and site localization within the vascular tree.
Animal Model: Rats (Sprague-Dawley).
Interpretation and Analysis: Autofluorescence of liver parenchyma is sufficient to distinguish hepatic sinusoids. Intravital microscopy is capable of differentiating early events in adhesion formation within hepatic sinosoids, adhesion stabilization, and extravasation of tumor cells into the liver parenchyma. Tumor cell adhesion occurs almost exclusively within sinusoidal capillaries; however, the diameter of these vessels is usually larger than that of tumor cells leaving remaining perfused lumen of the capillaries. Colon carcinoma cells rapidly migrate into the liver parenchyma after development of adhesion interactions within the sinusoids. In contrast to common end point assays of the metastatic cascade, this particular *in vivo* model allows investigations of metastatic colon carcinoma cell adhesion phenomenon within the liver microcirculation at specific steps during the formation of hematogenous metastasis and its underlying mechanisms.
Instrumentation: Intravital fluorescence microscopy.

An intravital model to monitor steps of metastatic tumor cell adhesion within the hepatic microcirculation. Haier J, Korb T, Hotz B, Spiegel HU, Senninger N. J Gastrointest Surg 2003 May–Jun; 7 (4), pp. 507–14; discussion 514–5.

Neutrophils and Macrophages/*In Vivo* Labeling of Tuftsin Complexes/Inflammation: Septic and Non-Septic/Central Nervous System (CNS): Multiple Sclerosis/Glucocorticoid Efficacy Evaluation/ Selective Delivery to Leukocytes or Sites of Inflammation/Experimental Allergic Encephalomyelitis
[^{99m}Technetium] ([^{99m}Tc])-RP128

Cellular/Molecular Mechanism of Action: The agent RP128 functions as a chelation agent for [^{99m}Tc], in addition to recognizing and binding to tuftsin receptors expressed by neutrophils and monocyte-macrophages.
Application: The agent [^{99m}Tc]-RP128 can be applied for the *in vivo* labeling of neutrophil and macrophage tuftsin receptor complexes for the purpose of detecting and characterizing focal sites of septic inflammation. Preparations can also potentially be applied for the detection and characterization of human multiple sclerosis conditions. Potentially could be applied to selectively deliver agents to macrophage and neutrophil populations.
Route of Administration: Intravascular.
Implementation: Administration of [^{99m}Tc]-RP128 for the *in vivo* detection of CNS inflammation (e.g., experimental allergic encephalomyelitis [EAE]).
Animal Model: Murine (EAE as a model for human multiple sclerosis).

Interpretation and Analysis: The radiopharmaceutical [^{99m}Tc]-RP128 can be detected at significantly increased levels within CNS tissues in conditions of EAE at 0.5, 1.0, and 3.0 hours postinjection using a dual radioisotope technique to correct for non-extravasated tracer ($p < 0.05$). Moreover, extravascular accumulation of [^{99m}Tc]-RP128 can be clearly demonstrated in inflammatory tissues with minimal loss of sensitivity when the secondary isotopic correction for blood volume is omitted. In addition, [^{99m}Tc]-RP128 can successfully monitor glucocorticoid suppression of inflammation ($p < 0.05$), detected as a typical dose-response to increasing steroid concentration. Clearly, [^{99m}Tc]-RP128 can quantitatively detect CNS inflammation and assess responses to therapy, indicating potential value as an imaging agent both clinically and as a research aid. Furthermore, the rapid *in vivo* labeling by [^{99m}Tc]-RP128 of specific inflammatory cells combined with the ability to monitor the progress of anti-inflammatory therapeutics may validate the use of this cellular radiolabeling agent for detecting a variety of inflammatory conditions.

The detection and quantitation of inflammation in the central nervous system during experimental allergic encephalomyelitis using the radiopharmaceutical [^{99m}Tc]-RP128. Paul C, Peers SH, Woodhouse LE, Thornback JR, Goodbody AE, Bolton C. J Neurosci Methods 2000 May 15; 98 (1), pp. 83–90.

Neutrophils and Monocytes/Consumption vs Production Abnormalities
[^{111}I]-Oxine Neutrophils/[^{111}I]-Merc Neutrophils Doxorubin (Chemotherapeutic "Loaded") Neutrophils

Physiological Mechanism of Action: Neutrophils and macrophages migrate into specific locations (tissues/organs) within the body in response to endogenous (certain cytokines) and exogenous (bacterial origin) agents perceived as chemotactic agents. In this manner, cells move toward an increasing concentration gradient.

Application: Facilitate differentiation between alterations in bone marrow leukocyte production compared to changes in consumption as a cause for neutropenia/monocytopenia or neutrophilia/monocytophia. Additionally, they can be used as an imaging carrier cell preparation for detecting regions of inflammation and sepsis or the selected delivery of diagnostic and therapeutic pharmaceuticals pretargeted with chemotactic agents conjugated with pH or enzyme-sensitive linking groups to site-specific monoclonal antibody fragments.

Route of Administration: Intravascular.

Considerations: Must be prepared fresh.

Semi-Synthetic Methods: Radioisotope markers or chemotherapeutic agents can be passively "loaded" into neutrophil populations at relatively high concentrations without affecting their response to chemotactic stimuli.

Implementation: Highly enriched leukocyte populations purified from fresh whole blood can be radiolabeled with a variety of agents including [^{51}Cr], [^{111}In]-merc, [^{111}In]-oxime, [^{111}In]-tropolone (detergent-free), and [^{99m}Tc]-HMPAO. In a similar manner, leukocytes can be "loaded" with a variety of antibiotic and chemotherapeutic agents, including doxorubicin, which passively penetrate across intact cell membranes and actually accumulate with the nucleus. Usually it is preferable to use neutrophil populations for the production of pharmaceutical-loaded cells, especially pertaining to chemotherapeutic agents, because this cell type does not undergo mitosis so it is unlikely to perpetuate any genetic defect that might possibly develop during the loading process.

Interpretation and Analysis: Declines in intravascular $T_{1/2}$ values for labeled neutrophils imply accelerated consumption or removal from the central compartment. In scenarios where neutrophil numbers are low but $T_{1/2}$ values are normal, this suggests the presence of abnormalities in hematopoeitic (bone marrow) production. Accumulations of radiolabeled cells at specific foci implies the presence of septic or non-septic inflammatory responses.

Antineoplastic activity of chemotherapeutic "loaded" neutrophils against human mammary carcinoma. Coyne CP, Fenwick BW, Ainsworth J. Biotherapy 1997; 10, pp. 145–59.

Neutrophil Leukotriene LTB_4 Receptors/ Induced Myocardial Ischemia
[^{99m}Technetium] ([^{99m}Tc])-RP517/[F]-RP517

Cellular/Molecular Mechanism of Action: In whole blood, [F]-RP517 (500 nmol/L) preferentially labels neutrophil LTB_4 receptor complexes.

Application: [^{99m}Tc]-RP517 is an LTB_4 receptor antagonist that has been developed for imaging acute inflammation or infection. A unique property of [^{99m}Tc]-RP517 is its ability to label white blood cells *in vivo* after intravenous injection.

Route of Administration: [^{99m}Tc]-RP517 (intravenous).

Implementation: Evaluate the relative [^{99m}Tc]-RP517 binding to human leukocyte subtypes and the [^{99m}Tc]-RP517 uptake pattern in myocardium with induced inflammatory lesions. One approach to assessing such conditions can involve delineation of RP517 binding to neutrophil populations *in vitro* within whole blood, and *in vivo* within the intravascular compartment. This can be achieved through the use of fluorescence-activated cell sorter analysis performed on whole human blood samples ($n = 2$) and isolated neutrophils ($n = 4$) with a fluorescent analogue of [^{99m}Tc]-RP517 referred to as [F]-RP517. Approaches *in vivo* can entail administration of [^{99m}Tc]-RP517 to determine its biodistribution within the myocardium before and after coronary occlusion. To facilitate *ex vivo* imaging of the myocardium, the left ventricle can be divided into 72 segments for flow and [^{99m}Tc]-RP517 uptake analyses.

Animal Model: Canine ($n = 9$ dogs; anesthetized, open-chest surgical exposure before coronary occlusion [90 minutes] and reperfusion [120 minutes]; or $n = 3$ dogs before intramyocardial tumor necrosis factor-alpha (TNF-α) administration). In this context, myocardium inflammation can be induced by either coronary occlusion and reperfusion or administration by injection of TNF-α preparations.

Interpretation and Analysis: In whole blood, [F]-RP517 (500 nmol/L) preferentially labels neutrophils. In isolated neutrophil populations, [F]-RP517 (10 nmol/L) binding is inhibited by 44% in the presence of LTB_4 (400 nmol/L). In the analysis of *ex vivo* cardiac samples, an inverse exponential relationship can be detected between [^{99m}Tc]-RP517 uptake and occlusion flow ($r = 0.73$). In the same 15 segments, [^{99m}Tc]-RP517 uptake is highly correlated with neutrophil myeloperoxidase enzyme levels ($r = 0.91$). *Ex vivo* images reveal tracer uptake in the reperfused area (ischemic to normal count ratio $= 2.7 \pm 0.2$). Therefore, it can be concluded that RP517 binds to the neutrophil LTB_4 receptor complex after intravenous injection. After reperfusion, [^{99m}Tc]-RP517 uptake correlates with myeloperoxidase levels and can be observed on *ex vivo* images, indicating that this tracer may have potential as an imaging agent for detecting inflammatory conditions.

Assessment of myocardial inflammation produced by experimental coronary occlusion and reperfusion with [^{99m}Tc]-RP517, a new leukotriene B_4 receptor antagonist that preferentially labels neutrophils *in vivo*. Riou LM, Ruiz M, Sullivan GW, Linden J, Leong-Poi H, Lindner JR, Harris TD, Beller GA, Glover DK. Circulation 2002 Jul 30; 106 (5), pp. 592–8.

Neutrophils/Gastrointestinal Inflammation: Colitis (Acute Chemically Induced) *[^{99m}Technetium] ([^{99m}Tc])-HYNIC-IL-8/ [^{99m}Tc]-Neutrophils*

Cellular/Molecular Mechanism of Action: Interleukin-8 (IL-8) binds to its corresponding membrane-associated receptor complex expressed at relatively high densities by neutrophil and monocyte cell types. Granulocytes are chemotactically attracted to sites of septic and non-septic inflammation.

Physiological Mechanism of Action: Interleukin-8 can function as a chemotactic agent for granulocyte populations.

Implementation: Evaluation of acute colitis conditions facilitated by scintigraphic image analysis with [^{99m}Tc]-HYNIC-IL-8 and [^{99m}Tc]-granulocytes (purified cell populations). Gamma camera images are acquired at 2 minutes and at 1, 2, and 4 hours postinjection. At 4 hours after injection, the uptake of the radiolabel within specific tissues and organs can be determined (postmortem analysis). The dissected colon can be imaged and the inflammatory lesions scored macroscopically. For each affected colon segment, the colitis index (affected colon-to-normal-colon uptake ratio, CI) is calculated and correlated with the macroscopically scored severity of inflammation.

Animal Model: Rabbit (chemically induced acute colitis).

Interpretation and Analysis: Both agents can facilitate visualization of colitis conditions within 1 hour after injection. [^{99m}Tc]-HYNIC-IL-8 images of colonic abnormalities are more accurate and the intensity of uptake in regions of affected colon continuously increase up to 4 hours after injection, whereas no further increase 1 hour after injection is noticed scintigraphically with [^{99m}Tc]-HMPAO-granulocytes. Absolute uptake within affected colon is much higher for IL-8 than for radiolabeled granulocytes with the percentage of injected dose per gram (%ID/g) equal to 0.41 $\pm$ 0.04 %ID/g and 0.09 $\pm$ 0.054 %ID/g hour after injection, respectively. With increasing severity, the CI at 4 hours after injection for [^{99m}Tc]-HYNIC-IL-8 is approximately 4.4 $\pm$ 0.6, 13.5 $\pm$ 0.5, and 25.8 $\pm$ 1.0; for granulocytes, the CI 4 hours after injection is 1.5 $\pm$ 0.1, 3.4 $\pm$ 0.2, and 6.4 $\pm$ 0.5, respectively. Values for CI correlate with the severity of tissue inflammation ($r = 0.95$, $p < 0.0001$ for IL-8; $r = 0.95$, $p < 0.0001$ for granulocytes). Therefore, within 1 hour after injection, visualization of the extent of colonic inflammation *in vivo* is possible with [^{99m}Tc]-HYNIC-IL-8 and [^{99m}Tc]-HMPAO-granulocytes. Within 2 hours after injection, [^{99m}Tc]-IL-8 allows a good evaluation, and within 4 hours after injection, a meticulous evaluation of the severity of inflammatory bowel disease (IBD) is possible. Although [^{99m}Tc]-HMPAO-granulocytes are able to delineate the extent of IBD within 2 hours after injection, an accurate estimation of the severity of inflammation is not possible. [^{99m}Tc]-HYNIC-IL-8 is an inflammation-imaging agent that has demonstrated promising results. [^{99m}Tc]-IL-8 can be prepared off-the-shelf and yields excellent imaging with high target-to-background ratios.

Instrumentation: Gamma camera imaging capabilities.

Rapid imaging of experimental colitis with [^{99m}Tc]-interleukin-8 in rabbits. Gratz S, Rennen HJ, Boerman OC, Oyen WJ, Corstens FH. J Nucl Med 2001 Jun; 42 (6), pp. 917–23.

Platelets/Thrombocytes/Cardiovascular: Arterial Thromboses/Pharmaceutical Efficacy Evaluation: Antiplatelet Therapy *[^{111}Indium] ([^{111}In])-Platelets/ 4-Cyano-5,5-Bis(Methoxyphenyl)-4-Pentenoic Acid*

Cellular/Molecular Mechanism of Action: The radioisotope [^{111}In] emits photons that can be detected as a tracer in a manner that allows it to be used to detect the biodistribution of labeled synthetic diagnostic pharmaceuticals, biological proteins, and purified cell populations. Platelets can accumulate at anatomical (e.g., intravascular) sites of platelet aggregation including vascular injury and arterial clot formation.

Application: Preparations of [^{111}In]-platelets can be utilized for the detection of intravascular thrombosis and evaluation of the efficacy of antithrombotic pharmaceutical agents. Aorta thromboses are composed of a higher percentage of platelets compared to venous thromboses, which contain predominantly polymerized ($\pm$ cross-linked) fibrin monomer.

Route of Administration: Intravascular.

Implementation: Combined application of [^{111}In]-platelet scintigraphy (PSG) and B-mode ultrasonography (US) to determine the short-term effects of antiplatelet agents [e.g., 4-cyano-5,5-bis (methoxyphenyl)-4-pentenoic acid (E5510)]. After 8 weeks, [^{111}In]-PSG and US are repeated. Platelet deposition is then assessed visually and semi-quantitatively using a platelet accumulation index.

Human Model: Cases with platelet deposition at either the carotid bifurcation or abdominal aorta (e.g., $n = 15$) detected by [^{111}In]-PSG. Half of affected cases are administered antiplatelet therapy (e.g., E5510 at 4 mg/day).

Interpretation and Analysis: Visual analysis reveals that a high percentage of cases become negative for platelet deposition after certain antithrombotic therapies (e.g., $n = 7/8$), while essentially no change occurs without the benefit of antithrombotic intervention. The platelet accumulation index in vessels with platelet deposition is significantly reduced after delayed antithrombotic treatment (6.0 $\pm$ 7.1% vs 12.4 $\pm$ 3.9%, $p < 0.01$), while there is no significant change in vessels without platelet deposition (2.9 $\pm$ 3.0% vs 2.9 $\pm$ 4.1%). In cases with early-phase antithrombotic therapy, vessels rarely demonstrate any changes (8.1 $\pm$ 6.4% vs 8.9 $\pm$ 7.3%). However, there is no significant reduction of carotid plaque size in either group. Short-term E5510 therapy inhibits platelet deposition in active atherothrombotic lesions, and the *in vivo* combination of PSG and US can be useful for evaluating the effectiveness of antithrombotic pharmaceuticals.

Effect of E5510, a novel antiplatelet agent, on platelet deposition in atherothrombotic lesions: Evaluation by [^{111}In] platelet scintigraphy. Moriwaki H, Matsumoto M, Handa N, Hashikawa K, Hori M, Nishimura T. Nucl Med Commun 2000 Nov; 21 (11), pp. 1051–8.

Platelets *Thrombogenic Lesions, Stimuli, or Devices/[^{111}Indium] ([^{111}In])-Platelets/[^{99m}Technetium] ([^{99m}Tc])-Platelets*

Physiological Mechanism of Action: The function of normal platelets includes maintenance of vascular endothelial integrity and active participation in thrombogenic responses. Thrombi formed within arterial structures have a higher platelet content than do thrombi found within the venous vasculature.

Application: Detection and characterization of vascular thromboses, the plasma half-life of platelets, and the thrombogenic properties of foreign materials.

Semi-Synthetic Methods: Radiolabeling of highly enriched populations of platelets with [^{111}In]-tropolone (detergent-free) and [^{99m}Tc]-HMPAO following their isolation from fresh whole blood.

Implementation: Evaluation of the platelet thrombogenicity of a hemodialyzer device quantified through the application of [^{99m}Tc]-platelets and [^{111}In]-platelets. Hemodialysis is performed with a hollow-fiber dialyzer (HFD) in a flow-loop, the temperature of which is maintained at 37°C, with flow-rates of 7, 150, and 270 mL/min. Following dialysis, the HFD radioactivity is measured with an ionization chamber and imaged with a gamma camera. The radioactivity of

samples of hollow fibers taken from the top, middle, and bottom of the dialyzer is quantitatively measured with a gamma counter.

Animal Model: Normal beagle dogs and Yorkshire pigs.

Human Model: Human volunteers.

Interpretation and Analysis: The canine platelets are more thrombogenic than porcine and human platelets. The adhesivity of porcine platelets to the biomaterial (cellulose-acetate) of the dialyzer approximates that of human platelets. The [^{99m}Tc] label underestimates thrombus formation ($p < 0.01$). The dynamic processes of thrombosis and embolization from the hemodialyzer device results in large standard deviations around the mean for values associated with the characteristics of adherent thrombi. In spite of this limitation of the dynamic pathology, quantitation of the comparative thrombogenicity utilizing [^{111}In]- and [^{99m}Tc]-labeled platelets suggests that both radionuclides may be used for detecting and quantifying device-induced thrombogenicity and may provide an estimation (extrapolation) of prosthesis-induced thrombogenicity of human platelets from animal studies.

Quantitation of thrombogenicity of hemodialyzer with technetium-99m and indium-111 labeled platelets. Dewanjee MK, Kapadvanjwala M, Ruzius K, Serafini AN, Zilleruelo GE, Sfakianakis GN. Nucl Med Biol 1993 Jul; 20 (5), pp. 579–87.

Platelet Consumption/Intravascular Kinetics/ Disseminated Intravascular Coagulation/ Autoimmune Thrombocytopenia
Platelets/[^{111}Indium] ([^{111}In])-Merc Platelets/ [^{111}In]-Oxine Platelets

Cellular/Molecular Mechanism of Action: Platelets, when exposed to activating agents like adenosine diphosphate and collagen monomer, become activated, undergo degranulation (phase I and II), and aggregate (reversible and irreversible phases).

Physiological Mechanism of Action: Function as active cellular participants in normal and abnormal coagulation events. Platelets, unrelated to their participation in coagulation events, also repair defects in the vascular endothelium.

Background Information: Labeled platelets can be utilized to characterize intravascular platelet kinetics or for diagnostic imaging purposes.

Application: Detection of alterations in platelet intravascular kinetics in conditions of increased consumption (e.g., disseminated intravascular coagulation, autoimmune thrombocytopenia) or decreased bone marrow production (radiation toxicity, chemotherapy sequela). Labeled platelets can also be utilized for diagnostic imaging for the detection of arterial and venous intravascular thromboses. In this context, intravascular arterial thrombi have a relatively higher platelet content than do thrombi formed within the venous vascular network. Lastly, platelets can be "loaded" with pharmaceutical agents for the therapeutic management of various disease states. One of the best examples of this strategy is the loading of platelets with vincristine for managing conditions of autoimmune thrombocytopenia, whereby phagocytized vincristine-loaded platelets by macrophage populations reduce accelerated consumption of immunoglobulin-coated platelets.

Route of Administration: Intravascular.

Semi-Synthetic Methods: Highly enriched platelet populations are isolated from fresh whole blood collected into citrate anticoagulant. Usually, chelating agents are used to label cell populations like platelets because agents like [^{111}In] have a cationic or anionic charge that substantially decreases their ability to penetrate intact cell membrane structures. Other radioisotope preparations that have been applied to label platelets include [^{51}Cr], [^{111}In]-merc, [^{111}In]-oxime, [^{111}In]-tropolone (detergent-free), and [^{99m}Tc]-HMPAO.

Interpretation and Analysis: Decreases in the intravascular kinetic profile for labeled platelet preparations suggest accelerated/elevated platelet consumption. In the event thrombocytopenia is present but platelet intravascular $T_{1/2}$ is normal, then a decrease in bone marrow platelet production should be considered. Regional intravascular accumulation of radiolabeled platelets suggests the presence of clot formation.

Instrumentation: Scintillation counter (intravascular kinetics), gamma camera (imaging).

Radiolabeling of equine platelets in plasma with [^{111}In]-(2-mercaptopyridine-N-oxide) and their *in vivo* survival in the horse. Coyne CP, Kelly AB, Hornof WJ, O'Brien TR, Phelps MS, Lamb JF. Am J Vet Res 1987; 48, pp. 385–91.

Stem Cells/Central Nervous System/ [Parkinson's Disease]

Background Information: The ability to track the patterns of stem cell migration within the brain by *in vivo* neuroimaging will improve current understanding about how these cell types mediate functional recovery after neural transplantation. One major challenge for the development and refinement of stem cell transplantation is to map the spatial distribution and rate of *in situ* migration.

Application: Tracking transplanted stem cells in the ischemia-damaged hippocampus utilizing magnetic resonance imaging (MRI) analysis.

Implementation: Assessment of stem cell migration applying *in vitro* labeling of populations with the novel bifunctional contrast agent gadolinium rhodamine dextran (GRID) prior to transplantation. Detection can be assessed applying either magnetic resonance imaging (MRI) and fluorescence microscopy or with PKH26 (visible exclusively under fluorescence microscopy). At different time points following engraftment, brain tissues can be evaluated applying *ex vivo* MRI techniques and histopathology analysis.

Animal Model: Rat (ischemia-damaged hippocampus).

Interpretation and Analysis: Transplanted stem cells can be identified by MRI only if prelabeled with GRID, whereas fluorescence microscopy can detect transplanted cells using either labeling reagent. Distribution images of GRID-labeled stem cells produced applying MRI closely correlate with results obtained using fluorescence microscopy analysis. Stem cells and their migration can therefore be reliably identified applying GRID-enhanced MRI methodologies.

Tracking transplanted stem cell migration using bifunctional, contrast agent-enhanced, magnetic resonance imaging. Modo M, Cash D, Mellodew K, Williams SC, Fraser SE, Meade TJ, Price J, Hodges H. Neuroimage 2002 Oct; 17 (2), pp. 803–11.

Stem Cells: Embryonic/Monitoring Stem Cell Traffic/Migration
Magnetic Resonance Imaging (MRI) Agent Cell Labeling/Lipofection Cell-Labeling Methodology

Background Information: *In vivo* monitoring of stem cells after grafting is essential for establishing an improved understanding about migration dynamics, cellular differentiation processes, and regeneration potential. Migration of endogenous or grafted stem cells and neurons has been described for vertebrate brain, both under normal conditions (involving the subventricular zone along the rostral migratory stream) and during pathophysiological events (e.g., conditions of degeneration and focal cerebral ischemia). However, previous investigations heavily relied upon invasive forms of analysis (e.g., brain sectioning) in combination with selective staining techniques.

Application: Embryonic stem cells could theoretically function as a selective delivery modality for diagnostic and therapeutic pharmaceutical agents. The concept of labeling stem-cell-like populations could also be utilized as a methodology for monitoring "therapeutic" cellular infiltration into specific regions of pathology (e.g., central nervous system [CNS] foci affected by ischemic insult or Parkinson's disease).

Route of Administration: Variable, including non-selective, selective, and highly selective delivery platforms (e.g., intravascular injection, vs specific catheterization of certain cerebral artery branches, vs direct implantation into tissue foci).

Implementation: External mapping of the *in vivo* dynamics of cell migration over a prolonged period of time. Embryonic stem (ES) cells can be modified to constitutively express green fluorescent protein (GFP) using a lipofection procedure employed in concert with a tracer MRI contrast agent. Contrast agent-labeled ES cells are then directly implanted into tissues, including the brain. To convincingly illustrate the capacity of ES cells to migrate into regions of pathology, they can be implanted into the healthy contralateral cerebral hemisphere.

Animal Model: Rat (e.g., focal lesions of cerebral ischemia induced 2 weeks before implantation of ES cells).

Analysis and Interpretation: Magnetic resonance imaging permits the observation of implanted cells with high contrast against normal tissue background. Confirmation can in part be established by analysis of GFP biodistribution patterns. During a 3-week period, cells migrate along the corpus callosum into the ventricular walls and massively populate the border-zone of damaged brain tissue within the hemisphere opposite to the original site of implantation. Embryonic stem cells can display highly desirable migration dynamics and are attracted to regions of induced cerebral ischemia in a relatively selective manner. Techniques employing labeled embryonic stem cells are ideally suited for the non-invasive imaging of cell migration events, engraftment, and morphological differentiation at high spatial and temporal resolution.

Instrumentation: Magnetic resonance imaging (MRI) at 78-microm isotropic spatial resolution.

Monitoring of implanted stem cell migration *in vivo*: A highly resolved *in vivo* magnetic resonance imaging investigation of experimental stroke in rat. Hoehn M, Kustermann E, Blunk J, Wiedermann D, Trapp T, Wecker S, Focking M, Arnold H, Hescheler J, Fleischmann BK, Schwindt W, Buhrle C. Proc Natl Acad Sci U S A. 2002 Dec 10; 99 (25), pp. 16267–72. Epub 2002 Nov 20.

Stem Cells: Hematopoietic Progenitor Cells
Magnetic Polysaccharide Nanoparticle-Transferrin/ Gadopentetate Dimeglumine Liposomes/P7228 Liposomes/Ferumoxides/Ferumoxtran

Application: Detection and characterization of the biodistribution of labeled human hematopoietic progenitor cells.

Route of Administration: Intravascular or direct implantation by injection (e.g., non-selective, selective, and highly selective delivery).

Semi-Synthetic Methods: Labeling of hematopoietic progenitor cells with ferumoxides, ferumoxtran, magnetic polysaccharide nanoparticles-transferrin, P7228 liposomes, and gadopentetate dimeglumine liposomes to facilitate detection by magnetic resonance imaging (MRI) analyses.

Implementation: Evaluation of the organ/tissue distribution of labeled hematopoietic progenitor cells using MRI (1.5 T). Data can be analyzed by measuring magnetic resonance signal intensities and R1 and R2* relaxation rates of labeled cells and non-labeled control cells. Mean quantitative data for the various contrast agents is then assessed for significant differences between experimental and reference controls utilizing the Scheffe statistical analysis test. As a reference standard, MRI data can be compared with electron microscopic and spectrometric analyses.

Interpretation and Analysis: For all contrast agents, intracellular cytoplasm uptake can be demonstrated by electron microscopy and quantified utilizing spectrometry. Compared to non-labeled control cells, progenitor cells labeled with iron oxides show significant increases in R2* ($p < 0.05$). Cells labeled with gadopentetate dimeglumine liposomes show significantly increased R1. Detection thresholds are approximately 5×10^5 cells for gadopentetate dimeglumine liposomes and ferumoxtran; 2.5×10^5 cells for ferumoxides and P7228 liposomes; and 1×10^5 cells for magnetic polysaccharide nanoparticles-transferrin. Hematopoietic progenitor cells therefore can be labeled with MR contrast agents and detected with a standard 1.5 T magnetic resonance imager.

Instrumentation: Magnetic resonance imaging with T_1- and T_2-weighted spin-echo and fast field-echo sequences (1.5 T).

Targeting of hematopoietic progenitor cells with MR contrast agents. Daldrup-Link HE, Rudelius M, Oostendorp RA, Settles M, Piontek G, Metz S, Rosenbrock H, Keller U, Heinzmann U, Rummeny EJ, Schlegel J, Link TM. Radiology 2003 Sep; 228 (3), pp. 760–7.

Stem Cells/Migration: Mesenchymal/Lymphocytes/ Oligodendrocyte Progenitor CG-4 Cells/Cervical Carcinoma/[Iron Load: *Ex Vivo*]
Mesenchymal Stem Cells/Transfection Agents/ Ferumoxides/MION-46L/[Prussian Blue]

Application: Labeling of mammalian and stem cells by combining commercially available transfection agents with superparamagnetic iron oxide (SPIO) magnetic resonance (MR) imaging enhancing contrast agents.

Route of Administration: Intended primarily for intravascular applications.

Semi-Synthetic Methods: Transfection agents can be incubated with ferumoxides and MION-46L in cell culture medium at various concentrations. Different viable cell types are subsequently incubated 2–48 hours with 25 μg of iron per milliliter of combined transfection agent/superparamagnetic iron oxide (TA/SPIO). Cellular labeling effectiveness can be assessed with T_2 relaxometry, MR imaging of labeled cell suspensions, and Prussian blue staining for detection of iron deposits.

Cellular Model: Human mesenchymal stem cells, mouse lymphocytes, rat oligodendrocyte progenitor CG-4 cells, and human cervical carcinoma cells.

Implementation: Evaluation of the proliferation and viability of mesenchymal stem cells and human cervical carcinoma cells labeled with a combination of transfection agents and ferumoxides.

Interpretation and Analysis: When ferumoxides-TA or MION-46L-TA are used, intracytoplasmic particles stained with Prussian blue stain can be detected in all cell line types with a labeling efficiency of nearly 100%. Limited or no uptake can be observed for cells incubated with ferumoxides or MION-46L alone. For TA-SPIO-labeled cells, MR images and relaxometry findings reveal a 50–90% decrease in signal intensity and a more than 40-fold increase in T_2s. Cell viability varies from 103.7% ± 9 to 123.0% ± 9 compared to corresponding values for reference control cells at 9 days, while cell proliferation is not affected by endosomal incorporation of SPIO nanoparticles. Iron concentrations vary with ferumoxides-TA combinations and cells with a maximum of 30.1 pg ± 3.7 of iron per cell for labeled mesenchymal stem cells. Therefore, in sum-

mary, magnetic labeling of mammalian cells aided by the use of ferumoxides and transfection agents is possible and may enable cellular MR imaging and tracking in experimental and clinical settings.

Clinically applicable labeling of mammalian and stem cells by combining superparamagnetic iron oxides and transfection agents. Frank JA, Miller BR, Arbab AS, Zywicke HA, Jordan EK, Lewis BK, Bryant LH Jr, Bulte JW. Radiology 2003 Aug; 228 (2), pp. 480–7.

Stem Cells: Myogenic Precursor Cells (MPC) (Iron Oxide-Loaded)
Myocardium Infarction/Detailed Segmental Left Ventricle (LV) Function

Cellular/Molecular Mechanism of Action: Myogenic precursor cells detect and accumulate within lesions of myocardial generation.
Application: Evaluation of myocardial implants of MPC loaded with iron oxide to determine how reliably they can be detected by *in vivo* cardiac magnetic resonance imaging (MRI).
Route of Administration: Direct catheterization.
Implementation: Electromechanical mapping of the LV and guided microinjections into normal and infarcted myocardium utilizing injected autologous MPC delivered through a percutaneous catheter. Cardiac MRI can be used to detect implanted MPC previously loaded with iron oxide nanoparticles.
Animal Model: Swine (n = 7 farm pigs; n = 4/7 with anterior myocardial infarction). Magnetic resonance imaging data are compared to LV electromechanical mapping findings and cross-registered pathology.
Interpretation and Analysis: The vast majority of injections into normal (e.g., n = 9/9) and locally damaged (e.g., n = 12/12) myocardium can be detected on T_2-weighted spin echo and inversion-recovery true-FISP MRI (low signal areas) with good anatomical concordance with sites of implantation on electromechanical maps. All sites of injection can be confirmed on pathology that can reveal in all infarct animals iron-loaded MPC at the center and periphery of the infarct, as expected from MRI. In summary, targeted catheter-based implantation of iron-loaded MPC into locally infarcted LV myocardium is accurate and can be reliably demonstrated by *in vivo* cardiac MRI. The ability to non-invasively identify intramyocardial cell implantation may be a determinant for future experimental studies designed to analyze subsequent effects of such therapy on detailed segmental LV function.

Magnetic resonance imaging of targeted catheter-based implantation of myogenic precursor cells into infarcted left ventricular myocardium. Garot J, Unterseeh T, Teiger E, Champagne S, Chazaud B, Gherardi R, Hittinger L, Guéret P, Rahmouni A. J Am Coll Cardiol 2003 May 21; 41 (10), pp. 1841–6.

Stem Cells/Oligodendrocyte Progenitor Cells/ Transferrin Magnetic Nanoparticles
Central Nervous System: Demyelinated Axons/ Transferrin Receptors

Cellular/Molecular Mechanism of Action: Oligodendrocyte progenitor cells naturally migrate to specific foci within the CNS, including demyelinated axons.
Background Information: Demyelination is a common pathological finding in human neurological diseases and frequently persists as a result of failure of endogenous repair. Transplanted oligodendrocytes and their precursor cells can (re)myelinate axons, raising the possibility of therapeutic intervention. The migratory capacity of transplanted cells is of key importance in determining the extent of (re)myelination and can, at present, be evaluated only by the use of invasive and irreversible procedures.
Application: Potential delivery modality that recognizes demyelinated axons.
Route of Administration: Presumably intravascular.
Implementation: Delineation of the capacity for transferrin receptors to function as an effective "target" that can facilitate the intracellular delivery of magnetic nanoparticles and guide the migration of transplanted tagged oligodendrocyte progenitor cells into the spinal cord with myelin-deficient neurons.
Animal Model: Rat (myelin-deficient neurons).
Interpretation and Analysis: Cell migration can be easily detected by using 3-dimensional magnetic resonance microscopy, which demonstrates a close correlation between the areas of contrast enhancement and degree of "new" myelination in an *in vivo* myelin-deficient model. In this context, investigations have demonstrated the feasibility of successfully using magnetic resonance to track transplanted oligodendrocyte progenitor cells. The technique has the potential of being easily extended into other neurotransplantation research investigations involving different precursor cell types.

Neurotransplantation of magnetically labeled oligodendrocyte progenitors: Magnetic resonance tracking of cell migration and myelination. Bulte JW, Zhang S, van Gelderen P, Herynek V, Jordan EK, Duncan ID, Frank JA. Proc Natl Acad Sci U S A 1999 Dec 21; 96 (26), pp. 15256–61.

VECTOR-MEDIATED GENE (OLIGONUCLEOTIDE) DELIVERY SYSTEMS

Cell Transfection: HEK293 and COS-7 Cell Lines/ Gene Expression: Facilitation of Phage Delivery
Plasmids (pBUDLacZ)/Pullulan (Hydrogel) Nanoparticles/Lipofectamine 2000

Application: Enhanced delivery of nucleic acid molecules to cells by encapsulation within hydrogel pullulan nanoparticles.
Route of Administration: Intravascular
Semi-Synthesis Method: Pullulan nanoparticles encapsulating pBUDLacZ plasmid can be prepared inside aqueous droplets of w/o microemulsions.
Implementation: Evalation of cellular viability/vitality (cytotoxicity response) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.
Interpretation and Analysis: Transmission electron microscopy (TEM) imaging reveals that particles are spherical in shape with a size of 45 ($\pm$0.80) nm diameter. Cell cytotoxicity studies reveal that cells incubated with nanoparticles remain more than 100% viable at nanoparticle concentration as high as 1000 μg/ml. From scanning electron microscope images, nanoparticles can be observed to be internalized and cells tend to exhibit vacuoles in the cell body due to nanoparticle internalisation. Endocytosis of nanoparticles results in disruption of F-actin and beta-tubulin cytoskeleton of human fibroblasts. The efficacy of transfection *in-vitro* on HEK293 and COS-7 cells is cell-type dependent, with COS cells demonstrating a higher level of gene expression. The beta-gal expression in COS-7 cells facilitated by pullulan nanoparticle is comparable to commercially available Lipofectamine 2000. The results of this study are encouraging for the development of pullulan nanoparticles as an intracellular delivery system for drugs and genes.

Hydrogel pullulan nanoparticles encapsulating pBUDLacZ plasmid as an efficient gene delivery carrier. Gupta M; Gupta AK J Control Release 2004 Sep 14; 99 (1), pp. 157–66.

Central Nervous System Capillary Endothelium Gene Transfer Model/Beta Galactosidase/ MBEC4 Cell Transfection
Hemagglutination Virus of Japan Liposomes [Liposome-Encapsulated Beta-Galactosidase Plasmid]

Background Information: Bioactive proteins or peptides cannot be effectively delivered into brain capillary endothelial cells (BCECs) or brain parenchyma.
Route of Administration: Super-selective internal carotid artery catheterization.
Implementation: Evaluation of the electiveness of transferring the *Escherichia coli* beta-galactosidase gene (beta-gal) as a model gene into brain capillary endothelial cells (BCEC) utilizing the hemagglutination virus of Japan (HVJ)-liposomes. HVJ-liposomes encapsulating a beta-gal plasmid can be used *in-vitro* to transfect MBEC4 cells, and also *in-vivo* by selective administration.
Animal Model: Rat via the internal carotid artery.
Interpretation and Analysis: Success of the procedure is confirmed by the detection of 116 kDa beta-gal protein in transfected MBEC4 cells and in brain capillaries isolated from transfected rats, by Western blot analysis and histological staining. The enzymatic activities of beta-galactosidase are 5- to 10-fold and 20-fold higher than when beta-gal-containing liposomes without fusogenic activity (uncoated liposomes) or plasmid alone are employed *in-vitro* and *in-vivo*, respectively. Thus, HVJ-liposomes have demonstrated applicability as a vector for the transfer of foreign genes into brain capillary endothelium *in-vivo* (transluminal route).

Transluminal gene transfer into brain capillary endothelial cells *in vivo* with HVJ-*liposomes*. Jiang C; Matsuo H; Koyabu N; Ohtani H; Fujimoto H; Yonemitsu Y; Kaneda Y; Narro M; Tsuruo T; Sawada Y Journal of drug targeting [J Drug Target] 2002 Jun; 10 (4), pp. 345–52.

Central Nervous System/Capillary Endothelium Cells/Brain Parenchyma Cells/Neurotrophic Factor (mGDNF Gene)
Hemagglutination Virus of Japan-Liposomes

Application: Gene delivery into the central nervous system (evaluation of the protective effect for dopamine neurons against a retrograde 6-hydroxydopamine (6-OHDA))
Route of Administration: HVJ-liposomes (internal carotid artery).
Semi-Synthesis Method: The gene encoding mouse glial cell-derived neurotrophic factor (mGDNF gene) can be transfected into brain capillary endothelial cells (BCECs) with the aim of delivering the gene product extensively into the brain parenchyma by making use of BCEC secretory function. First, the mGDNF gene is transfected *in-vitro* into cultured BCECs (MBEC4; mouse brain capillary endothelial cells). The amount of mGDNF protein secreted from the transfected cells into the medium is about 1500 to 3200 pg/mg of cell protein/day, or about sevenfold higher than that accumulated intracellularly. Furthermore, the basolateral-directed secretion of mGDNF protein from the transfected MBEC4 cells is fivefold higher than the apical-directed secretion.
Implementation: Evaluation of the capacity of the hemagglutination virus of Japan (HVJ)-liposomes to deliver the encapsulated mGDNF gene into the central nervous system.
Animal Model: Rat
Interpretation and Analysis: Transfected rats show a marked increase in the brain level of GDNF as assessed by means of enzyme-linked immunosorbent assay (ELISA) and Western blotting on day 3 after the administration, and the level remains significantly elevated for at least 12 days. Furthermore, immunohistochemical staining reveals an increase in GDNF immunoreactivity throughout the transfected forebrain. Such profiles indicate that the gene can be successfully transferred *in-vivo* from HVJ-liposomes into BCECs, where it is expressed, and the gene product is secreted into the brain. Using this delivery method, the protective effect provided to dopamine neurons against a retrograde 6-hydroxydopamine (6-OHDA) lesion can be evaluated (e.g. assessed as a function behavioral and neurochemical indices).

In-vivo delivery of glial cell-derived neurotrophic factor across the blood-brain barrier by gene transfer into brain capillary endothelial cells. Jiang C, Koyabu N, Yonemitsu Y, Shimazoe T, Watanabe S, Naito M, Tsuruo T, Ohtani H, Sawada Y. Hum Gene Ther. 2003 Aug 10;14(12):1181–91.

Central Nervous System Gene Delivery/Cerebral Ishchemia Lesions
Anti-Apototic Transgenes/Human bcl-2 Protein Plasmid DNA Complexes: Liposome Facilitated [Lipoplexes]

Background Information: Acute cerebral ischemia causes hypoxic neuronal cell death by necrosis and apoptosis. Expression of anti-apoptotic transgenes in ischemic brain may provide a useful therapeutic strategy for alleviation of postischemic damage.
Route of Administration: Lipoplexes (intrathecal)
Implementation: Evaluate the potential of liposomes to mediate transfer of human bcl-2 protein into the CNS during conditions of focal transient cerebral ischemia. Two different types of plasmid vectors can be used for bcl-2 expression; [i] one driven by the constitutive cytomegalovirus promoter (pCMV) and [ii] a second based on the hypoxia-inducible human vascular endothelial growth factor promoter (pHRE). Cationic liposome/plasmid DNA complexes (lipoplexes) are injected directly into the cerebrospinal fluid (CSF) of rats immediately after MCA occlusion. The brains of treated and control animals are analyzed 48 hours later. Infarct volumes and numbers of apoptotic cells are then quantified.
Animal Model: Rat (focal transient ischemia created by middle cerebral artery (MCA) occlusion).
Interpretation and Analysis: Occlusion of the MCA results in ipsilateral cerebral infarcts. Transfer of the bcl-2 gene results in high level widespread protein expression in the case of the pCMV-bcl2 plasmid, while animals treated with the pHRE-bcl2 vector show lower expression levels of bcl2 that are limited to the ischemic area. Treatment with pCMV-bcl2, but not with pHRE-bcl2, is able to significantly reduce infarct volumes [109 ± 8 mm^3 for pCMV-bcl2; 152 ± 29 mm^3 for pHRE-bcl2; and 155 ± 18 mm^3 for control animals]. Animals transfected with either of the two vectors have significant reductions in apoptotic cell numbers within infarcts and penumbra area compared to controls. There is no short-term neurological side-effects from CSF lipoplex injection or of bcl-2 expression. Hypoxia-inducible bcl-2 expression mediated by intrathecal lipoplexes may represent a novel, biologically safe and lesion-selective therapeutic approach for neuroprotection after acute cerebral ischemia.

Liposome-mediated transfer of the bcl-2 gene results in neuroprotection after in vivo transient focal cerebral ischemia in an animal model. Cao YJ; Shibata T; Rainov NG Gene therapy [Gene Ther] 2002 Mar; 9 (6), pp. 415–9.

Green Fluorescent Protein (GFP)/Stem Cell: Embryonic/Monitoring Stem Cell Traffic/Migration
Lipofection Cell Labeling Methodology/Magnetic Resonance Imaging Agent Cell

Background Information: *In vivo* monitoring of stem cells after grafting is essential for a better understanding of their migra-

tional dynamics, differentiation characteristics, and their potential for regeneration. Migration of endogenous or grafted stem cells and neurons has been described in the vertebrate brain, both under normal conditions from the subventricular zone along the rostral migratory stream, and under pathophysiological conditions, such as neuronal degeneration or focal cerebral ischemia. However, many studies conducted for such purposes have relied upon the invasive analysis of brain sections in combination with appropriate staining techniques.

Application: Embryonic stem cells could theoretically function as a selective delivery modality for diagnostic and therapeutic pharmaceutical agents. In concept, the labeling of stem-cell like populations could also function as a methodology for monitoring the "therapeutic" infiltration of desirable cell types into specific lesion sites (e.g., CNS focal cerebral ischemia, Parkinson's disease, epilepsy).

Route of Administration: Variable but usually involving intravascular or possibly direct injection delivery into pathologic lesions.

Implementation: Mapping the *in vivo* dynamics of cell migration (e.g. CNS biodistribution) over a prolonged period of time. Embryonic stem (ES) cells, constitutively expressing GFP are labeled with a lipofection procedure employing a MRI contrast agent. Contrast agent-labeled cells are then directly implanted into brains.

Animal Model: Rat. (focal cerebral ischemia induced 2 weeks before implantation of ES cells into the healthy, contralateral cerebral hemispheres).

Interpretation and Analysis: Magnetic resonance imaging (MRI) at 78-μm isotropic spatial resolution permits the observation of implanted cells with high contrast against host tissue background that can be confirmed by GFP detection patterns. During a 3-week period, cells migrate along the corpus callosum into the ventricular walls, and massively populate the borderzone of damaged brain tissue on the hemisphere opposite to the implantation sites. Embryonic stem cells (ES) have high migrational dynamics and are attracted to regions of created cerebral lesions in a relatively selective manner. Techniques employing labeled embryonic stem cells are ideally suited for the non-invasive imaging of cell migration, engraftment, and morphological differentiation at high spatial and temporal resolution.

Monitoring of implanted stem cell migration in vivo: A highly resolved *in vivo* magnetic resonance imaging investigation of experimental stroke in rat. Hoehn M, Kustermann E, Blunk J, Wiedermann D, Trapp T, Wecker S, Focking M, Arnold H, Hescheler J, Fleischmann BK, Schwindt W, Buhrle C. Proc Natl Acad Sci U S A 2002 Dec 10; 99 (25), pp. 16267–72. Epub 2002 Nov 20.

Hunter Syndrome: Mucopolysaccharidosis Type II/ Iduronate-2-Sulfatase/Lymphocyte-Mediated Selective Delivery

L2SN and L2/Avian β-Actin Promoter in LB2/CMV early Promoter in LNC2

Cellular /Molecular Mechanism of Action: Utilization of the natural (native) homing properties of peripheral blood lymphocytes (PBL) to selectively deliver diagnostic agents.

Route of Administration: Intravenous.

Considerations: Measurement of trans-gene lysosomal enzyme soon after exposure of target cells to vector supernatant may yield a gross overestimate of long-term trans-gene expression by transduced cells. Nevertheless, fibroblasts from clinical cases cocultured with transduced PBL(MPS) have reduced [$^{35}SO^{4}$]-GAG accumulation, levels similar to those of normal fibroblasts.

Implementation: Characterization of IDS expression from 3 different promoters in 4 retroviral vectors performed in peripheral blood lymphocytes (PBL) from cases with Hunter's syndrome (PBL[MPS]). Relevant vectors for LTR include L2SN and L2, avian β-actin promoter in LB2, and the CMV early promoter in LNC2. The production of PBL(MPS) in this manner involves their exposure to packaging cell supernatant.

Interpretation and Analysis: Surprisingly, IDS activities are equally high in all transduced lymphocyte populations: 515 U/mg/h in PBL(MPS)-L2SN, 734 in PBL(MPS)-LB2, 352 in PBL(MPS)-L2, and 389 in PBL(MPS)-LNC2 compared to controls ($<$ 10 in PBL[MPS]-LXSN or PBL[MPS]). The half-life of endocytosed IDS in PBL(MPS) is approximately 1.9 days. However, the level of lymphocyte IDS activity from proviral expression is only a fraction of the total, a large portion being derived from reuptake to enzyme from murine packaging cells, that is, a "2nd source" of enzyme.

Cells can be charged with a lysosomal enzyme to levels much higher than those found in nature. By "supercharging" cells with a lysosomal protein (or other molecule bearing the mannose-6-phosphate ligand), such cells may be exploited as vehicles for systemic delivery of therapeutic or diagnostic agents.

"Supercharged cells" for delivery of recombinant human iduronate-2-sulfatase. Pan D, Jonsson JJ, Braun SE, McIvor RS, Whitley CB. Mol Genet Metab 2000 Jul: 70 (3), pp. 170–8.

Hunter's Syndrome: Mucopolysaccharidosis Type II/ Lymphocyte-Mediated Selective Delivery

Retroviral Vectors (L2SN) /Iduronate-2-Sulfatase (IDS)/ Neomycin Resistance Genes

Cellular/Molecular Mechanism of Action: Vectors "transfect" host cells in a manner that leads to the transfer of genetic material (nucleotides) thereby facilitating subsequent gene expression by the host cell population.

Background Information: The process of growing and transducing large quantities of human primary peripheral blood lymphocytes (PBLs) with high gene transfer efficiency continues to be one of the major challenges for clinical and experimental therapy.

Implementation: Development of a clinical trial of lymphocyte gene therapy for mucopolysaccharidosis type II (i.e., Hunter's syndrome). One approach to such an objective can entail investigating methods that exploited the innate capability of a hollow-fiber bioreactor system to filter large quantities of vector supernatant and facilitate transduction. An aliquot (5×10^7) of PBL apheresis product can be precultured in a gas-permeable culture bag or bioreactor, and then transduced with a retroviral vector L2SN containing the iduronate-2-sulfatase (IDS) and neomycin resistance genes.

Interpretation and Analysis: The total number of PBLs could be expanded up to 187-fold, ielding up to 10^{10} cells at the end of a 7-day culture period. The multiplicity of infection can be increased (up to 20-fold) by ultrafiltrating a large volume of vector supernatant through a semipermeable membrane of this system. A high level of transduction efficiency (up to 57%) can be achieved, resulting in IDS enzyme activity as high as 1250 U/mg/h in transduced PBL(MPS) 15 days after transduction. This level is markedly increased from that of non-transduced cells ($<$ 3 U/mg/h) and is even greater than that of normal PBLs (mean, 809; n = 10). After 12 days of G418 selection, PBL(MPS) transductants exhibits a proviral IDS enzyme level approximately 3-fold higher than that in normal PBLs. These results indicated t hat a hollow-fiber bioreactor could be used to culture and transduce human primary PBLs in clinically useful quantities with relatively high gene transfer efficiency and transgene expression.

Combined ultrafiltration-transduction in a hollow-figer bioreactor facilitates retrovirus-mediated gene transfer into peripheral blood lymphocytes from patients with mucopolysaccharidosis type II. Pan D, Shankar R, Stroncek DF, Whitley CB, Hum Gene Ther 1999 Nov 20; 10 (17), pp.2799–810.

Macrophages: Kupffer Cell Selective Delivery/ Antigen Processing Inhibition
Adenoviral Vector Medicated Delivery (Enhanced High Capacity)/Clodronate Liposomes

Background Information: Tissue macrophages, in particular hepatic Kupffer cells (KCs), contribute to early inflammatory responses following adenoviral vector administration.
Application: Characterization of hepatic Kupffer cells and enhancement of adenoviral vector delivery.
Route of Administration: Intravascular.
Implementation: Evaluate the effect of selective and transient (3 days) depletion of hepatic Kupffer Cells (KC) by a single injection of clodronate liposomes on the *in vivo* performance of high-capacity adenoviral (HC-Ad) vectors.
Interpretation and Analysis: Interestingly, in the majority of animals of both strains, the depletion of KCs is sufficient to prevent the generation of anti-hAAT antibodies, resulting in prolonged transgene expression. Thus, short-term and selective depletion of hepatic macrophages at the same time significantly increases hepatic transgene expression and reduces the humoral immune response to the transgenic protein.

Selective depletion or blockade of Kupffer cells leads to enhanced and prolonged hepatic transgene expression using high-capacity adenoviral vectors. Schiedner G, Hertel S, Johnston M, Dries V, van Rooijen N, Kochanek S. Mol Ther 2003 Jan; 7 (1), pp. 35–43.

Neoplastic Disease: Renal Solid Tumors
Plasmid-Microspheres/Plasmids Complexed Liposomes/Selective Tumor DNA Delivery Via Vasculature

Application: Selective targeting of genetic DNA incorporated into plasmid for neoplastic lesions (selective delivery is in part facilitated by selective catheterization of the major arterial network supplying a neoplastic tissue mass.
Route of Administration: Arterial catheterisation
Implementation: Evaluation of plasmid DNA delivery into to an established rat renal solid tumor model involving the utilization of plasmid bound to microspheres, and plasmids complexed with liposomes to evaluate their potential to function as a selective delivery modality for tumors (via arterial catheterization).
Ex-Vivo: Rat renal solid tumor.
Interpretation and Analysis: Forty-eight hours post-delivery, tumor to normal kidney tissue chloramphenicol acetyltransferase expression ratios are as follows: free (1.8:1), microspherical (3.9:1), and liposomal (1.2:1). Microspheres are able to selectively deliver plasmids to tumours, whereas cationic liposomes distribute plasmids to both kidney parenchymal and tumor cells. The tumor model has the potential of screening delivery vehicles as well as therapeutic agents for the capacity of selective delivery to tumors via the vasculature.

A model for evaluating *selective* delivery of plasmid DNA to tumours via the vasculature. Dass CR; Burton MA Cancer biotherapy & radiopharmaceuticals [Cancer Biother Radiopharm] 2002 Oct; 17 (5), pp. 501–5.

Stem Cell Populations: Hematopoietic ($CD34^+$)/ Gene Delivery: EGFP and LNGFR Coding Sequence: Reporter Sequences
Retroviral Vectors (MFG Retroviral Backbone)

Background Information: Retroviral vectors are widely used to deliver foreign genes to hematopoietic stem cells (HSC). Improvement of marking protocols requires reporter genes to facilitate rapid detection and efficient selection of transduced cells. Both EGFP and LNGFR hold great potential as reporter systems and can be used simultaneously to compare their properties using the same retroviral backbone and the same gene transfer procedures. Note: [i] EGFP = enhanced green fluorescent protein; [ii] LNGFR = low-affinity nerve growth factor receptor; [iii] MFG = milk fat globulin; and [iv] ATG = antithymocyte globulin.
Application: Delivery of genetic sequences for their expression in mammalian cell systems. Such strategies could theoretically be utilized to deliver sense or antisense nucleotide strands for various diagnostic purposes.
Implementation: The EGFP and LNGFR coding sequences are separately cloned into the MFG retroviral backbone. A cloning strategy that assures that both genes utilize the same ATG as the start codon can be applied. Marker gene expression, viral titers, transduction efficiency, and vector stability can be evaluated in expanded amphotropic packaging clones and human hematopoietic cell lines by flow cytometry and PCR analysis. Vectors can also be tested for their ability to transduce different cell populations (e.g. CD34+ peripheral blood cells).
Interpretation and Analysis: A significantly larger number of MFG- LNGFR packaging clones can be obtained that produced high viral titers. A direct correlation between viral titer and marker gene expression in packaging clones can be demonstrated for both constructs. Similar expression kinetics and absence of *in vitro* toxicity in transduced cells are also observed for both constructs. Successful infection of CD34+ cells can be achieved even after a short time of exposure to recombinant viruses.

The marker genes, EGFP and LNGFR have been demonstrated to be equally useful for a rapid, specific and non-toxic detection of transduced cells. The MFG-EGFP construct appears useful to optimize gene transfer protocols *in vitro*. Alternatively, the MFG-LNGFR construct, for making possible a more efficient selection of high titer producer clones, as well as for safety and adaptability for *in vivo* use, is more suitable for clinical applications.

A comparative evaluation of gene transfer into blood cells using the same retroviral backbone for independent expression of the EGFP and deltaL-NGFR marker genes. Giaretta I, Madeo D, Bonaguro R, Cappellari A, Rodeghiero F, Giorgio P. Haematologica 2000 Jul; 85 (7), pp. 680–9.

MACRO-SYNTHETIC PLATFORMS

LIPOSOMES

Concepts and Perspectives: Liposomes
Synthetic Liposomes

Cellular/Molecular Mechanism of Action: Liposomes function as a nano-scale reservoirs and mobile *in vivo* delivery platforms for diagnostic agents.
Application: Delivery of *in vivo* diagnostic agents to the lymphatic system.
Route of Administration: Subcutaneous (s.c.) injection resulting in the assimilation of liposomes into the lymphatic system.
Dosage: Designed to establish a correlation between the specific biological activity (efficacy) of aptamer preparations and the corresponding pathological condition. Factors that influence lymphatic absorption and lymph node uptake of liposome administered subcutaneously include mean size and the anatomical site of injection (e.g., regional perfusion variable). Generally, other factors such as lipid composition, charge, and the presence of a hydrophilic poly (ethylene glycol) (PEG)-coating on the liposome surface do not sub-

stantially affect lymphatic absorption and lymph node uptake of s.c. administered liposomes.

Considerations: Subcutaneous injection is the route of delivery that is most effective for characterizing lymph node drainage basins with liposome preparations.

Interpretation and Analysis: Studies on the intranodal fate of liposomes demonstrate that phagocytosis by macrophages is the most important mechanism for lymph node uptake of liposomes. The observation of relatively high uptake of liposomes in regional lymph nodes after s.c. administration has stimulated research on lymphatic targeting of liposomes for diagnostic and therapeutic applications.

Role of macrophages in the localization of liposomes in lymph nodes after subcutaneous administration. Oussoren C, Storm G. Int J Pharm 1999 Jun 10; 183 (1), pp. 37–41.

Concepts and Perspectives: Liposomes/ Clearance Properties

Cellular/Molecular Mechanism of Action: Encapsulation can protect pharmaceutical agents from biochemical degradation and serve as a format for delivering high concentrations to specific organ/tissue targets or as a means for achieving slow-release pharmacokinetic patterns.

Application: Encapsulation can protect pharmaceutical agents from biochemical degradation and serve as a format for delivering high concentrations to specific organ/tissue targets or a platform for achieving slow-release pharmacokinetic patterns.

Route of Administration: Variable but most frequently intravascular.

Considerations: The clearance rate of liposomal drugs from the circulation is determined by the rate and extent of both drug release and uptake of liposomes by cells within the mononuclear phagocyte system (MPS). Intravenously injected liposomes initially come into contact with serum proteins. The interaction of liposomes with serum proteins is thought to play a critical role in the liposome clearance. In contrast to conventional (non-surface modification) liposomes, accelerated clearance of poly(ethylene glycol) (PEG)ylated-liposomes (sterically stabilized long-circulating liposomes) on repeated injection is a phenomenon that has been observed in previous investigations.

Implementation: Evaluation of the role of opsonin-independent and so-called opsonin-dependent mechanisms in promoting enhanced clearance of liposomes.

Interpretation and Analysis: Certain serum proteins can function as so-called opsonins in a manner that enhances the clearance of liposomes, when bound to their exterior surface membrane. In addition to opsonin-dependent liposome clearance, opsonin-independent liposome clearance is also an important consideration.

Liposome clearance. Ishida T, Harashima H, Kiwada H. Biosci Rep 2002 Apr; 22 (2), pp. 197–224.

Concepts and Perspectives/Diagnostic Pharmaceuticals Delivery
Liposomes

Cellular/Molecular Mechanism of Action: Encapsulation of relatively large volumes of diagnostic pharmaceutical agents to reduce biological degradation and diffusion and enhance selective delivery to specific cell types or tissues/organs.

Application: Variable and flexible.

Considerations: Major components of liposomes can include lipids, water, drugs, electrolytes, antioxidants, preservatives, and viscosity-inducing agents. Various methods and techniques have been developed for preparing different types of liposomes, including unilamellar, multilamellar, and immunoliposomes.

Interpretation and Analysis: The effectiveness and extent of liposomal drug delivery systems is directly influenced by vesicle size distribution; concentration of residual organic phase in the phospholipid bilayer system; surface characteristics and properties; efficiency and extent of drug encapsulation; chronological stability; and selective homing properties.

Entrapment of small molecules and nucleic acid-based drugs in liposomes. Fenske DB, Cullis PR. Methods Enzymol 2005; 391, pp. 7–40.

Recent trends in drug delivery systems: Liposomal drug delivery system—preparation and characterization. Chandran S, Roy A, Mishra B. Indian J Exp Biol 1997 Aug; 35 (8), pp. 801–9.

Concepts and Perspectives: Liposomes/ Membrane Fluidity
Diphenylhexatriene (DPH) Fluorescence Anisotropy

Background Information: Some reports have suggested that selective uptake of photosensitizers is due to significantly lower pH levels in interstitial fluid of tumors in contrast to normal tissue.

Application: Diphenylhexatriene can be applied to measure membrane fluidity by fluorescence anisotropy.

Implementation: Evaluation of the cellular uptake of merocyanine 540 (MC 540) examined at two pH levels: 6.8 ± 0.1 and 7.4 ± 0.1. There is no difference in spectral properties (absorption and fluorescence maxima positions, fluorescence intensity) of the drug in the presence of increasing amounts of either human blood plasma or fetal calf serum (FCS) (0–2%) at the 2 pH values evaluated.

Interpretation and Analysis: Nevertheless, significantly higher amounts of the drug are taken up by WiDr cells at pH 6.8 ± 0.1, both in the presence and absence of 10% FCS formulations. The absorption spectra of MC 540 in the presence of egg phosphatidylcholine (PC) liposomes are NaCl concentration-dependent (0.00–0.30 mol/L). Membrane fluidity, as measured by fluorescence anisotropy of DPH, is unchanged within the experimental error for NaCl concentration ranges between 0.01 and 0.30 mol l/L. Spectral changes indicate an enhancement of the incorporation of MC 540 into lipid membranes with increasing ionic strength. Such a concentration-dependent phenomenon for salt suggests a possible involvement of the surface potential in the interaction of MC 540 with lipid membranes. The results might provide an explanation of the pH dependency of the cellular uptake observed for MC 540 preparations.

pH, serum proteins, and ionic strength influence the uptake of merocyanine 540 by WiDr cells and its interaction with membrane structures. Cunderlíková B, Sikurová L, Moan J. Bioelectrochemistry 2003 Apr; 59 (1–2), pp. 1–10.

Concepts and Perspectives: Liposomes/ Protein-Lipid Membrane Interactions
N-[10-(1-Pyrenyl)Decanoyl]Sphingomyelin

Cellular/Molecular Mechanism of Action: The agent N-[10-(1-pyrenyl)decanoyl]sphingomyelin is a fluorescent sphingomyelin derivative.

Background Information: Selective enzymatic hydrolysis, lipid compositional analyses, and fluorescence studies have been performed on acetylcholine receptor (AChR)-rich membranes from Torpedinidae to investigate the topology of sphingomyelin (SM) in the native membrane and its relationship with the AChR protein.

Controlled sphingomyelinase hydrolysis of native membranes shows that SM is predominantly (approximately 60%) localized in the outer half of the lipid bilayer. Differences can also observed in the distribution of SM fatty acid molecular species in the 2 bilayer leaflets.

Application: Characterization of membrane protein-lipid interactions.

Implementation: A fluorescent SM derivative (N-[10-(1-pyrenyl)decanoyl]sphingomyelin; Py-SM) can be used to study protein-lipid interactions in AChR-rich membrane preparations and in affinity-purified Torpedo AChR reconstituted in liposomes made from Torpedo electrocyte lipid extracts. The efficiency of Förster resonance energy transfer (FRET) from the protein to the pyrenyl-labeled lipid as a function of acceptor surface density can be used to estimate distances and topography of the SM derivative relative to the protein. The dynamics of the lipid acyl chains can be explored by measuring the thermal dependence of Py-SM excimer formation, sensitive to the fluidity of the membrane.

Interpretation and Analysis: Differences can be observed in the concentration dependence of excimer/monomer pyrenyl fluorescence when measured by direct excitation of the probe under FRET conditions, indicating differences in the intermolecular collisional frequency of the fluorophores between bulk and protein-vicinal lipid environments, respectively. Py-SM exhibits a moderate selectivity for the protein-vicinal lipid domain, with a calculated relative affinity K_r of approximately 0.55. Upon sphingomyelinase digestion of the membrane, FRET efficiency increases by about 50%, indicating that the resulting pyrenyl-ceramide species have higher affinity for the protein than the parental SM derivative.

Sphingomyelin composition and physical asymmetries in native acetylcholine receptor-rich membranes. Bonini IC, Antollini SS, Gutiérrez-Merino C, Barrantes FJ. Eur Biophys J 2002 Oct; 31 (6), pp. 417–27.

Liposomes: Cardiovascular/Atherosclerosis (Ultrasonography)/Atheroma: Regions of Fibrous Content/Atheroma: Regions of Early Phase Deposition

Antifibrinogen-Liposomes/Anti-ICAM-Liposomes

Background Information: Acoustically reflective liposomes that can be conjugated for site-specific acoustic enhancement.

Application: Selective targeting of acoustically reflective liposomes for atherosclerotic plaques *in vivo* in order to achieve ultrasound image enhancement.

Semi-Synthetic Methods: Liposomes were prepared with phospholipids and cholesterol using a dehydration/rehydration method. Antibodies can be thiolated for liposome conjugation with *N*-succinimidyl 3-(2-pyridyldithio) propionate, resulting in the creation of a thioether linkage between the protein and phospholipid group. Liposomes can also be conjugated to antifibrinogen or anti-intercellular adhesion molecule-1 (anti-ICAM-1).

Implementation: Evaluation of the ability of liposomes coupled to antibodies specific for different components of atherosclerotic plaques and thrombi to target and enhance ultrasonic images *in vivo*. Imaging of arterial plaques can be performed utilizing a 20-MHz intravascular ultrasound catheter and 7.5-MHz trans-vascular linear probe following injection of saline (reference control), unconjugated liposomes (reference control), and antibody-conjugated liposomes.

Animal Model: Swine (Yucatan miniswine model for atherosclerosis developed by crush injury of 1 carotid artery and 1 femoral artery in concert with the ingestion of a hypercholesterolemic diet).

Interpretation and Analysis: Conjugated liposomes retain their acoustically reflective properties and provide ultrasonic image enhancement of their targeted structures. Liposomes conjugated to antifibrinogen attach to thrombi and fibrous portions of atheromas, whereas liposomes conjugated to anti-ICAM-1 attach to early atheromas. Such findings demonstrate that these novel acoustic agent can provide targeting flexibility facilitated with the application of different antibodies with preparations retaining intravascular and trans-vascular acoustic properties.

Instrumentation: 20-MHz intravascular ultrasound catheter and 7.5-MHz trans-vascular linear probe.

In vivo targeting of acoustically reflective liposomes for intravascular and trans-vascular ultrasonic enhancement. Demos SM, Alkan-Onyuksel H, Kane BJ, Ramani K, Nagaraj A, Greene R, Klegerman M, McPherson DD. J Am Coll Cardiol 1999 Mar; 33 (3), pp. 867–75.

Liposomes: Cardiovascular/Blood-Pool Imaging Agent

Iohexol Liposomes

Background Information: Although soluble non-ionic iodine compounds with low systemic toxic effects have been developed for use in computed tomography (CT), they have short residence times of a few minutes or mere seconds, which is insufficient time for blood-pool imaging, even with high-speed multidetector row spiral CT. Moreover, potential renal toxic effects preclude repeated administration of these contrast agents during imaging, as well as their use in cases with compromised renal function.

Implementation: Development and evaluation of a CT contrast agent for blood-pool imaging that remains in the blood for more than 3 hours and that is relatively non-toxic to the kidneys. One candidate agent includes liposomal iohexol formulations and determination of encapsulation efficiency parameters in terms of milligrams of iodine per milliliter of lipid formulation, stability in phosphate buffer solution, and stability in human plasma *in vitro*. Complementary investigations can be devoted to assessing *in vivo* stability, residence time, and enhancement of contrast on images of various organ systems.

Animal Model: Rabbit.

Interpretation and Analysis: Formulations containing 34.8 mg of iodine per milliliter of liposomal iohexol solution retain stability in blood plasma both *in vitro* and *in vivo* postinjection into the central vascular compartment. An intravenous dose of 475 mg of iodine per kilogram of body weight produces contrast enhancement of approximately 130 Hounsfield units (HU) in the aorta and liver cortex and approximately 100 HU in the kidney cortex. Contrast enhancement can be maintained for 3 hours postinjection, and minimal clearance of the contrast agent via the kidneys can be observed. The liposomal iohexol formulation has a sufficient residence time for blood-pool imaging in a rabbit model. Future experiments with long-residence-time iohexol formulations may lead eventually to applications in cardiac imaging and in early tumor detection.

Long-residence-time nano-scale liposomal iohexol for x-ray-based blood-pool imaging. Kao CY, Hoffman EA, Beck KC, Bellamkonda RV, Annapragada AV. Acad Radiol 2003 May; 10 (5), pp. 475–83.

Liposomes: Cardiovascular Endothelial Cells

Liposomes: E-Selectin Directed/[Immunoliposomes]

Cellular/Molecular Mechanism of Action: E-selectin is expressed on the exterior surface membrane of endothelial cell populations.

Background Information: Previous *in vitro* experiments demonstrated that the targeting of immunoliposomes for vascular selectins represents a potential avenue for selective drug delivery sites of inflammation.

Route of Administration: Intravenous.
Implementation: Evaluation of the cellular uptake of targeted immunoliposomes by interleukin-1-activated human endothelial cells detected by spectroscopy and microscopic fluorescence techniques. In this manner it is possible to follow the fate of liposomes after target binding and the route and degree of liposome internalization. Analytical focus is directed at delineating the state of cell activation facilitated by modifying various liposomal parameters (e.g., sterical stabilization, type of antibody or antibody coupling strategy).
Interpretation and Analysis: In attempts to further adapt the targeting experiments to physiological conditions, it has been established that E-selectin-directed immunoliposomes can cumulatively bind to target cells under the simulated shear force conditions of capillary blood flow for up to 18 hours. The use of liposomes labeled with NBD (7-nitrobenz-2-oxa-1,3-diazole or 7-nitro-2-1,3-benzoxadiazol-4-yl) and subsequent fluorescence quenching outside cells with dithionite shows that circa 25% of the targeted immunoliposomes become internalized. According to inhibition experiments with agents that interfere with the endocytotic pathway, it has been determined that the major mechanism of liposome entry is endocytic. The entry of liposomes into cells involves, at least in part, receptor-mediated endocytosis facilitated by E-selectin, followed by liposome accumulation within endosomes and their subsequent acidification, which can be characterized through the use of pyranine spectroscopy techniques. Furthermore, microscopy has revealed that a fusion of liposomes with the cell membrane occurs, followed by a release of entrapped calcein into the cytoplasm. Such observations have provided greater insight into the behavior of E-selectin-targeted immunoliposomes and indicate that this type of immunoliposomes have great potential for use as drug carriers for intracellular drug delivery at inflammatory sites (diagnostic/therapeutic).

Investigation of the cellular uptake of E-selectin-targeted immunoliposomes by activated human endothelial cells. Kessner S, Krause A, Rothe U, Bendas G. Biochim Biophys Acta 2001 Oct 1; 1514 (2), pp. 177–90.

Liposomes: Central Nervous System (CNS) Inflammation/Macrophages/Microglial Cells/ Astrocytes
Poly(Ethylene Glycol) (PEG)-Long-Circulating Liposomes/[^{3}H]-Labeled Prednisolone Liposomes (PL)

Cellular/Molecular Mechanism of Action: Selective delivery into the CNS.
Background Information: High-dose glucocorticosteroid hormones are a mainstay of treatment during episodes of relapse in conditions of multiple sclerosis.
Application: [^{3}H]-labeled PL display selective targeting of the inflamed CNS.
Implementation: Evaluation of liposome as an ultra-high-dose delivery modality for glucocorticosteroids to the CNS using a formulation of PEG-coated long-circulating liposomes encapsulating prednisolone (PL).
Animal Model: Rat (experimental autoimmune encephalomyelitis [EAE]).
Interpretation and Analysis: [^{3}H]-labeled PL possess an ability to selectively target inflamed CNS tissues, where up to 4.5-fold higher radioactivity can be achieved compared to healthy control animals. Analysis by HPLC reveals much higher and more persistent levels of prednisolone within the spinal cord after PL, compared with an equal dose of free prednisolone.

Gold-labeled liposomes can be detected in the target tissue, mostly taken up by macrophages (Mphi), microglial cells, and astrocytes. Blood-brain barrier disruption is greatly reduced by 10 mg/kg PL, which is superior to a 5-fold higher dose of free methylprednisolone (MP). Prednisolone liposomes are also superior to MP in diminishing T cell infiltration by induction of T cell apoptosis within spinal cord. Macrophage infiltration is clearly decreased only by prednisolone liposomes. The percentage of tumor necrosis factor-alpha (TNF-α)-positive T cells or Mphi is greatly reduced by PL and by MP. No adverse effects on glial cells can be detected. A single injection of prednisolone liposomes clearly ameliorates the course of adoptive transfer EAE and EAE induced by immunization. Therefore, PL is a highly effective drug in treatment of EAE and is superior to a 5-fold higher dose of free MP, possibly by means of drug targeting. These findings may have implications for future diagnosis and therapy of autoimmune disorders such as multiple sclerosis.

Drug targeting by long-circulating liposomal glucocorticosteroids increases therapeutic efficacy in a model of multiple sclerosis. Schmidt J, Metselaar JM, Wauben MH, Toyka KV, Storm G, Gold R. Brain 2003 Aug; 126 (Pt 8), pp. 1895–904.

Liposomes: Central Nervous System (CNS)/ Macrophage Selective Depletion/Meningeal and Perivascular/Microglial Cells (Spared)
Clodronate Liposomes

Background Information: Perivascular (PVM) and meningeal (MM) macrophages form a distinct population that is resident within the CNS that selectively expresses the ED2 mature macrophage marker (rat).
Route of Administration: Clodronate liposomes (intraventricular injection).
Implementation: Elucidate the role of PVM and MM during normal functioning of the brain and pathology conditions by employing a single intraventricular injection of clodronate liposomes.
Animal Model: Rat.
Interpretation and Analysis: Intraventricular injection of clodronate liposomes completely depletes PVM and MM. Clodronate liposomes do not deplete the microglial cells. In other parts of the body, a temporal and mild depletion effect can be appreciated, which is restored within 1 week. Detailed analysis of the elimination and repopulation kinetics of the PVM and MM reveals a slow repopulation of the CNS, starting at 14 days postdepletion. Such selective depletion methods for PVM and MM can facilitate increasing the level of knowledge about their functions during normal and pathologic conditions of the CNS.

A method for the selective depletion of perivascular and meningeal macrophages in the central nervous system. Polfliet MM, Goede PH, van Kesteren-Hendrikx EM, van Rooijen N, Dijkstra CD, van den Berg TK. J Neuroimmunol 2001 Jun 1; 116 (2), pp. 188–95.

Liposomes: Central Nervous System (CNS)/ Macrophage Selective Depletion/Meningeal and Perivascular Macrophages
Mannosylated Clodronate Liposomes

Background Information: Meningeal (MM) and perivascular (PVM) macrophages constitute major populations of resident macrophages in the CNS that can be distinguished from microglial cells. So far, there is no direct evidence that demonstrates a possible role of MM and PVM in the CNS during normal or pathologic conditions.
Route of Administration: Mannosylated clodronate liposomes (intraventricular injection).
Implementation: Elucidation of the role of the MM and PVM during CNS inflammation through the application of a single intraventricular injection of mannosylated clodronate liposomes.

Interpretation and Analysis: Mannosylated clodronate liposome injection results in a complete and selective depletion of the PVM and MM from the CNS. Depletion of MM and PVM during experimental pneumococcal meningitis results in increased illness, which correlates with higher bacterial counts within the cerebrospinal fluid and blood. This is associated with a decreased in the influx of leukocytes into the cerebrospinal fluid, which occurs despite elevated production of relevant chemokines (e.g., macrophage-inflammatory protein-2) and higher expression of vascular adhesion molecules (e.g., VCAM-1). Alternatively, higher bacterial counts correlates with elevated production of local and systemic inflammatory mediators (e.g., IL-6) indicating enhanced local leukocyte and systemic immune activation, and this may explain worsening of clinical signs. Such findings show that PVM and MM play a protective role during bacterial meningitis and suggests that 1 of the primary functions of these macrophages is to facilitate the influx of leukocytes at the blood-brain barrier.

Meningeal and perivascular macrophages of the central nervous system play a protective role during bacterial meningitis. Polfliet MM, Zwijnenburg PJ, van Furth AM, van der Poll T, Döpp EA, Renardel de Lavalette C, van Kesteren-Hendrikx EM, van Rooijen N, Dijkstra CD, van den Berg TK. J Immunol 2001 Oct 15; 167 (8), pp. 4644–50.

Liposomes: Central Nervous System (CNS)/ Macrophage Selective Depletion/Meningeal and Perivascular/Microglial Cells (Spared): Encephalitis Research Model
Clodronate Liposomes

Background Information: The perivascular (PVM) and meningeal (MM) macrophages constitute a major population of resident macrophages in the CNS.

Implementation: Evaluation of the possible role of PVM and MM during CNS inflammation in conditions of experimental allergic encephalomyelitis (EAE). Investigations can be facilitated by the selective depletion of CNS PVM and MM utilizing clodronate liposomes.

Animal Model: Rat (EAE).

Interpretation and Analysis: There is a remarkable increase in the expression of the ED2 antigen on PVM and MM (already present at day 9 post-EAE induction), which precedes the onset of clinical symptoms and infiltration of leukocytes into the CNS (occurs at day 13). Therefore, the onset of EAE is accompanied by alterations of PVM and MM, and the ED2 antigen provides an early indicator/marker of pathology during episodes of CNS inflammation. Moreover, selective depletion of the ED2-positive macrophages in the CNS using clodronate liposomes results in a suppression of clinical symptoms. These observations indicate that PVM and MM play a role during the early stages of EAE development.

The role of perivascular and meningeal macrophages in experimental allergic encephalomyelitis. Polfliet MM, van de Veerdonk F, Döpp EA, van Kesteren-Hendrikx EM, van Rooijen N, Dijkstra CD, van den Berg TK. J Neuroimmunol 2002 Jan; 122 (1–2), pp. 1–8.

Liposomes/Central Nervous System (CNS)/ Monocytes and Neutrophils Delivery/CNS Inflammation/Cerebrovascular Disease
Anionic Magnetic Liposomes/Soya Lecithin/Cholesterol/Phosphatidyl Serine/[Arginine-Glycine-Aspartic Acid (RGD)] Anionic PC/Cholesterol/PS/Phopsphatidyl Ethanolamine

Cellular/Molecular Mechanism of Action: Preferential uptake by neutrophils and macrophages.

Application: Selective delivery of diagnostic and therapeutic agents into the CNS facilitated by neutrophil/macrophage-mediated delivery.

Semi-Synthetic Methods: Negatively charged magnetic liposomes can be prepared using soya lecithin (Soya PC), cholesterol, and phosphatidyl serine (PS) in a manner that can preferentially enhance phagocytosis by monocytes and neutrophils within the intravascular compartment. The PS ratio can be optimized in terms of drug and magnetite loading, *in vitro* magnetic responsiveness, and *ex vivo* monocytes/neutrophils uptake. RGD peptide can be covalently coupled to negatively charged liposomes composed of PC, cholesterol, PS, and phosphatidyl ethanolamine (PE) via carbodiimide-mediated coupling.

Implementation: *In vivo* cellular sorting studies performed under magnetic guard indicate an increase in relative count of neutrophils and monocytes. Results suggest that selective uptake of RGD-anchored magnetic liposomes by these cells imparts magnetic properties.

In the brain, high levels of diclofenac sodium can be detected. Negatively charged uncoated magnetic liposome brain levels of diclofenac are 5.95-fold compared to free drug and 7.58-fold when compared to non-magnetic formulations, while for RGD-coated magnetic liposomes this ratio is 9.1-fold compared to free drug solution, 6.62-fold compared to non-magnetic RGD-coated liposomes, and 1.5-fold when compared to uncoated magnetic liposomes. Liver uptake can be significantly enhanced (37.2% and 48.3% for uncoated and RGD-coated magnetic liposomes, respectively). Therefore, negatively charged and RGD-coated magnetic liposomes can serve as a strategy for monocytes/neutrophils-mediated delivery of pharmaceuticals to relatively inaccessible sites of inflammation (e.g., brain). Liposomes prepared in such a fashion represent a strategy for the active delivery of drugs in a manner possibly suitable for the diagnosis and selective treatment of cerebrovascular disease.

RGD-anchored magnetic liposomes for monocytes/neutrophils-mediated brain targeting. Jain S, Mishra V, Singh P, Dubey PK, Saraf DK, Vyas SP. Int J Pharm 2003 Aug 11; 261 (1–2), pp. 43–55.

Liposomes/Central Nervous System (CNS): Vascular Capillary Endothelium/Gene Transfer Model
Hemagglutination Virus of Japan (HVJ)-Liposomes/ Liposome-Encapsulated Beta-Gal Plasmid

Background Information: Bioactive proteins or peptides cannot be effectively delivered into brain capillary endothelial cells (BCECs) or brain parenchyma. Alternatively, cells referred to as MBEC are BCEC cells can be transfected with the mGDNF gene (glial cell-derived neurotrophic factor).

Route of Administration: Superselective internal carotid artery catheterization.

Implementation: Evaluation of the effectiveness of transferring the *Escherichia coli* beta-galactosidase gene (beta-gal) as a model gene into BCEC utilizing the HVJ-liposomes. HVJ-liposomes encapsulating a beta-gal plasmid can be used both *in vitro* to transfect MBEC4 cells and *in vivo* by selective administration/delivery.

Animal Model: Rat (internal carotid artery catheterization).

Interpretation and Analysis: Success of the procedure is confirmed by the (i) detection of 116 kDa beta-gal protein in transfected MBEC4 cells and in brain capillaries isolated from transfected rats; and (ii) Western blot analysis and histological staining. The enzymatic activities of beta-gal are 5- to 10-fold and 20-fold higher when beta-gal-containing liposomes without fusogenic activity (uncoated liposomes) or plasmid alone are employed *in vitro* and *in vivo*, respectively. Thus, HVJ-liposomes have demonstrated applicability

as a vector for the transfer of foreign genes into brain capillary endothelium *in vivo* (trans-luminal route).

Trans-luminal gene transfer into brain capillary endothelial cells *in vivo* with HVJ-liposomes. Jiang C, Matsuo H, Koyabu N, Ohtani H, Fujimoto H, Yonemitsu Y, Kaneda Y, Narro M, Tsuruo T, Sawada Y. J Drug Target 2002 Jun; 10 (4), pp. 345–52.

Liposomes/Central Nervous System (CNS): Vasculature of the Choriocapillaris/Choroidal Angiogram Imaging
Liposomes: Heat Sensitive/Carboxyfluorescein (Encapsulated)/[Laser Targeted Angiography]

Background Information: Preparations can be applied to obtain high-quality angiograms of the rat choriocapillaris with continuous laser-targeted angiography (LTA), for the *in vivo* assessment of choroidal vascular structures.
Application: Characterization of choroidal circulation by angiogram imaging.
Route of Administration: Heat-sensitive liposomes (intravascular).
Implementation: Evaluation of a carboxyfluorescein compound encapsulated into heat-sensitive liposomes and injected intravenously. Local release of encapsulated carboxyfluorescein is induced by applying a continuous heat beam utilizing a diode laser (810 nm) of variable power. Video angiograms are generated with excitation illumination provided by argon laser (488 and 514 nm) to observe highly selective images of the choriocapillaris.
Animal Model: Rat.
Interpretation and Analysis: Three distinct phases (filling, plateau, and draining) can be observed in fluorescent images of choriocapillaris by applying a continuous diode laser. In the plateau phase, a lobe-shaped area of choriocapillaris peripheral to the laser site can be illuminated, and this finite area does not change in size with continuous laser application to the same spot. When laser power is increased, a larger area of choriocapillaris can be illuminated in the plateau phase. The filling and draining phases demonstrate the flow patterns within choriocapillaris lobules, which fill from a central spot and drain along a peripheral ring. Choriocapillaris (rat) is divided into independent functional units and the choroidal circulation is segmentally organized under normal conditions. In LTA, the diode laser warms up a choroidal artery, and released fluorescein flows downstream to an area of choriocapillaris fed by the same artery. Laser-targeted angiography (LTA) appears to be a powerful method for analyzing *in vivo* choroidal circulation.
Instrumentation: Modified slit lamp that incorporates 2 kinds of lasers (argon and diode).

In vivo analysis of choroidal circulation by continuous laser-targeted angiography in the rat. Hirata Y, Nishiwaki H, Miura S, Ieki Y, Kiryu J, Honda Y. Invest Ophthalmol Vis Sci 2003 Jul; 44 (7), pp. 3103–9.

Liposomes/Dermal: Trans-Cutaneous Delivery Modality
Hydrogels: Cross-Linked Chitosan/(Lauric, Myristic, Palmitic, or Stearic Acids)

Application: Protected, selective delivery of diagnostic and therapeutic pharmaceutical agents.
Route of Administration: Topical delivery.
Semi-Synthetic Methods: Chitosan hydrogels can be physically cross-linked with lauric, myristic, palmitic, or stearic acid. Preservation can be achieved by freeze-drying. Propranolol hydrochloride functions as a suitable hydrophilic model drug to facilitate the design of trans-dermal delivery systems and the evaluation of such preparations. In this manner, the nature of the effect of cross-linker on drug permeation through porcine skin and the main permeation parameters (diffusion coefficient, flux, and lag time) can be established.
Interpretation and Analysis: All chitosan hydrogels can provide more trans-cutaneous permeation of propranolol hydrochloride than the corresponding solution of the commercial drug. Among the different chitosan vehicles, chitosan-laurate and chitosan-myristate hydrogels enhance lyophilized drug diffusion through the skin with respect to chitosan-palmitate and chitosan-stearate hydrogels. This can be explained by the interaction of the hydrogels with the stratum corneum, increasing the solubility of the drug within the skin.

Physically cross-linked chitosan hydrogels as topical vehicles for hydrophilic drugs. Cerchiara T, Luppi B, Bigucci F, Orienti I, Zecchi V. J Pharm Pharmacol 2002 Nov; 54 (11), pp. 1453–9.

Liposomes/Endothelial Intercellular Adhesion Molecule (ICAM)/Microvascular (Endothelial) Leukocyte Receptor Complex Expression
[Anti-ICAM]-Paramagnetic Liposomes

Application: The *in vivo* targeting of molecules expressed by vascular endothelium, while providing sufficient signal enhancement at these sites for detection by magnetic resonance imaging (MRI).
Implementation: [Anti-ICAM]-paramagnetic liposomes (ACPL) targeted to ICAM-1 administered *in vivo* for delineating endothelial leukocyte receptor upregulation in the cerebral microvasculature during conditions of autoimmune encephalitis.
Animal Model: Mouse (autoimmune encephalitis).
Interpretation and Analysis: Fluorescence microscopy analysis reveals that fluorescently-tagged ACPLs are localized within the central nervous system (CNS) microvasculature in a pattern consistent with ICAM-1 upregulation. Such findings closely correlate with immunohistochemical analyses. High resolution MRI of mouse brains *ex vivo* demonstrate that ACPL binding patterns confer significant enhancement of signal intensity (SI) as compared to control images. Preparations of ACPLs therefore appear to be a useful MRI contrast agent for visualization of specific molecules expressed on the exterior surface membrane of vascular endothelium during various disease states.
Instrumentation: Magnetic resonance imaging instrumentation.

ICAM-1 expression in autoimmune encephalitis visualized using magnetic resonance imaging. Sipkins DA, Gijbels K, Tropper FD, Bednarski M, Li KC, Steinman L. J Neuroimmunol 2000 Apr 3; 104 (1), pp. 1–9.

Liposomes/Gastrointestinal: Colitis
[^{99m}Technetium] ([^{99m}Tc])-Liposomes

Cellular/Molecular Mechanism of Action: Liposomes can protect encapsulated pharmaceutical agents from biological degradation and facilitate variable levels of selective delivery.
Application: Selective accumulation of diagnostic agents or therapeutic pharmaceuticals within inflamed regions of the colon.
Considerations: Therapeutic intervention in inflammatory bowel diseases (IBDs) is often associated with severe toxicity related to non-specific and ubiquitous interaction of drugs with organs and tissues. In order to prevent side effects from aggressive and prolonged treatment with glucocorticoids and immunosuppressive agents, preferential accumulation of these potent drugs in diseased tissue is desired.
Implementation: Evaluation of the disposition of [^{99m}Tc]-liposomes monitored by gamma camera imaging, and determination of the biodistribution of radioactivity within various organs.

Animal Model: Rat.
Interpretation and Analysis: Images reveal distinct accumulation of radioactivity in the colon within regions of colitis, while the abdomen of normal rats is conspicuously free of any visible radioactivity. Although images acquired 4 hours after [^{99m}Tc]-liposome injection are clear enough for diagnostic purposes, the real potential of liposomes for drug delivery is evident in 24-hour images, where it is evident that the major organs of liposome accumulation are dwarfed by intense colon activity in animals with colitis. On necropsy, 13.5% ± 5.48 of the activity is within the inflamed colon compared to only 0.1% in the normal colon, giving a target-to-nontarget ratio of 135. Blood-borne radioactivity is 9% ± 2.12 (colitis) and 25.7% ± 4.27 (normal), indicating that the decrease in circulating liposomes is associated with an increase in liposome accumulation in the inflammatory site. The other 2 major organs that accumulate liposomes are the spleen (10.7% normal vs 11% colitis) and liver (8% normal vs 10.1% colitis). Liposomes therefore have an innate propensity to accumulate in the sites of inflammation, which demonstrates the potential of liposomes loaded with therapeutic drugs or diagnostic agents to selectively target colitis conditions.

Accumulation of PEG-liposomes in the inflamed colon of rats: Potential for therapeutic and diagnostic targeting of inflammatory bowel diseases. Awasthi VD, Goins B, Klipper R, Phillips WT. J Drug Target 2002 Aug; 10 (5), pp. 419–27.

Liposomes/Gene Delivery/Nucleic Acid Sequences
Glycosylated Cationic Liposomes

Cationic liposomes have been considered as a potential non-viral vector for gene delivery because, unlike viral vectors, they possess a low level of immunogenicity. The gene transfer efficiency of cationic liposomes is lower than that of viral vectors, but recent advances have shown that it is possible to enhance the gene expression levels facilitated by cationic liposomes. The main problem with cationic liposomes seems to be the lack of organ or cell selectivity, because the lung has the highest level of gene expression after non-selective intravenous injection. Applying cell-specific targeting technology to liposomes would improve *in vivo* gene delivery and reduce any unexpected side effects. Both liver parenchymal and non-parenchymal cells exclusively express large numbers of high-affinity asialoglycoprotein and mannose receptors, respectively. Receptor-mediated gene delivery systems *in vivo* are able to introduce foreign DNA into specific cell types. Not only the nature of the ligands grafted to carriers but also the overall physicochemical properties of the complexes need to be optimized for effective cell-selective targeting of plasmid DNA (e.g., plasmid DNA/glycosylated cationic complexes).

Glycosylated cationic liposomes for cell-selective gene delivery. Kawakami S, Yamashita F, Nishida K, Nakamura J, Hashida M. Crit Rev Ther Drug Carrier Syst 2002; 19 (2), pp. 171–90.

Liposomes/Gene Delivery/Nucleic Acid Sequences
Cationic Liposome-Mediated Gene Delivery/Mechanisms of Intracellular Incorporation

Cellular/Molecular Mechanism of Action: Liposomes diminish biological degradation and elimination of encapsulated pharmaceuticals and biopharmaceutical agents. Cationic properties of liposomes can be used as an approach for selectively targeting liposome preparations for predetermined anatomical sites (e.g., anionic tumor microvascular structures).
Application: Cationic liposome-mediated gene delivery as an alternative approach for promoting internalization of large lipoplex particles.
Interpretation and Analysis: Significant changes in particle size and zeta potential of liposomes and their physical structures (assessed by atomic force microscopy) are created with addition of DNA sequences. These alterations appear to be dependent on the relative (±) charge ratio created. Atomic force microscopy images reveal that lipoplexes are formed from extensively fused and apparently homogeneous lipid particles encapsulating DNA sequences. Lipoplexes are internalized within cells through the endocytosis pathways. Lipoplex-cell fusion mainly occurs at the plasma membrane level; however, this lipoplex-cell membrane fusion is essential for the uptake of large particles.
Instrumentation: Atomic force microscopy.

Cationic liposome-mediated gene delivery: Biophysical study and mechanism of internalization. Almofti MR, Harashima H, Shinohara Y, Almofti A, Baba Y, Kiwada H. Arch Biochem Biophys 2003 Feb 15; 410 (2), pp. 246–53.

Liposomes/Gene Delivery Model: Nucleic Acid Sequences/Brain Capillary Endothelium
Hemagglutination Virus of Japan (HVJ) Liposomes/ Liposome-Encapsulated (Escherichia Coli Beta-Galactosidase [Beta-Gal]) Plasmid

Background Information: Bioactive proteins or peptides cannot be effectively delivered into brain capillary endothelial cells (BCECs) or brain parenchyma.
Route of Administration: Superselective internal carotid artery catheterization.
Implementation: Evaluation of the selectiveness of the *E. coli* beta-gal plasmid as a model for gene transfer into BCECs utilizing the HVJ-liposomes as a vector modality. Such preparations of HVJ-liposomes encapsulating a beta-gal plasmid can subsequently be tested *in vitro* to transfect MBEC4 cells, and also evaluated *in vivo* utilizing selective catheter administration techniques.
Animal Model: Rat (internal carotid artery selective delivery).
Interpretation and Analysis: Success of the procedure has been confirmed by the detection of 116 kDa beta-gal protein in transfected MBEC4 cells and in brain capillaries isolated from transfected rats, using Western blot analysis and histological staining. The enzymatic activities of beta-gal are 5- to 10-fold and 20-fold higher than when beta-gal-containing liposomes without fusogenic activity (uncoated liposomes) or plasmid alone are employed *in vitro* or *in vivo*, respectively. Thus, HVJ-liposomes have demonstrated applicability as a vector platform for the *in vivo* transfer of foreign genes into brain capillary endothelium (trans-luminal route).

Trans-luminal gene transfer into brain capillary endothelial cells *in vivo* with HVJ-liposomes. Jiang C, Matsuo H, Koyabu N, Ohtani H, Fujimoto H, Yonemitsu Y, Kaneda Y, Narro M, Tsuruo T, Sawada Y. J Drug Target 2002 Jun; 10 (4), pp. 345–52.

Liposomes/Gene Delivery/Nucleic Acid Sequences/ Central Nervous System (CNS)/Capillary Endothelium Cells/Brain Parenchyma Cells
Hemagglutination Virus of Japan (HVJ) Liposomes

Application: Facilitated gene delivery into the CNS in a manner that enhances expression.
Route of Administration: HVJ-liposomes (internal carotid artery).
Semi-Synthetic Methods: Evaluation of the protective effect for dopamine neurons against a retrograde 6-hydroxydopamine [6-OHDA]). The gene encoding mouse glial cell-derived neurotrophic

factor (mGDNF gene) can be transfected into brain capillary endothelial cells (BCECs) with the aim of delivering the gene product extensively into brain parenchyma by making use of BCEC secretory function. First, the mGDNF gene is transfected *in vitro* into cultured BCECs (MBEC4; mouse brain capillary endothelial cells). The amount of mGDNF protein secreted by transfected cells into the medium is about 1,500 to 3,200 pg/mg of cell protein/day, or about 7-fold higher than that accumulated intracellularly. Furthermore, the basolateral-directed secretion of mGDNF protein from the transfected MBEC4 cells is 5-fold higher than the apical-directed secretion.

Implementation: Evaluation of the capacity of the HVJ-liposomes to deliver the encapsulated mGDNF gene into CNS tissues.

Animal Model: Rat.

Interpretation and Analysis: Transfected rats show a marked increase in brain levels of GDNF as assessed by means of enzyme-linked immunosorbent assay (ELISA) and Western blotting analysis on day 3 after administration, and the level remains significantly elevated for at least 12 days. Furthermore, immunohistochemical staining reveals an increase in GDNF immunoreactivity throughout the transfected forebrain. Such profiles indicate that the gene can be successfully transferred into BCECs *in vivo* utilizing HVJ-liposomes, where it is expressed, and the gene product is secreted into the brain. Using this delivery method, the protective effect for dopamine neuron against a retrograde 6-OHDA lesion can be evaluated (assessed as a function of observed behavioral and neurochemical indices).

In vivo delivery of glial cell-derived neurotrophic factor across the blood-brain barrier by gene transfer into brain capillary endothelial cells. Jiang C, Koyabu N, Yonemitsu Y, Shimazoe T, Watanabe S, Naito M, Tsuruo T, Ohtani H, Sawada Y. Hum Gene Ther 2003 Aug 10; 14 (12), pp. 1181–91.

Liposomes/Gene Delivery/Nucleic Acid Sequences/ Central Nervous System (CNS) Tumor/Gliosarcoma

Liposomes: Cationic/Triethyl Aminopropane Carbamoyl Cholesterol (TEAPC-Chol)-Dioleoyl Phosphatidyl Ethanolamine (DOPE) Cationic Liposomes/ Poly-L-Lysine Plasma DNA Preconditioning/ Poly(Ethylene Glycol) (PEG)

Application: Delivery and transfection of genetic DNA sequences into cells within the CNS.

Semi-Synthetic Methods: Cationic liposome can be prepared from TEAPC-Chol and a helper lipid in the form of DOPE.

Implementation: Evaluation of a strategy for transfecting genes into gliosarcoma cell lines (9L) using an original cholesterol-based cationic liposome as a carrier platform.

Animal Model: Murine (nude mice with subcutaneous neoplastic lesions).

Interpretation and Analysis: Liposomes at specific concentrations are not cytotoxic based on MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cytoxic staining analysis. TEAPC-Chol/DOPE liposomes allow plasmids encoding reporter genes to enter the nucleus as validated both by electron microscopy and functionality tests using fluorescence detection of green fluorescent protein (GFP) and luminometric measurements of luciferase activity. By changing the cationic lipid/DNA molar charge ratio, optimal properties can be determined. Further, improvement of the transfection level can be obtained by either precondensing plasmid DNA with poly-L-lysine or by adding PEG in the transfection medium. The optimal conditions determined are different depending on whether transfection can be achieved within cells in culture or with tumors induced by subcutaneous injection of cells into *in vivo* animal models. For *in vivo* assays, a simple method to overcome the interference of hemoglobin with the chemiluminescence intensity of luciferase has been used. These results would be useful for gaining knowledge about the potential for the cationic liposome TEAPC-Chol/DOPE to transfect brain tumors efficiently.

Evaluation and optimization of DNA delivery into gliosarcoma 9L cells by a cholesterol-based cationic liposome. Lesage D, Cao A, Briane D, Lievre N, Coudert R, Raphael M, Salzmann J, Taillandier E. Biochim Biophys Acta 2002 Aug 31; 1564 (2), pp. 393–402.

Liposomes/Gene Delivery/Nucleic Acid Sequences/ Central Nervous System (CNS)/Hippocamus Neurons/Neuronal Nucleoli Entry

Anionic Liposomes/Liposome-Encapsulated Cy3-Labeled Oligonucleotides

Background Information: Anionic liposomes can efficiently introduce foreign DNA into postmitotic neurons and other cell types.

Application: Liposome-encapsulated Cy3-labeled oligonucleotides can be applied to deliver DNA sequences into the nucleus of CNS neurons.

Implementation: Evaluation of the mechanism of liposome uptake established through characterization of internalization processes for anionic liposome-encapsulated Cy3-labeled oligonucleotides (AL-Cy3ONs) in hippocampal neuron populations.

Interpretation and Analysis: Uptake of AL-Cy3ONs is widespread and occurs as a function of time and temperature(e.g., dependent variables), which is indicative of receptor-mediated endocytosis. The low-density lipoprotein receptor-related protein (LRP) is crucial for anionic liposome endocytosis because the receptor-associated protein or an anti-LRP antibody inhibits internalization, and fibroblasts lacking LRP do not internalize AL-Cy3ONs. Using selective endocytosis inhibitors, it can be determined that liposome endocytosis and intracellular transport require clathrin, dynamin, an intact cytoskeletal network, and phosphatidylinositol 3-kinase activity. Cy3ONs does not significantly co-localize with recycling endosomal/lysosomal markers and enter neuronal nuclei within 1 to 3 hours of incubation. Approximately 50% of the internalized liposomal phospholipids are recycled back to the cell surface, in keeping with the fluidity of their acyl chains. Liposome endocytosis does not require heparan sulfate proteoglycans or cause calcium influx into neurons. Thus, constitutive endocytosis of anionic liposomes by LRP utilizes only 1 component, in contrast to the more involved heparan sulfate proteoglycan-LRP pathway implicated in the pathogenesis of Alzheimer's disease.

Instrumentation: Confocal microscopy.

Low-density lipoprotein receptor-related protein mediates the endocytosis of anionic liposomes in neurons. Lakkaraju A, Rahman YE, Dubinsky JM. J Biol Chem 2002 Apr 26; 277 (17), pp. 15085–92.

Neurons are protected from excitotoxic death by p53 antisense oligonucleotides delivered in anionic liposomes. Lakkaraju A, Dubinsky JM, Low WC, Rahman YE. J Biol Chem 2001; 276, pp. 32000–7.

Liposomes/Gene Delivery/Nucleic Acid Sequences/ Central Nervous System (CNS)/Ischemia: Cerebral (Focal)

Liposome/Plasmid DNA Complexes/[Lipoplexes]

Background Information: Acute cerebral ischemia causes hypoxic neuronal cell death by necrosis and apoptosis. Expression of antiapoptotic transgenes in ischemic brain may provide a useful therapeutic strategy for alleviation of postischemic damage.

Route of Administration: Lipoplexes (intrathecal).

Implementation: Evaluation of the potential of liposomes to mediate transfer of human bcl-2 protein into the CNS during conditions of focal transient cerebral ischemia. Two different types of

plasmid vectors can be used for bcl-2 expression, including (i) constitutive cytomegalovirus promoter (pCMV) and the (ii) hypoxia-inducible human vascular endothelial growth factor promoter (pHRE). Cationic liposome/plasmid DNA complexes (lipoplexes) can be injected directly into the cerebrospinal fluid (e.g., rat CSF) immediately after middle cerebral artery (MCA) occlusion. The brains of treated and control *in vivo* models are then analyzed 48 hours later. Infarct volumes and numbers of apoptotic cells can then be quantified.

Animal Model: Rat (focal transient ischemia created by MCA occlusion).

Interpretation and Analysis: Occlusion of the MCA results in ipsilateral cerebral infarcts. Transfer of the bcl-2 gene results in high-level widespread protein expression in the case of the pCMV-bcl2 plasmid, while animals treated with the pHRE-bcl2 vector show lower expression levels of bcl2 that are limited to ischemic regions. Treatment with pCMV-bcl2, but not with pHRE-bcl2, is able to significantly reduce infarct volumes (109 ± 8 mm^3 for pCMV-bcl2; 152 ± 29 mm^3 for pHRE-bcl2; and 155 ± 18 mm^3 for controls). The transfection with either of the 2 vectors results in significant reductions in apoptotic cell numbers within infarcts and penumbra areas compared to controls. There are no short-term neurological side effects from CSF lipoplex injection or bcl-2 expression. Hypoxia-inducible bcl-2 expression mediated by intrathecal lipoplexes may represent a novel, biologically safe, and lesion-selective therapeutic approach for neuroprotection after acute cerebral ischemia.

Liposome-mediated transfer of the bcl-2 gene results in neuroprotection after *in vivo* transient focal cerebral ischemia in an animal model. Cao YJ, Shibata T, Rainov NG. Gene Ther 2002 Mar; 9 (6), pp. 415–9.

Liposomes/Gene Delivery/Nucleic Acid Sequences/Hepatic Sinusoidal Gene Delivery/Antisense Oligonucleotide/[*In Vivo* Genetic Messenger RNA (mRNA) Expression]

Albumin-Coated Cationic Lipoplexes (CCLs)/Anti-ICAM-1/Antisense Oligonucleotides (ODN)/Cationic Lipid (DOTAP)/Poly(Ethylene Glycol) (PEG)/Aconitylated Albumin (AcoHSA)/Anti-ICAM-1-ODN-[DOTAP]-PEG-Neutral Lipid (Aco-HAS)

Application: Massive uptake of liposomes that have been surface modified with negatively charged AcoHSA by liver sinusoidal endothelial cells (EC) *in vivo* has previously been reported for targeting various cell types.

Route of Administration: Albumin-CCLs (intravascular).

Semi-Synthetic Methods: Anti ICAM-1 ODN can be complexed with the cationic lipid DOTAP and the complex coated with an excess of neutral lipids including a lipid-anchored PEG. Aco-HSA is coupled to the CCLs.

Implementation: Plasma disappearance and organ and intrahepatic distribution of Aco-HSA modified CCLs can be determined using [^{3}H]-cholesteryl oleyl ether and [^{32}P]-labeled ODN as markers.

Animal Model: Rat.

Interpretation and Analysis: Aco-HSA coupled CCLs are $<$ 160 nm in size and contain 1.03 ± 0.35 nmol ODN and 54 ± 18 μg Aco-HSA per micromol total lipid. Such CCLs are rapidly eliminated from plasma with about 60% the injected dose of [^{3}H]- or [^{32}P]-label entering the liver after 30 minutes. Within the liver, the EC account for two-thirds of total liver uptake. Control non-targeted CCLs are eliminated very slowly because after 30 minutes, $>$ 90% of the particles are still present in the blood. Laboratory findings have demonstrated efficient targeting of antisense ODN to EC *in vivo*, employing plasma-stable CCLs, surface modified with negatively charged albumin. Over a period of 30 minutes, approximately 40% of injected ODN is delivered to predetermined "target cell" populations.

Massive and selective delivery of lipid-coated cationic lipoplexes of oligonucleotides targeted *in vivo* to hepatic endothelial cells. Bartsch M, Weeke-Klimp AH, Meijer DK, Scherphof GL, Kamps JA. Pharm Res 2002 May; 19 (5), pp. 676–80.

Liposomes/Gene Delivery and Expression/Neoplastic Disease/Lewis Lung Carcinoma Model/Fibroblasts (NIH-3T3)/Cardiomyocytes (H9C2)/Nucleic Acid Sequences/*Ex Vivo* and *In Vivo* Cell Transfection/Gene Transfer and Detection/pEGFP-N1 Plasmid Encoding Green Fluorescent Protein (GFP)

Liposomes (TAT-Modified) Complexed with DNA Sequences

Application: Preparations of TATp-liposome-DNA complexes can be utilized in protocols for *in vitro* and *in vivo* transfection techniques. Such procedures have utility as a form of *in vitro* or *ex vivo* cell treatment schemes as well as for facilitating localized *in vivo* gene therapy.

Laboratory Methodologies: Mouse NIH/3T3 fibroblasts and rat H9C2 cardiomyocytes can be transfected *in vitro* with TATp-liposome-DNA complexes. Both *in vitro* and *in vivo* transfection with TATp-liposome-DNA complexes is possible.

Interpretation and Analysis: Liposomes modified with TAT peptide (TATp-liposomes) show fast and efficient translocation into the cell cytoplasm with subsequent migration into the perinuclear zone. TATp-liposomes containing a small quantity ($\leq$ 10 mol %) of a cationic lipid form "firm" non-covalent complexes with DNA.

Transfection with the TATp-liposome-associated pEGFP-N1 plasmid encoding for GFP is high, whereas the cytotoxicity is lower than that of commonly used cationic lipid-based gene-delivery systems. Intratumoral injection of TATp-liposome-DNA complexes into the Lewis lung carcinoma tumor of mice also results in the expression of GFP within tumor cells.

Cell transfection *in vitro* and *in vivo* with non-toxic TAT peptide-liposome-DNA complexes. Torchilin VP, Levchenko TS, Rammohan R, Volodina N, Papahadjopoulos-Sternberg B, D'Souza GG. Proc Natl Acad Sci U S A 2003 Feb 18; 100 (4), pp. 1972–7. Epub 2003 Feb 05.

Liposomes/Gene Delivery/Nucleic Acid Sequences/Neoplastic Disease/Melanoma/Vascular Endothelium of Tumors/Melanocyte-Specific Tyrosinase Promoter/$\alpha_V\beta_3$-Integrins

Liposomes: Artificial Retroviral Envelope Structure Mimics

Semi-Synthetic Methods: A semi-synthetic liposome can be designed to function in a vectorlike fashion, in part due to formulation of its composition so that it mimics the properties of retroviral envelope structures (serum-resistant, non-toxic, $\leq$ 200 nm in size). The DNA is condensed using low molecular weight branched polyethylenimine (PEI).

Interpretation and Analysis: Addition of a cyclic arginine-glycine-aspartic acid (RGD) peptide ligand to these particles as a targeting device (i) renders them selective for tumor endothelial and melanoma cells that express high levels of $\alpha_V\beta_3$-integrins; and (ii) allows for an efficient delivery of the enclosed genetic material. The

specificity of the vector system for melanoma cells theoretically can be further improved by using a melanocyte-specific tyrosinase promoter to drive trans-gene expression.

A new colloidal lipidic system for gene therapy. Fahr A, Muller K, Nahde T, Muller R, Brusselbach S. J Liposome Res 2002 Feb–May; 12 (1–2), pp. 37–44.

Liposomes/Gene Delivery/Nucleic Acid Sequences/ Neoplastic Disease/Ovarian Carcinoma Plasmid Transfection of a (Mammalian Cell Type)/Non-Viral Vector Platform/Green Phosphorous Protein/DDC/ DNA Complex

Cationic DOTAP:DOPE:Cholin Liposomes

Application: Various strategies have been attempted to design efficient protocols for ovarian cancer gene therapy, but there has been little progress toward their clinical application.

Semi-Synthetic Methods: Preparations of DDC liposome are produced by mixing DOTAP:DOPE:Cholin at a 1:0.7:0.3 molar ratio using the extrusion method.

Implementation: Evaluation of cationic liposomes prepared with dioleoyltrimethylaminopropane (DOTAP), 1,2-dioleoyl-3-phosphophatidylethanolamine (DOPE), and cholesterol (Chol) DDC for plasmid DNA transfer into ovarian cancer cells. Plasmid DNA (pEGFP-C1) and DDC are complexed at various weight ratios to find the optimum conditions followed by determining the percentage of transfected cells created. Detection can be appreciated through the application of a green fluorescence protein (GFP) expressing cell line in combination with flow cytometry analysis. The transfection efficiency of the DDC liposome is compared with 3[*N*-(*N*,*N*-dimethylaminoethylene) carbamoyl] cholesterol (DC-Chol)/DOPE liposome and commercially available lifopectin.

Interpretation and Analysis: The optimal transfection of plasmid DNA can be achieved at a 1:4 (w/w) ratio of DDC to DNA. The DDC/DNA complex exhibits higher transfection efficiency in human ovarian cancer cells (OVCAR-3 and SK-OV-3 cells) compared to that observed in other types of cell lines (NCI-NIH:522 and HepG2 cells). Flow cytometric analysis reveals that the DDC/DNA complex exhibits an over 4-fold increase in GFP expression compared with DC-Chol/DOPE or lipofectin in OVCAR-3 cells. Such findings can be further confirmed by confocal microscopy and reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. In summary, cationic liposome (DDC) appears to be a promising non-viral vector for treating ovarian adenocarcinoma because of its high level of selective gene transfer capabilities in ovarian cancer cells.

Instrumentation: Flow cytometry.

Non-viral vector for efficient gene transfer to human ovarian adenocarcinoma cells. Kim CK, Haider KhH, Choi SH, Choi EJ, Ahn WS, Kim YB. Gynecol Oncol 2002 Jan; 84 (1), pp. 85–93.

Liposomes/Gene Delivery/Nucleic Acid Sequences/ Neoplastic Disease/Solid Tumor Masses

Liposome: Cationic (100–200 nm)/Porphyrin Derivatives (< 1 nm)/Branched Chain Dendrimers (10 nm)/ Polyethylenimine Polymers (10 nm)/Nanoparticles (20–1,000 nm)/Microspheres (> 1 μm)/ Cyclodextrins (CyDs 1.5 nm)

Background Information: The vascular network of a tumor provides the most effective route by which neoplastic cells may be reached and eradicated with drugs. The fact that a tumor's vasculature is relatively more permeable than healthy host tissue should enable selective delivery of drugs to tumor tissue. Such delivery is relevant to carrier-mediated delivery of gene therapy to tumors. Cationic liposomes have been used for the introduction of nucleic acids into mammalian cells.

Application: Nucleic acid delivery into cells (*in vitro* and *in vivo*).

Semi-Synthetic Methods: Cationic liposomes are generally 100–200 nm in diameter, whereas CyDs typically span 1.5 nm across.

Implementation: Evaluate the potential for delivering therapeutic oligonucleotides (ONs) to tumors using cationic liposomes and CyDs, in addition to assessing the major hindrances posed by the tumor itself on such forms of delivery.

Interpretation and Analysis: CyD molecules are routinely used as agents that engender cholesterol efflux from lipid-laden cells, thus having an efficacious potential in the management of atherosclerosis. A recent trend is to employ these oligosaccharide molecules for both *in vitro* and *in vivo* delivery of nucleic acids to cell populations. Comparisons have been made with other oligonucleotide delivery agents, such as porphyrin derivatives (< 1 nm), branched chain dendrimers (approximately 10 nm), polyethylenimine polymers (approximately 10 nm), nanoparticles (20–1,000 nm), and microspheres (> 1 μm), in the context of delivery to solid tumors. The chemical and physical properties of these carriers and how they may affect the uptake of oligonucleotides into cells, particularly *in vivo,* have been described.

Vehicles for oligonucleotide delivery to tumors. Dass CR. J Pharm Pharmacol 2002 Jan; 54 (1), pp. 3–27.

Liposomes/Gene Delivery/Nucleic Acid Sequences/ Neoplastic Disease/Transferrin Receptors

Transferrin-Poly(Ethylene Glycol) (PEG)-Polyethylenimine/TNF-cDNA

Application: Selective delivery of specific cDNA sequences to "target" cell populations.

Route of Administration: Intravenous.

Considerations: Tumor-targeting DNA complexes that can readily be generated by mixing stable, freeze-thawed preparations would be advantageous for diagnostic/therapeutic applications.

Semi-Synthetic Methods: Complexes can be formulated by mixing plasmid DNA, linear polyethylenimine (PEI22, 22 kDa as the main DNA condensing agent), PEG-PEI (PEG-conjugated PEI for surface shielding), and Tf-PEG-PEI (transferrin-PEG-PEI) to provide a ligand for receptor-mediated cell uptake.

Within the shielding conjugates, PEG chains of varying size (5, 20, or 40 kDa) are conjugated with either linear PEI22 (22 kDa) or branched PEI25 (25 kDa). The 3 polymer components are mixed together at various ratios with DNA; particle size, surface charge, and then the *in vitro* transfection activity, and systemic gene delivery to tumors is delineated.

Interpretation and Analysis: The particle size or surface charge of the complexes containing the PEG-PEI conjugate does not significantly change after freeze-thawing, while complexes without the shielding conjugate aggregate.

In general, increasing the proportion of shielding conjugate in the complex reduces surface charge, particle size, and *in vitro* transfection efficiency in transferrin receptor-rich K562 cells. Complexes containing PEG-PEI conjugate efficiently transfect K562 cells after freeze-thawing. Furthermore, the systemic application of freeze-thawed complexes exhibits *in vivo* tumor targeted expression. For complexes containing the luciferase reporter gene the highest expression is found in tumor tissue of mice. An optimum formulation for

in vivo application, PEI22/Tf-PEG-PEI/PEI22-PEG5, containing plasmid DNA encoding for the tumor necrosis factor-alpha (TNF-α), can inhibit tumor growth in 3 different murine tumor models. These new DNA complexes offer simplicity and convenience, with tumor targeting activity *in vivo* after freeze-thawing.

Novel shielded transferrin-polyethylene glycol-polyethylenimine/DNA complexes for systemic tumor-targeted gene transfer. Kursa M, Walker GF, Roessler V, Ogris M, Roedl W, Kircheis R, Wagner E. Bioconjug Chem 2003 Jan–Feb; 14 (1), pp. 222–31.

Liposomes/Gene Delivery/Nucleic Acid Sequences/ Pulmonary/Cystic Fibrosis (CF) Trans-Membrane Conductance Regulator (CFTR) Gene *(CFTR-DNA)-DC-Chol/DOPE Cationic Liposomes/ Genetic DNA Sequences*

Background Information: The major cause of mortality in patients with CF is lung disease. Expression of the CFTR gene product in the airways is a potential avenue for treatment. Clinical studies in which the CFTR cDNA is delivered to the respiratory epithelia of CF patients have resulted in modest, transient gene expression. It seems likely that repeated administration of the gene transfer vector is required in order to achieve long-term gene expression.
Application: Delivery of genetic DNA sequences (*in vitro* and *in vivo*) into cell populations to induce gene expression.
Route of Administration: DNA/liposome formulation (variable but primarily intravascular for clinical purposes).
Implementation: Evaluation of multiple doses of a DNA/liposome formulation to determine its ability to serve as a delivery platform into nasal epithelium (double blinded study). Complementary *in vivo* investigations can employ the administration of plasmid DNA expressing the CFTR cDNA complexed with DC-Chol/DOPE cationic liposomes (e.g., n = 10; n = 2 placebo controls). Each case receives 3 doses, administered 4 weeks apart.

Nasal epithelial cells can be collected 4 days after each dose for a series of efficacy assays, including quantitation of vector-specific DNA and mRNA, immunohistochemistry of CFTR protein, bacterial adherence, and detection of halide efflux *ex vivo*. Airway ion transport can also be assessed *in vivo* by repeated nasal potential difference (PD) measurements.

***Ex Vivo* Model:** Nasal epithelium harvested from cases of CF.
Human Model: Cases of CF.
Interpretation and Analysis: There is no evidence of inflammation, toxicity, or an immune response toward the DNA/liposomes or the expressed CFTR gene. On average, over half of treated subjects are positive for CFTR gene transfer after each dose (e.g., n = 6/10). All subjects positive for CFTR function are also positive for plasmid DNA, plasmid-derived mRNA, and CFTR protein.

Repeat administration of DNA/liposomes to the nasal epithelium of patients with cystic fibrosis. Hyde SC, Southern KW, Gileadi U, Fitzjohn EM, Mofford KA, Waddell BE, Gooi HC, Goddard CA, Hannavy K, Smyth SE, Egan JJ, Sorgi FL, Huang L, Cuthbert AW, Evans MJ, Colledge WH, Higgins CF, Webb AK, Gill DR. Gene Ther 2000 Jul; 7 (13), pp. 1156–65.

Liposomes/Gene Transfer/Nucleic Acid Sequences/ Neoplastic Disease/Ovarian Cancer *O-Chol:DNA Lipoplex (Catioinic)/3-β-[l-Ornithinamide-Carbamoyl] Cholesterol/Luciferase and β-Galactosidase Reporter Genes*

Background Information: Synthesis of cationic liposomes that displays high efficiency and low toxicity in the form of 3 β[l-ornithinamide-carbamoyl] cholesterol (O-Chol) using solid-phase synthesis techniques has been described previously.
Application: The preparation O-Chol can function as a highly effective trans-fection reagent. Nucleotide sequences encoding for luciferase and β-galactosidase are effective reporter genes.
Implementation: Evaluation of O-Chol to facilitate gene delivery applying *in vitro* and *in vivo* models for ovarian cancer. Intraperitoneal gene delivery into peritoneal disseminated ovarian cancer populations can be assessed using a stable chloramphenicol acetyl transferase (CAT)-expressing ovarian cancer cell line (OV-CA-2774/CAT), which allows quantification of the exact tumor burden of organs.

***Ex Vivo* Model:** Stable ovarian carcinoma cell type expressing CAT in cell line OV-CA-2774/CAT.

Animal Model: Murine (nude mice). An intraperitoneal route of administration can be applied *in vivo* to create disseminated populations of ovarian carcinoma within the peritoneal cavity.
Interpretation and Analysis: Applying luciferase and β-galactosidase genes as reporter genes, O-Chol shows better efficiency than other commercial transfection reagents such as lipofectin, lipofect AMINE, DC-Chol, and FuGENE 6, in both *in vitro* and *in vivo* models. Moreover, the transfection efficiency of the cationic lipid reagent remains high in serum-containing medium and under serum-free conditions. Furthermore, *in vivo* transfection with O-Chol shows high levels of gene expression specific for peritoneal tumor cells. Consequently, O-Chol:DNA lipoplexes appear to offer several potential advantages over other commercial transfection reagents because of its (i) higher level of gene expression *in vitro* and *in vivo*; (ii) reduced susceptibility to serum inhibition; and (iii) highly selective transfection into tumor cells. Therefore, O-Chol:DNA lipoplex is a promising tool in gene therapy for conditions of peritoneal disseminated ovarian cancer.

Intraperitoneal gene delivery mediated by a novel cationic liposome in a peritoneal disseminated ovarian cancer model. Lee MJ, Cho SS, You JR, Lee Y, Kang BD, Choi JS, Park JW, Suh YL, Kim JA, Kim DK, Park JS. Gene Ther 2002 Jul; 9 (13), pp. 859–66.

Liposomes/Glucose Binding Avidity *Di-Branched Amino Acid Derivative Liposome/ Dihexadecyl-Glutamate-Glutamine/ Dihexadecyl-Glutamate-Asparagine/Dihexadecyl-Glutamate-Glutamate*

Cellular/Molecular Mechanism of Action: Di-branched amino acids possess binding avidity for glucose moieties.
Application: Detection and physical binding to glucose moieties, including glycosylated moieties of cell receptor complexes.
Semi-Synthetic Methods: Di-branched amino acid derivatives, such as dihexadecyl-glutamate-glutamine (DHD-glu-gln), dihexadecyl-glutamate-asparagine (DHD-glu-asn), and dihexadecyl-glutamate-glutamic acid (DHD-glu-glu), can be synthesized and then incorporated into lipid vesicles (liposomes) using dipalmitoylphosphatidylcholine (DPPC). To form binding sites for glucose, the liposomes containing amino acid derivatives are mixed with glucose above the phase transition temperature (PTT) of DPPC, and subsequently the temperature is lowered below the PTT.
Implementation: Evaluation of liposomes with amino acid derivatives possessing glucose-binding affinity (± glucose imprinting properties) incorporated into their membrane layer utilizing surface plasmon resonance (SPR) and equilibrium dialysis techniques.
Interpretation and Analysis: Surface plasmon resonance of liposomes containing individual amino acid derivative or a combination of all 3 amino acid derivatives reveals that only liposomes con-

taining all 3 amino acid derivatives have glucose-binding affinity. The glucose-imprinting process is essential to fix the amino acid derivatives into a glucose binding site on liposomes. Equilibrium dialysis studies of glucose-imprinted liposomes produces curvilinear Scatchard plots, indicating that the amino acid derivatives play a direct role in glucose binding. Therefore, synthesized di-branched amino acid derivatives are promising agents for the development of biocompatible synthetic glucose binding materials.

Preparation of liposomes with glucose binding sites: Liposomes containing di-branched amino acid derivatives. Seong H, Choi WM, Kim JC, Thompson DH, Park K. Biomaterials 2003 Nov; 24 (24), pp. 4487–93.

Liposomes/Hematology: Platelets (Activated)/ Platelet GPIIb-IIIa Receptor Complexes *[Arginine-Glycine-Aspartate]-Oligodextran-Liposome*

Background Information: Cardiovascular disease processes such as atherosclerosis, restenosis, and inflammation are typically localized within discrete regions of the vasculature, which affords a great opportunity for developing targeted diagnostic and therapeutic pharmaceutical agents. Liposomes are potentially advantageous targeted drug carriers for such intravascular applications
Route of Administration: [Arginine-glycine-aspartate]-oligodextran-liposome (intravascular).
Implementation: Evaluation of 3 potential components of liposome design that can function as delivery vehicles, including (i) candidate cell surface receptors for targeting; (ii) ligands that maintain binding specificity and affinity; and (iii) prevention of rapid nonspecific clearance of liposomes by the reticuloendothelial organs.
Interpretation and Analysis: Surface modifications can simultaneously influence both the clearance/longevity and targeting specificity of liposome preparations. In this context, peptides can be applied to guide liposomes to desired receptor complexes and influence *in vivo* liposome stability. One example is the use of arginine-glycine-aspartic acid (RGD) containing peptides as a model ligand for targeting liposomes to integrin GPIIb-IIIa complexes expressed on the exterior surface membrane of activated platelets. Additionally, oligodextran surfactants incorporated into liposomes can provide insight into the effect of vesicle perturbations on liposome clearance and the importance of molecular geometry in designing oligosaccharide surface modifications.

Surface modification of liposomes for selective cell targeting in cardiovascular drug delivery. Lestini BJ, Sagnella SM, Xu Z, Shive MS, Richter NJ, Jayaseharan J, Case AJ, Kottke-Marchant K, Anderson JM, Marchant RE. J Control Release 2002 Jan 17; 78 (1–3), pp. 235–47.

Liposomes/Hepatic Kupffer Cells (Macrophages): Selective Delivery/Hepatic Trans-Gene Expression/ Antigen Processing Inhibition: Enhanced Adenoviral Vector Delivery *Clodronate Liposomes/High-Capacity Adenoviral (HC-Ad) Vectors*

Background Information: Tissue macrophages, in particular hepatic Kupffer cells (KCs), contribute to early inflammatory responses following adenoviral vector administration.
Implementation: Evaluate the effect of selective and transient (3 days) depletion of hepatic KCs by a single injection of clodronate liposomes on the *in vivo* activity of HC-Ad vectors.
Interpretation and Analysis: Interestingly, in the majority of animals of both strains, the depletion of KCs is sufficient to prevent the generation of anti-hAAT antibodies, resulting in prolonged transgene expression. Thus, short-term and selective depletion of hepatic macrophages at the same time significantly increases hepatic transgene expression and reduces the humoral immune response to transgenic protein.

Selective depletion or blockade of Kupffer cells leads to enhanced and prolonged hepatic transgene expression using high-capacity adenoviral vectors. Schiedner G, Hertel S, Johnston M, Dries V, van Rooijen N, Kochanek S. Mol Ther 2003 Jan; 7 (1), pp. 35–43.

Liposomes/Hepatic Parenchymal Cells/ Non-Parenchymal Hepatic Cell Assimilation/ Manipulation of Hepatic Targeting Strategies *Liposomes: Distearoyl-L-Phosphatidylcholine/DSPC/ Chol/Gal-C4-Chol (60:35:5) (Gal)/DSPC/Chol/ Gal-C4-Chol/Tween 20/DSPC/Chol/Gal-C4-Chol/ PEG_{2000}-DSPE (59:35:5:1) (PEG_{2000}-Gal)/DSPC/Chol/ Gal-C4-Chol/PEG_{350}-DSPE (59:35:5:1)/(PEG_{350}-Gal)*

Application: Control the delivery rate of liposome-encapsulated diagnostic and therapeutic pharmaceutical agents to hepatic parenchyma.
Route of Administration: Modified liposomes (intravascular).
Semi-Synthetic Methods: Modification of DSPC/Chol (60:40) liposomes with a galactosylated cholesterol derivative (Gal-C4-Chol) and polysorbate (Tween-20 or 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-polyethylene glycol (PEG(x)-DSPE).
Implementation: Evaluate the capacity of PEG_{350}-DSPE to control the targeted and sustained delivery rate of Gal-liposomes to liver parenchymal cells without losing targeting capabilities.
Interpretation and Analysis: Following intravenous injection, DSPC/Chol/Gal-C4-Chol (60:35:5) (Gal) liposomes are rapidly eliminated from the blood circulation and are mostly recovered from the liver. The blood elimination of DSPC/Chol/Gal-C4-Chol/Tween 20 (55:35:5:5) (Tween 20-Gal) liposomes is slightly reduced compared to Gal-liposomes. Alternatively, a significant reduction in the blood elimination can be observed with DSPC/Chol/Gal-C4-Chol/ PEG_{2000}-DSPE (59:35:5:1) (PEG_{2000}-Gal) liposomes. Hepatic uptake of DSPC/Chol/Gal-C4-Chol/PEG_{350}-DSPE (59:35:5:1) (PEG_{350}-Gal) liposomes is intermediate between PEG_{2000}-Gal-liposomes and Tween 20-Gal-liposomes. The uptake of PEG_{350}-Gal-liposomes by liver parenchymal cells is 7.7-fold higher than that by non-parenchymal cells. Such findings imply that PEG_{350}-DSPE can control the delivery rate of Gal-liposomes to liver parenchymal cells without losing their targeting capabilities.

Targeted and sustained drug delivery using PEGylated galactosylated liposomes. Managit C, Kawakami S, Nishikawa M, Yamashita F, Hashida M. Int J Pharm 2003 Nov 6; 266 (1–2), pp. 77–84.

Liposomes/Immunity: Antigen Delivery/(IgE Selective Unresponsiveness) *OVA-Liposome Conjugate Pretreatment*

Background Information: Previous investigations have reported the capacity of intraperitoneal injections of OVA-liposome conjugates to induce OVA-specific and IgE-selective unresponsiveness in mice.
Application: Antigen-liposome conjugates can potentially be administered orally to control antigen-specific IgE antibody production, without affecting IgG antibody production.
Implementation: Evaluate the effects of oral pretreatment with OVA-liposome conjugates compared to plain OVA solution on anti-OVA IgE antibody production.

Animal Model: Murine (mice immunized with alum-adsorbed OVA; control mice receive only the immunization).
Interpretation and Analysis: Serum levels of anti-OVA IgG antibody following oral administration of OVA-liposome are comparable to those observed in reference controls. However, with the oral administration of the same dose of plain OVA, levels of serum anti-OVA IgE antibody are significantly lower than those in control mice. Surprisingly, anti-OVA IgE antibody production is completely inhibited with the oral administration of OVA-liposome conjugates. Splenic $CD4^+$ T cells of mice receiving orally administered OVA-liposome and those of reference controls result in the production of comparable levels of cytokines, while those of mice receiving oral plain OVA solution produce significantly lower levels of cytokines than those in the other 2 groups. Orally administered OVA-liposomes do not affect anti-OVA IgG production but do not inhibit anti-OVA IgE antibody production, while orally administered OVA solution inhibits production of both IgG and IgE antibodies.

Selective inhibition of systemic anti-OVA IgE production in response to oral pretreatment with OVA-liposome conjugates. Naito S, Taneichi M, Kato H, Tanaka Y, Ami Y, Suzaki Y, Mori M, Nakano Y, Yamamura H, Morokuma K, Ohkuma K, Miyake H, Kiniwa M, Komuro K, Uchida T. Int Arch Allergy Immunol 2002 Dec; 129 (4), pp. 314–9.

Liposomes/Immunity: Antigen Delivery to Dendritic Cells/Balanced $CD8^+$ and $CD4^+$ T Cell Responses
Sterically Stabilized Liposomes

Cellular/Molecular Mechanism of Action: Liposomes function as a modality that can delay the metabolism and elimination of pharmaceuticals, in addition to protecting innocent organ systems from unwanted exposure and promoting or facilitating selective delivery.
Background Information: Liposomes have been proposed as a potential vehicle for delivering proteins to antigen-presenting cells (APC) including dendritic cells (DC) in a manner that can stimulate strong T cell–mediated immune responses. Unfortunately, because of their *in vivo* instability and their rapid uptake by cells of the mononuclear phagocyte system on intravenous administration, most types of conventional liposomes lack clinical applicability.
Application: Selective delivery to APC types, including DC.
Route of Administration: Intravascular.
Animal Model: Murine
Interpretation and Analysis: Sterically stabilized liposomes (SL) have increased *in vivo* stability. Both immature and mature dendritic cells take up sterically stabilized liposomes into neutral or mildly acidic compartments distinct from endocytic vacuoles. These DC present SL-encapsulated protein to both $CD4^+$ and $CD8^+$ T cells (*in vitro*). Although $CD4^+$ T cell responses were comparable to those induced by soluble protein, $CD8^+$ T cell proliferation is up to 300-fold stronger when dendritic cells are pulsed with SL containing encapsulated ovalbumin. Dendritic cells process SL with encapsulated antigen through a TAP-dependent mechanism. Immunization with SL with encapsulated ovalbumin facilitates antigen presentation by DC *in vivo* and stimulates greater $CD8^+$ T cell responses than immunization with soluble protein or with conventional or positively charged liposomes carrying ovalbumin. Application of SL with encapsulated antigens offers a novel, effective, safe vaccination approach if a combination of $CD8^+$ and $CD4^+$ T cell responses is desired (i.e., in antiviral or antitumor immunity).

Presentation of proteins encapsulated in sterically stabilized liposomes by dendritic cells initiates $CD8^+$ T cell responses *in vivo*. Ignatius R, Mahnke K, Rivera M, Hong K, Isdell F, Steinman RM, Pope M, Stamatatos L. Blood 2000 Nov 15; 96 (10), pp. 3505–13.

Liposomes/Immunity: Antigen Delivery to Dendritic Cells/Lymph Node Antigen Processing
[Fluorescein Isothiocyanate (FITC)]-H-2K_b CTL Epitope (SIINFEKL)/pH-Sensitive Liposomes

Cellular/Molecular Mechanism of Action: Fluorescein isothiocyanate emits fluorescent light when excited by certain wavelengths of light energy. Liposomes that are pH sensitive provide a modality for protecting their internal contents from metabolic degradation or accelerated elimination but also have the property of degrading upon exposure to environments within a specific hydrogen ion concentration.
Background Information: Cytotoxic lymphocytes (CTL)
Application: Fluorescein isothiocyanate can be applied as a fluorescent tracer molecule in biological systems. Fluorescein isothiocyanate-conjugated H-$2K_b$ CTL epitope (SIINFEKL) liposomes that have pH-sensitive properties have been designed so that they release their internal contents through mechanisms of lysis when exposed to environments within a specific pH range.
Interpretation and Analysis: Fluorescence can initially be detected in lymph nodes 3 hours after immunization, and its intensity reaches a peak level in superficial inguinal lymph nodes at 9 hours. No trace amounts can be detected in the spleen, even with prolonged monitoring for up to 24 hours. These results strongly suggest that the presentation of CTL-peptide antigen delivered by pH-sensitive liposomes exclusively occurs in lymph nodes. In mice immunized with the H-$2K_b$ CTL epitope–encapsulated pH-sensitive liposomes, peptide-specific CTL response can be detected at day 3. The response is strongly augmented by a 2nd immunization and persists up to at least 45 days. Therefore, liposomes formulated to be pH-sensitive can function as potential adjuvants of peptide antigens and are useful for the *in vivo* induction of antigen-specific CTLsv.

Investigation of antigen delivery route *in vivo* and immune-boosting effects mediated by pH-sensitive liposomes encapsulated with K_b-restricted CTL epitope. Lee KY, Chun E, Seong BL. Biochem Biophys Res Commun 2002 Apr 5; 292 (3), pp. 682–8.

Liposomes: Immunity/Macrophages: Mannosyl-Fucosyl Receptors
Mannosylated Liposomes

Background Information: Despite the rapid development in medicinal and pharmaceutical technology, the targeting of drugs to phagocytic cells in macrophage-related disease states still remains a major unsolved problem.
Application: Selective liposome-mediated delivery to macrophage populations.
Route of Administration: Mannosylated liposomes (intravascular).
Implementation: Evaluation of liposome modalities for their ability to selectively target mannosyl-fucosyl receptors on macrophages as a potential model for targeting antileishmanial pharmaceuticals encapsulated within mannosylated or fucosylated liposomes to treat leishmaniasis.

One approach for achieving such an objective can involve loading liposomes with an indigenous drug, andrographolide, a labdane diterpenoid isolated from the Indian medicinal plant *Andrographis paniculata*, which can be prepared and tested against experimental leishmaniasis.

Animal Model: Hamster (experimental leishmaniasis model).
Interpretation and Analysis: Mannosylated liposomes are relatively efficient in delivering antileishmanial drugs to phagocytic cells. Mannosylated liposomes loaded with the drug are found to be

most potent in reducing the parasitic burden within the spleen as well as in reducing the hepatic and renal toxicity. In addition, mannosylated drug-loaded liposome-treated *in vivo* models show a normal blood picture and splenic tissue histoarchitecture when compared with those treated with free drug or regular liposomal drug preparations. Such a drug-vehicle formulation may have considerable applicability for clinical environments.

Targeting of liposomal andrographolide to L. donovani-infected macrophages *in vivo*. Sinha J, Mukhopadhyay S, Das N, Basu MK. Drug Deliv 2000 Oct–Dec; 7 (4), pp. 209–13.

Liposomes: Immunity/Macrophages: Phagocytic Clearance Capacity and Enhancement/ [Altered Phagocytic Activity in Neoplasia] *[^{99m}Technetium] ([^{99m}Tc])-Liposomes/ Methoxypoly(Ethylene Glycol)2000/Poloxamine-908*

Background Information: Intravenous injection of an endotoxin-free solution of poloxamine-908 can enhance the phagocytic clearance capacity of tissue macrophages, particularly those of the liver and the spleen.
Semi-Synthetic Methods: Methoxypoly(ethylene glycol)2000 liposomes (mean size of 87 nm) can be labeled with [^{99m}Tc] utilizing the *N*-hydroxysuccinimidyl hydrazine nicotinate hydrochloride derivative of distearoyl phosphatidylethanolamine.
Animal Model: Rat.
Interpretation and Analysis: Poloxamine-908 stimulated macrophages cells are able to clear a significant portion of intravenously injected methoxypoly(ethylene glycol)2000 liposomes (labeled with [^{99m}Tc] via the N-hydroxysuccinimidyl hydrazine nicotinate hydrochloride derivative of distearoyl phosphatidylethanolamine) within 4 hours postadministration. Liposomes prepared in this fashion otherwise exhibit long circulatory behavior in control *in vivo* models, with poor localization within the liver and spleen. Such [^{99m}Tc]-labeled engineered vesicles may be of aid for detection of the liver and spleen macrophages with enhanced phagocytic clearance capacity by gamma scintigraphy. Alterations in the phagocytic activity of liver and spleen macrophages is known to occur during cancer. Therefore, such diagnostic procedures may prove useful for case selection or for monitoring the progress of treatment with long circulating nanoparticles carrying anticancer agents, thus minimizing damage to this important line of the body's defense mechanism.

Recognition and clearance of methoxypoly(ethylene glycol) 2000-grafted liposomes by macrophages with enhanced phagocytic capacity. Implications in experimental and clinical oncology. Laverman P, Carstens MG, Storm G, Moghimi SM. Biochim Biophys Acta 2001 Jun 15; 1526 (3), pp. 227–9.

Liposomes: Immunity/Macrophages: Selective Depletion/Alveolar Macrophages (Pulmonary) *Liposomal Dichloromethylene Diphosphonate*

Application: Relatively selective depletion of macrophage populations.
Implementation: Evaluation of the alveolar macrophages participation in host defense mechanisms directed against *Pneumocystis carinii*, their role in organism degradation, and their clearance from the lung utilizing liposomal dichloromethylene diphosphonate (L-Cl2MDP) as a modality for depleting macrophage populations.
Animal Model: Rats.
Interpretation and Analysis: Over a period of 72 hours, L-Cl2MDP exposure creates a loss of $>$ 85% of alveolar macrophages. Alternatively, L-PBS-treated *in vivo* models have cellular differentials identical to negative reference controls (e.g., phosphate buffered saline [PBS] administration).

The role of alveolar macrophages in *Pneumocystis carinii* degradation and clearance from the lung. Limper AH, Hoyte JS, Standing JE. J Clin Invest 1997 May 1; 99 (9), pp. 2110–7.

Liposomes/Immunity: Macrophage Selective Depletion/Hepatic and Spleen Populations/Alveolar Macrophage Sparing/Platelet Kinetics/ Biodistribution *Cl2MBP-Liposomes/5-Hydroxytryptamine (5HT or Serotonin)*

Background Information: Liposomes encapsulating dichloromethylene bisphosphonate (Cl2MBP-liposomes) have been shown to cause selective depletion of phagocytic macrophages.
Route of Administration: Cl2MBP-liposomes (intravascular/intravenous).
Implementation: Evaluate the capacity of Cl2MBP-liposomes to induce an almost complete depletion of F4/80-positive cells (mature macrophages) within the liver and in the splenic red pulp, but not in the lung.

Platelets contain a large amount of 5HT and so, by measuring 5HT, it is possible to assess the translocation of platelets to tissues.

Animal Model: Murine
Interpretation and Analysis: Injection of Cl2MBP-liposomes induces a prolonged and marked increase in 5HT that occurs selectively within the spleen. Alternatively, 5HT in the blood decreases by as much as 50%. These changes in 5HT correspond well with each other in terms of both time course and dose-response relationship. To judge from measurements made at the peak of the response, the 5HT increase within the spleen corresponds with about 80% of the 5HT lost from the blood. Electron microscopic analysis reveals a great accumulation of platelets in splenic cords. It has been shown that aggregation and degranulation of platelets in the lung is involved in rapid anaphylactoid shock within 10 minutes of intravenous injection into mice of a lipopolysaccharide fraction. Biphasic, organ-specific, and strain-specific accumulation of platelets can be induced by *Escherichia coli* lipopolysaccharide and in part respresents a component of shock responses. Contrasting effects have been observed for lipopolysaccharides (endotoxins) from oral black-pigmented bacteria and *Enterobacteriaceae* on platelets (major source of serotonin) and on histamine-forming enzyme. Such shock is almost completely prevented in those mice in which platelets are displaced from the blood by Cl2MBP-liposomes. These results suggest that in the spleen the depletion of phagocytic macrophages may impair the function or structure of this organ. This may lead to the entry of platelets into the spleen in such large numbers as to reduce their level within the blood and result in their prolonged accumulation in the spleen. The Cl2MBP-liposome may be an excellent tool for the *in vivo* investigation of the role of platelets, as well as that of macrophages.

Biphasic, organ-specific, and strain-specific accumulation of platelets induced in mice by a lipopolysaccharide from *Escherichia coli* and its possible involvement in shock. Shibazaki M, Nakamura M, Endo Y. Infect Immun 1996 Dec; 64 (12), pp. 5290–4.

Contrasting effects of lipopolysaccharides (endotoxins) from oral black-pigmented bacteria and Enterobacteriaceae on platelets, a major source of serotonin, and on histamine-forming enzyme in mice. Endo Y, Shibazaki M, Nakamura M, Takada H. J Infect Dis; 175, 1404–1412.

Displacement of platelets from blood to spleen following intravenous injection of liposomes encapsulating dichloromethylene bisphosphonate. Shibazaki M, Nakamura M, Nitta Y, Endo Y. Immunopharmacology 1998 Mar; 39 (1), pp. 1–7.

Liposomes/Neoplastic Disease: Assimilation Enhancement/Enhanced Selective Systemic/Local-Regional Uptake/Slowing of Local-Regional Blood Flow

Small Unilamellar Vesicles (SUV)-Poly(Ethylene Glycol) (PEG) Liposomes/Starch Microspheres

Physiological Mechanism of Action: Starch microspheres can be used to slow local-regional blood flow in a manner that can maximize assimilation of encapsulated liposomes within neoplastic lesions.

Background Information: Systemic adjuvant chemotherapy achieves unsatisfactory results for inoperable liver tumors and metastases. Various clinical studies have shown that loco-regional chemotherapy increases survival rate by a few months.

Application: Enhancement of systemic and local-regional uptake of diagnostic and therapeutic pharmaceutical agents.

Route of Administration: Systemic and local-regional.

Implementation: Evaluate the capacity of liposome encapsulation to selectively facilitate local-regional/systemic targeting of neoplastic cell populations with cytostatic 5-fluouracil (5-FU).

Interpretation and Analysis: Tumor concentrations of 5-FU encapsulated within SUV-PEG liposomes increases by a factor of 27 with systemic injection, and by a factor of 90 with loco-regional delivery. The tumor concentration of 5-FU increases by a factor of 8,000, if vascular blood flow is additionally slowed by starch microspheres (Spherex) during loco-regional therapy with 5-FU encapsulated liposomes.

Liposome encapsulation of cystostatic drugs and starch microspheres improve tumor targeting in loco-regional therapy. An animal experiment study of CC 531 liver tumor. Berger G, Reszka R, Pohlen U, Jung M, Lucas S, Buhr HJ. Langenbecks Arch Chir Suppl Kongressbd 1998; 115 (Suppl I), pp. 693–7.

Liposomes/Neoplastic Disease: Carbohydrate Binding Proteins/Stability of Radiolabeling Techniques

[^{125}I]-Glycoprotein Liposome Conjugate (GCL)

Cellular/Molecular Mechanism of Action: Specific glycoproteins can be designed that selectively bind to complexes uniquely expressed by neoplastic cell types.

Application: Selective delivery of diagnostic and therapeutic pharmaceuticals/radiopharmaceuticals to neoplastic cell populations.

Route of Administration: [^{125}I]-GCL (intravascular).

Semi-Synthetic Methods: In order to obtain radiolabeled GCL at a high labeling efficiency, *p*-hydroxyphenylpropyl groups can be introduced into liposome membranes through the amine moiety of a constitutive phospholipid, dipalmitoylphosphatidylethanolamine (DPPE), by using the Bolton-Hunter reagent (BHR). Radioiodination of the introduced tyrosyl groups can be performed using the Chloramine-T method.

Implementation: Evaluate the suitability of a radiolabeling method for GCL, in order to investigate its potential utility as a drug carrier modality that can target functional cellular carbohydrate-binding proteins.

Interpretation and Analysis: The labeling efficiency of BHR-treated liposome conjugate is high compared to that of the BHR-untreated liposome conjugate. Inhibition studies (*in vitro*) show that the binding affinity of [^{125}I]-labeled BHR-treated GCL ([^{125}I]-F3S-BH) with lectin is twice as high as that of untreated conjugate ([^{125}I]-F3S). The biodistribution of [^{125}I]-F3S-BH in mice is considerably different from that of [^{125}I]-F3S. [^{125}I]-F3S-BH is more rapidly taken up by the liver and is more rapidly excreted from the liver than [^{125}I]-F3S. Moreover, [^{125}I]-F3S-BH accumulates more rapidly within the kidney, resulting in lower radioactivity in the blood circulation at an earlier time point than is observed with [^{125}I]-F3S preparations. The characteristics of tumor [^{125}I]-F3S-BH and [^{125}I]-F3S accumulation are similar to those in blood. If F3S is to be employed as an *in vivo* targeting ligand in biodistribution studies, BHR would be a suitable tool for radiolabeling because it allows GCL to retain the biological activity and characteristics of the unmodified conjugate.

Radioiodination of glycoprotein-conjugated liposomes by using the Bolton-Hunter reagent and biodistribution in tumor-bearing mice. Shimura N, Sogawa Y, Kawakita Y, Ikekita M, Yamazaki N, Kojima S. Nucl Med Biol 2002 May; 29 (4), pp. 491–6.

Liposomes/Neoplastic Disease: Carcinoma of the Colon/Selective Delivery/5-Fluoro-2′-Deoxyuridine (FUdR)

Immunoliposomes

Background Information: Immunoliposomes, liposomes with monoclonal antibodies attached, have been developed for targeting the anticancer drug FUdR to colon cancer cells.

Application: Selective delivery of pharmaceuticals to neoplastic cell populations.

Semi-Synthetic Methods: A monoclonal antibody directed against colon carcinoma can be covalently coupled to liposomes containing a dipalmitoylated derivative of the anticancer drug FUdR (FUdR-dP) as a prodrug in their bilayers.

Implementation: Evaluation of different types of immunoliposomes that have antibody incorporated into their exterior surface at various positions and orientations, and delineate how these features affect their interaction with neoplastic cell types. Complementary investigations can assess the *in vitro* antitumor activity of liposome preparations and the mechanism by which the active drug FUdR is delivered intracellularly.

Cell Type: Rat colon carcinoma (CC531 cell type).

Interpretation and Analysis: Specific binding of the immunoliposomes to tumor cells can be observed. Immunoliposomes containing FUdR-dP cause a much stronger inhibition of CC531 cell growth *in vitro* than FUdR-dP in non-targeted liposomes. After binding to the cell surface, only a limited number of immunoliposomes become internalized. Alternatively, immunoliposome-incorporated FUdR-dP is intracellularly hydrolyzed virtually completely to the parent drug within 24 hours. The mechanism of intracellular delivery of the drug most likely involves a selective transfer of lipophilic prodrug from the liposomes to the cell membrane and subsequent intracellular processing. In conclusion, it is entirely possible to formulate targeted liposomal preparations that can deliver chemotherapeutics (e.g., FUdR) to colon carcinoma cells intracellularly at a high level of efficiency, without the need for cells to internalize liposomes as such. Such an approach may be attractive for lipophilic diagnostic pharmaceuticals or other lipophilic anticancer (pro)drugs. In this sense the system described may also serve as a model for the development of new lipid-based drug delivery systems for anticancer therapy.

Efficient intracellular delivery of 5-fluorodeoxyuridine into colon cancer cells by targeted immunoliposomes. Koning GA, Kamps JA, Scherphof GL. Cancer Detect Prev 2002; 26 (4), pp. 299–307.

Liposomes/Neoplastic Disease: Carcinoma Preferential Uptake/Mammary Carcinoma/ Ovarian Carcinoma/Prostatic Carcinoma
PEGylated Liposomes

PEGylated liposomal doxorubicin (doxorubicin HCl liposome injection; Doxil or Caelyx) is a liposomal formulation of doxorubicin that has a lower level of uptake by the reticuloendothelial system due to the attachment of poly(ethylene glycol) polymers to a lipid anchor. The chemotherapeutic is stable and retained to a relatively high degree as a result of liposomal entrapment via an ammonium sulfate chemical gradient. These features result in a pharmacokinetic profile characterized by an extended circulation time and a reduced volume of distribution, thereby enhancing tumor uptake. Preclinical studies have demonstrated the presence of a 1- or 2-phase plasma concentration-time profile. Most of the drug is cleared with an elimination half-life of 20 to 30 hours. The volume of distribution is close to the blood volume, and the area-under-the-concentration-time-curve (AUC) is increased at least 60-fold compared with free doxorubicin. Studies of tissue distribution indicate preferential accumulation into various implanted tumors and human tumor xenografts, with an enhancement of drug concentrations in tumors compared with free drug. PEGylated liposomal doxorubicin has been employed in several human disease states, including AIDS-related Kaposi's sarcoma (ARKS) and a variety of solid tumors, including ovarian, breast, and prostate carcinomas. The pharmacokinetic profile in humans at doses between 10 and 80 mg/m^2 is similar to that in animal models, with 1 or 2 distribution phases: an initial phase with a half-life of 1 to 3 hours, and a 2nd phase with a half-life of 30 to 90 hours. The AUC after a dose of 50 mg/m^2 is approximately 300-fold greater than that with free drug. Clearance and volume of distribution are drastically reduced (at least 250-fold and 60-fold, respectively). Preliminary observations indicate that utilizing the distinct pharmacokinetic parameters of PEGylated liposomal doxorubicin in dose scheduling is an attractive option. In agreement with the preclinical findings, the ability of PEGylated liposomes to extravasate through the leaky vasculature structures of tumors, as well as their extended circulation time, results in enhanced delivery of liposomal drugs and/or radiotracers to the tumor sites in cancer patients. There is evidence of selective tumor uptake in malignant effusions, ARKS skin lesions, and a variety of solid tumors. The toxicity profile of PEGylated liposomal doxorubicin is characterized by dose-limiting mucosal and cutaneous toxicities, mild myelosuppression, decreased cardiotoxicity compared with free doxorubicin, and minimal alopecia. The mucocutaneous toxicities are dose limiting per injection; however, the reduced cardiotoxicity allows a larger cumulative dose than that acceptable for "free" doxorubicin administration. Thus, PEGylated liposomal doxorubicin represents a new class of chemotherapy delivery system that may significantly improve the therapeutic index of doxorubicin.

Pharmacokinetics of PEGylated liposomal doxorubicin: Review of animal and human studies. Gabizon A, Shmeeda H, Barenholz Y. Clin Pharmacokinet 2003; 42 (5), pp. 419–36.

Liposomes/Neoplastic Disease/Carcinoma: Mammary/Vasoactive Intestinal Peptide (VIP) Receptor Complexes
[^{99m}Technetium] ([^{99m}Tc])-HMPAO-[SSL-VIP] Liposomes

Cellular/Molecular Mechanism of Action: Vasoactive intestinal peptide recognizes and physically binds to its respective receptor complex.

Background Information: Targeted delivery of radionuclides and therapeutic agents to specific biomarkers of breast cancer has important implications for the diagnosis and therapy of breast cancer. Vasoactive intestinal peptide receptors (VIP-R) are expressed at levels that are approximately 5-fold higher in human breast cancer lesions, compared to normal breast tissue.

Semi-Synthetic Methods: Incorporation of VIP (28 amino acid mammalian neuropeptide), as a breast cancer targeting moiety for selective imaging of breast cancer. VIP can be covalently attached to the surface of sterically stabilized liposomes (SSL) encapsulating [^{99m}Tc]-HMPAO radionuclide.

Implementation: Evaluation of liposome preparations as a potential delivery platform for imaging agents to facilitate detection and characterization of mammary carcinoma. Complementary investigations can delineate the pharmacokinetics and biodistribution of [^{99m}Tc]-HMPAO encapsulating SSL with and without VIP.

Animal Model: Rats with *n*-methyl nitrosourea (MNU)-induced *in situ* breast cancers.

Interpretation and Analysis: The presence of VIP does not alter the size and [^{99m}Tc]-HMPAO encapsulation ability of SSL. It also does not alter the pharmacokinetic profile of SSL. Long-circulating liposomes with and without VIP on their surface accumulate at significantly higher concentrations in breast cancer compared to normal breast, indicating passive targeting of these constructs for neoplastic lesions. Importantly, in breast cancer, [^{99m}Tc]-HMPAO encapsulating SSL with VIP show significantly more accumulation than SSL without VIP. The tumor to non-tumor ratio is also significantly higher for [^{99m}Tc]-HMPAO encapsulating VIP-SSL than [^{99m}Tc]-HMPAO encapsulating SSL without VIP, suggesting active targeting of VIP-SSL for mammary carcinoma lesions. Collectively, these data show that [^{99m}Tc]-HMPAO encapsulating VIP-SSL can be successfully used for targeted imaging of breast cancer.

VIP grafted sterically stabilized liposomes for targeted imaging of breast cancer: *In vivo* studies. Dagar S, Krishnadas A, Rubinstein I, Blend MJ, Onyuksel H. J Control Release 2003 Aug 28; 91 (1–2), pp. 123–33.

Liposomes/Neoplastic Disease/Carcinoma: Ovarian
Virosomes/Immunoliposomes/425 MoAb-PEG-PE/ Fusogenic Di-Peptide/Diphtheria Toxin Chain A

Application: Facilitated delivery of therapeutically active macromolecules into the cytosol of target cell populations.

Semi-Synthetic Methods: Detergent solubilized influenza virus envelopes can be reconstituted to form virosomes. Cell specificity can be created by incorporating poly(ethylene glycol (PEG)-derivatized lipids with mAB 323/A3 (Fab′ fragments) connected to their distal PEG end. These cell-specific, modified virosomes maintain their fusogenic activity at lower pH levels. Most importantly, antibody-mediated binding is a prerequisite for low pH-induced membrane fusion. Basic problems that can be encountered include (i) extensive virosome leakage; and (ii) usually immunogenic/antigenic properties of virosome preparations. A solution to correct leakage and potential immunogenicity of site-specific liposomal structures is to use immuno-PEG-liposomes with a pH-dependent fusogen inside the liposome. One approach to achieving this objective includes the use or incorporation of (i) a fusogenic di-peptide dINF-7; (ii) the monoclonal antibody 425 connected to the distal end of PEG-PE (for site-specific binding and endosomal uptake); (iii) diphtheria toxin chain A (DTA, as carrier-dependent active compound); and (iv) phosphatidylcholine/cholesterol as "bilayer backbone."

Implementation: Evaluate the degree of selective binding and pH-dependent destabilization of (endosomal) membranes. To test the

cytotoxic activity of DTA-loaded liposomes, OVCAR-3 cells can be used as an *ex vivo* testing model. OVCAR-3 cells express the epidermal growth factor receptor, which is the ligand for antibody 425.

Interpretation and Analysis: *In vitro*, these site-specific and fusogenic liposomes show a remarkable, cell-specific cytotoxic effect.

Targeted liposomes for delivery of protein-based drugs into the cytoplasm of tumor cells. Mastrobattista E, Crommelin DJ, Wilschut J, Storm G. J Liposome Res 2002 Feb–May; 12 (1–2), pp. 57–65.

Liposomes/Neoplastic Disease/Carcinoma: Prostatic/Prostate-Specific Membrane Antigen (PSMA)/Glutamyl Carboxypeptidase/ Endothelial Tumor Antigen

Prostate-Specific Membrane Antigens/Soluble Extracellular Tissue Factor (PSMA Catalytic Site)

Cellular/Molecular Mechanism of Action: Prostate-specific membrane antigen is a glutamyl-preferring carboxypeptidase.

Background Information: Prostate-specific membrane antigen is present in prostate and other carcinomas where it is expressed on both tumor cells and endothelial cells of associated microvascular structures. It has been shown that the channel structures delineated by PSMA-expressing cells in human and rat prostate tumors are in functional continuity with the vasculature and thus form part of the tumor microvasculature. The PSMA-positive cell-outlined channels are CD31 negative and mutually exclusive of CD31-positive cell-lined channels elsewhere in the tumor consistent with tumor cells adapted to a pseudoendothelial phenotype in vasculogenic mimicry.

Application: Molecular strategy for selectively targeting the microvasculature network of neoplastic lesions.

Implementation: Evaluation of the functional aspects of (i) PSMA-lined microvasculature to selectively direct infarctive tumor therapy combined with the application of (ii) soluble extracellular domain of tissue factor to a PSMA catalytic site inhibitor to create a PSMA-directed selective tumor vascular thrombogen (STVT).

Interpretation and Analysis: The semi-synthetic protein induces selective local *in vivo* infarctive necrosis of the rat Mat Lu prostate tumor when administered intravenously. The combined administration of this STVT with low-dose doxorubicin produces a profound tumoricidal effect, resulting in complete eradication of some tumors. This is consistent with the therapeutic potential for a PSMA-directed STVT and expands the potential for selective infarctive ablation of tumors.

Prostate-specific membrane antigen directed selective thrombotic infarction of tumors. Liu C, Huang H, Doñate F, Dickinson C, Santucci R, El-Sheikh A, Vessella R, Edgington TS. Cancer Res 2002 Oct 1; 62 (19), pp. 5470–5.

Liposomes/Neoplastic Disease: Chondroitin Sulfate (CS) Expression/Solid Tumors

Cationic Poly(Ethylene Glycol) (PEG)-TRX-20 Liposomes/ Cationic 3,5-Dipentadecycloxybenzamidine

Cellular/Molecular Mechanism of Action: The cationic lipid 3,5-dipentadecycloxybenzamidine hydrochloride (TRX-20) binds to CS complexes expressed on the exterior surface membrane of neoplastic cell types.

Background Information: An increased level of CS expression on the cell surface is often associated with malignant transformation and the pathological progression of tumor cells.

***Ex Vivo* Model:** Neoplastic cells (LM8G5 and ACHN cell lines).

Implementation: Evaluation of the capacity for CS expressed on highly metastatic tumor cells to function as a unique "target" to facilitate selective delivery of anticancer drugs by PEG-coated liposomes that contain a cationic lipid (e.g. 3,5-dipentadecycloxybenzamidine hydrochloride or TRX-20).

Animal Model: Murine (mice bearing solid subcutaneous LM8G5 tumors).

Interpretation and Analysis: PEG-coated TRX-20 liposomes (TRX-20 liposomes) bind preferentially to certain CS, including CS B, CS D, and CS E, whereas PEG-coated liposomes lacking TRX-20 show no significant binding to any of the glycosaminoglycans tested. *In vitro*, TRX-20 liposomes, but not plain PEG-liposomes, avidly bind to and are readily internalized by highly metastatic tumor cells such as LM8G5 and ACHN cells, which express large amounts of CS on their exterior surface membrane. When TRX-20 liposomes are loaded with cisplatin, they effectively kill CS-expressing tumor cells *in vitro*, whereas cisplatin-PEG liposomes lacking TRX-20 are totally ineffective. When injected systemically, TRX-20 liposomes preferentially accumulate in the liver and in solid LM8G5 tumors. Therapeutic experiments in mice bearing M8G5 tumors reveal that cisplatin-loaded TRX-20 liposomes are significantly more effective in reducing local tumor growth than cisplatin-loaded plain PEG-liposomes or free cisplatin. Furthermore, cisplatin-loaded TRX-20 liposomes markedly suppress metastatic spreading of LM8G5 tumor cells to the liver, and significantly increase overall survival time (e.g., tumor-bearing mice). Therefore, CS-targeted delivery of anticancer drugs by novel cationic liposomes represents a potentially useful strategy for preventing local growth and metastasis, particularly to the liver, relevant to tumor cells that display enhanced CS expression.

Novel chondroitin sulfate-binding cationic liposomes loaded with cisplatin efficiently suppress the local growth and liver metastasis of tumor cells *in vivo*. Lee CM, Tanaka T, Murai T, Kondo M, Kimura J, Su W, Kitagawa T, Ito T, Matsuda H, Miyasaka M. Cancer Res 2002 Aug 1; 62 (15), pp. 4282–8.

Liposomes/Neoplastic Disease: Delivery and Extravasation Potential

Poly(Ethylene Glycol) (PEG)-Liposomes (100–200 nm)/ PEG-Liposomes (400 nm)

Route of Administration: PEG-liposomes (intravascular).

Semi-Synthetic Methods: Liposomes composed of distearoyl phosphatidylcholine, cholesterol, and distearoylphosphatidylethanolamine derivative of PEG can be prepared in various size ranges.

Implementation: Evaluation of the size dependence of extravasation and interstitial localization of PEG-liposomes within solid tumor tissue by means of electron microscopic observation. Of particular interest is the assessement of the degree of tumor accumulation.

Animal Model: Murine.

Interpretation and Analysis: PEG-liposomes with an average diameter of 100 to 200 nm have the most prolonged circulation time and the greatest tumor accumulation in all the solid tumors employed in this experiment. Although large PEG-liposomes with a diameter of 400 nm show a short circulation time in normal mice, the results in splenectomized mice indicate that they do have an intrinsically prolonged *in vivo* circulation profile. However, large PEG-liposomes do not extravasate into solid tumor tissue. These results indicate that the size of liposomes is critical for extravasation. The electron microscopic observations reveal the almost exclusive engulfment of extravasated liposomes by tumor-associated macrophages; very few are taken up by tumor cells.

Size-dependent extravasation and interstitial localization of polyethyleneglycol liposomes in solid tumor-bearing mice. Ishida O, Maruyama K, Sasaki K, Iwatsuru M. Int J Pharm 1999 Nov 10; 190 (1), pp. 49–56.

Liposomes/Neoplastic Disease/Delivery of Chemotherapeutics/Doxorubicin (Example)
Liposomes

Cellular/Molecular Mechanism of Action: Liposomes function as a carrier modality for pharmaceutical agents.
Background Information: The optimization of drug disposition in the body leads to an increase in therapeutic effect and a decrease in adverse effects involving innocent tissues and organ systems. Liposomes can serve as a potential drug carrier system that can be utilized for such purposes. However, the behavior of a drug carrier system under *in vivo* conditions is complex. Therefore, a more complete understanding of the pharmacokinetics of liposomes themselves, as well as that of the encapsulated drug, is a mandatory requirement.
Route of Administration: Intravenous.
Dosage: Dependent upon procedure and the type of pharmaceutical incorporated into liposome preparations.
Implementation: The optimization of the pharmacokinetic profile of liposomes can be performed by linking a pharmacodynamic model of free drugs encapsulated into liposome preparations. Sensitivity analysis based on maximizing the antitumor effects of liposomal doxorubicin can be applied as a guide for optimizing delivery systems. Advanced forms of biotechnology for ligand-mediated selective targeting and intracellular targeting have also been developed to facilitate superior intracellular introduction of antitumor agents and gene delivery systems.

Pharmacokinetics of targeting with liposomes. Harashima H, Ishida T, Kamiya H, Kiwada H. Crit Rev Ther Drug Carrier Syst 2002; 19 (3), pp. 235–75.

Liposomes/Neoplastic Disease/Endothelium of Tumor Microvasculature Structures/ Anionic Targeting of the Endothelial Surface Membrane/Melanoma
Liposomes: Cationic/Rhodamine-Labeled Cationic Liposomes

Cellular/Molecular Mechanism of Action: The microvasculature of neoplastic lesions appears to express anionic characteristics that can be physically recognized by cationic liposomes.
Background Information: Cationic liposomes have been shown to selectively target tumor endothelial cells. Therefore, the encapsulation of diagnostic and antineoplastic pharmaceuticals into cationic liposomes is a promising tool for improving selective drug delivery by targeting tumor vasculature.
Application: Cationic liposomes can be applied to selectively deliver pharmaceutical agents to microvasculature structures of neoplastic cell populations.
Route of Administration: Intravenous infusion over a period of 90 minutes.
Dosage: Lipid dose, 150 mg/kg body weight; paclitaxel dose, 5 mg/kg body weight.
Implementation: Evaluate the capacity for cationic liposomes (LipoPac) to enhance the *in vivo* chemotherapeutic properties of encapsulated paclitaxel (Taxol) by targeting the anionic exterior surface membrane of endothelial cells associated with tumor microvascular structures. Reference controls can include the administration of free drug paclitaxel (Taxol); glucose 5%; and unloaded cationic liposomes in order to accurately determine selectivity for tumor cells and relative ability to modify cancer cell growth, tumor volume, and presence of regional lymph node metastases quantified.
Animal Model: Hamsters (Syrian golden strain bearing hamster melanoma A-Mel-3 in dorsal skinfold preparations). Growth of tumor cells can be observed after subcutaneous inoculation (day 0).
Interpretation and Analysis: Vascular targeting of rhodamine-labeled cationic liposomes is achieved after encapsulation of paclitaxel when evaluated by *in vivo* fluorescence microscopy (ratio of dye concentration, tumor:normal tissue = 3:1). Subcutaneous tumor growth evaluation reveals a remarkable retardation of tumor growth after treatment with LipoPac (1.7 ± 0.3 cm^3). Alternatively, control tumors show exponential tumor growth (tumor volume at the end of the observation period [mean $\pm$ standard error]: 5% glucose, 17.7 ± 1.9 cm^3; unloaded cationic liposomes, 10.0 ± 1.6 cm^3; Taxol, 10.7 ± 1.7 cm^3). In addition, the appearance of regional lymph node metastases is significantly delayed by treatment with paclitaxel encapsulated into cationic liposomes compared to all other test groups. Therefore, cationic liposomes are a powerful tool for selective and efficient drug delivery to tumor microvasculature structures. This may serve as proof of the concept of neovascular tumor targeting diagnostic therapeutic pharmaceuticals by cationic liposomes.

Neovascular targeting therapy: Paclitaxel encapsulated in cationic liposomes improves antitumoral efficacy. Schmitt-Sody M, Strieth S, Krasnici S, Sauer B, Schulze B, Teifel M, Michaelis U, Naujoks K, Dellian M. Clin Cancer Res 2003 Jun; 9 (6), pp. 2335–41.

Liposomes/Neoplastic Disease/Endothelium of Tumor Microvascular/Vascular Marker System
Liposomes: Fluorescent Encapsulation/ Fluorescent-Lectin

Cellular/Molecular Mechanism of Action: Cationic liposomes display binding avidity for unique anionic domains expressed by the vascular endothelium with neoplastic lesions.
Background Information: Cationic liposomes have been shown to preferentially target the angiogenic endothelium of tumors.
Application: Cationic liposomes can be applied for selective delivery to angiogenic tumor vessels. Anionic and neutral liposomes may be used as carriers of drugs to the extravascular compartment of tumors by processes of extravasation. Fluorescently labeled lectin can be utilized as a vascular marker for either validation of other reagents or as a diagnostic tool.
Route of Administration: Liposomes (intravenous).
Implementation: Evaluation of the influence of liposomal surface charge on the uptake and kinetics of liposomes into solid tumors and tumor vasculature. In this context, fluorescently labeled liposomes prepared with different surface charge properties can be evaluated *in vivo* for their ability to localize within amelanotic melanoma lesions. Accumulation of liposomes can be detected utilizing fluorescence microscopy of tumor and surrounding normal healthy tissue. The histological distribution of liposomes can be analyzed by double-fluorescence microscopy 20 minutes after application of fluorescently labeled lectin as a vascular marker.
Animal Model: Syrian Golden Hamster (male animals with amelanotic hamster melanoma A-Mel-3 growing in a dorsal skin fold chamber preparation).
Interpretation and Analysis: Following intravenous application of anionic and neutral liposomes, an almost homogeneous distribution can be appreciated for liposome-induced fluorescence throughout the chamber preparation without specific targeting to tumor tissue. Alternatively, cationic liposomes exhibit a significant level of enhanced accumulation within tumor tissue and tumor

vasculature up to 3-fold higher compared to surrounding tissues ($p < 0.05$). The histological distribution of neutral and anionic liposomes reveals extravasation 20 minutes after intravenous injection, while cationic liposomes display a highly selective accumulation on vascular endothelium. Cationic liposomes exhibit a preferential uptake in angiogenic tumor vessels and they therefore provide an efficient tool for the selective delivery of diagnostic or therapeutic agents to angiogenic blood vessels of solid tumors. Additionally, anionic and neutral liposomes may be used as carriers of drugs to the extravascular compartment of tumors due to their extravasation.

Instrumentation: Quantitative intravital fluorescence microscopy.

Effect of the surface charge of liposomes on their uptake by angiogenic tumor vessels. Krasnici S, Werner A, Eichhorn ME, Schmitt-Sody M, Pahernik SA, Sauer B, Schulze B, Teifel M, Michaelis U, Naujoks K, Dellian M. Int J Cancer 2003 Jul 1; 105 (4), pp. 561–7.

Liposomes/Neoplastic Disease/Folate Receptor Expression
Folate Liposomes

The folate receptor is a cell surface protein that has recently been identified as a tumor marker due to its differential overexpression in several malignancies. Current research indicates that folate can be covalently attached to the surface of liposomes to mediate their selective internalization by tumor cells through the folate receptor-mediated endocytic pathway. Optimized liposome formulations, characterized by improvements in drug loading, extended residence times within the intravascular compartment, and improved drug release, have been developed to improve the biodistribution of therapeutic molecules. Theoretically, folate receptor targeting can be combined with liposome encapsulation to synergistically affect disease outcome by enhancing the delivery of chemotherapeutic agents to neoplastic cells, while reducing systemic toxicities to normal tissues.

Folate receptor-targeted liposomes as vectors for therapeutic agents. Gosselin MA, Lee RJ. Biotechnol Annu Rev 2002; 8, pp. 103–31.

Liposomes/Neoplastic Disease/Folate Receptors/ Ovarian Carcinoma/Acute Myelogenous Leukemia (AML)/Granulocytes/Neutrophils/Donor Bone Marrow
Folate-Coated Liposomes [Fluorescent Cacein Marker]/ All-Trans-Retinoic Acid (ATRA)

Background Information: Upregulation of folate receptor (FR) type-beta in AML by ATRA and its restricted normal tissue distribution makes it a potential target for therapeutic intervention.

Implementation: Evaluation of multicomponent folate liposomes to selectively bind to folate receptor complexes expressed by peripheral blood granulocytes.

***Ex Vivo* Model:** Granulocytes; donor bone marrow cell preparations; folate receptor negative (FR^-) cell lines (e.g., KG-1a murine leukemia cells, L1210, and Chinese hamster ovary [CHO]); and folate receptor positive (FR^+) cell lines (e.g., FR-beta expression KG-1 human AML cells, L1210 JF, and recombinant CHO-FR-beta).

Animal Model: Murine (mouse ascites leukemia) using L1210JF or KG-1 cell lines.

Interpretation and Analysis: The FR-beta in peripheral blood granulocytes is unable to bind folate and appears to have a variant glycosylphosphatidylinositol (GPI) membrane anchor, evident from its insensitivity to phosphatidylinositol-specific phospholipase C but not nitrous acid. Granulocyte FR-beta lack mutations, and neither deglycosylation nor detergent solubilization restores folate binding. The posttranslational modification causing its non-functionality is evidently absent in FR-beta from AML cells from donor marrow that binds folate. From flow cytometric analysis of 78 AML bone marrow specimens of different subtypes, 68% express FR-beta, most of which are also $CD34^+$. In model cell lines that are FR^- (KG-1a, L1210, and CHO) or FR^+ (KG-1, L1210 JF, and recombinant CHO-FR-beta), selective FR^--mediated binding and cytotoxicity can be obtained using folate-coated liposomes encapsulating fluorescent calcein (f-L-calcein) and doxorubicin (f-L-DOX), respectively, which can be blocked by free folic acid (1 mM). In the FR-beta-expressing KG-1 human AML cells, treatment with ATRA further increases this specificity. In mouse ascites leukemia models generated using L1210JF or KG-1 cells, increases in median survival times can be obtained with f-L-DOX treatment compared to non-targeted L-DOX. In the KG-1 model, ATRA treatment increases the cure rate with f-L-DOX from 10% to 60%. Appreciation of these data serves to further support the feasibility and potential usefulness of selective ATRA-facilitated liposomal drug delivery in FR-$beta^+$ AMLs.

Strategy for the treatment of acute myelogenous leukemia based on folate receptor beta-targeted liposomal doxorubicin combined with receptor induction using all-trans-retinoic acid. Pan XQ, Zheng X, Shi G, Wang H, Ratnam M, Lee RJ. Blood 2002 Jul 15; 100 (2), pp. 594–602.

Liposomes/Neoplastic Disease/Melanoma c-myc Oncogene/Neuroblastoma (GD_2 Positive)
Cationic Immunoliposomes/Antidisialoganglioside (GD_2)/Antisense c-myb Olidodeoxynucleotide

Background Information: Melanoma is a highly malignant and increasingly common tumor. Because the cure rate of metastatic melanoma by conventional treatment is very low, new therapeutic approaches are needed. Reports have previously described that coated cationic liposomes (CCL) targeted with a monoclonal antibody against the disialoganglioside (GD_2) and containing c-myb antisense oligodeoxynucleotides (asODNs) result in a selective inhibition of (GD_2)-positive neuroblastoma cells *in vitro* proliferation.

Considerations: Melanoma is a neuroectodermal tumor that, along with neuroblastoma cell types, shares the expression of GD_2 complexes.

Application: Selective delivery to melanoma and neuroblastoma neoplastic cell types.

Route of Administration: Intravascular.

Implementation: Evaluation of the selective *in vivo* delivery capabilities and antineoplastic properties of liposomes encapsulating antisense oligonucleotides for the c-myc oncogene of melanoma and designed to target GD_2-positive cell lines (e.g., melanoma).

Interpretation and Analysis: Preparations of GD_2-targeted liposomes can be produced that entrap in a stable manner 90% of added c-myc asODNs. Such liposomes show a selective binding *in vitro* for GD_2-positive melanoma cells. Melanoma cell proliferation is inhibited to a greater extent by GD_2-targeted liposomes containing c-myc asODNs (aGD_2-CCL-myc-as) than by non-targeted liposomes or free asODNs. The pharmacokinetic results obtained after intravenous injection of [^{3}H]-myc-asODNs, free or encapsulated in non-targeted CCLs or GD_2-targeted CCLs, reveals that free c-myc-asODNs are rapidly cleared, with less than 10% of the injected dose remaining in the blood 30 minutes after injection. Sequences of c-myc-asODNs encapsulated within either CCL or aGD_2-CCL demonstrate a more favorable profile within the vascular compartment, with about 20% of the injected dose of each preparation remaining *in vivo* at 24 hours after injection. In *in vivo* melanoma

experimental metastatic models, aGD_2-CCL-myc-as, at a total dose of only 10 mg of asODN per kilogram, significantly inhibits the development of microscopic metastases into the lung compared with animals treated with myc-asODNs, free or entrapped in non-targeted liposomes, or aGD_2-CCL encapsulating scrambled asODNs ($p < 0.01$). Moreover, mice bearing established subcutaneous human melanoma xenografts treated with aGD_2-CCL-myc-as exhibit significantly lower levels of tumor growth and increased survival ($p < 0.01$ vs control mice). The mechanism for the antitumor effects appears to be downregulation of c-myc protein expression and interruption of c-myc-mediated signaling: induction of p53 and inhibition of Bcl-2 proteins, leading to extensive tumor cell apoptosis. Such observations suggest that inhibition of c-myc proto-oncogene by GD_2-targeted antisense therapy could provide an effective approach for the treatment of melanoma in an adjuvant setting.

Targeted liposomal c-myc antisense oligodeoxynucleotides induce apoptosis and inhibit tumor growth and metastases in human melanoma models. Pastorino F, Brignole C, Marimpietri D, Pagnan G, Morando A, Ribatti D, Semple SC, Gambini C, Allen TM, Ponzoni M. Clin Cancer Res 2003 Oct 1; 9 (12), pp. 4595–605.

Liposomes/Neoplastic Disease/Photoexposure (Ultraviolet [UV]) Induced Selective Delivery
Sterically Stabilized Liposomes/Poly(Ethylene Glycol) (PEG)-Liposomes/Photoperturbed PEG-Liposomes/ Photoexposure-Induced Delivery

The use of liposomes for the delivery of therapeutic agents to tumor sites took a major step forward with the introduction of sterically stabilized liposomes (PEG-liposomes). Several research groups reported the increased localization of PEG-liposomes at tumor sites. Once PEG-liposomes reach these sites, they may demonstrate the desirable feature of releasing encapsulated compound(s) at a relatively rapid rate. The use of radiation for this purpose is an attractive option because it can allow delivery in a spatially and temporally selective manner. An effective strategy for the photoperturbation of PEG-liposomes relies on the photoinitiated polymerization of reactive lipids in the liposomal bilayer. Previous studies have revealed that the inclusion of the photoreactive 1,2-bis[10-(2′,4′-hexadienoyloxy)decanonyl]-sn-glycero-3-phosphocholine (bis-SorbPC(17,17)) among the lipids of PEG-liposomes has little effect on their permeability until the PEG-liposomes are exposed to UV light. Photoexposure increases the permeability of the PEG-liposomes 200-fold. Additional investigations characterizing this phenomena have revealed that PEG-liposomes can be designed that have extremely low permeabilities to water-soluble fluorescent probes at 37°C in the dark, yet the permeability can be increased 28,000-fold upon exposure to UV irradiation. The large increase in the rate of photoinitiated release of the encapsulated contents may be a consequence of increased phase separation between photoreactive and saturated phospholipids used in the PEG-liposomes.

Rapid release of liposomal contents upon photoinitiated destabilization with UV exposure. Spratt T, Bondurant B, O'Brien DF. Biochim Biophys Acta 2003 Apr 1; 1611 (1–2), pp. 35–43.

Liposomes/Neoplastic Disease/Proliferating Populations/Low-Density Lipoprotein (LDL) Receptor Complexes
LDL Lipsomes

Cellular/Molecular Mechanism of Action: Low-density lipoprotein fractions recognize and physically bind to LDL receptor complexes.

Background Information: In photodynamic therapy, 1 of the problems limiting the use of many photosensitizers (PS) is the difficulty in preparing pharmaceutical formulations that enable their parenteral administration. Due to their low water solubility, the hydrophobic PS cannot be simply injected intravenously. Different strategies, including polymer-PS conjugation or encapsulation of the drug in colloidal carriers such as oil-dispersions, liposomes, and polymeric particles, have been investigated. Although these colloidal carriers tend to accumulate selectively in tumor tissues, they are rapidly taken up by the mononuclear phagocytic system. In order to reduce this undesirable uptake by phagocytic cells, long-circulating carriers that consist of surface-modified carriers have been developed. Moreover, considerable effort has been directed toward using other types of carriers to improve tumor targeting and to minimize the side effects.
Application: Selective targeting of proliferating cell populations.
Semi-Synthetic Methods: Lipophilic agents (e.g., photosensitizing therapeutics) can be entrapped within the lipophilic core of LDL without altering their biological properties.
Interpretation and Analysis: The LDL receptor pathway is an important factor in the selective accumulation of lipophilic agents (e.g., photosensitizing therapeutics) within tumor tissues owing to the increased number of LDL receptors on the proliferating cell surface. Specific targeting can also be achieved by binding of monoclonal antibodies or specific tumor-seeking molecules to PS or by the coating of lipophilic agent-loaded carriers.

State of the art in the delivery of photosensitizers for photodynamic therapy. Konan YN, Gurny R, Allémann E. J Photochem Photobiol B 2002 Mar; 66 (2), pp. 89–106.

Liposomes/Neoplastic Disease: Transferrin Receptor Expressing Cancers/(HeLa Cells)
Transferrin Poly(Ethylene Glycol) (PEG) Liposomes

Cellular/Molecular Mechanism of Action: Transferrin recognizes and physically binds to transferrin receptor complexes.
Background Information: Many tumor cells, including HeLa cells, overexpress the transferrin receptor complexes on the exterior surface membranes.
Application: Selective targeting of neoplastic cell populations that overexpress transferrin receptor complexes.
Route of Administration: Transferrin-conjugated PEG-liposomes (intravascular).
Considerations: Photodynamic therapy has attracted increasing interest over the last few years, whereby the activation of photosensitizers by light causes the production of reactive oxygen species (ROS), such as cytotoxin singlet oxygen species.
Semi-Synthetic Methods: Production of transferrin-conjugated PEG-liposomes that contain AlPcS4 photodynamic agent.
Implementation: Evaluation of liposome cellular internalization facilitated by tranferrin receptor-mediated endocytosis. The antiproliferative activity of the targeted liposomes is then evaluated and compared to native AlPcS4 and non-targeted liposome preparations. Laboratory data are then correlated with findings regarding the intracellular concentration of the photoactive compounds. Accumulation together with ROS production after irradiation is then visualized to confirm data found in antiproliferative and accumulation assays.
Interpretation and Analysis: Transferrin liposomes containing ALPcS4 phododynamic agent (Tf-Lip-AlPcS4) are 10 times more photocytotoxic (IC_{50}, 0.63 μM) than free AlPcS4 at a light dose of 45 kJ/m, whereas Lip-AlPcS4 displays no photocytotoxicity at all. The high photocytotoxicity of Tf-Lip-AlPcS4 is the result of a high

intracellular concentration (136.5 μM) in HeLa cells, which can be lowered dramatically by incubating the conjugate with a competing transferrin concentration. The images of intracellular accumulation and ROS production match the accumulation and photocytotoxicity profile of the different photo-active compounds. The photodynamic activity of the Tf-Lip-AlPcS4 conjugate on HeLa cells is much more potent than free AlPcS4 as a result of selective transferrin receptor-mediated uptake.

Instrumentation: Confocal microscopy.

Targeting of the photocytotoxic compound AlPcS4 to HeLa cells by transferrin-conjugated PEG-liposomes. Gijsens A, Derycke A, Missiaen L, De Vos D, Huwyler J, Eberle A, de Witte P. Int J Cancer 2002 Sep 1; 101 (1), pp. 78–85.

Liposomes/Neoplastic Disease: Vasculature Supply/ Selective Tumor DNA Delivery
Plasmid-Microspheres/Plasmid-Complexed Liposomes

Application: Selective targeting of genetic DNA sequences incorporated into plasmid vectors for neoplastic lesions (selective delivery is in part facilitated by selective catheterization of the major arterial network supplying a neoplastic tissue mass).

Route of Administration: Selective catheterization of major arterial branches of neoplastic lesions.

Implementation: Evaluation of the capacity for a plasmid vector bound to liposomes and microsphere to mediate delivery of DNA sequences (chloramphenicol acetyltransferase) into an established rat renal solid tumor model. Preparations are selectively administered via an arterial catheter system.

***Ex Vivo* Model:** Rat renal solid tumor.

Interpretation and Analysis: Forty-eight hours postdelivery, tumor to normal kidney tissue chloramphenicol acetyltransferase expression ratios are as follows: free (1.8:1), microspherical (3.9:1), and liposomal (1.2:1). Microspheres are able to selectively deliver plasmids to tumors, whereas cationic liposomes distribute plasmids to both kidney parenchymal and tumor cells. The tumor model has the potential of screening delivery vehicles as well as therapeutic agents for the capacity of selective delivery to tumors via the vasculature.

A model for evaluating selective delivery of plasmid DNA to tumours via the vasculature. Dass CR, Burton MA. Cancer Biother Radiopharm 2002 Oct; 17 (5), pp. 501–5.

Liposomes/Peptide (Protein) Delivery Modality: Oral Route/Biological Peptides/Vaccine Antigen
Liposomes

Cellular/Molecular Mechanism of Action: Liposomes can protect peptides/proteins from degradation within the gastrointestinal tract and facilitate their absorption across mucosal surfaces and subsequent entry into the intravascular compartment.

Application: Delivery of biological proteins and synthetic peptides across the gastrointestinal mucosa following oral administration.

Route of Administration: Oral.

Semi-Synthetic Methods: The tested mixtures include [EggPC/Chol] 1:1 (formula I), [EggPC/Chol/SM] 1:1:1 (formula II), [EggPC/Chol/SM/GM type III] 1:1:1:0.14 (formula III), [EggPC/Chol/SM/GM1] 1:1:1:0.14 (formula IV), and [DIAPC/DMPC] 1:1 non-polymerized (formula V) and polymerized (formula VI); all mole ratio.

Implementation: Evaluation of the usefulness of liposomes as possible vaccine vehicles (oral and subcutaneous). Stability of liposomes can be assessed in buffer, plasma, and saliva at 25 and 37°C, followed by analysis using fluorescence and enzymatic methodologies. Complementary investigations can delineate blood and feces anti-GM1 responses to antigen-associated liposomes after subcutaneous and oral administration.

Animal Model: Murine.

Interpretation and Analysis: Liposome formulations I and II are more stable in buffer at 25°C. Formulations III and IV are more stable in plasma at 37°C; formula VI is more stable in plasma at 37°C than in buffer or saliva. Formulations IV and V liposomes are both stable in saliva for at least 1 hour. After immunization of formula IV, no detectable antiganglioside GM1 antibody response can be detected. Negative stain transmission electron microscopy shows that liposomes containing SM, GM1, GM type III, and DIAPC: DMPC are twice the size of those made with EggPC/Chol. The hydrophobicity factor expressed as A(570/500) is obtained using the probe merocynine 540 (MC540). The order of fluidity increases from mixture II < mixture I < mixture III < mixture IV < mixture V < mixture VI. Although the high hydrophobicity factor for polymerizable lipids is an important characteristic, there are other factors like stability that must be taken into account according to the desired route of administration. The hydrophilicity of groups protruding from the membrane interphase into the surrounding solution in the case of subcutaneous inoculation is very relevant, and for oral administration, stability is an important property that needs to be characterized due to exposure to different biologically active fluids within the gastrointestinal tract. Liposome preparations containing GM1, GM type III, or DIAPC/DMPC are therefore stable in saliva and plasma at 37°C and can potentially serve effectively as a delivery vehicle for oral and subcutaneously administered non-viral vaccines.

Study of *in vitro* stability of liposomes and *in vivo* antibody response to antigen associated with liposomes containing GM1 after oral and subcutaneous immunization. Fabani MM, Gargini R, Taira MC, Iacono R, Alonso-Romanowski S. J Liposome Res 2002 Feb–May; 12(1–2), pp. 13–27.

Liposomes/Peptide Delivery/Biological Proteins: Anti-Inflammatory Interleukins
Liposome-(Interleukin [IL]-4)/Liposome-(IL-10)

Inflammatory bowel disease (IBD) is comprised of the 2 disorders ulcerative colitis (UC) and Crohn's disease (CD). Although the etiology is still unclear, initiation and aggravation of the inflammatory processes seem to be due to a massive local mucosal immune response. An increased number of greatly activated macrophages seems to contribute to the onset of IBD by expressing upregulated co-stimulatory molecules (e.g., CD80/CD86) and a cytokine profile favoring a type I proinflammatory response. The release of IL-2 and interferon-gamma (IFN-γ) by naive T lymphocytes predominantly stimulates cytotoxic T lymphocytes, macrophages, and natural killer (NK) cells and increases the antigen-presenting potential of all these cell types. Opposite this proinflammatory immune reaction a compensatory Type II anti-inflammatory response has been suggested to occur within inflamed mucosa, involving mainly IL-4 and IL-10. Both cytokines are able to downregulate inflammatory mediators, including tumor necrosis factor-alpha (TNF-α) and IL-1, and favor a humoral immune response. Previous clinical trials have evaluated local liposome-mediated gene transfer of these 2 anti-inflammatory cytokines, IL-4 and IL-10, in patients with severe IBD of the rectum. Local administration of anti-inflammatory cytokines avoids toxic systemic side effects and prevents blocking of the beneficial effects of proinflammatory cytokines (e.g., TNF-α in other tissue compartments and increases the local concentration of IL-4 and IL-10 over a prolonged period of time). The combined effects of IL-4 and IL-10

have been shown to shift the Th1/Th2 cell activation in favor of a Th2 immune response, which seems to be essential for fighting against the inflammation and facilitating healing.

Transfer of interleukin-4 and interleukin-10 in patients with severe inflammatory bowel disease of the rectum. Rogy MA, Beinhauer BG, Reinisch W, Huang L, Pokieser P. Hum Gene Ther 2000 Aug 10; 11 (12), pp. 1731–41.

Liposomes/Peptide Delivery/Biological Proteins
Liposomes/Example: Liposome-Encapsulated Interferon-Alpha-2b (IFN-α-2b)

Cellular/Molecular Mechanism of Action: Liposomes can be applied for the purpose of selective delivery, protection against premature excretion or metabolism, and transport across biological membrane barriers.

Background Information: Systemic administration of IFN-α-2b by injection for treatment of dermal fibrosis is uncomfortable, requires a large quantity of the cytokine, and cannot be easily used in children. IFN-α-2b can potentially be encapsulated within liposomes without compromising its *in vitro* antifibrogenic effects on dermal fibroblasts. In this context, liposomes are a potentially useful vehicle for the topical delivery of various pharmaceuticals and biological agents.

Application: Modality for the delivery of biologically active peptides across cellular membranes and cutanous barriers.

Route of Administration: Topical application of an IFN-α-2b liposome cream preparation.

Dosage: IFN-α-2b-encapsulated liposomes (100 mg/wound containing 3,000 units of liposome-encapsulated IFN-α-2b).

Semi-Synthetic Methods: Empty sonicated liposome vesicles are mixed with various concentrations of IFN-α-2b and then dried and rehydrated. An enzyme-linked immunosorbent assay (ELISA) can be used to determine the efficiency of encapsulation and the stability of the preparation under experimental conditions.

Implementation: Topical application and evaluation of the the ability of liposomes to effectively deliver encapsulated IFN-α-2b as a function of reduced scar tissue formation. Efficacy of peptide delivery can be assessed by applying liposome preparations on wounds (e.g., right side receiving a cream of liposome-encapsulated IFN-α-2b, while wounds on the left side receiving cream containing empty liposomes). Northern analysis can be applied to evaluate the messenger RNA (mRNA) expression for Type I and Type III collagens. Cellularity can be assessed by measurement of cellular vimentin content.

Animal Model: Guinea pig (n = 36 full thickness skin wounds; n = 6/animal, n = 3 on each side that are made with an 8 mm disposable punch).

Interpretation and Analysis: There is a significant reduction in rate of contraction of wounds treated with IFN-α-2b liposomes as early as 5 days after wounding. Reductions remain significant for a period of up to 10 days. Northern analysis reveals that expression of mRNA transcripts for Type I and Type III collagens in response to IFN-α-2b is markedly reduced in abundance for the pro-α1(I) chain of type 1 collagen on days 11 and 14 after wound production. Similarly, the level of mRNA for Type III procollagen is markedly reduced as early as day 7 and remains depressed up to day 14. These findings were consistent with results obtained for the total collagen content in tissue samples. Cellularity of IFN-α-2b-treated wounds, assessed by measurement of vimentin content, is also markedly reduced at day 7 and remains depressed for up 14 days. Liposome-associated IFN-α-2b applied 5 days after completion of epithelialization reduces mRNA for the pro-α1(I) chain of Type 1 collagen, confirming its trans-epidermal penetration and effectiveness. The activity of liposome-associated IFN-α-2b *in vivo* supports the concept of the topical use of antifibrogenic agent of this type for treatment of fibroproliferative disorders.

Liposome-associated interferon-alpha-2b functions as an antifibrogenic factor in dermal wounds in the guinea pig. Ghahary A, Tredget EE, Shen Q, Kilani RT, Scott PG, Takeuchi M. Mol Cell Biochem 2000 May; 208 (1–2), pp. 129–37.

Liposomes/Pharmacokinetic Profile/Prolongation
[^{51}Cr]-D-Mannose Surface-Modified Liposomes/ [^{51}Cr]-6-Amino-6-Deoxy-D-Mannose Surface-Modified Liposomes

Cellular/Molecular Mechanism of Action: The relatively massive size of liposomes compared to simple molecules prevents the elimination of pharmaceutical or biological peptides contained within their interior. The size of liposomes prevents their passage into the glomerular filtrate. Liposomes also prevent biological degradation and can facilitate selective delivery. Modification of the chemical composition of the external surface of liposomes with *D*-mannose and 6-amino-6-deoxy-*D*-mannose can facilitate the selective delivery and site-specific release/deposition of diagnostic radioisotopes (e.g., [^{51}Cr]-).

Application: Selective delivery of diagnostic pharmaceutical agents facilitated by molecular determinant incorporated into the exterior surface of liposome membranes.

Route of Administration: Intravenous.

Synthetic Chemistry and Analysis: Liposomes with synthetic saccharide determinants can be prepared from synthetic cholesterol conjugates of *D*-mannose and 6-amino-6-deoxy-*D*-mannose and labeled with [^{51}Cr]-chromate.

Implementation: The kinetics and tissue distribution of the radiolabel can be determined after footpad and subcutaneous injection (mice). These results show that incorporation of synthetic glycolipids can substantially alter the *in vivo* lifespan and distribution of liposomes outside the bloodstream. Such surface-modified liposomes may be useful for sustained release or selective delivery of therapeutic or diagnostic agents.

Animal Model: Murine.

Interpretation and Analysis: Liposomes bearing either of the saccharides determinants *D*-mannose and 6-amino-6-deoxy-*D*-mannose greatly increase retention of label at the injection sites compared to control liposomes, which contain no glycolipid, and to free [^{51}Cr]-chromate. Draining lymph nodes contain small fractions of the injected radioactivity but in some cases the retention is saccharide dependent and highly concentrated. Incorporation of synthetic glycolipids can substantially alter the *in vivo* lifespan and distribution of liposomes outside the intravascular compartment.

Modified *in vivo* behavior of liposomes containing synthetic glycolipids. Wu MS, Robbins JC, Bugianesi RL, Ponpipom MM, Shen TY. Biochim Biophys Acta 1981 Apr 17; 674 (1), pp. 19–29.

Liposomes/Sepsis and Inflammation: Peptide Delivery/Anti-Inflammatory Interleukins (Ils)
Liposome-(IL-4)/Liposome-(IL-10)

Inflammatory bowel disease (IBD) is comprised of the 2 disorders ulcerative colitis (UC) and Crohn's disease (CD). Although the etiology is still unclear, initiation and aggravation of the inflammatory processes seem to be due to a massive local mucosal immune response. An increased number of greatly activated macrophages seems to contribute to the onset of IBD by expressing upregulated co-stimulatory molecules (e.g., CD80/CD86) and a cytokine profile

favoring a type I proinflammatory response. The release of IL-2 and interferon-gamma (IFN-γ) by naive T lymphocytes predominantly stimulates cytotoxic T lymphocytes, macrophages, and natural killer (NK) cells and increases the antigen-presenting potential of all these cell types. Opposite this proinflammatory immune reaction a compensatory Type II anti-inflammatory response has been suggested to occur within inflamed mucosa, involving mainly IL-4 and IL-10. Both cytokines are able to downregulate inflammatory mediators, including tumor necrosis factor-alpha (TNF-α) and IL-1, and favor a humoral immune response. Previous clinical trials have evaluated local liposome-mediated gene transfer of the two anti-inflammatory cytokines, IL-4 and IL-10, in patients with severe IBD of the rectum. Local administration of anti-inflammatory cytokines avoids toxic systemic side effects and prevents blocking of the beneficial effects of proinflammatory cytokines (e.g., TNF-α in other tissue compartments and increases the local concentration of IL-4 and IL-10 over a prolonged period of time). The combined effects of IL-4 and IL-10 have been shown to shift the Th1/Th2 cell activation in favor of a Th2 immune response, which seems to be essential for fighting against the inflammation and facilitating healing.

Transfer of interleukin-4 and interleukin-10 in patients with severe inflammatory bowel disease of the rectum. Rogy MA, Beinhauer BG, Reinisch W, Huang L, Pokieser P. Hum Gene Ther 2000 Aug 10; 11 (12), pp. 1731–41.

Liposomes/Septic Abscesses: *Staphylococcus Aureus Biotin-Poly(Ethylene Glycol) (PEG)-Distearoylphosphatidylethanolamine/Avidin*

Cellular/Molecular Mechanism of Action: Avidin binds with high affinity to biotin to form a stable complex.
Application: Detection and characterization of localized regions of septic inflammation (e.g., abscess).
Route of Administration: Intravascular.
Semi-Synthetic Methods: Biotinylated liposomes were radiolabeled with [^{99m}technetium] ([^{99m}Tc])-hydrazinonicotinamide.
Implementation: Evaluation of avidin to determine if it can be applied to accelerate the clearance of biotinlyated liposomes from the intravascular compartment after a portion of the dose has accumulated within a septic lesion. Biotin can then be coupled to PEG-distearoylphosphatidylethanolamine (DSPE) and subsequently incorporated into PEG-liposomes followed by radiolabeling with [^{99m}Tc]-hydrazinonicotinamide. Efficacy of preparations can be assessed by performing *in vitro* binding studies to determine the optimal liposome biotin concentration. In biodistribution analyses performed *in vivo* with [^{99m}Tc]-biotin-PEG liposomes, the findings are then compared with the biodistribution profiles corresponding to non-biotinylated [^{99m}Tc]-PEG liposomes. Complementary *in vivo* investigations can be employed to assess both the effect of the biotin content and the optimal avidin dose that allows for the most efficient clearance of liposomes. Other analysis that can be peformed includes the application of liposomes containing 0.5 or 1.0 mol% biotin-PEG-DSPE in order to compare their *in vivo* ability to accumulate within septic foci. Avidin is injected 4 hours after injection of liposomes.
Animal Model: Rat biodistribution studies (septic foci created by injection of *S. aureus* into the left calf musculature). Avidin is then injected 4 hours after liposome injections.
Interpretation and Analysis: Biotinylation of the liposomes does not affect their *in vivo* behavior. All biotin-PEG liposome formulations display good *in vitro* avidin binding with 50% inhibitory concentrations ranging from 36 to 8 μmol/L. With avidin doses higher than 100 μg, both preparations are rapidly cleared from the circulation. As a result, abscess-to-blood ratios increase 5-fold. In an *in vivo* environment, avidin promotes clearance of radiolabeled PEG-liposomes and improves the detection of subcutaneous *S. aureus* abscesses applying [^{99m}Tc]-biotin-PEG liposomes as an imaging agent. The infection can best be visualized only after injection of 100 μg avidin. Therefore, biotin-coated [^{99m}Tc]-PEG liposomes in combination with the injection of avidin can lead to improved imaging of infection or localized inflammation especially in regions with high blood-pool activity.

Improved imaging of infections by avidin-induced clearance of [^{99m}Tc]-biotin-PEG liposomes. Laverman P, Zalipsky S, Oyen WJ, Dams ET, Storm G, Mullah N, Corstens FH, Boerman OC. J Nucl Med 2000 May; 41 (5), pp. 912–8.

Liposomes/Thermosensitive Delivery/Melanoma Plumbagin Delivery
Liposomes: Thermosensitive Preparations/Localized Hyperthermia

Semi-Synthetic Methods: Small-sized, thermosensitive liposomes can be prepared by thin film hydration in combination with sonication. Liposome products are then evaluated for the characteristics of size, phase transition temperature, *in vitro* drug release, and stability. The results of particle size analysis indicate that almost 90% of the vesicles are below 0.19 microm in size. The phase transition temperature of liposomes as determined by differential scanning calorimetry is approximately 41.32°C. The results of *in vitro* release studies in phosphate buffered saline with plasma (e.g., murine) indicate that maximum drug release (51.25%) occurs at 42°C, compared to the less than 9% release at 37°C. Superior stability profiles are observed when plumbagin liposomes are stored at 4°C.
Implementation: Evaluation of the capacity for thermosensitive liposomes and the application of local hyperthermia to facilitate selective *in vivo* delivery of plumbagin to melanoma lesions.
Animal Model: Murine (mice bearing C57B:/6J melanoma)
Interpretation and Analsis: When combined with the application of localized hyperthermia (43°C, 30 minutes or 1 hour), liposomal plumbagin administered intravenously in conditions of melanoma exhibits better anticancer activity compared to in vivo models treated with an equivalent dose of free plumbagin with or without hyperthermia, which is evident by enhanced tumor volume doubling time and growth delay.

Temperature sensitive liposomes of plumbagin: Characterization and *in vivo* evaluation in mice bearing melanoma B16F1. Tiwari SB, Pai RM, Udupa N. J Drug Target 2002 Dec; 10 (8), pp. 585–91.

Liposome Production/Cryoprotection: Hyaluronan/Sustained Release
Liposomes: Lyophilized/Unilamellar

Cellular/Molecular Mechanism of Action: Liposomes function as a form of encapsulation for various diagnostic and therapeutic agents, thereby diminishing the rate of biological degradation and premature elimination and potentially facilitating selective delivery. Hyaluronan incorporation into liposomes can enhance their bioadhesive properties but also serves as a cryoprotectant involving the function of substitute structure-stabilizing H-bonds in a manner similar to cryoprotectant sugars.
Physiological Mechanism of Action: Liposomes protect pharmaceutical agents from certain forms of degradation, enhance high-concentration delivery to specific organ/tissues, and provide time-delayed release in a manner that creates improved pharmacokinetic parameters.
Background Information: Lyophilized unilamellar liposomes (ULV) revert upon reconstitution to larger multilamellar liposomes (MLV). Such confirmational alterations are detrimental to the many carrier-mediated agents that require delivery facilitated by particles

of small size. High doses of sugars such as trehalose, sucrose, and others, included in the original formulations for cryoprotection, have also been shown to prevent conversion to multilamellar liposomes.

Application: Selective, protected delivery of diagnostic and pharmaceutical agents.

Route of Administration: Usually intravenous.

Dosage: Dependent upon intent and nature of encapsulated pharmaceutical agent.

Implementation: Characterization and evaluation of liposomes pertaining to assessement of structural and physicochemical properties in order to identify differences between reconstituted hyaluronan-unilamellar liposomes (HA-ULV) and similar regular ULV (RL-ULV) liposomes (e.g., reference controls). In the evaluation of liposomes, several properties need to be delineated, including particle size, zeta potential, encapsulation efficiency, and half-life of drug release ($tau_{1/2}$) for each individual pharmaceutical agent (e.g., chloramphenicol [CAM], vinblastine [VIN,] and mitomycin C [MMC]].

Interpretation and Analysis: Encapsulation efficiencies are very similar for RV-ULV liposomes and HA-ULV liposomes (range 25–70%). All liposome preparations are capable of acting as sustained-release drug depots with drug-release ($tau_{1/2}$) ranging from 1.3 to 5.3 days. Drug species and lipid composition are the major determinants that influence encapsulation and release properties. Lyophilization of liposomes creates several significant changes in the reconstituted RV-ULV liposome (17-fold increase in diameter; tripling of zeta potential; 25–60% drop in encapsulation efficiencies; 25–30% decrease in drug-release [$tau_{1/2}$]). Alternatively, reconstituted HA-ULV preparations retain the same physical dimensions, zeta potentials, encapsulation efficiencies, and drug-release ($tau_{1/2}$) of the original systems. In this context, hyaluronan appears to function as a cryoprotectant, and these properties are believed to be provided by substitute structure-stabilizing H-bonds in a manner similar to cryoprotectant sugars.

Hyaluronan is a key component in cryoprotection and formulation of targeted unilamellar liposomes. Peer D, Florentin A, Margalit R. Biochim Biophys Acta 2003 May 2; 1612 (1), pp. 76–82.

Liposome Production/Cryoprotection
Liposomes: Sodium Diclofenac/Lactose Cryoprotectant/ Mannitol Cryoprotectant

Semi-Synthetic Methods: Large liposomes (1–10 microm) containing sodium diclofenac can be prepared in lyophilized form using lactose or mannitol (7.5% in respect to the lipid content) as cryoprotectants.

Interpretation and Analysis: Physical studies on liposomes performed during 30 days of storage in a dry or resuspended form reveals that lyophilization of large liposomes and storage in dry form at 5°C increases their physical stability. Lactose is a cryoprotectant that does not create changes in liposome physical properties pertaining to size, encapsulation efficacy, and release rate. Large liposomes lyophilized in the presence of mannitol tend to increase in size and encapsulation efficacy, but the lipid bilayers are stabilized and less permeable to sodium diclofenac.

The effect of cryoprotectants on the physical properties of large liposomes containing sodium diclofenac. Janicki S, Jankowski J, Szulc J, Woyczikowski B, Sznitowska M. Acta Pol Pharm 2002 May–Jun; 59 (3), pp. 187–91.

Liposome Production/Determinants of Structure
Liposomes: Calcein

Semi-Synthetic Methods: Extrusion methods can be used to prepare liposomes composed of egg phosphatidylcholine (EggPC) or dimyristoylphosphatidylcholine (DMPC) and containing poly(ethylene glycol) (PEG)-lipids covering a range of 0 to 30 mol%. Physicochemical properties including size evolution and calcein permeation can be evaluated to determine the effect of PEG-lipids on bilayer structure.

Interpretation and Analysis: The results from quasielasetic light scattering (QELS), freeze-fracture microscopy, and gel exclusion chromatography reveal that low PEG-lipid concentrations results in declines in vesicle size. Increases in PEG-lipid concentrations lead to a transition from the production of lamellar membranes to micelles. The permeability for calcein increases with increases in the concentration of distearoylphosphatidylethanolamine (DSPE)-PEG. Conversely, permeability decreases with low amounts of cholesterol-PEG (below 20% cholesterol-PEG) and increases with high amounts of it. The maximum concentration of PEG-lipid that may be incorporated without alteration of the liposome structure depends on the bilayer composition. The concentration of DSPE-PEG2000 incorporated into vesicles without damaging vesicle structures is < 20 mol% for EggPC and < 10% for DMPC.

Physicochemical properties of PEG-grafted liposomes. Sriwongsitanont S, Ueno M. Chem Pharm Bull (Tokyo) 2002 Sep; 50 (9), pp. 1238–44.

Liposome Production/Dispersion: Delayed Pharmaceutical Release
Dispersions (Concentrated): Vesicular Phospholipid Gels/Chemotherapeutics: 5-Fluorouracil (5-FU)

Applications: Vesicular phospholipid gels (VPG), also known as highly concentrated liposomal dispersions, are suitable for entrapping substances such as anticancer drugs with particular high-encapsulation efficiencies (EE).

Semi-Synthetic Methods: Different formulations of VPG can be prepared with 30% (w/w) lipid containing 5-FU applying high-pressure homogenization. Analysis of preparations involves assessment of EE and drug release capabilities. Using mixtures of hydrogenated soy phosphatidylcholine and cholesterol with molar ratios ranging from 55/45 to 75/25, a decrease in cholesterol content correlates with an increase in EE that is probably due to a reduced amount of smaller vesicles and number of lamellae.

Interpretation and Analysis: Using a 5-FU solution at a pH 8.6 for VPG preparation, an EE of approximately 40% can be achieved after redispersion of the gel to a liposomal dispersion and separation of free drug from liposomal drug by size exclusion chromatography. The reduced encapsulation efficiency for preparations at lower pH levels is attributed to a fast initial drug release due to the increased drug lipophilicity below an 8 pKa value. After redispersion of a VPG of pH 8.0, an initially faster release of about a 3rd of the entrapped drug is detected during the 1st 20 minutes, followed by stable entrapment over many hours. The rapid initial release may be due to the portion of liposomes smaller than 40 nm in diameter, detected by photon correlation spectroscopy. Cryo electron microscopic images reveal that these small liposomes have a lentil-like shape. The membrane defects on the edges are probably the reason for the very high initial drug release rate. The half-life time for the release of 5-FU from intact FU-VPG at both pH 7.4 and 8.0 is in the order of 4 to 5 hours and the kinetics are typical for matrix-controlled drug diffusion. The *in vitro* data of 5-FU loaded VPG suggest their applicability as implants with controlled release properties or, after redispersion, as intravenously injected liposomal formulations.

5-fluorouracil in vesicular phospholipid gels for anticancer treatment: Entrapment and release properties. Kaiser N, Kimpfler A, Massing U, Burger AM, Fiebig HH, Brandl M, Schubert R. Int J Pharm 2003 Apr 30; 256 (1–2), pp. 123–31.

Liposome Production / Lyophilization of Pharmaceuticals/Example: Doxorubicin *Liposomes*

Background Information: Doxorubicin can be loaded into preformed liposome by remote loading. Lyophilization of liposomes results in particle size increase and content leakage. Cryoprotectants have been used to improve the stability of liposomal formulations during lyophilization.
Semi-Synthetic Methods: Liposomes composed of egg phosphatidylcholine/cholesterol and containing either glucose or sucrose as a cryoprotectant can be prepared by polycarbonate membrane extrusion. Doxorubicin can be loaded into liposome preparations by a pH-gradient-based technique performed either before or after lyophilization and reconstitution. The loading efficiency of doxorubicin can be established by gel-filtration chromatography.
Implementation: Evaluation of a formulation kit for liposomal doxorubicin production based on lyophilized liposomes incorporating specific cryoprotectants.
Route of Administration: Intravenous.
Interpretation and Analysis: Cryoprotectants are effective in maintaining liposome size distribution but not drug retention during lyophilization. Doxorubicin loading efficiency of reconstituted liposomes is nearly quantitative and comparable to that of freshly prepared liposomes. In this context, liposomal doxorubicin preparations can be stored as a lyophilized product that can be reconstituted without significant changes to critical formulation properties.

Formulation kit for liposomal doxorubicin composed of lyophilized liposomes. Stevens PJ, Lee RJ. Anticancer Res 2003 Jan–Feb; 23 (1A), pp. 439–42.

Liposome Production/Membrane Characterization/ Optimization of Properties *ANS Fluorescent Probe/Diphenylhexatriene (DPH) Fluorescent Probe/Poly-L-Lysine Backbone*

Cellular/Molecular Mechanism of Action: Fluorescent probes emit detectable light energy when excited by specific wavelengths of radiant energy.
Application: Fluorescent probes can be applied as visible molecular makers for assessing various *in vitro* and *in vivo* properties associated with liposome preparations.
Implementation: Evaluation of the systematic physicochemical characteristics of 2 branched chain polymers (based on a poly-*L*-lysine backbone) with a general formula poly[Lys-(DL-Ala_m-X_i)], where X = Orn (OAK) or *N*-acetyl-Glu (Ac-EAK) and m congruent with 3, using surface pressure and fluorescence polarization methods. Experimental findings are compared with those of the linear poly-*L*-Lys from which OAK and Ac-EAK are derived. Complementary investigations can involve the delineation of interactions between these polymers with phospholipid bilayers (neutral or negatively charged) with vesicles labeled with 2 fluorescent probes: ANS and DPH.
Interpretation and Analysis: The 2 polymers show a moderate surface activity, able to form stable monomolecular layers at the air-water interface. Poly-*L*-Lys, the most hydrophilic, has the lowest surface activity. Results indicate that the polymers are able to accommodate in their internal structure, mainly through electrostatic interactions, a certain amount of ANS marker molecule, but fluorescence increases of the ANS-polypeptide complexes are so low that its influence in further polarization measurements can be discarded. After interaction with liposomes, polymers induce an increase in the polarization of the probes, thus indicating a rigidification of the bilayers. Electrostatic forces seem to be very important in this interaction; cationic polymers are clearly more active, with PG-containing liposomes, than Ac-EAK. Moreover, in assays, poly-*L*-Lys behaves as the more active compound. This fact is probably due to its major ability to form alpha-helical structures that can insert readily into bilayers. Such findings indicate that the polymeric structures studied can be used as carriers for biologically active molecules, because their interactions with bilayers remain soft and have a positive effect on the stability of the membranes.

Physicochemical characterization of branched chain polymeric polypeptide carriers based on a poly-lysine backbone. Nagy IB, Hudecz F, Alsina MA, Reig F. Biopolymers 2003 Nov; 70 (3), pp. 323–35.

Liposome Production/Stability and Biodistribution/ Radiolabeling Reagents *[^{99m}Technetium] ([^{99m}Tc])-BMEDA/[^{99m}Tc]-BMEDA + BT/ [^{99m}Tc]-BMBuA/[^{99m}Tc]-BMBuA + BT*

Background Information: Liposomes are important carriers for controlled drug release, for gene or antisense therapy, and for immunization. Radiolabeled liposomes can be used to evaluate the *in vivo* behavior of different liposome formulations as well as their applicability for diagnostic imaging and radionuclide therapy.
Application: Radiolabeling of liposomes can be performed in order to delineate their *in vivo* stability and biodistrubution for research or clinical purposes.
Route of Administration: Intravenous.
Semi-Synthetic Methods: Methods have been described for radiolabeling liposomes with [^{99m}Tc]-"SNS/S" complexes, in addition to [^{186}Re] and [^{188}Re] (e.g., therapeutic radionuclides). One production strategy involves [^{99m}Tc] labeling of *N*,*N*-bis(2-mercaptoethyl)-*N*′, *N*′-diethyl-ethylenediamine (BMEDA), *N*,*N*-bis(2-mercaptoethyl)-1-butylamine (BMBuA), and benzene thiol (BT), yielding [^{99m}Tc]-BMEDA, [^{99m}Tc]-BMEDA + BT, [^{99m}Tc]-BMBuA, and [^{99m}Tc]-BMBuA + BT, which can ultimately be applied for the labeling of glutathione-encapsulated liposomes.
Implementation: Evaluation of the *in vitro* properties and *in vivo* biodistribution of [^{99m}Tc]-liposomes composed of distearoyl phosphatidylcholine (DSPC) and cholesterol.
Interpretation and Analysis: Labeling efficiencies for [^{99m}Tc]-reduced glutathione (GSH) liposomes is approximately 36.9–69.2%. After incubation in serum, [^{99m}Tc]-GSH liposomes labeled with [^{99m}Tc]-BMEDA or [^{99m}Tc]-BMEDA + BT have the best labeling stability. Distribution studies after intravenous injection of [^{99m}Tc]-liposomes composed of distearoyl phosphatidylcholine (DSPC) and cholesterol have a slow blood clearance and high spleen accumulation, demonstrating the *in vivo* labeling stability of the radiolabeled liposomes. Preparations of [^{99m}Tc]-liposomes have great potential as a radiopharmaceutical agent applicable for evaluating various kinds of liposomes of different lipid compositions, for evaluating in advance a subsequent radionuclide therapy strategy using [^{186}Re]- or [^{188}Re]-labeled liposomes, and for specialized diagnostic imaging procedures.

A novel liposome radiolabeling method using 99mTc-"SNS/S" complexes: *In vitro* and *in vivo* evaluation. Bao A, Goins B, Klipper R, Negrete G, Mahindaratne M, Phillips WT. J Pharm Sci 2003 Sep; 92 (9), pp. 1893–904.

Liposome Production/Aqueous and Oily Phase Preparations

Application: Modification of the rate at which pharmaceuticals are absorbed from the gastrointestinal tract.
Route of Administration: Oral.

Dosage: Not applicable.
Semi-Synthetic Methods: Ampicillin trihydrate can be formulated in different oily and aqueous suspensions.
Implementation: Evaluation of the bioavailability of aqueous and oily phase ampicillin in plasma samples determined by using microbiological-based biological assay techniques.
Interpretation and Analysis: Oily and sucrose-containing aqueous formulations enhance the extent of ampicillin absorption (nearly statistically significant). Ampicillin appears to be absorbed at essentially the same rate from both aqueous and oily formulations. The latter shows plasma-level time curves with biphasic absorption profiles and is likely to produce prolonged plasma concentrations of ampicillin because of the effects of enterohepatic recycling. Viscosity appears to play an insignificant role in the results obtained because the bioavailability parameters correlate poorly with the viscosity, except Cmax. It has been suggested that enhancement in ampicillin bioavailability is due to a decrease in the gut transit rate brought about by the oil, which predominates and masks the other effects of viscosity and osmotic effects of sucrose. Existence of a correlation between the *in vitro* drug-release rate (t50%) and viscosity, in addition to the lack of correlation between *in vivo* and *in vitro* parameters, supports the suggestion and indicates that traditional dissolution rate tests, such as flask-stirrer method, are unsatisfactory as bioavailability indicators when applied in dosage forms that cause marked changes in physiological factors like gastroesophogeal reflux (GER) and biliary excretion.

Effects of oils and pharmaceutical excipients on the bioavailability of ampicillin orally administered, different oily and aqueous suspensions in rabbit. Alhamami OM. Drug Dev Ind Pharm 2003 Jan; 29 (1), pp. 51–60.

Liposome Production/Biopolymers/Microcapsules/Casein
Sustained Release of Pharmaceuticals

Cellular/Molecular Mechanism of Action: Casein microcapsulation acts like a molecular matrix that can incorporate pharmaceuticals in a manner that facilitates delayed/sustained release.
Application: Casein microcapsules can function as a sustained-release system (e.g., acetaminophen drug model).
Semi-Synthetic Methods: Casein microcapsules can be prepared by aqueous coacervation in lactate buffer containing gelatin, hydroxypropyl cellulose (HPC), and lecithin. After preparation, microcapsules (± glutaraldehyde) can be treated with a cross-linking agent. Casein microcapsules are subsequently loaded using 2 distinctly different procedures, either by (i) dissolving 50% of the drug (w/w), relative to casein, in the polymer dispersion; or by (ii) dissolving the drug in a coacervating solution. The drug introduced into casein/microcapsules can be quantified applying high-performance liquid chromatography (HPLC) following an enzymatic degradation assay, complemented by scanning electron microscopy and thermal analysis (differential scanning calorimetry and thermogravimetrical analysis) of casein/microcapsule preparations.
Considerations: The use of biopolymers in sustained-release systems has been studied by many research groups because of the bioavailability and biodegradability of these compounds. Casein is a natural biopolymer whose degradation results in biologically utilizable compounds.
Interpretation and Analysis: Loading of the pharmaceutical agent is approximately 8% (w/w) and is associated with a high resistance to enzymatic degradation. The absence of an acetaminophen melting peak indicates that there is no drug present on the surface of the cross-linked systems. In addition, pharmaceutical loading is accompanied by a reduction of the specific heat capacity of the systems and suggests a decrease in stability. The outer morphology of the encapsulating polymer is affected by the process of microencapsulation. Therefore, the microencapsulation process of aqueous coacervation and cross-linking appears to be appropriate for the preparation of microencapsulated systems for sustained drug delivery.

Physicochemical characterization and enzymatic degradation of casein microcapsules prepared by aqueous coacervation. Santinho AJ, Ueta JM, Freitas O, Pereira NL. J Microencapsul 2002 Sep–Oct;19 (5), pp. 549–58.

Liposome Production/Solubilization of Lipophilic Pharmaceuticals and Biopharmaceuticals/Hydrophilic Compound Coating/Evaporative Precipitation into Aqueous Solution
Dichloromethane/Carbamazepine

Cellular/Molecular Mechanism of Action: Evaporative precipitation into aqueous solution (EPAS) in order to coat poorly water-soluble drugs.
Application: "Coating" poorly water-soluble drugs.
Methodology: A heated organic solution of the drug in dichloromethane is sprayed though a fine nozzle into a heated aqueous solution.
Interpretation and Analysis: The rapid evaporation of the organic solvent produces high supersaturation and rapid precipitation of the drug in the form of a colloidal suspension that is stabilized by a variety of low molecular weight and polymeric surfactants. The stabilizer adsorbs to the drug surface and prevents particle growth and crystallization during the spray process. The suspensions are dehydrated by spray drying or ultra-rapid freezing. The high dissolution rates are a consequence of the following advantages of the EPAS process: a small primary particle size, a hydrophilic coating on the particles that enhances wetting, and low crystallinity.

Enhanced drug dissolution using evaporative precipitation into aqueous solution. Sarkari M, Brown J, Chen X, Swinnea S, Williams RO 3rd, Johnston KP. Int J Pharm 2002 Aug 28; 243 (1–2), pp. 17–31.

MICROBUBBLES AND MICROSPHERES

Acoustic Parameters
Definity/Optison/SonoVue/Sonazoid

Implementation: Evaluation of the acoustic properties of 4 ultrasonic contrast agents (Optison, Definity, SonoVue, and Sonazoid) at 30 MHz. A range of contrast agent concentrations can be studied using either saline or glucose as a diluting media. Mean backscatter power is measured within regions-of-interest (ROIs) at distances of 1, 1.5, 2, 3, 4, and 5 mm from the center of the intravascular probe and normalized to the ultrasound (US) data collected from a standard glass reflector.
Interpretation and Analysis: For all of the agents, the mean backscatter power at 30 MHz varies in a linear manner with concentration between 0.01 million microbubbles/mL and 1 million microbubbles/mL. Furthermore, for 2 of the agents, mean backscatter enhancement is detectable at concentrations as low as 2 microbubbles/sample volume.
Instrumentation: Boston Scientific ClearView Ultra intravascular US scanner modified to allow access to the unprocessed US data.

In vitro acoustic characterization of 4 intravenous ultrasonic contrast agents at 30 MHz. Moran CM, Watson RJ, Fox KA, McDicken WN. Ultrasound Med Biol 2002 Jun; 28 (6), pp. 785–91.

Arthritis/Rheumatoid Arthritis of the Knee/ Inflammatory vs Non-Inflammatory Pannus Vascularity
Levovist

Application: Characterization of vascular structures including the intra-articular vascularity of knee-joint synovial pannus in conditions of rheumatoid arthritis (RA) using power Doppler ultrasonography (PDS) and an echo-contrast-enhancing agent.
Implementation: Evaluation of arthritic knee joints. Disease activity is evaluated using the disease activity score (DAS) and the chronic arthritis systemic index (CASI) for each case. Examinations are performed with conventional ultrasonography and PDS before injection of an intravenous ultrasound contrast agent (Levovist). Quantitative estimation of synovial membrane vascularity is performed utilizing time-intensity curves and calculation of the area-under-the-curves (AUCs) for specific regions-of-interest (ROIs). Parameters calculated from time-intensity curves are then correlated with measures of disease activity, including clinical and functional assessments (e.g., number of swollen joints, intensity of pain, general health-visual analogue scale, disability index-Health Assessment Questionnaire, Ritchie articular index) and clinical pathology analyses. Disease activity is evaluated using the DAS and the CASI for each case.

Human Model: Cases with signs and history of arthritis of the knee ($n = 42$ rheumatoid arthritis; $n = 31$ women, $n = 11$ men) classified according to a modified index of synovitis activity (active, moderately active, and inactive).
Interpretation and Analysis: The mean (standard deviation [SD]) value of the area underlying time-intensity curves is 216.2 (33.4) in cases with active synovitis, 186.8 (25.8) in cases with moderately active synovitis, and 169.6 (20.6) in those with inactive synovitis. The mean value for AUCs differs significantly between cases with active and those with inactive synovitis ($p < 0.01$). The mean value of the AUC of the entire group weakly correlates with the number of swollen joints ($p = 0.038$), but a strong correlation is found with composite indexes of disease activity such as the DAS ($p = 0.006$) and CASI ($p = 0.01$). No correlation can be appreciated for age, disease duration, and other laboratory and clinical variables. Power Doppler ultrasonography may be a valuable tool to detect fractional vascular volume and to assist clinicians in distinguishing between inflammatory and non-inflammatory pannus. The transit of microbubbles of ultrasound contrast across a tissue can be used to estimate hemodynamic alterations and may have a role in assessing synovial activity and the therapeutic response to treatment of synovitis of the knee joint.

Power Doppler sonography in the assessment of synovial tissue of the knee joint in rheumatoid arthritis: A preliminary experience. Carotti M, Salaffi F, Manganelli P, Salera D, Simonetti B, Grassi W. Ann Rheum Dis 2002 Oct; 61 (10), pp. 877–82.

Cardiovascular Endothelium: ICAM-1 Expression/ Optimization of Binding/Antibody-Density/ Wall Shear Rate
Anti-ICAM-1 Microbubbles/Lipid-Based Perfluorobutane-Filled Microbubble

Background Information: The early stages of atherosclerosis are characterized by increased endothelial cell (EC) surface expression of leukocyte adhesion molecules (LAMs). Ultrasound detection of acoustically active LAM-targeted microbubbles might provide a means to non-invasively assess the functional status of vascular endothelium.
Application: Non-invasive ultrasound assessment of the functional status of vascular endothelium.
Route of Administration: Intravascular.
Semi-Synthetic Methods: Production of a lipid-based perfluorobutane-filled microbubbles synthesized with various densities of anti-ICAM-1 monoclonal antibodies conjugated to the bubble shell (1%, 5%, 10%, 50%, 75%, and 100% of maximum coverage) and possessing various wall shear rates (100, 175, 250, 350, and 500 s^{-1}).
Implementation: Evaluation of the hypothesis that modulating the surface antibody density can permit regulation of the endothelial adhesion characteristics of microbubbles, and that microbubble adhesion is dependent on local wall shear rate. Such a hypothesis can be addressed by exposing coronary artery endothelials cells to microbubbles produced with different surface antibody densities and various wall shear rates in a parallel plate perfusion chamber.

***Ex Vivo* Model:** Human coronary artery ECs cultured on coverslips. Endothelial cells are either normal or activated by interleukin-1 beta (IL-1β) in a manner that induces ICAM-1 overexpression.
Interpretation and Analysis: Immunomicrobubble adhesion is greater in the presence of activated vs normal endothelial cell ($p < 0.001$); increases with increasing surface antibody density ($p < 0.01$); and decreases with increasing wall shear rate ($p = 0.02$). In conclusion, shell antibody density and wall shear rate are critical parameters controlling differential microbubble adhesion. This phenomenon might ultimately permit imaging of clinically relevant (*in vivo*) LAM expression.

Modulating targeted adhesion of an ultrasound contrast agent to dysfunctional endothelium. Weller GE, Villanueva FS, Klibanov AL, Wagner WR. Ann Biomed Eng 2002 Sep; 30 (8), pp. 1012–9.

Cardiovascular Endothelium: ICAM-1 Expression/ Endothelial Dysfunction: Coronary Ischemia
Microbubbles

Background Information: Clinical signs and symptoms of coronary artery disease are predated in decades by endothelial dysfunction, an aberration in the vascular lining that permits the development and propagation of atherosclerotic lesions, and vasomotor dysfunction in the arterial circulation. These ultimately lead to acute and chronic coronary ischemic syndromes. Other pathophysiologic scenarios encountered in clinical cardiology practice, such as cardiac transplant rejection and the period following coronary angioplasty or cardiac surgery, also are associated with endothelial dysfunction. Endothelial dysfunction parallels coronary risk factors and is potentially reversible, rendering early identification of the phenomenon a clinically important end point. Current methods for detecting endothelial dysfunction are limited.
Application: Characterization of vascular endothelial integrity based on the detection of alterations in the expression of surface membrane complexes, including intracellular adhesion molecule 1 (ICAM-1) using targeted ultrasound imaging techniques.
Route of Administration: Intravascular.
Considerations: Implications pertain to other disease processes besides coronary ischemia and encompass both diagnostic and therapeutic delivery strategies.
Implementation: Evaluation of endothelial cell viability and biology as a function of unique surface marker expression (e.g., ICAM-1).
Interpretation and Analysis: Myocardial contrast echocardiography using microbubbles targeted to bind to cell surface markers uniquely expressed by dysfunctional endothelial cells may offer an approach for the non-invasive detection of endothelial disease using clinical ultrasound imaging techniques.

Microbubble-endothelial cell interactions as a basis for assessing endothelial function. Villanueva FS, Klibanov A, Wagner WR. Echocardiography 2002 Jul; 19 (5), pp. 427–38.

Cardiovascular Thromboses
[Lys-Gla-Ala-Gly-Asp-Val]-Aerosomes-Entrapped Microbubbles

Cellular/Molecular Mechanism of Action: Microbubbles can function as a means for delaying excretion, suppressing biological degradation, and enhancing site-specific accumulation of diagnostic and therapeutic pharmaceutical agents.
Application: Ultrasound can be used as a primary diagnostic technique for the detection of deep venous thrombosis. Microbubble preparations can potentially enhance the detection and characterization of intravascular thrombi.
Route of Administration: Intravascular.
Semi-Synthetic Methods: In the 1st phase of production, the synthetic linear hexapeptide (lysine-glutamine-alanine-glycine-aspartate-valine) can be coupled to a lipid moiety. The targeted lipid moiety is then incorporated into a lipid blend, followed by entrapment of Aerosomes contrast agent (ImaRx) yielding perfluorobutane-entrapped microbubbles. Prior to administration microbubble preparations are then sized for uniformity.
Implementation: Evaluation of the *in vitro* capacity of sized perfluorobutane-entrapped microbubbles to (i) retain high levels of acoustic stability; (ii) recognize (bind) to blood clots; and (iii) enhance ultrasound image analysis of intravascular thromboses (blood clots).
Interpretation and Analysis: Experimental results reveal that the mean size of the specific ultrasound contrast agent (MRX-408) is about 2.0 microm. Microbubbles appear as smooth spherical structures. Microscopic analysis reveals that targeted bubbles bind to blood clots, whereas control, non-targeted bubbles do not bind to blood clots. *In vitro* acoustic studies reveal similar levels of stability for modified microbubbles compared with control microbubbles. Targeted microbubbles enhance *in vitro* blood clot visualization, whereas non-targeted microbubbles do not increase clot detection. Therefore, this new thrombus-specific ultrasound contrast agent could potentially improve the detection of thrombosis by ultrasound and may be useful for distinguishing between new and old thrombosis. Investigations performed *in vivo* have been initiated to further characterize the applicability of these preparations.

In vitro studies of a new thrombus-specific ultrasound contrast agent. Unger EC, McCreery TP, Sweitzer RH, Shen D, Wu G. Am J Cardiol 1998 Jun 18; 81 (12A), pp. 58G–61G.

Controlled/Delayed/Sustained Release of Diagnostic Agents
Microcapsules: Casein

Cellular/Molecular Mechanism of Action: Casein microencapsulation functions like a molecular matrix that can incorporate pharmaceutical agents in a manner that facilitates delayed/sustained release.
Application: Casein microcapsules can function as a sustained-release system (acetaminophen drug model).
Semi-Synthetic Methods: Casein microcapsules can be prepared by aqueous coacervation in lactate buffer containing gelatin, hydroxypropyl cellulose (HPC), and lecithin. After preparation, microcapsules (± glutarldehyde) can be treated with a cross-linking agent. Casein microcapsules are subsequently loaded using 2 distinct procedures, involving either (i) dissolving 50% of the drug (w/w), relative to casein, in the polymer dispersion; or by (ii) dissolving the drug in the coacervating solution. The drug introduced into casein/microcapsules can be quantified applying high-performance liquid chromatography (HPLC) following an enzymatic degradation assay, in concert with scanning electron microscopy and thermal analysis (differential scanning calorimetry and thermogravimetrical analysis) of casein/microcapsule preparations.
Considerations: The use of biopolymers in sustained release systems has been studied by many research groups because of the bioavailability and biodegradability of these compounds. Casein is a natural biopolymer whose degradation results in biologically relevant compounds.
Interpretation and Analysis: Loading of the drug is approximately 8% (w/w), and preparations have a high resistance to enzymatic degradation. Absence of an acetaminophen melting peak indicates that there was no drug present on the surface of the cross-linked systems. In addition, pharmaceutical loading is accompanied by a reduction of the specific heat capacity of the systems and suggests a decrease in stability. The outer morphology of the encapsulating polymer is affected by the process of microencapsulation. Laboratory data suggests that the microencapsulation processes of aqueous coacervation and cross-linking are appropriate for the preparation of microencapsulated systems for sustained drug delivery.

Physicochemical characterization and enzymatic degradation of casein microcapsules prepared by aqueous coacervation. Santinho AJ, Ueta JM, Freitas O, Pereira NL. J Microencapsul 2002 Sep–Oct; 19 (5), pp. 549–58.

Controlled/Sustained/Delayed Release/Systemic Delivery Post-Intramuscular (IM) Injection
Gelatin Microspheres/Genipin-Cross-Linked Gelatin Microspheres

Cellular/Molecular Mechanism of Action: Gradually, gelatin microspheres are degraded when implanted into tissues, thereby facilitating the release of their contents for systemic absorption from the site of deposition (e.g., IM injection).
Background Information: Gelatin microspheres have been widely evaluated as a drug carrier. Nevertheless, gelatin dissolves rather rapidly in aqueous environments, making the use of the polymer difficult for the production of long-term delivery systems. Such an undesirable feature requires the use of a cross-linking agent to form a non-soluble network within microspheres. However, the use of cross-linking agents such as formaldehyde and glutaraldehyde can lead to toxic side effects due to residual amounts of cross-linkers.
Application: Slow, prolonged release of diagnostic and therapeutic pharmaceutical agents from a site of administration (e.g., IM injection).
Route of Administration: Intramuscular (example) with potential applications for delivery to other organ systems, tissues sites, and body compartments (e.g., gastrointestinal tract, subcutaneous tissues, intrathorax, intraperitoneal, intrathecal, intravascular, and intra-articular).
Considerations: Glutaraldehyde can be used as a reference control.
Semi-Synthetic Methods: In an attempt to overcome the problem of rapid decomposition, a naturally occurring cross-linking agent (genipin) can be used to cross-link gelatin microspheres to produce a biodegradable drug-delivery system for intramuscular administration.
Implementation: Evaluate the (i) *in vitro* morphology, dynamic swelling, and antienzymatic degradation properties of test microspheres; and (ii) biocompatibility and degradability of test microspheres implanted in skeletal muscle following intramuscular injection.
Animal Model: Rat (intramuscular injection).

Interpretation and Analysis: Results obtained under controlled conditions suggest that cross-linking of gelatin microspheres with glutaraldehyde or genipin produces distinct cross-linking structures. The water transport mechanism in both glutaraldehyde- and genipin-cross-linked gelatin microspheres exhibits anomalous behavior ranging from Fickian to case II extremes. Increases in the swelling diameter for the genipin-cross-linked microspheres is significantly less than that observed for the glutaraldehyde-cross-linked microspheres. In an *in vivo* (skeletal muscle) environment, the degree of induced inflammatory reaction for tissues implanted with genipin-cross-linked microspheres is significantly less than muscle implanted with the glutaraldehyde-cross-linked microspheres. Additionally, the degradation rate for genipin-cross-linked microspheres is significantly slower than their glutaraldehyde-cross-linked counterparts. Such findings indicate that the genipin-cross-linked gelatin microspheres may be used as a long-acting drug carrier for intramuscular administration.

Genipin-cross-linked gelatin microspheres as a drug carrier for intramuscular administration: *In vitro* and *in vivo* studies. Liang HC, Chang WH, Lin KJ, Sung HW. J Biomed Mater Res. 2003 May 1; 65A (2), pp. 271–82.

Estradiol
Microspheres: Biodegradable Production

Cellular/Molecular Mechanism of Action: Encapsulation of pharmaceutical agents for protection against degradation and to delay excretion, in addition to promoting selective distribution at high concentrations to specific tissue/organ targets.
Semi-Synthetic Methods: Preparations of poly (D, L-lactic acid) (PLA)/D, L-lactide/glycolide copolymer (PLGA) can be used to produce biodegradable and biocompatible carriers to achieve sustained release of estradiol. Microspheres can be formed by an emulsification-solvent evaporation method. Quality control measures include the measurement of various parameters and properties, including *in vitro* drug release behavior; effects of polyvinyl alcohol (PVA) concentration in the aqueous phase; concentration of PLA in the organic phase; stirring speed; volume O/W ratio; weight ratio of E2/PLA fed; and polymer type and molecular weight.

Preparation and evaluation of poly (D, L-lactic acid) (PLA) or D, L-lactide/glycolide copolymer (PLGA) microspheres with estradiol. Xinteng Z, Weisan P, Ruhua Z, Feng Z. Pharmazie 2002 Oct; 57 (10), pp. 695–7.

Hysterosalpingography (Tubal Patency)/ Infertility (Female)
Levovist

Background Information: Previous experimental and clinical data suggest that insonation of echo-enhancing contrast agents with high acoustic power produces disintegration of microbubbles, resulting in a phenomenon called simulated acoustic emission (SAE).
Implementation: Evaluation of female infertility using x-ray hysterosalpingography (HSG) and SAE-HyCoSy. The order of the 2 procedures is randomly established for each case after placement of a trans-cervical balloon catheter. For SAE-HyCoSy, the ultrasound contrast medium Levovist is injected, with the acoustic power set at the maximum level permitted on ultrasound machines employing dedicated algorithms. Conventional HSG is performed for comparison.

Human Model: Cases of female infertility (n = 41 and n = 77 fallopian tubes). X-ray hysterosalpingography for infertility evaluation also receive SAE-HyCoSy.

Interpretation and Analysis: In the majority of cases, it is possible to appreciate the SAE phenomenon. In 13% of tubes, (e.g., n = 10) proximal filling is not observed by either SAE-HyCoSy or HSG. In the remaining 87% of tubes (e.g., n = 67), free spill from the distal end of the lumen can be demonstrated in 96% of cases (e.g., n = 64/67) with SAE-HyCoSy and in 97% of cases (e.g., n = 65/67) with HSG. Disagreement between the 2 techniques may be observed in only a few instances (e.g., n = 5/77; Cohen's kappa coefficient of 0.76; 95% confidence interval, 0.56–0.96). Therefore, SAE techniques can successfully be applied to HyCoSy, and they allow visualization of the "free spill" of contrast agent into the peritoneal cavity in the majority of cases. SAE-HyCoSy has shown good agreement with HSG in previous preliminary studies.

The sonographic evaluation of tubal patency with stimulated acoustic emission imaging. Prefumo F, Serafini G, Martinoli C, Gandolfo N, Gandolfo NG, Derchi LE. Ultrasound Obstet Gynecol 2002 Oct; 20 (4), pp. 386–9.

Lymph Nodes: Regional
Poly(Ethylene Glycol) (PEG)-Coated Magnetite Nanospheres

Semi-Synthetic Methods: Magnetite nanospheres can be prepared with a grafted PEG layer using various PEG lengths from 350 to 1,000 Da. Thermogravimetric analysis can be utilized to measure adsorbed amounts of PEG. Colloid stability is confirmed by measurement of particle size and electrophoretic mobility.
Implementation: Evaluation of the kinetics of injection site drainage and lymph node retention 2 hours after subcutaneous administration of nanospheres coated with PEG lengths of 350, 550, 750, and 1,000 Da. For 750 PEG-coated nanospheres, the kinetics of distribution can be determined over a 48-hour time course.
Interpretation and Analysis: The distribution of nanospheres is modified and lymph node localization is enhanced by altering the surface coverage of PEG on the magnetic surface. PEG-coated magnetite nanospheres with different surface characteristics can be utilized to target regional lymph nodes with diagnostic agent.

Development of systems for targeting the regional lymph nodes for diagnostic imaging: *In vivo* behavior of colloidal PEG-coated magnetite nanospheres in the rat following interstitial administration. Illum L, Church AE, Butterworth MD, Arien A, Whetstone J, Davis SS. Pharm Res 2001 May; 18 (5), pp. 640–5.

Lymph Nodes (Sentinel)/Normal vs Metastatic
Carbon Dioxide Microbubbles

Background Information: Although endoscopic ultrasonography is considered the most useful diagnostic modality for regional staging, the capability of diagnosing lymph node metastasis based on endoscopic ultrasonography images alone is not sufficient. To improve the capability of establishing a differential diagnosis of lymph node enlargement, contrast-enhanced echolymphography can be performed using endoscopic ultrasonography-guided puncture.
Implementation: Evaluation of the ability of contrast-enhanced echolymphography to differentiate between normal and metastatic lymph nodes. Guided puncture of lymph nodes can be performed under real-time endoscopic ultrasonography, followed by carbon dioxide microbubble injection to evaluate echo features before and after microbubble injection.

***Ex Vivo* Model:** Metastatic lymph nodes (n = 8) surgically removed from cases with gastrointestinal cancer (*in vitro* study).

Human Model: Cases with abdominal lymph node swelling detected by endoscopic ultrasonography (n = 55).

Interpretation and Analysis: Contrast-enhanced echolymphography of freshly resected metastatic lymph nodes display nonhomogeneous echo patterns. In regions demonstrating filling defects detected by contrast-enhanced echolymphography, neoplastic infiltration pathology can be observed. In the vast majority of the malignant lymph nodes studied *in vivo*, filling defects and heterogeneous enhancements can be observed by contrast-enhanced echolymphography. However, contrast-enhanced echolymphography demonstrates uniform patterns for most lesions classified as benign. Sensitivity, specificity, positive and negative predictive value, and accuracy of differential diagnosis by contrast-enhanced echolymphography are 95.8%, 90.3%, 88.5%, 96.6%, and 92.7%, respectively. Contrast-enhanced echolymphography is a useful method for differentiating between reactive and malignant lymph node alterations.

Differentiation of benign and malignant lymph nodes with contrast-enhanced echolymphography using endoscopic ultrasound-guided puncture. Kojima S, Goto H, Hirooka Y, Itoh A, Ishiguro Y, Hashimoto S, Hirai T, Hayakawa T. Hepatogastroenterology 2003 Sep–Oct; 50 (53), pp. 1285–91.

Neoplastic Disease/Gene Delivery/Selective Tumor DNA Delivery via Vasculature
Plasmid-Microspheres/Plasmid-Complexed Liposomes

Application: Selective targeting of plasmid-incorporated genetic DNA sequences for neoplastic cell types.
Route of Administration: Arterial catheterization.
Implementation: Evaluation of plasmid DNA delivery into a renal solid tumor involving the utilization of plasmid bound to microspheres and plasmids complexed with liposomes to evaluate their potential to function as a selective delivery modality to tumors. Selective delivery is in part facilitated by selective catheterization of the major arterial network supplying individual neoplastic tissue masses.

***Ex Vivo* Model:** Rat (established renal solid tumor model).
Interpretation and Analysis: Forty-eight hours postdelivery, tumor-to-normal kidney tissue chloramphenicol acetyltransferase expression ratios are as follows: free (1.8:1), microspherical (3.9:1), and liposomal (1.2:1). Microspheres are able to selectively deliver plasmids to tumors, whereas cationic liposomes distribute plasmids to the parenchyma of both kidneys and tumor cells. The tumor model has the potential of screening delivery vehicles as well as therapeutic agents for the capacity of selective delivery to tumors via vascular structures.

A model for evaluating selective delivery of plasmid DNA to tumors via the vasculature. Dass CR, Burton MA. Cancer Biother Radiopharm 2002 Oct; 17 (5), pp. 501–5.

Neoplastic Disease: Liposome Assimilation Enhancement/Enhanced Selective Systemic/Local-Regional Uptake/Slowing of Local-Regional Blood Flow
Starch Microspheres/Small Unilamellar Vesicles (SUV)-Poly(Ethylene Glycol) (PEG) Liposomes

Physiological Mechanism of Action: Starch microspheres can be used to slow local-regional blood flow in a manner that can maximize assimilation of encapsulated liposomes into neoplastic lesions.
Background Information: Systemic adjuvant chemotherapy achieves unsatisfactory results for inoperable liver tumors and metastases. Various clinical studies have shown that loco-regional chemotherapy increases survival rate by several months.
Application: Enhancement of systemic and local-regional uptake of diagnostic and therapeutic pharmaceutical agents.
Route of Administration: Systemic and direct local-regional injection.
Implementation: Evaluate the capacity of liposome encapsulation to selectively facilitate local-regional/systemic targeting of neoplastic cell populations with cytostatic 5-fluouracil (5-FU).
Interpretation and Analysis: Tumor concentrations of 5-FU encapsulated in SUV-PEG liposomes increases by a factor of 27 in systemic therapy and a factor of 90 in loco-regional therapy. The tumor concentration of 5-FU increases by a factor of 8,000 if the blood flow is additionally slowed by starch microspheres (Spherex) during loco-regional therapy with 5-FU encapsulated within liposome preparations.

Liposome encapsulation of cystostatic drugs and starch microspheres improve tumor targeting in loco-regional therapy. An animal experiment study of CC 531 liver tumor. Berger G, Reszka R, Pohlen U, Jung M, Lucas S, Buhr HJ. Langenbecks Arch Chir Suppl Kongressbd 1998; 115 (Suppl I), pp. 693–7.

Neoplastic Disease/Malignant Glioma ($\alpha_v\beta_3$)
Echistatin Microbubbles

Background Information: Angiogenesis is a critical determinant that influences tumor growth and metastasis. It has been hypothesized that contrast-enhanced ultrasound (CEU) with microbubbles targeted to α_V-integrins expressed on the neovascular endothelium could be used to image angiogenesis phenomenon.
Implementation: Evaluation of malignant glioma CEU utilizing contrast-enhanced ultrasound in combination with microbubbles targeted to $\alpha_V\beta_3$ by surface conjugation with echistatin. Contrast-enhanced ultrasound perfusion imaging with non-targeted microbubbles can be used to derive tumor microvascular blood volume and blood velocity. Vascular α_V-integrin expression can be assessed by immunohistochemistry, and microbubble adhesion characterized by confocal microscopy.

Animal Model: Rat (athymic; $n = 14$). Malignant gliomas are produced by intracerebral implantation of U87MG human glioma cell populations. On day 14 or day 28 after implantation, CEU is performed.
Interpretation and Analysis: Mean tumor size increases markedly from 14 to 28 days (2 ± 1 vs 35 ± 14 mm^2, $p < 0.001$). Tumor blood volume increases by approximately 35% from day 14 to day 28, whereas microvascular blood velocity decreases, especially at the central portions of the tumors. On confocal microscopy, $\alpha_V\beta_3$-targeted but not control microbubbles are retained preferentially within tumor microcirculation structures. Contrast-enhanced ultrasound signals produced by $\alpha_V\beta_3$-targeted microbubbles in tumors increase significantly from 14 to 28 days (1.7 ± 0.4 vs 3.3 ± 1.0 relative units, $p < 0.05$). Contrast-enhanced ultrasound signals from $\alpha_V\beta_3$-targeted microbubbles is greatest at the periphery of tumors, where α_V-integrin expression is the most prominent, and correlates well with tumor microvascular blood volume ($r = 0.86$). Contrast-enhanced ultrasound with $\alpha_V\beta_3$-targeted microbubbles can non-invasively detect early tumor angiogenesis. Such a technique, when coupled with changes in blood volume and velocity, may provide insights into the biology of tumor angiogenesis and be used for various diagnostic applications.

Imaging tumor angiogenesis with contrast ultrasound and microbubbles targeted to $\alpha_V\beta_3$. Ellegala DB, Leong-Poi H, Carpenter JE, Klibanov AL, Kaul S, Shaffrey ME, Sklenar J, Lindner JR. Circulation 2003 Jul 22; 108 (3), pp. 336–41.

Neoplastic Disease/Prostatic Carcinoma
Galactose-Based Air Microbubbles

Implementation: Evaluate and compare gray-scale and color Doppler before and during intravenous injection of ultrasound contrast agent made of galactose-based air microbubbles. Biopsy samples can be harvested during contrast injection or directed cores can be collected based on contrast-enhanced imaging (e.g., n = 18). Diagnostic efficiency with and without contrast medium injection for detecting prostate neoplasia are then compared and validated based on biopsy results.

Human Model: Cases evaluated by biopsy of the prostate (n = 85 suspects).

Implications and Analysis: Cancer can be identified in a large number of suspected cases (e.g., n = 58 biopsies/54 cases). Gray-scale imaging can detect a large number of abnormal hypoechoic nodules or irregular zones inside the outer gland (e.g., n = 96/54 cases), of which approximately 50% are found to be malignant on pathological evaluation (e.g., n = 48/96). Contrast-enhanced color Doppler has a higher sensitivity (93%) than unenhanced color Doppler (54%), while specificity increases only 79% to 87% for enhanced imaging. Approximately 90% (e.g., n = 9/10) of isoechoic suspicious zones can be depicted with enhancement, while unenhanced Doppler detects approximately 70% (e.g., n = 7/10). There is no significant difference between the intensity of enhancement and tumor Gleason scores. Contrast-enhanced color Doppler endorectal sonography increases the detection of prostate cancer. Improvement in sensitivity is high, while the difference in specificity is not as pertinent. It is accurate when using a common and routine application ultrasound unit. Such a technique is easy to perform and not time-consuming. Obtaining additional biopsy cores of suspicious enhancing foci significantly improves the detection rate of cancer.

Contrast-enhanced color Doppler endorectal sonography of prostate: Efficiency for detecting peripheral zone tumors and role for biopsy procedure. Roy C, Buy X, Lang H, Saussine C, Jacqmin D. J Urol 2003 Jul; 170 (1), pp. 69–72.

Nucleic Acid (Gene) Delivery/Epidermal Growth Factor Receptor Messenger RNA (mRNA)/ Antisense cDNA
[5-Fluorouracil (5-FU)] Microspheres/[Antisense Oligodeoxynucleotides] Microspheres

Cellular/Molecular Mechanism of Action: Liposomes, due to their low ionic character, are able to traverse across intact cell membranes, thereby facilitating the intracellular release of encapsulated contents.

Background Information: Antisense oligonucleotides (AODNs) can selectively inhibit oncogene expression by Watson-Crick hybridization to target mRNA sequences. Such strategies are being increasingly considered for use in combination with conventional drugs for potential anticancer therapy.

Application: Selective delivery to neoplastic lesions.

Route of Administration: Intravascular (1° intravenous).

Considerations: Combination therapy of AODNs and cytotoxic agents using biodegradable polymeric delivery systems potentially offers several advantages, including site-specific or organ-directed targeting, protection from digesting enzymes, and improved pharmacokinetics/pharmacodynamics resulting from sustained delivery of the entrapped drugs.

Semi-Synthetic Methods: Production of poly-(lactide-co-glycolide) (P(LA-GA)) microsphere formulations. Antisense oligodeoxynucleotides and 5-FU can be either co-entrapped in a single microsphere formulation or individually entrapped in 2 separate microsphere formulations. Release profiles over time can be determined *in vitro*.

Implementation: Evaluate the effectiveness of P(LA-GA) microsphere formulations to deliver (i) a model AODN for targeting epidermal growth factor receptor mRNA sequences (overexpressed by several cancers including breast and brain neoplasias); in combination with (ii) the cytotoxic chemotherapeutic agent 5-FU.

Interpretation and Analysis: Using a double emulsion method for preparing P(LA-GA) microspheres, suitable entrapment and sustained release can be achieved for both types of formulations over a period of 35 days. Release of AODN and 5-FU from all formulations appears to be biphasic. However, the release rates of the 2 agents are significantly slower when co-entrapped as a single microsphere formulation compared to those obtained with 2 separate formulations. Electrophoretic mobility shift assays suggested that this might be, in part, due to an interaction of 5-FU with the oligodeoxynucleotide (ODN). Such findings suggest that mixing individual formulations of 5-FU and ODNs at different mass ratios allows for greater flexibility in achieving a desired release profile, as well as avoiding potential drug-drug interactions. Thus, co-administration of individual P(LA-GA) microsphere formulations containing AODNs and 5-FU, at appropriate mass ratios, appears worthy of further investigation for the potential *in vivo* co-delivery of these anticancer agents.

Co-delivery of an antisense oligonucleotide and 5-fluorouracil using sustained release poly (lactide-co-glycolide) microsphere formulations for potential combination therapy in cancer. Hussain M, Beale G, Hughes M, Akhtar S. Int J Pharm. 2002 Mar 2; 234(1–2), pp. 129–38.

Nucleic Acid (Gene) Delivery/Leukocyte Depletion of Neoplastic Tissues Prior to Genetic Analysis
Magnetic Microspheres

Application #1: Selective "targeted" delivery and controlled or sustained release of encapsulated diagnostic agents.

Example #1: A method of delivering recombinant adeno-associated virus 2 (rAAV), resulting in increased vector transduction efficiencies using microspheres reversibly conjugated to rAAV vectors. A major hurdle in most current gene-delivery modalities is the ability to transduce target tissues at very high efficiencies sufficient to ultimately lead to therapeutic levels of trans-gene expression.

Example #2: Enhanced Kuppfer cell phagocytosis of superparamagnetic particles. The evolution of the proton transverse magnetization of an organ perfused with increasing doses of starch-coated magnetic microspheres is followed by analysis utilizing the Carr-Purcell-Meiboom-Gill sequence with various echo times for images acquired using contrast media preparations.

Example #3: Magnetic microsphere-methoxtrexate conjugates.

Example #4: Microspheres coated with CD45 for the purpose of depleting tumor-infiltrating T lymphocytes. Due to masking or dilution of infrequent tumor cell events, the presence of normal cell types, such as inflammatory cells and fibroblasts, can interfere with accurate DNA analysis of solid tumor samples. In conditions simulating human breast tumor samples employing mixed cell populations (e.g., MDA-MB-175-VII human breast carcinoma cells, WI-38 human lung fibroblast cells, and peripheral blood leukocytes), preparations of CD45-conjugated magnetic microspheres can be used to selectively deplete tumor-infiltrating leukocytes, thus producing a sample that is highly enriched with neoplastic cells.

Example #5: Purging of bone marrow in children with acute lymphocytic leukemia with monoclonal antibody conjugated magnetic microspheres in concert with high-dose VP-16 60 mg/kg; fractionated total body irradiation (12 Gy: 2 × 2 Gy daily); and autologous bone marrow rescue.

Example #6: Selective recognition of neurblastoma cell populations utilizing monoclonal antibody conjugated to microsphere preparations. Depending on the monoclonal antibody, cell lines can be selected that have specific differences in size, morphology, phenotype, neurite process development, and proliferation rate. Due in part to the specificity of the monoclonal antibody applied, all cell lines express the M_r 145,000 neurofilament, and a few express the M_r 200,000 neurofilament, with very little or no expression of the M_r 68,000 neurofilament. Approximately 80% of all cells lines have near-diploid DNA content. High expression of MDR-1 protein is present in about 27% of isolated cell lines. Great heterogeneity can be observed in the expression of N-myc oncoprotein, with a large percentage overexpressing protein fractions. Such findings are accompanied by the expression of c-myc oncoprotein in all cell lines (4- to 10-fold lower than the N-myc oncoprotein). Localization studies of c-myc and N-myc oncoproteins on the level of light microscopy and electron microscopy reveal exclusive nuclear localization of c-myc, while N-myc is localized within the nucleus and cytoplasm.

Route of Administration: Intravenous but depends on each individual preparation.

Synthetic Chemistry and Analyses: Example methodology: Magnetic microsphere-methotrexate (MM-MTX) can be prepared by the following methods: (i) reaction of MTX with poly(ethylene glycol) 1500 (PEG) to form a PEG-methotrexate conjugate (PEGMTX), which is then added to a ferrous/ferric ion salt solution to give MM-MTX I; (ii) reaction of ferrous/ferric ion salts with PEG to give a ferromagnetic polymer complex, which is then coupled with MTX to give MM-MTX II; (iii) MM-MTX IIIA can be prepared by reacting MTX with amino-terminated magnetic microspheres in the presence of 1-ethyl-3,3-bis(methylamino)propylcarbodiimide (EDCI); (iv) reaction of aminohexanol with di-tert-butyl dicarbonate to form an [N-(tert-butoxycarbonyl)amino]hexanol (t-Boc-AH), which is then coupled with MTX in the presence of 1,3-dicyclohexylcarbodiimide and 4-pyrrolidinopyridine to give a t-Boc-AH-MTX conjugate, which is then saturated with hydrogen chloride to give an aminohexanol-methotrexate (AH-MTX) conjugate. MM-MTX IIIB can then be prepared by reacting AH-MTX with carboxyl-terminated magnetic microspheres in the presence of EDCI and 4-(dimethylamino)pyridine. The identity of MTX conjugates is confirmed using ultraviolet, infrared, and nuclear magnetic resonance spectroscopy. Drug content of the magnetic microsphere-methotrexate conjugates as determined by high-performance liquid chromatography (HPLC) is 0.45% (w/w), 4.0% (w/w), and 6.3% (w/w) MTX for MM-MTX I, MM-MTX II, and MM-MTX IIIB, respectively.

Considerations: A major hurdle in most current diagnostic/therapeutic gene modalities is the ability to transduce target tissues at very high efficiencies. Conjugation of agents to microspheres should result in a higher effective concentration within predetermined tissue or organ targets as it moves through tissue vasculature, as well as providing a relatively longer retention time of diagnostic agents within a given site or lesion of interest. Depending on the objective and agent being applied, *in vitro* investigations have demonstrated that preparations conjugated to microspheres can be 100 times more efficient in reaching a predetermined target site compared to a scenario where the preparation is evaluated without the benefits or attributes associated with microspheres. Application of magnetic microspheres allows the advantage of enhancing regional accumulation. Furthermore, *in vivo* microsphere-mediated strategies can result in higher efficiencies of delivery and induced biological effect compared to agents in "free" form administered either intramuscularly or intravenously. Often, this can be accompanied by prolonged retention of microsphere preparations within capillary bed structures.

Application #2: From the multiexponential fit of the echo train, the amplitudes and the relaxation rates R2 of the liver tissue can be obtained. Results from such studies clearly indicate that shortly after contrast medium administration, internalization takes place, which can be followed by the rapid and biphasic evolution of the transverse relaxation rate of water protons. A very fast decaying component looking like an initial loss of the magnetization can be observed together with an increase in the relaxation rate of the remaining water tissue. Such a regime is strongly dependent on both the echo time and iron concentration (e.g., behavior characteristic of agglomerations of magnetic particles). The examination of liver tissues by electron microscopy shows that this clustering arises within cytoplasmic vacuoles.

Improved method of recombinant AAV2 delivery for systemic targeted gene therapy. Mah C, Fraites TJ Jr, Zolotukhin I, Song S, Flotte TR, Dobson J, Batich C, Byrne BJ. Mol Ther 2002 Jul; 6 (1), pp. 106–12.

Nucleic Acid (Gene) Delivery/Solid Tumors
Microspheres (> 1 μm)/Liposome: Cationic (100–200 nm)/Porphyrin Derivatives (< 1 nm)/Branched-Chain Dendrimers (10 nm)/ Polyethylenimine Polymers (10 nm)/Nanoparticles (20–1,000 nm)/Microspheres (> 1 μm)/ Cyclodextrins (CyDs 1.5 nm)

Background Information: The vasculature of a tumor provides the most effective route by which neoplastic cells may be reached and eradicated by drugs. The fact that a tumor's vasculature is relatively more permeable than healthy host tissue should enable selective delivery of drugs to tumor tissue. Such delivery is relevant to carrier-mediated delivery of genetic medicine to tumors. Cationic liposomes have been used for the introduction of nucleic acids into mammalian cells for more than a decade.

Application: Nucleic acid delivery into cells (*in vitro* and *in vivo*).

Semi-Synthetic Methods: Cationic liposomes are generally 100–200 nm in diameter, whereas CyDs typically span 1.5 nm across.

Implementation: Evaluate the potential of delivering therapeutic oligonucleotides (ONs) to tumors using cationic liposomes and cyclodextrins (CyDs) and the major hindrances posed by the tumor itself on such delivery.

Interpretation and Analysis: CyD molecules are routinely used as agents that engender cholesterol efflux from lipid-laden cells, thus having an efficacious potential in the management of atherosclerosis. A recent trend is to employ these oligosaccharide molecules for delivering nucleic acids in cells both *in vitro* and *in vivo*. Comparisons have been made with other oligonucleotide delivery agents, such as porphyrin derivatives (< 1 nm), branched-chain dendrimers (approximately 10 nm), polyethylenimine polymers (approximately 10 nm), nanoparticles (20–1,000 nm), and microspheres (> 1 μm), in the context of delivery to solid tumor. The chemical and physical properties of these carriers and how they may affect the uptake of oligonucleotides into cells, particularly *in vivo*, have been reviewed.

Vehicles for oligonucleotide delivery to tumours. Dass CR. J Pharm Pharmacol 2002 Jan; 54 (1), pp. 3–27.

Ocular Tissues and Adnexa: Budesonide Delivery/ Retinal Pigment Epithelial Cells/(Ocular Globe Delivery: Subconjunctival Injection)
Microparticles and Nanoparticles

Route of Administration: Topically (*in vitro* tissue culture populations) and subconjunctivally by injection (*in vivo*) into 1 eyelid (Sprague-Dawley rats).
Dosage: Budesonide (100 pM to 10 μM in exposed tissue culture cell populations).
Semi-Synthetic Methods: DL-polylactide (PLA) nanoparticles and microparticles containing budesonide can be prepared using a solvent evaporation technique, and the particles can then be profiled for the characteristics of size, morphology, encapsulation efficiency, and *in vitro* release.
Implementation: Evaluation of the influence that budesonide has on vascular endothelial growth factor (VEGF) secretion, VEGF messenger RNA (mRNA) expression, and cytotoxicity in cell populations assessed by ELISA, real-time polymerase chain reaction (RT-PCR), and cell-viability assays, respectively.
***Ex Vivo* Model:** ARPE-19 cell lines.
Animal Model: Rat (Sprague-Dawley).
Interpretation and Analysis: At concentrations devoid of cytotoxicity, budesonide inhibits VEGF secretion as well as VEGF mRNA expression in ARPE-19 cell lines in a dose-dependent manner. Glucocorticoid receptor antagonists (e.g., RU486) suppress budesonide-mediated inhibition of VEGF secretion and VEGF mRNA expression, thereby implying a role of this receptor type in the observed effects mediated by budesonide.

Budesonide-PLA nanoparticles (345 nm) and microparticles (3.6 μm), with an encapsulation efficiency of 65% and 99%, respectively, possess properties of sustained *in vitro* release of budesonide. In an *in vivo* model, subconjunctival administration of both budesonide-PLA nanoparticles and microparticles produces sustained budesonide levels within the retina and other ocular tissues (measured in the retina, vitreous, lens, and cornea at 1, 7, and 14 days postinjection).

Subconjunctival nano- and microparticles sustain retinal delivery of budesonide, a corticosteroid capable of inhibiting VEGF expression. Kompella UB, Bandi N, Ayalasomayajula SP. Invest Ophthalmol Vis Sci 2003 Mar; 44 (3), pp. 1192–201.

Parkinson's Disease (Bromocryptine Mesylate)
Poly(L-Lactide)/Poly(D,L-Lactide)/Poly(D,L-Lactide-co-Glycolide)/Microspheres (Biodegradable)

Cellular/Molecular Mechanism of Action: Encapsulation of pharmaceutical agents for protection against degradation and premature elimination in a manner that can enhance accumulation of preparations within a specific organ or tissue.
Application: Delivery of pharmaceutical agents in a manner that is protected from degradation and a means of selectively concentrating preparations within a specific organ/tissue lesion.
Semi-Synthetic Methods: Bromocryptine mesylate can be encapsulated into biodegradable polymers using the polymers poly(L-lactide), poly(D,L-lactide), and poly(D,L-lactide-co-glycolide).
Interpretation and Analysis: Photomicrographs (scanning electron microscopy [SEM]) have shown that microsphere morphology depends significantly on the polymer and emulsifying agent. Regardless of the polymer type, increases in emulsifying agent concentration from 0.25 to 0.75% w/v markedly decreases microsphere particle size. Delineation of particle size characteristics reveals that the use of 0.75% w/v of emulsifying agent concentration and a polymer solution concentration of 10% w/v results in the production of particles of optimum proportions and size. In order to prepare biodegradable microspheres with high drug content and small particle size, selection of polymer concentration, as well as emulsifying agent concentration, is critical. Polymer type has a less pronounced effect on the percent encapsulation efficiency and particle size of microspheres than on the (t50%). Microspheres prepared utilizing all 3 polymers, at a polymer concentration of 10% w/v and an emulsifying agent concentration of 0.75% w/v with NaCMC:SO (4:1, w/v), produce a formulation that produces optimum characteristics.

Biodegradable bromocryptine mesylate microspheres prepared by a solvent evaporation technique. I: Evaluation of formulation variables on microspheres characteristics for brain delivery. Arica B, Kas HS, Orman MN, Hincal AA. J Microencapsul 2002 Jul–Aug; 19 (4), pp. 473–84.

Protein Delivery
Microparticles (Solvent-Free)

Preparations: Solvent-free microparticles, loaded with protein fractions, can be produced using a supercritical (SC) fluid-based coating technology.
Semi-Synthetic Methods: Coating material consists of either trimyristin (Dynasan 114) or Gelucire 50-02. Microparticles produced in this manner are categorized according to the characteristics of morphology, protein content, and *in vitro* release profile. A discontinuous coating made of trimyristin micro-needles leads to an initial burst release (approximately 70% within 30 minutes). However, a prolonged release of reference protein fractions (e.g., bovine serum albumin [BSA]) can be achieved in a phosphate buffer solution at 37°C over a 24-hour period from particles coated with Gelucire 50-02. In addition, it has been shown that BSA does not undergo degradation after supercritical CO_2 treatment conditions used during the coating process.

A supercritical fluid-based coating technology. 3: Preparation and characterization of bovine serum albumin particles coated with lipids. Ribeiro Dos Santos I, Richard J, Thies C, Pech B, Benoit JP. J Microencapsul 2003 Jan–Feb; 20 (1), pp. 110–28.

Protein Delivery
Microencapsulation

Application: Delivery of peptide, lipoproteins, and glycoprotein diagnostic biopharmaceuticals.
Semi-Synthetic Methods: Preparations are made using a mixer that consists of an array of microchannels. Advantages of the system are that it is simple to assemble, requires only a very small work space, lacks moving parts, and offers simple control of microsphere size. Scale-up from lab bench to industrial production is easily feasible through parallel installation of a sufficient number of micromixers. Poly(lactic-co-glycolic acid) microspheres can be loaded with a model reference protein (e.g., bovine serum albumin [BSA]) and the influence of various process and formulation parameters on the characteristics of the microspheres can be examined, particularly with respect to the properties of particle size and distribution. Because of its simple setup and its suitability for continuous production, static micromixing is preferred for the automated and aseptic production of protein-loaded microspheres.
Implementation: Evaluate the potential of a static micromixer to be utilized in the production of protein-loaded biodegradable polymeric microspheres by a modified solvent extraction process.

Interpretation and Analysis: Microspheres with monomodal size distributions having mean diameters of 5 to 30 μmeters can be produced with excellent consistency (reproducibility). Particle size distributions are largely unaffected by polymer solution concentration, polymer type, and nominal protein fraction load with BSA, but this characteristic is dependent on the polymer solvent. Moreover, particle mean diameters can be varied over a considerable range by modulating the flow rates of the mixed fluids. Protein fraction encapsulation efficiencies are mostly in the range of 75 to 85% (e.g., BSA) and product yields range from 90 to 100% (e.g., BSA).

Solvent extraction employing a static micromixer: A simple, robust and versatile technology for the microencapsulation of proteins. Freitas S, Walz A, Merkle HP, Gander B. J Microencapsul 2003 Jan–Feb; 20 (1), pp. 67–85.

Pharmaceutical Delivery: Eudragit RS 100/ Eudragit RL 100/Microencapsulation Coatings *Microcapsules: Sulphamethoxazole*

Physiological Mechanism of Action: Facilitates sustained release of compounds from microcapsule formulation.
Application: Eudragit is used as a coating material in microencapsulation techniques.
Analytical Methodology: Evaluation of preparation characteristics for sulphamethoxazole microcapsules coated with Eudragit RS 100 and RL 100 stored at room temperature for 10 years for comparison with freshly formulated microcapsules. Determination of the various storage conditions over a 6-month period can also be used as a strategy for assessing stability and retained integrity. Critical properties of interest include analysis for total drug content, dissolution, surface properties, and physical properties.
Interpretation and Analysis: The parameters of drug content, dissolution, surface properties, and physical properties evaluated for sample preparations stored at room temperature for 10 years compared to fresh preparations, and formulations subjected to various storage conditions for a period of 6 months, reveal that 70% of the core material is still intact in 10-year-old samples. Similarly, dissolution studies of 10-year-old sustained-release microcapsule samples show that drug release ranges from 4% in the 1st hour to 65% at the end of 8 hours. Drug release from the freshly prepared microcapsule (keeping the manufacturing parameters the same as that followed for older sample) ranges from 25% in the 1st hour to 98% at the end of the 8th hour. Infrared spectra of freshly prepared microcapsules and that of the 10-year-old microcapsules are identical and indicate that no interaction occurs between the coating polymer and core substance even after 10 years.

Accelerated stability of sulphamethoxazole microcapsules coated with Eudragit RS 100 and Eudragit RL 100. Basnet S, Ganesan M, Pal TK. Boll Chim Farm 2002 May–Jun; 141 (3), pp. 202–9.

Pharmaceutical Delivery/Example: Eudragit RL *Microencapsulation/Preparation (Solvent Evaporation Technique)*

Cellular/Molecular Mechanism of Action: Functions as a delivery modality that can protect pharmaceutical agents from degradation and premature elimination, in addition to facilitating selective accumulation of preparations within a given target tissue or organ system.
Application: Delivery of pharmaceutical.
Semi-Synthetic Methods: Microcapsules composed of Eudragit RL can be prepared applying a solvent evaporation techniques. In example preparations using Diclofenac sodium as a model reference pharmaceutical for encapsulation, a solution of drug and Eudragit RL is dissolved in acetone-isopropyl alcohol (1:1) and sprayed into liquid paraffin.
Route of Administration: Usually intravenous.
Dosage: Dependent upon encapsulated pharmaceutical and intended application.
Interpretation and Analysis: The microcapsules obtained are uniform and free-flowing particles. The release rate is more sustained if the polymer concentration is increased. As an experimental procedure, this technique has promise as a rapid and convenient method for the preparation of Eudragit RL microcapsules.

A novel method for preparation of Eudragit RL microcapsules. Satturwar PM, Mandaogade PM, Dorle AK. J Microencapsul 2002 Jul–Aug; 19 (4), pp. 407–13.

Pharmaceutical Delivery/Controlled Release *Microspheres: Reacetylated Chitosan*

Cellular/Molecular Mechanism of Action: Functions as a delivery modality that can protect pharmaceutical agents from degradation,and premature elimination, in addition to facilitating selective accumulation of preparations within a given target tissue or organ system.
Considerations: The high aqueous solubility of chitosan restricts the utility of chitosan microspheres for gastric drug delivery.
Semi-Synthetic Methods: Two different microencapsulation techniques can be optimized in order to efficiently encapsulate hydrophilic (e.g., amoxycillin) and hydrophobic (e.g., metronidazole) compounds. Reacetylated chitosan microspheres exhibit a controlled water swelling capacity and gelify at acidic pH levels, resulting in prolonged release of encapsulated antibiotics.
Implementation: Evaluation of reacetylated chitosan microspheres possessing desirable properties to determine their capacity to facilitate controlled release of pharmaceutical agents within the gastrointestinal system (e.g., active antimicrobial agents, including amoxycillin and metronidazole).
Interpretation and Analysis: The reacetylation time is an important variable affecting not only drug release but also encapsulation efficiency and antimicrobial activity of encapsulated compounds. Encapsulation efficiency and biological activity is also dependent on drug solubility within the reacetylating agent. Using short reacetylation time periods, it is possible to efficiently control the release of both hydrophilic and lipophilic antibiotics, while maintaining their biological activity (antimicrobial properties). Based on preliminary findings, reacetylated chitosan microspheres appear to be promising vehicles for the controlled delivery of pharmaceutical agents (e.g., antimicrobial agents) within the gastric lumen for the purpose of achieving a specific outcome (e.g., *Helicobacter pylori* eradication or imaging/treatment of gastric carcinoma).

Reacetylated chitosan microspheres for controlled delivery of antimicrobial agents to the gastric mucosa. Portero A, Remunan-Lopez C, Criado MT, Alonso MJ. J Microencapsul 2002 Nov–Dec; 19 (6), pp. 797–809.

Pharmaceutical Delivery/Ultrasound-Triggered Focal Delivery/Acoustic Power: Optimization/ Signal Intensity Maximization/Bulk Modulus/ Influence of Difference in Shell Characteristics *Microbubbles/Acoustic Lability*

Background Information: Low mechanical index perfusion imaging relies on the detection of signals produced by microbubble oscillation at low acoustic powers that results in minimal microbubble destruction.

Route of Administration: Intravascular.
Consideration: Localized ultrasound-mediated disruption of microbubble preparations containing encapsulated pharmaceutical agents has been employed as a modality for selective delivery.
Implementation: Evaluation of the hypothesis that the optimal acoustic power for real-time imaging differs for microbubbles with different shell characteristics. One approach for pursuing this hypothesis can involve the evaluation of 3 microbubble agents with varying shell elastic properties according to their polymer composition. Differences in the elastic properties of these microbubbles can be demonstrated by (i) measurement of their bulk modulus, and (ii) evaluation of their acoustic lability by microscopic visualization of microbubble destruction during insonification at incremental acoustic powers. The ultrasound signal generated by these microbubbles at various mechanical indexes and the degree of microbubble destruction during continuous imaging is then determined both in an *in vitro* flow system and during *in vivo* open-thorax imaging.

Animal Model: Canine (open-thorax preparations).
Interpretation and Analysis: Optimal power for achieving maximal signal intensity with minimal microbubble destruction is influenced by the shell elastic properties. Acoustic power for maximizing acoustic signal without destroying microbubbles during low mechanical index imaging varies according to specific shell characteristics.

Influence of microbubble shell properties on ultrasound signal: Implications for low-power perfusion imaging. Leong-Poi H, Song J, Rim SJ, Christiansen J, Kaul S, Lindner JR. J Am Soc Echocardiogr 2002 Oct; 15 (10 Pt 2), pp. 1269–76.

Pharmaceutical Delivery/Ultrasound (US)-Triggered Focal Delivery/Cardiovascular Endothelium: Endothelium Injury/Endothelial Cell (EC) Viability: *Ex Vivo*/[Local Delivery]
Microbubbles/Propidium Iodine (PI)

Background Information: Microvascular EC may be subject to the bioeffects induced by contrast US because of their proximity to the circulating microbubbles.
Application: Potential modality for the selective delivery of diagnostic or therapeutic pharmaceutical agents contained with microbubble preparations that can subsequently be liberated by exposure to ultrasound-induced disruption (lysis).
Route of Administration: Intravascular.
Implementation: Evaluation of EC injury produced in mesentery microvessel sections (e.g., arteriole, capillary, or venule) by exposure of ultrasound microbubble contrast agents to ultrasound energy. Assessment of such phenomenon can involve injection of microbubble preparations, followed by US exposure at a 1-Hz or 30-Hz frame rate. Propidium iodide, a fluorescent indicator of cell injury, can then be employed to visualize impaired endothelial cell integrity. Note: PI-positive nuclei should be equally few among the 3 control preparations.

Animal Model: Rat (mesenteric vasculature) in 5 experimental groups, including 3 controls (sham operation, microbubble injection alone, US exposure with saline injection); and 2 contrast US groups (1-Hz or 30-Hz frame rates).
Interpretation and Analysis: Contrast US increases the number of PI-positive cells in capillaries (1-Hz frame rate, 2.4 ± 2.2 cells per 0.1 mm vessel length, $p = 0.09$; 30-Hz frame rate, 4.3 ± 1.8 cells, $p < 0.01$) and in venules (1-Hz frame rate, 4.1 ± 2.5 cells, $p < 0.05$; 30-Hz frame rate, 13.8 ± 3.6 cells, $p < 0.01$) compared with sham preparations (0.10 ± 0.22 cells). Such findings indicate that diagnostic contrast ultrasound potentially causes endothelial injury, particularly within venules and capillaries.

Endothelial cell injury in venule and capillary induced by contrast ultrasonography. Kobayashi N, Yasu T, Yamada S, Kudo N, Kuroki M, Kawakami M, Miyatake K, Saito M. Ultrasound Med Biol 2002 Jul; 28 (7), pp. 949–56.

Pharmaceutical Delivery/Estrogen/Systemic Delivery (Protected)
Estradiol Microspheres: Biodegradable

Cellular/Molecular Mechanism of Action: Encapsulates pharmaceutical agents for protection against degradation and selective distribution at high concentrations to specific tissue/organ targets.
Semi-Synthetic Methods: Preparations of poly (D, L-lactic acid) (PLA)/D, L-lactide/glycolide copolymer (PLGA) can be used to produce biodegradable and biocompatible carriers to achieve sustained release of estradiol. Microspheres can be formed by an emulsification-solvent evaporation method. Quality control measures include the measurement of various parameters and properties, including *in vitro* drug release behavior; effects of polyvinyl alcohol (PVA) concentration in the aqueous phase; concentration of PLA in the organic phase; stirring speed; volume oil/water (O/W) ratio; weight ratio of E2/PLA fed; and polymer type and molecular weight.

Preparation and evaluation of poly (D, L-lactic acid) (PLA) or D, L-lactide/glycolide copolymer (PLGA) microspheres with estradiol. Xinteng Z, Weisan P, Ruhua Z, Feng Z. Pharmazie 2002 Oct; 57 (10), pp. 695–7.

Protein Delivery/Enzymes: Preparation Optimization
Microparticulates Enzyme Prolidase Stability/ Poly[Lactide-Co-Glycolide] (PLGA)/Microparticulate Enzyme Formulations

Semi-Synthetic Methods: Various strategies can be applied to determine the stability of prolidase and its interaction with reagents, in addition to delineating the effectiveness of various condition variables applied during the emulsification of prolidase-loaded PLGA microparticulate systems. Such forms of evaluation are necessary in order to characterize optimal semi-synthetic techniques. Enzyme stability can be tested pertaining to contact with methylene chloride, ethyl acetate, PLGA polymers, and several agents used as emulsifiers such as polyvinyl alcohol (PVA), polyvinyl pyrolidone (PVP), carboxymethyl cellulose (CMC), and sodium oleate (NaOl). Evaluation of enzyme stability to temperature and mechanical stirring of prolidase-loaded PLGA microspheres ideally should be performed in order to detect alterations in protein activity.
Interpretation and Analysis: Prolidase-loaded PLGA microspheres can be prepared only upon enzyme stabilization by addition of both bovine serum albumin (BSA) and $MnCl_2$ into TRIS formulations. Methylene chloride is a suitable organic solvent for use in double emulsion techniques, together with PVA as a dispersing agent in the outer aqueous phase. Low temperatures during the emulsification step and very short process times are recommended, in order to maintain maximum enzyme activity. In these conditions spherical microspheres can be obtained, releasing active prolidase for up to 15 days.

Evaluation of enzyme stability during preparation of polylactide-co-glycolide microspheres. Perugini P, Genta I, Pavanetto F, Modena T, Maculotti K, Conti B. J Microencapsul 2002 Sep–Oct; 19 (5), pp. 591–602.

Protein Delivery/Immunity and Inflammatory Mediators (Endogenous)

Poly(Lactic/Glycolic Acid) Microspheres/Interleukin (IL)-1α

Physiological Mechanism of Action: Interleukin-1α is a pleiotropic cytokine mediator with multiple antitumor activities.

Background Information: Microspheres encapsulating IL-1α have the potential of functioning as an immunotherapy platform for the management of neoplastic disease.

Application: Microspheres can be applied to deliver labile biological protein fractions, including endogenous mediators of immunity and inflammation (e.g., IL-1α).

Semi-Synthetic Methods: Encapsulation of IL-1α within biodegradable poly(lactic/glycolic acid) microspheres, 1–5 μm diameter in size.

Implementation: Enhance IL-1α-mediated activation of macrophages using microspheres as a delivery vehicle.

Animal Model: Murine.

Interpretation and Analysis: Preparations of IL-1α microspheres are efficiently internalized by macrophages in tissue culture and after intraperitoneal injection. In culture, phagocytosis of the microspheres reaches a saturation level within 3 hours and there is no apparent effect of polymer type on the extent of uptake. *In vivo* uptake of human IL-1α microspheres by macrophages leads to cell activation reflected as an enhanced level of murine IL-1α, IL-6, and IL-12 production. Control microspheres, containing bovine serum albumin, induce only background, to low levels of cytokine production. These cytokines, when expressed or secreted from macrophages, may *in situ* stimulate diverse immune and inflammatory responses, including T cell–mediated immune responses such as those involved in the development of Th1 cells and cytotoxic lymphocytes. Thus, directed delivery of IL-1α to macrophage populations via appropriate microsphere preparations may serve as a unique approach for modulating the immunoregulatory properties of this cell type and enhancing their *in situ* participation in antitumor immune responses.

Macrophage activation for the production of immunostimulatory cytokines by delivering interleukin-1 via biodegradable microspheres. Mullerad J, Cohen S, Voronov E, Apte RN. Cytokine 2000 Nov; 12 (11), pp. 1683–90.

Protein Delivery/Sustained Peptide Release (*In Vivo*)/Example: Insulin

Microspheres: Inherently Fluorescent

Cellular/Molecular Mechanism of Action: Microspheres can function as a delivery platform that can prevent degradation, delay the rate of elimination, control systemic release, and/or facilitate selective delivery in a manner that minimizes innocent organ/tissue exposure and enhances concentrations at desired target regions.

Application: Selective or protracted pharmaceutical release.

Route of Administration: Intravenous.

Semi-Synthetic Methods: Inherently fluorescent microspheres composed of a fluorescent polyanhydride (e.g., poly(p-(carboxyethylformamido) benzoic anhydride, or PCEFB, and poly(lactide-co-glycolide) [PLGA]) can be prepared using a water-in-oil-in-water (w/o/w) emulsion solvent evaporation technique. Characterization of microsphere blends can be achieved applying combinations of gel permeation chromatography (GPC), infrared spectrometry (IR), fluorometry, and scanning electron microscopy (SEM) analyses.

Implementation: Evaluation of the effect of the PCEFB/PLGA feed ratio and composition of the oil phase on insulin entrapment and microsphere diameter.

Interpretation and Analysis: Insulin entrapment efficiency increases with PCEFB content and acetone percent in the oil phase. Microsphere diameter decreases as acetone is added into the oil phase. Although slight degradation of PCEFB during the fabrication process can be identified by GPC and IR, PCEFB/PLGA microspheres can still be clearly visualized by either confocal laser scanning microscopy (CLSM) or fluorescent microscopy in a manner that makes it possible to directly detect microspheres *in vivo* by fluorometry without the need for fluorescent dye labeling techniques. The surface of PCEFB/PLGA (1:2) microspheres is smooth, while PCEFB/PLGA (2:1) microspheres have rough and uneven surfaces. Sustained release of insulin from the microspheres can be achieved over a period of approximately 4 days.

Bioadhesive fluorescent microspheres as visible carriers for local delivery of drugs. I: Preparation and characterization of insulin-loaded PCEFB/PLGA microspheres. Jiang HL, Zhu KJ. J Microencapsul 2002 Jul–Aug; 19 (4), pp. 451–61.

Release Evaluation

Brilliant Blue Microspheres/Tumor Necrosis Factor-Alpha (TNF-α) Microspheres/Poly(D,L-Lactic-Co-Glycolic Acid) Multiphase Microspheres

Cellular/Molecular Mechanism of Action: Brilliant blue functions as a dye marker reagent that is compatible with biological systems.

Application: Brilliant blue can be applied to evaluate the rate of release of compounds incorporated into microsphere preparations.

Route of Administration: Intravascularly or within individual body cavities.

Semi-Synthetic Methods: Microspheres can be prepared using an anhydrous multiple-emulsion solvent evaporation method.

Implementation: Evaluation of the *in vivo* rate of release within the peritoneal cavity.

Animal Model: Murine (intraperitoneal).

Interpretation and Analysis: Multiphase microspheres containing brilliant blue exhibit a 3-phase release profile *in vitro* and display a significantly lower level of dye released during the initial phase compared to conventional matrix-type microspheres. Slow release of the dye is observed during the 2nd phase, which is followed by disintegration of the polymer wall during the 3rd phase of the release process. *In vitro* dissolution profiles of TNF-α can be calculated following compensation for the simultaneous degradation of the protein that occurs in the dissolution medium. The initial burst release of TNF-α is significantly reduced with multiphase microspheres. The 3-phase release profile, as seen with the dye, is not observed for microspheres containing TNF-α. The rate of release of protein from microspheres can be determined *in vivo* by analyzing the residual level of TNF-α remaining in the particles following intraperitoneal administration of microspheres. The release of protein from microspheres *in vivo* is significantly faster than predicted from the results observed during *in vitro* studies. The absence of an initial burst release of TNF-α from multiphase microspheres is recognized as a significant reduction in plasma TNF-α levels when compared to matrix-type microspheres and a solution of the biological protein. The controlled release property of multiphase microspheres is expected to overcome adverse reactions due to dose dumping that can occur following local TNF-α administration.

In vitro and *in vivo* release properties of brilliant blue and tumor necrosis factor-alpha (TNF-α) from poly(D,L-lactic-co-glycolic acid) multiphase microspheres. Iwata M, Nakamura Y, McGinity JW. J Microencapsul 1999 Nov–Dec; 16 (6), pp. 777–92.

Respiratory System: Pulmonary Delivery (Passive)
Albumin Microspheres/[Terbutaline]

Background Information: Terbutaline sulfate is widely used as a bronchodilator for the therapeutic management of bronchial asthma, chronic bronchitis, and emphysema. As it has a short biological half-life, a long-acting terbutaline sulfate formulation would considerably improve clinical compliance.
Application: Passive pulmonary delivery. Speculation suggests that preferential uptake of albumin microspheres is due to a combination of several variables, including (i) partial wedging within the lumen of microvascular structures of a specific diameter if preparations are given by jugular vein injection because the pulmonary vasculature is the first capillary bed they would encounter following administration; and/or (ii) if preparations were given by jugular vein injection, the first physical contact microspheres would have with large populations of cells belonging to the mononuclear phagocytic system would be within the lung (e.g., intravascular and alveolar macrophage populations). So in reality, the preferential distribution pattern may instead be a direct result of the route of administration in concert with the anatomical and functional features of the lung instead of a true molecular-based form of selective delivery.
Semi-Synthetic Methods: Preparation of bovine serum albumin (BSA) microspheres by an emulsion polymerization method using glutaraldehyde as a cross-linking agent. All microspheres are spherical and smooth with a mean particle size in the range of 22 to 30 μm.
Interpretation and Analysis: Drug release from BSA microspheres displays a biphasic pattern characterized by an initial fast release, followed by a slower release phase. The amount released decreases with increases in glutaraldehyde concentration. In the absence of trypsin, the time required for complete degradation of microspheres is increased from 144 to 264 hours when the glutaraldehyde concentration increases from 0.1 to 0.7 ml. In the presence of trypsin, a linear relationship can be observed between degradation rate and trypsin concentration, which indicates that saturation of the biological system has not yet been reached. Biodistribution studies indicate that the degree of uptake by the lungs is higher than that for other organ systems. Therefore, terbutaline sulfate–loaded microspheres can be used for passive lung targeting.

Preparation, characterization and *in vivo* distribution of terbutaline sulfate–loaded albumin microspheres. Sahin S, Selek H, Ponchel G, Ercan MT, Sargon M, Hincal AA, Kas HS. J Control Release 2002 Aug 21; 82 (2–3), pp. 345–58.

Semi-Synthetic Technique Optimization
Microparticulates/Enzyme Prolidase Stability

Implementation: Various strategies can be applied to determine the stability of prolidase and its interaction with reagents, in addition to determining the influence of various condition variables applied during the emulsification of prolidase-loaded poly(lactide-co-glycolide) (PLGA) microparticulate systems. Such information is of benefit for characterizing optimal semi-synthetic techniques. Enzyme stability can be tested following contact with methylene chloride, ethyl acetate, PLGA polymers, and several agents used as emulsifiers such as polyvinyl alcohol (PVA), polyvinyl pyrolidone (PVP), carboxymethyl cellulose (CMC), and sodium oleate (NaOl). Evaluation of enzyme stability to temperature and mechanical stirring of prolidase-loaded PLGA microspheres ideally should be performed in order to detect alterations in protein activity.
Interpretation and Analysis: Prolidase-loaded PLGA microspheres can be prepared effectively if the enzyme fraction is stabilized by addition of both bovine serum albumin (BSA) and $MnCl_2$ into TRIS formulations. Methylene chloride is a suitable organic solvent for use in double emulsion techniques, together with PVA as a dispersing agent in the outer aqueous phase. Low temperatures during the emulsification step and very brief processing times are recommended in order to maintain maximum enzyme activity. Under such conditions, spherical microspheres can be generated releasing active prolidase for up to 15 days.

Evaluation of enzyme stability during preparation of polylactide-co-glycolide microspheres. Perugini P, Genta I, Pavanetto F, Modena T, Maculotti K, Conti B. J Microencapsul 2002 Sep–Oct; 19 (5), pp. 591–602.

Stability Evaluation of Microbubble Formulations/Saline in Plastic vs Glass Vials/Saline in Vacuum vs Non-Vacuum Vials/Bladder Contrast Enhanced Voiding Urosonograpy
Microbubbles (Galactose-Based Formulations: Levovist)

Background Information: The sonographic diagnosis of vesicoureteral reflux with intravesical administration of ultrasound contrast media is increasingly more frequent.
Application: Lower urinary tract examination.
Route of Administration: Urethral catheter.
Considerations: Rapid dissolution of microbubbles in the bladder filled with normal saline can occasionally be appreciated during the course of ultrasound examinations.
Implementation: Evaluate the degree of interaction between normal saline and ultrasound contrast media in concert with elucidating any potential causes for such interactions. Such an objective can be addressed applying an *in vitro* experimental model to characterize normal saline solution and a galactose-based ultrasound contrast medium (Levovist) formulated at gradient concentrations and then scanned utilizing predefined parameters. As a point of reference, the oxygen concentration of the solutions are also measured (pO_2 and O_2 mg/l).
Interpretation and Analysis: Ultrasound contrast medium has a significantly longer contrast duration when mixed with normal saline from plastic containers rather than glass containers. The contrast duration difference is even more marked between normal saline from containers sealed under vacuum and those not sealed under vacuum (e.g., 0.7 and 12 minutes, respectively). The oxygen concentration in normal saline from vacuum-sealed containers is one-third or less that from non-vacuum-sealed containers. The high concentration of dissolved oxygen in normal saline seems to prevent the diffusion of air from the microbubbles into the solution and thus their collapse. Desaturated normal saline has a detrimental effect on microbubbles of galactose-based ultrasound contrast agents. For the purpose of filling the bladder during contrast-enhanced voiding urosonography, only normal saline solution from non-vacuum-sealed containers should be used. For practical purposes, normal saline from plastic containers is safest.

Interaction of normal saline solution with ultrasound contrast medium: Significant implication for sonographic diagnosis of vesicoureteral reflux. Darge K, Bruchelt W, Roessling G, Troeger J. Eur Radiol 2003 Jan; 13 (1), pp. 213–8.

Systemic Delivery
Polymer Millrods: Doxorubicin Loaded

Cellular/Molecular Mechanism of Action: Pharmaceutical delivery modality.

Application: Quantify the key parameters governing drug transport processes in radiofrequency (RF) thermoablated and non-ablated liver tissues.
Route of Administration: Variable.
Dosage: Not applicable (delivery modality).
Implementation: Determination of the spatial distribution of doxorubicin by measuring fluorescence imaging from 1 to 96 hours after implantation of doxorubicin-containing polymer millirods. A mathematical model can be applied to quantitatively describe the transport processes in ablated and non-ablated rat livers.

Animal Model: Rat (ablated and non-ablated liver tissue).
Interpretation and Analysis: At all time points, doxorubicin has significantly higher tissue penetration and retention in ablated tissues than in non-ablated tissues. Based on the experimental data and mathematical models, the optimal estimates of apparent drug diffusivities in ablated and non-ablated tissues are 1.1×10^{-7} and 6.7×10^{-7} cm^2/s, respectively, and the apparent drug elimination rate coefficient is $9.6 \times 10^{-4}/s$ in non-ablated tissues. Results from this study contribute to the fundamental understanding of *in vivo* drug transport in liver tissues and provide the quantitative parameters for the rational design of polymer millirods for liver cancer treatment and diagnosis.

Quantification of *in vivo* doxorubicin transport from PLGA millirods in thermoablated rat livers. Qian F, Stowe N, Liu EH, Saidel GM, Gao J. J Control Release 2003 Aug 28; 91 (1–2), pp. 157–66.

Urinary: Vesicoureteral Reflux Grading
Levovist

Background Information: The sonographic diagnosis of vesicoureteral reflux (VUR) with contrast-enhanced voiding urosonography (VUS) is gradually increasing. Introduction of VUS as part of the routine diagnostic imaging modalities for reflux has significantly reduced the number of voiding cystourethrographies (VCUG) necessary in a clinical environment. Similar to VCUG, the grading of reflux in VUS is becoming more and more clinically relevant.
Implementation: Determine if there are any sonomorphologic and sonomorphometric parameters that correlate with reflux grading in VCUG. Complementary investigations involve testing the degree of correlation between the reflux grading system proposed for VUS and the grading system used for VCUG.

The VUS is conducted by the intravesical administration of ultrasound (US) contrast medium (Levovist). Ureteral and pelvicalyceal dilatations before administration of US contrast medium and during reflux are then documented and the renal pelvic diameter measured. The density of microbubbles in the renal pelves is scored on a scale of 1 to 3 (low to high). A grading system for reflux in VUS can be established similar to the international reflux grading system for VCUG with the addition of 1 more differentiation parameter, namely whether the reflux is primarily in a dilated or non-dilated urinary tract. Reflux grades in VUS are then compared with those in VCUG.

Human Model: Children undergoing both VUS and VCUG (n = 186 children, n = 89/186 with VUR in at least 1 and the same kidney-ureter-unit (KUU) in both diagnostic imagings).
Interpretation and Analysis: None of the sonomorphologic and sonomorphometric parameters demonstrate any clear-cut finding that would simplify reflux grading in VUS. In 62% (e.g., n = 59/95) KUUs the reflux grades are the same in both examinations. In 11% (e.g., n = 10/95) and 27% (e.g., n = 26/95) KUUs, the reflux is graded lower or higher, respectively, in VUS than in VCUG. Approximately 57% are in a primarily dilated system and the remaining 43% in a non-dilated one. Approximately 70% of KUUs diagnosed as having grade I reflux in VCUG show as grade 2 VUS procedures. A reflux grading system similar to the 1 used in VCUG can be applied in VUS. Adding the parameter of reflux into a primarily dilated or non-dilated ureter and/or pelvicalyceal system may contribute a further dimension to the reflux grading in VUS. Most of the refluxes labeled as grade 1 in VCUG are actually grade 2 or higher.

Vesicoureteral reflux grading in contrast-enhanced voiding urosonography. Darge K, Troeger J. Eur J Radiol 2002 Aug; 43 (2), pp. 122–8.

Urinary Bladder Rupture: Ultrasonography
Microbubbles

Background Information: Because urinary bladder rupture can become life threatening, a simple, safe technique for detecting such conditions is clinically desirable. Current diagnostic protocols involve radiographic imaging, but ultrasound contrast-based techniques have not been thoroughly evaluated for applicability in conditions of urologic trauma.
Implementation: Ultrasound contrast cystography (contrast cystosonography) can be performed by the infusion of microbubbles suspended in saline solution through a urinary catheter.

Animal Model: Canine (naturally occurring clinical cases of urinary bladder rupture).
Interpretation and Analysis: A positive result consists of visualizing microbubbles sonographically in fluid (urine) contained within the bladder immediately after infusion of contrast into the urinary catheter. A positive result can be easily appreciated in both an *in vitro* model and *in vivo* (n = 2 dogs). Confirmation can be established by radiographic examination and exploratory surgery of the abdomen for conditions of naturally occurring intraperitoneal urinary bladder rupture. Based on previous clinical experience, ultrasound contrast cystography appears to be more sensitive than 2-dimensional abdominal sonography for detecting naturally occurring urinary bladder rupture in dogs.

Diagnosis of urinary bladder rupture using ultrasound contrast cystography: *In vitro* model and 2 case-history reports. Ccoté E, Carroll MC, Beck KA, Good L, Gannon K. Vet Radiol Ultrasound 2002 May–Jun; 43 (3), pp. 281–6.

NANOPARTICLES AND NANOSPHERES

Cardiovascular/Endothelial $\alpha_V\beta_3$ Integrin
Anti-$\alpha_V\beta_3$ Integrin Linked [Gd]-Perfluorocarbon Nanoparticles

Cellular/Molecular Mechanism of Action: Anti-α_V β_3 integrin monoclonal antibody recognizes and physically binds to $\alpha_V\beta_3$ integrin complexes. Gadolinium is an element that has its special orientation modified by magnetic fields. Nanoparticles can serve as a selective delivery modality for a compound and/or diminish elimination and metabolism within the plasma compartment.
Background Information: $\alpha_V\beta_3$ integrin is a molecular marker that is a biological characteristic of angiogenic endothelium.
Application: Site-directed contrast image enhancement of (micro)angiogenic vessels. Gadolinium can be detected by magnetic resonance (MR) imaging capabilities.
Route of Administration: Intravenous.

Animal Model: Rabbit (corneal micropocket with neovascularization induced using basic fibroblast growth factor).
Interpretation and Analysis: Selectively targeted contrast agent *in vivo* displays a 25% increase in the average MR signal intensity 90 minutes postinjection. Control groups in which nanoparticles are either used alone, linked to an isotype-matched antibody, or linked to DM101 and administered following receptor blocking do

not display MR contrast enhancement at similar dose levels. Antibody-targeted agents enhance MR signal intensity of the capillary bed in a corneal micropocket model of angiogenesis and are selectively retained within the angiogenic region via specific interaction with $\alpha_V\beta_3$ epitopes.

Magnetic resonance contrast enhancement of neovasculature with $\alpha_v\beta_3$-targeted nanoparticles. Anderson SA, Rader RK, Westlin WF, Null C, Jackson D, Lanza GM, Wickline SA, Kotyk JJ. Magn Reson Med 2000 Sep; 44 (3), pp. 433–9.

Cardiovascular Endothelium: Angiogenesis/ Atherosclerosis
$\alpha_v\beta_3$-Integrin-Targeted Paramagnetic Nanoparticles

Background Information: Angiogenesis is a critical feature of plaque development in atherosclerosis and might play a key role in both the initiation and subsequent rupture of plaques that lead to myocardial infarction and stroke. The precursory molecular or cellular events that initiate plaque growth and that ultimately contribute to plaque instability, however, cannot be detected directly with any current diagnostic modality.
Application: Detection of alterations in vascular angiogenesis.
Route of Administration: Intravascular.
Implementation: Evaluation of angiogenesis phenomenon using $\alpha_V\beta_3$-integrin-targeted paramagnetic nanoparticles injected intravenously.
Animal Model: New Zealand White rabbits (atherosclerosis model dietarily induced by feeding 1% cholesterol for approximately 80 days).
Interpretation and Analysis: $\alpha_V\beta_3$-integrin-targeted paramagnetic nanoparticles can specifically detect neovasculature structures within 2 hours by routine magnetic resonance imaging (MRI) at a clinically relevant field strength (1.5 T). Increased angiogenesis can be detected as a 47 $\pm$ 5% enhancement in MRI signal averaged throughout the abdominal aortic wall following $\alpha_V\beta_3$-targeted paramagnetic nanoparticle administration. Pretreatment of atherosclerotic rabbits with $\alpha_V\beta_3$-targeted non-paramagnetic nanoparticles competitively blocks specific contrast enhancement of the $\alpha_V\beta_3$-targeted paramagnetic agent. Magnetic resonance imaging analysis can detect patterns of increased $\alpha_V\beta_3$-integrin distribution within the atherosclerotic wall that are spatially heterogeneous along both transverse and longitudinal planes of the abdominal aorta. Histology and immunohistochemistry can detect marked proliferation of angiogenic vessels within the aortic adventitia, coincident with prominent, neointimal proliferation among cholesterol-fed, atherosclerotic *in vivo* models in comparison with a sparse incidence of neovasculature in reference controls. Such approaches for molecular imaging may provide a method for defining the burden and evolution of atherosclerosis in susceptible individuals as well as responsiveness of individual conditions to antiatherosclerotic therapies.

Molecular imaging of angiogenesis in early-stage atherosclerosis with $\alpha_v\beta_3$-integrin-targeted nanoparticles. Winter PM, Morawski AM, Caruthers SD, Fuhrhop RW, Zhang H, Williams TA, Allen JS, Lacy EK, Robertson JD, Lanza GM, Wickline SA. Circulation 2003 Nov 4; 108 (18), pp. 2270–4.

Cardiovascular Endothelium: Selective Targeting/ Inflammation/Ischemia/Neoplastic Disease
Polymerized Nanoparticle

Background Information: Successful formulation of personalizing treatment regimens requires the implementation of protocols for appropriate case selection, discovery of surrogate diagnostic biomarkers for monitoring the effects of treatment, and development of an efficacious targeted form of therapy.
Application: Selective detection and targeting of disease-specific biomarker systems.
Route of Administration: Intravascular.
Implementation: Development of a polymerized nanoparticle platform technology that will allow the application of different targeting moieties on the surface of particle platforms, in addition to loading the particles with different contrast and therapeutic agents.
Interpretation and Analysis: Nanoparticles can be targeted to endothelial receptors for the delivery of different payloads of contrast agents and therapeutic pharmaceuticals in a manner that provides a high target-to-background ratio. Using this combined vascular targeted imaging and therapy approach, it is highly possible that personalized/individualized treatment regimens can be developed for a variety of disease processes such as cancer, inflammation, and ischemia.

Combined vascular targeted imaging and therapy: A paradigm for personalized treatment. Li KC, Guccione S, Bednarski MD. J Cell Biochem Suppl 2002; 39, pp. 65–71.

Central Nervous System (CNS): Blood-Brain Barrier (BBB) Penetration
Nanoparticles (Solid Lipid)/Superparamagnetic Solid Lipid Nanoparticles

Cellular/Molecular Mechanism of Action: Nanoparticles function as a carrier modality that is capable of changing the pharmacokinetic parameters and biodistribution of various pharmaceutical agents.
Application: Solid lipid nanoparticles (SLN) are able to target drugs to lymph after duodenal administration and diffuse across the BBB of the CNS. In addition, SLN have the potential of functioning as an effective carrier/contrast agent modality for CNS magnetic resonance imaging (MRI) analyses.
Semi-Synthetic Methods: Superparamagnetic SLN can be prepared to possess colloidal size characteristics and have *in vitro* relaxometric properties (e.g., similar to Endorem).
Route of Administration: Intravascular.
Interpretation and Analysis: *In vivo* MRI of the CNS with both SLN and Endorem shows that superparamagnetic SLN have slower blood clearance than Endorem. Results from MRI analysis are consistent with the CNS uptake of SLN that lasts up to 135 minutes. Based on these observations, SLN can traverse across the BBB of the CNS.

In vitro and *in vivo* study of solid lipid nanoparticles loaded with superparamagnetic iron oxide. Peira E, Marzola P, Podio V, Aime S, Sbarbati A, Gasco MR. J Drug Target 2003 Jan; 11 (1), pp. 19–24.

Central Nervous System (CNS)/Blood-Brain Barrier (BBB) Penetration
[^{14}C]-PEGylated Polyalkylcyanoacrylate Nanoparticles/ Poloxamine 908-Coated Polyalkylcyanoacrylate Nanoparticles/Non-Long-Circulating Polyalkylcyanoacrylate Nanoparticles

Background Information: Under healthy conditions, the BBB limits the passage of solutes and cells from the blood to the CNS. During neurological diseases, BBB permeability increases dramatically, and it has been hypothesized that drug carrier systems such as polymeric nanoparticles could cross the BBB and penetrate into the CNS.

Application: PEGylated polyalkylcyanoacrylate nanoparticles (long-circulating carrier) can potentially be applied as a carrier modality for the transport of agents across the BBB, as has been demonstrated in preliminary investigations for conditions of experimental allergic encephalomyelitis (murine EAE).
Route of Administration: Intravascular.
Implementation: Evaluation of brain and spinal cord concentrations of [^{14}C]-radiolabeled PEGylated polyalkylcyanoacrylate nanoparticles compared to another blood long-circulating carrier (poloxamine 908-coated polyalkylcyanoacrylate nanoparticles) and conventional non-long-circulating polyalkylcyanoacrylate nanoparticles. To better characterize deposition/localization within neurologic tissues, microscopic examination can be performed to detect fluorescent nanoparticles in order to further understand the mechanism by which they penetrate across the BBB.
Interpretation and Analysis: Concentrations of PEGylated nanoparticles in the CNS, especially within white matter regions, is greatly increased compared to profiles noted for conventional non-PEGylated nanoparticles. In addition, this increase is significantly higher in pathological situations where BBB permeability is augmented and/or macrophage populations have infiltrated. Passive diffusion and macrophage uptake in inflammatory lesions seems to be the mechanism underlying the brain penetration of such particles. Due to their long-circulating properties within blood and on their surface characteristics that promote cell interactions, PEGylated nanoparticles penetrate into the CNS to a greater extent than all other related formulations tested to date. Thus, PEGylated polycyanoacrylate nanoparticles can potentially function as a new brain delivery system during conditions of neuroinflammatory diseases.

Quantification and localization of PEGylated polycyanoacrylate nanoparticles in brain and spinal cord during experimental allergic encephalomyelitis in the rat. Calvo P, Gouritin B, Villarroya H, Eclancher F, Giannavola C, Klein C, Andreux JP, Couvreur P. Eur J Neurosci 2002 Apr; 15 (8), pp. 1317–26.

Central Nervous System (CNS)/Blood-Brain Barrier (BBB)/Transferrin Receptor Complexes/ Demyelinated Axons
Oligodendrocyte Progenitor Cells/ [Transferrin Magnetic Nanoparticles]

Cellular/Molecular Mechanism of Action: Oligodendrocyte progenitor cells naturally migrate to specific foci within the CNS, including demyelinated axons.
Background Information: Demyelination is a common pathological finding in human neurological diseases and frequently persists as a result of failure of endogenous repair. Transplanted oligodendrocytes and their precursor cells can (re)myelinate axons, raising the possibility of therapeutic intervention. The migratory capacity of transplanted cells is of key importance in determining the extent of (re)myelination and can, at present, be evaluated only by the use of invasive and irreversible procedures.
Application: Potential delivery modality that recognizes demyelinated axons.
Route of Administration: Presumably intravascular.
Implementation: Delineation of the capacity for transferrin receptors to function as an effective "target" that can facilitate the intracellular delivery of magnetic nanoparticles and guide the migration of transplanted tagged oligodendrocyte progenitor cells into the spinal cord containing myelin-deficient neurons.
Animal Model: Rat (myelin-deficient neurons).
Interpretation and Analysis: Cell migration can be easily detected by using 3-dimensional magnetic resonance microscopy, which demonstrates a close correlation between the areas of contrast enhancement and degree of "new" myelination in an *in vivo* myelin deficient model. In this context, investigations have demonstrated the feasibility of successfully using magnetic resonance to track transplanted oligodendrocyte progenitor cells. The technique has the potential of being easily extended into other neurotransplantation research investigations involving different precursor cell types.

Neurotransplantation of magnetically labeled oligodendrocyte progenitors: Magnetic resonance tracking of cell migration and myelination. Bulte JW, Zhang S, van Gelderen P, Herynek V, Jordan EK, Duncan ID, Frank JA. Proc Natl Acad Sci U S A 1999 Dec 21; 96 (26), pp. 15256–61.

Central Nervous System (CNS)/Infarction/ Discrimination of Normal vs Infarctive Lesions/ Blood-Brain Barrier (BBB)/Discrimination: Normal vs Infarcted Myocardium
Peptide Delivery/Polysorbate 80-Coated Polybutylcyanoacrylate Nanoparticles

Background Information: The BBB limits the penetration of substances into the brain. Because many drugs, particularly peptides, therefore can't be delivered to the brain, carrier systems need to be developed to overcome this problem. Previous investigations have demonstrated the central analgesic effects of the peptide dalargin (dal) after systemic administration, when this substance is bound to the exterior surface of polybutylcyanoacrylate nanoparticles and coated with polysorbate 80, but not when given alone.
Application: Delivery of polypeptides across the BBB.
Route of Administration: Intended for intravascular administration, but preparations have the potential of facilitating oral routes of administration for biologically active peptides.
Implementation: Determine the body distribution of [^{3}H]-labeled dal bound to nanoparticles compared to unbound dal after intravenous injection. The radioactivity in several tissues, including the brain, is separated into subcellular preparations and measured over time after a single intravenous injection.
Animal Model: Murine.
Interpretation and Analysis: Dal radioactivity levels in brain preparations is 3 times higher when the drug is bound to nanoparticles, whereas the 1st-pass pathway in liver is reduced. Such results support previous reports that nanoparticles can be used to transport peptides across the BBB.

Body distribution of [^{3}H]-labeled dalargin bound to poly(butyl cyanoacrylate) nanoparticles after i.v. injections to mice. Schroeder U, Schroeder H, Sabel BA. Life Sci 2000; 66 (6), pp. 495–502.

Central Nervous System (CNS) Neoplasia/Tumor Proliferation Rate/Tumor-Associated Macrophages/ Tumor-Associated Angiogenesis
Dextran-Coated Iron Oxide Particles (LCDIO)/ Rhodaminated LCDIO Green Fluorescent Protein (GFP)/ Hoechst 33258

Cellular/Molecular Mechanism of Action: Hoechst 33258 dye can be applied as an intravital endothelial stain. Rhodaminated LCDIO particles can preferentially localize intracellularly within certain malignant neoplastic cell types (e.g., gliosarcoma).
Application: Detection and characterization of CNS neoplastic lesions (e.g., gliosarcoma).
Route of Administration: Intravascular.
Implementation: Determination of the distribution of LCDIO preparation in tumors. Accumulation of LCDIO in tissue sections is

evaluated using multichannel fluorescence microscopy in combination with rhodaminated LCDIO, GFP functioning as a tumor marker, and Hoechst 33258 dye serving as an intravital endothelial stain. Uptake into tumor cells is corroborated with results from immunohistochemical and cell culture uptake analyses. The effect of intratumoral LCDIO uptake on magnetic resonance imaging (MRI) signal intensity is evaluated with a 1.5-T superconducting magnet.

Animal Model: Rodent gliosarcoma.

Interpretation and Analysis: Tumoral accumulation of LCDIO is 0.11% ± 0.06 of the injected dose per gram of tissue in brain tumors and is sufficient for detection using MR imaging. In tumor sections, LCDIO preferentially localizes in tumor cells (49.0% ± 4.6) but is also taken up by macrophages within tumors (21.0% ± 3.1) and by endothelial cells in the areas of active angiogenesis (6.5% ± 1.4). In cell culture, LCDIO uptake strongly correlates with growth rate of tumor cell lines. Tumoral LCDIO accumulation is not negligible and helps explain MRI signal intensity changes observed in clinical trials. Microscopically, LCDIO accumulates predominantly in tumor cells and tumor-associated macrophages. Uptake into tumor cells appears to be directly proportional to cellular proliferation rate.

Instrumentation: Magnetic resonance imaging signal intensity evaluated with a 1.5-T superconducting magnet.

Tumoral distribution of long-circulating dextran-coated iron oxide nanoparticles in a rodent model. Moore A, Marecos E, Bogdanov A Jr, Weissleder R. Radiology 2000 Feb; 214 (2), pp. 568–74.

Fluorescent Probe Delivery
Nanospheres: Fluorescent Probe Protected Delivery/ PEBBLEs: Probes Encapsulated by Biologically Localized Embedding

Cellular/Molecular Mechanism of Action: Nanospheres can influence the pharmacodynamics of a compound by delaying elimination, slowing biological degradation, and facilitating selective delivery.

Route of Administration: Intravascular injection.

Semi-Synthetic Methods: Spherical optical nanosensors, or PEBBLEs, can be produced in various sizes, including ranges between 20 nm and 200 nm (diameter). The sensors are fabricated in a microemulsion and consist of fluorescent indicators entrapped in a polyacrylamide matrix. A generalized polymerization method has been developed that permits production of sensors containing any hydrophilic dye or combination of dyes within the matrix.

Interpretation and Analysis: The PEBBLE matrix protects fluorescent dyes from interference by proteins, allowing reliable *in vivo* dye calibration. Sensor response times are less than 1 ms. Cell viability assays indicate that PEBBLEs are biocompatible and exert negligible biological effects compared to reference controls. Several sensor delivery methods have been studied, including liposomal delivery, gene gun bombardment, and *pico*-injection into single living cells.

Optical nanosensors for chemical analysis inside single living cells. 1. Fabrication, characterization, and methods for intracellular delivery of PEBBLE sensors. Clark HA, Hoyer M, Philbert MA, Kopelman R. Anal Chem 1999 Nov 1; 71 (21), pp. 4831–6.

Gastrointestinal: Trans-Mucosal Delivery/ Epithelial Cell Tight Junctions: Induced Opening
Poly[Methacrylic Acid-Grafted-Poly(Ethylene Glycol)]/Nanospheres/[Trypan Blue Exclusion]

Background Information: The challenges of oral delivery for therapeutic agents with unfavorable physicochemical properties, such as proteins, have been the primary motivating factor leading to the design and development of novel oral delivery systems that could circumvent biological barriers.

Application: Facilitate delivery of diagnostic and therapeutic agents across anatomical barriers such as the mucosa of the gastrointestinal tract. Trypan blue is applied as a cell vitality stain reagent (exclusion vitality dye reagent).

Route of Administration: Oral (nanospheres); topical (trypan blue).

Implementation: Examine the influence of complexation-sensitive hydrogel nanospheres composed of poly[methacrylic acid-grafted-poly(ethylene glycol)] (P(MAA-g-EG)) on a model biological environment. An example of how to evaluate such properties can entail determination of the cytotoxic effects of the polymeric carrier and its effects on the cell monolayer integrity. The determination of the cytotoxic effects of the polymer network on the cell monolayer can be performed by a colorimetric assay and by the counting of viable cells using the trypan blue exclusion method. Electrophysiological measurements can also be performed to measure the transepithelial electrical resistance changes in the monolayers in the presence and absence of the nanosphere suspension.

***Ex Vivo* Model:** Intestinal mucosal cells propagated *in vitro* in a tissue culture environment (Caco-2 cell line).

Interpretation and Analysis: The examination of the physicochemical interactions of P(MAA-g-EG) nanosphere system with Caco-2 cell monolayers reveals that these systems possess low cytotoxicity and are capable of opening the tight junctions between epithelial cells, therefore significantly reducing trans-epithelial electrical resistance.

Physicochemical behavior and cytotoxic effects of p(methacrylic acid-g-ethylene glycol) nanospheres for oral delivery of proteins. Torres-Lugo M, García M, Record R, Peppas NA. J Control Release 2002 Apr 23; 80 (1–3), pp. 197–205.

Gene Delivery/Metalloproteases/Influence on Inflammatory Response/Tumor Necrosis Factor-Alpha (TNF-α), Interleukin (IL)-1β, IL-6, and IL-10 and Metalloproteinases (MMP-2 and MMP-9)
Chytosan-DNA Nanoparticles

Physiological Mechanism of Action: Macrophages play an important and active role in inflammatory processes.

Background Information: The use of nanoparticles for gene therapy is gaining more and more interest for medical applications. Chitosan is among the candidate polymers that can potentially be applied as a gene delivery modality.

Application: Chitosan is a candidate compound potentially suitable for facilitating gene (e.g., olidonucleotide) delivery.

Considerations: Before using chitosan-DNA nanoparticles *in vivo*, it is necessary to first thoroughly characterize their interactions with cells and determine how such phenomenon influences their biological properties.

Implementation: Evaluation of the influence of chitosan-DNA nanoparticles on macrophage-mediated inflammatory responses (e.g., human THP-1 cell lines). Profiling over time the production and release of cytokines (TNF-α, IL-1β, IL-6, and IL-10) and metalloproteinase (MMP-2 and MMP-9), as well as their inhibitors (TIMP-1 and TIMP-2) in the presence of different amounts of nanoparticles is 1 approach to determining the biological properties of such preparations. Concentration of these mediators of immunity and inflammation can be quantified by enzyme-linked immunosorbent assay (ELISA). Gelatinolytic activity of MMP-2 and MMP-9 can be determined by zymography in cell supernatants and lysates.

Interpretation and Analysis: Cytokine secretion can usually not be detected even in the presence of high amounts of nanoparti-

cles. Alternatively, the secretion of MMP-9 in cell supernatants increases significantly after 24 hours and 48 hours compared to negative reference control cell populations. MMP-2 secretion is augmented only after 48 hours with the highest concentrations of nanoparticles (10 and 20 μg/ml DNA content). However, zymography studies have shown that secreted MMPs are in the zymogen (proenzyme) forms, while the active form of MMP-9, but not MMP-2, can be detected in cell lysates when 10 and 20 μg/ml DNA containing nanoparticles are used. In conclusion, exposure of THP-1 macrophages to Ch-DNA nanoparticles does not induce the release of proinflammatory cytokines. The presence of active MMP-9 within macrophages may be related to nanoparticle phagocytosis and degradation rather than to inflammatory reactions.

Metalloproteinase and cytokine production by THP-1 macrophages following exposure to chitosan-DNA nanoparticles. Chellat F, Grandjean-Laquerriere A, Naour RL, Fernandes J, Yahia L, Guenounou M, Laurent-Maguin D. Biomaterials 2005 Mar; 26 (9), pp. 961–70.

Gene Delivery: Oligonucleotide (Hybridization)
Magnetic Bead-Linked Oligonucleotide Probes

Cellular/Molecular Mechanism of Action: Oligonucelotides (e.g., antisense strands) incorporated into magnetic bead-linked oligonucleotide probes will hybridize with sense oligonucleotide strands (e.g., form cDNA/cDNA diamers or mRNA/cDNA heteriomers).
Application: Methodology for the detection of gene expression that could potentially be developed or adapted as a technique for the non-invasive detection of *in vivo* gene expression.
Route of Administration: Intravascular most commonly (selective and non-selective delivery).
Implementation: Hybridization of a target oligonucleotide to magnetic bead-linked oligonucleotide probes is followed by binding of the streptavidin-coated metal nanoparticles to the captured DNA, dissolution of the nanometer-sized gold tag, and potentiometric stripping measurements of the dissolved metal tag at single-use thick-film carbon electrodes.

An advanced magnetic processing technique is used to isolate the DNA duplex and to provide low-volume mixing. The influence of relevant experimental variables, including the amounts of gold nanoparticles and the magnetic beads, the duration of the hybridization and gold dissolution steps, and the parameters of the potentiometric stripping operation, on the hybridization signal can be effectively optimized and have been previously described by several laboratories.
Interpretation and Analysis: Transmission electron microscopy micrographs indicate that hybridization leads to the bridging of the gold nanoparticles to the magnetic beads. Further signal amplification, and lowering of the detection limits to the nanomolar and picomolar range, are achieved by precipitating gold or silver, respectively, onto the colloidal gold label. The new electrochemical stripping metallogenomagnetic protocol couples the inherent signal amplification of stripping metal analysis with discrimination against non-hybridized DNA and the use of microliter sample volumes and disposable transducers, and hence offers great promise for decentralized genetic testing.

Metal nanoparticle-based electrochemical stripping potentiometric detection of DNA hybridization. Wang J, Xu D, Kawde AN, Polsky R. Anal Chem 2001 Nov 15; 73 (22), pp. 5576–81.

Gene Expression: Phage Facilitated Delivery
Pullulan (Hydrogel) Nanoparticles/ [Lipofectamine 2000]

Application: Nanoparticles can facilitate the transport of vectors across intact cell membranes. Vectors (e.g., phage) can be utilized to transfect cells with "foreign" DNA sequences in a manner that allows amplified production of the desired gene and the biological protein translated from its corresponding messenger RNA (mRNA) sequence.
Route of Administration: Intravascular.
Semi-Synthetic Methods: Pullulan nanoparticles encapsulating pBUDLacZ plasmid can be prepared inside aqueous droplets with or without the use of microemulsions.
Implementation: Evaluation of cellular viability/vitality (cytotoxicity response) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay following cellular transfection.
Interpretation and Analysis: Transmission electron microscopy (TEM) imaging reveals that particles are spherical in shape with a size of 45 (± 0.80) nm diameter. Cell cytotoxicity studies reveal that cells incubated with nanoparticles remain more than 100% viable at nanoparticle concentration as high as 1,000 μg/ml. From scanning electron microscope images, nanoparticles can be observed internalized within cells that tend to exhibit vacuole changes within their cell body. Endocytosis of nanoparticles results in disruption of F-actin and beta-tubulin cytoskeleton of human fibroblasts. The effect of *in vitro* tranfection on HEK293 and COS-7 cells is cell-type dependent, with COS cells demonstrating a higher level of gene expression. The beta-gal expression in COS-7 cells facilitated by pullulan nanoparticle is comparable to commercially available Lipofectamine 2000. Such findings are encouraging for the development of pullulan nanoparticles as an intracellular delivery system for drugs and genes.

Hydrogel pullulan nanoparticles encapsulating pBUDLacZ plasmid as an efficient gene delivery carrier. Gupta M, Gupta AK. J Control Release 2004 Sep 14; 99 (1), pp. 157–66.

Gene Delivery/Solid Tumor Nucleic Acid Delivery
Nanoparticles (20–1,000 nm)/Liposome: Cationic (100–200 nm)/Porphyrin Derivatives (< 1 nm)/ Branched-Chain Dendrimers (10 nm)/ Polyethylenimine Polymers (10 nm)/Nanoparticles (20–1,000 nm)/Microspheres (> 1 μm)/Cyclodextrins (CyDs 1.5 nm)

Background Information: The vasculature of a tumor provides the most effective route by which neoplastic cells may be reached and eradicated by chemotherapeutics and biopharmaceuticals. The fact that a tumor's vasculature is relatively more permeable than healthy host tissue should enable selective delivery of diagnostic and therapeutic agents to neoplastic tissues. Such approaches to delivery are relevant to the carrier-mediated delivery of genetic-based therapies to tumors. Cationic liposomes have been used for the introduction of nucleic acids into mammalian cells for more than a decade.
Application: Nucleic acid delivery into cells (in vitro and in vivo).
Semi-Synthetic Methods: Cationic liposomes are generally 100–200 nm in diameter, whereas CyDs typically have a diameter span of 1.5 nm.
Implementation: Evaluate the potential of delivering therapeutic oligonucleotides (ONs) to tumors using cationic liposomes and CyDs, and determine the major biological properties of tumors that hinder such delivery modalities.
Interpretation and Analysis: CyD molecules are routinely used as agents that engender cholesterol efflux from lipid-laden cells, thus having efficacious potential in the management of atherosclerosis. A recent trend is to employ these oligosaccharide molecules for delivering nucleic acids into the interior of cells in both *in vitro* and *in vivo* environments. Comparisons have been made with other oligonucleotide delivery agents, such as porphyrin derivatives

(< 1 nm), branched-chain dendrimers (approximately 10 nm), polyethylenimine polymers (approximately 10 nm), nanoparticles (20–1,000 nm), and microspheres (> 1 μm), in the context of delivery formats for solid tumors. The chemical and physical properties of these carriers and how they may affect the uptake of oligonucleotides into cells, particularly *in vivo*, have been described in published reports.

Vehicles for oligonucleotide delivery to tumors. Dass CR. J Pharm Pharmacol 2002 Jan; 54 (1), pp. 3–27.

Lymphatic System Delivery
Nanospheres

Synthetic Chemistry and Analysis: Development and production of a sub-100 nm biodegradable colloidal system for the efficient delivery of drugs and diagnostic agents to the lymphatic system. Nanospheres of poly(lactide-co-glycolide) can be prepared by interfacial polymer deposition. Nanospheres are coated with block copolymers in order to modify their surface characteristics. Radiolabeling of the nanospheres for *in vivo* tracing can be achieved by incorporation of the lipophilic complex [^{111}In]-oxine during the preparation of nanospheres. Stability of radiolabeled nanospheres *in vitro* can be determined at 37°C (e.g., rat serum).
Interpretation and Analysis: The lymphatic distribution of nanospheres determined after subcutaneous administration (rat) reveals lymphatic uptake of all coated systems is enhanced compared to non-coated nanospheres. Maximal uptake of 17% of the administered dose in the regional lymph nodes can realistically be achieved and suggests that nanospheres are a suitable modality for diagnostic and therapeutic applications in clinical and experimental medicine.

Lymph node localization of biodegradable nanospheres surface modified with poloxamer and poloxamine block copolymers. Hawley AE, Illum L, Davis SS. FEBS Lett 1997 Jan 6; 400 (3), pp. 319–23.

Lymphatic System Delivery: Selective
Biodegradable Nanoparticulate Systems/ (Nanospheres, Emulsions, Liposomes)

Cellular/Molecular Mechanism of Action: Nanospheres and nanoparticles can influence the pharmacodynamics of a compound by delaying elimination, slowing biological degradation, and facilitating selective delivery.
Application: Selective delivery and accumulation of agents (diagnostic pharmaceuticals/chemotherapeutic agents, antigens) within regional lymph node structures. Localization of diagnostic agents in the regional lymph nodes can be of benefit as an anatomical/pathological guide during surgical procedures. Furthermore, they potentially serve as a modality that can enhance the oral bioavailability of macromolecular drugs, like polypeptides or biological proteins, that are known to selectively localize within the Peyer's patches of the intestine.
Route of Administration: Subcutaneous.
Implementation: Nanocapsules (ultrafine oily droplet-coated polymeric substances) are probably 1 of the most promising candidates of colloidal carriers. Surface engineering by the interfacial deposition method can create a suitable size distribution and the necessary surface characteristics required of nanocapsules.
Diagnostic Interpretation and Implications: Recent *in vivo* studies have demonstrated that polyisobutylcyanoacrylate nanocapsules possess enhanced accumulation of biological compounds and pharmaceuticals within lymph nodes, compared with other carriers such as emulsions and liposomes.

Lymphatic targeting with nanoparticulate system. Nishioka Y, Yoshino H. Adv Drug Deliv Rev 2001 Mar 23; 47 (1), pp. 55–64.

Lymphatic System Delivery: Selective
Nanoparticulate Systems: Biodegradable/ (Nanospheres, Emulsions, Liposomes)

Cellular/Molecular Mechanism of Action: Nanospheres and nanoparticles can influence the pharmacodynamics of a compound by delaying elimination, slowing biological degradation, and facilitating selective delivery.
Application: Selective delivery and accumulation of agents (diagnostic pharmaceuticals/chemotherapeutic agents) within structures of the lymphatic system (regional lymph nodes and lymphatic vessels). Localization of diagnostic agents in the regional lymph nodes can be of benefit as an anatomical/pathological guide during surgical procedures. Additionally, they can potentially serve as a modality for enhancing the oral bioavailability of macromolecular drugs, like polypeptides or proteins that are known to selectively accumulate within the Peyer's patches of the intestine.
Route of Administration: Subcutaneous.
Synthetic Chemistry and Analysis: The surface modification of model polystyrene (PS) and poly(lactide-co-glycolide) (PLGA) nanospheres by poly(lactide)-poly(ethylene glycol) (PLA:PEG) copolymers has been assessed by *in vitro* laboratory analyses and *in vivo* biodistribution studies following subcutaneous administration of the nanospheres (rat). Preparations of PLA:PEG copolymers have been characterized that possess PEG chain lengths of 750, 2,000, and 5,000 Da.

Preparations of PS and PLGA nanospheres can subsequently be "coated" with PLA:PEG copolymers or the latter can be co-precipitated in the formation of PLGA-PLA:PEG nanospheres. Validation of PLA:PEG "coating" of nanospheres can be established by detecting an increase in their overall particle size and a corresponding decrease in surface potential.
Considerations: A substantial amount of research has previously been devoted to delineating strategies capable of selectively "targeting" diagnostic pharmaceutical agents for the lymphatic system using colloidal carrier compounds. Nanocapsules, which are ultrafine oily droplet-coated polymeric substances, are probably one of the most promising candidates of colloidal carriers. Surface engineering utilizing the interfacial deposition method can produce a suitable size distribution and necessary surface characteristics to the nanocapsules. Observations noted in preliminary investigations have demonstrated the feasibility of producing fully biodegradable synthetic nanosphere systems that possess excellent lymph node targeting characteristics.
Interpretation and Analysis: Investigations conducted *in vivo* have demonstrated that polyisobutylcyanoacrylate nanocapsules can enhance the accumulation of pharmaceuticals within lymph nodes to a relatively greater degree compared to other carriers such as emulsions and liposomes. The kinetics of injection site drainage and lymph node retention determined over a 24-hour time period for non-"coated," "coated," and co-precipitated nanosphere systems demonstrate that their distribution is dependent upon the composition of their exterior surface. Significant modification of their characteristics can be achieved through such strategies for the purpose of achieving variable degrees of lymph node accumulation. Such features are extensively enhanced by "coating" their exterior surface with PLA:PEG copolymers or through their production as co-precipitated PLGA or PLA:PEG nanospheres. Dependent on the surface characteristics, the distribution of nanospheres can be significantly modified and the lymph node localization dramatically enhanced by coat-

ing their surfaces with PLA:PEG copolymers or by producing coprecipitate nanospheres of PLGA and PLA:PEG.

Preparation of biodegradable, surface engineered PLGA nanospheres with enhanced lymphatic drainage and lymph node uptake. Hawley AE, Illum L, Davis SS. Pharm Res 1997 May; 14 (5), pp. 657–61.

Lymph Node (Regional)/Selective Delivery
Poly(Ethylene Glycol) (PEG)-Modified Magnetite Nanospheres

Application: Selective diagnostic imaging or selective pharmaceutical delivery to regional lymph nodes.
Route of Administration: Subcutaneous injection (rat).
Synthetic Chemistry and Analysis: Magnetite nanospheres can be prepared with a grafted PEG layer using various PEG lengths ranging from 350 to 1,000 Da. Thermogravimetric analysis can be utilized to measure the adsorbed amount of PEG. Colloid stability can be confirmed by measuring particle size and electrophoretic mobility.
Considerations: Effectiveness of surface modifications of magnetite nanospheres with PEG can be assessed by *in vitro* characterization and *in vivo* biodistribution studies following subcutaneous administration (rat).
Implementation: The kinetics of injection site drainage and lymph node retention can be determined 2 hours after subcutaneous administration for nanospheres coated with PEG lengths of 350, 550, 750, and 1,000 Da, while for 750 PEG-coated nanospheres, the kinetics of distribution can be determined over a 48-hour time period.
Implications and Analyses: The distribution of nanospheres is modified and the lymph node localization enhanced by altering the surface coverage of PEG on the magnetic surface. PEG-coated magnetite nanospheres with different surface characteristics can be utilized to target diagnostic agents to regional lymph nodes.

Development of systems for targeting the regional lymph nodes for diagnostic imaging: *In vivo* behavior of colloidal PEG-coated magnetite nanospheres in the rat following interstitial administration. Illum L, Church AE, Butterworth MD, Arien A, Whetstone J, Davis SS. Pharm Res 2001 May; 18 (5), pp. 640–5.

Macrophage Function/Hepatic Mononuclear Phagocytic Activity
Superparamagnetic Iron Oxide (AMI-25)/ [^{99m}Technetium] ([^{99m}Tc])-Phytate

Cellular/Molecular Mechanism of Action: Superparamagnetic iron oxide (AMI-25) is non-selectively assimilated intracellularly by the hepatic mononuclear phagocytic system.
Application: Assessment of hepatic phagocytic activity with potential applications for also evaluating the pulmonary phagocytic system.
Route of Administration: Intravascular.
Implementation: Evaluation of the potential for measuring hepatic phagocytic activity by dynamic superparamagnetic iron oxide-enhanced magnetic resonance (MR) imaging. One approach that can be utilized for achieving this objective includes the application of dynamic MR imaging analysis in concert with performing a radioisotope study with [^{99m}Tc]-phytate. In a dynamic MR study of this nature, the ratio (Rv) of the distribution volumes of iron oxide (Vm/Ve) can be obtained by applying a 3-compartment model to data obtained simultaneously from the kidney and liver. Changes in Rv caused by silica injection and by dosing superparamagnetic iron oxide, AMI-25, can subsequently be evaluated. In the dynamic MR study using an *in vivo* model, the input function can be calculated from data obtained from the hepatic artery and portal vein.

Animal Model: Rabbits (New Zealand White) randomly allocated to normal and silica-treated groups.

Animal Model: Beagle dog (hepatic artery and portal vein).
Interpretation and Analysis: The ratio of the distribution volumes for iron oxide (Vm/Ve) reaches a maximum value at around 80 minutes after AMI-25 injection. The Rv of the normal controls groups is approximately 5.06 $\pm$ 1.53, whereas the Rv of the silica-treated group is approximately 2.13 $\pm$ 1.20. The results are similar to tissue count data obtained in radioisotope study. The Rv value is not dependent on the injected dose of AMI-25. The rate of transport constants (k1, k2, k3) cannot be estimated with a 3-compartment model regardless of obtaining the input function. It has therefore been concluded that the ratio of the distribution volumes for iron oxide (Vm/Ve) may be a quantitative index reflecting decreases in phagocytic activity within the liver as determined by dynamic superparamagnetic iron oxide-enhanced MR imaging.

Pharmacokinetic modeling of phagocytic activity of the liver using superparamagnetic iron oxide nanoparticles in dynamic MR imaging. Na JB, Suh JS, Huh YM, Kim SJ, Kim SH, Cha SH, Lee SH. Yonsei Med J 2003 Jun 30; 44 (3), pp. 429–37.

Macrophages: Vascular Lesions/Atherosclerotic Plaques
Ultrasmall Superparamagnetic Iron Oxide Particles (USPIOs)/(Sinerem, Guerbet)

Cellular/Molecular Mechanism of Action: Iron oxide particles change their spatial orientation when placed within an intense magnetic field. Macrophages perceive the particles as foreign and internalize them by phagocytic mechanisms.
Application: Detection and characterization of atherosclerotic plaques.
Dosage: Ultrasmall superparamagnetic particles of iron oxide (1 mmol Fe/kg).
Implementation: Evaluation of USPIOs for their ability to be internalized by macrophages and detect atherosclerosis-associated inflammatory changes in vessel walls before progressing to luminal narrowing. One approach to addressing this objective can entail characterization of the thoracic aorta using 3-dimensional magnetic resonance angiography (MRA) with enhancement of images using a paramagnetic contrast agent. One week later, USPIO (Sinerem, Guerbet) are then injected.

***Ex Vivo* Model:** Rabbit aorta.

Animal Model: Rabbit (n = 6 heritable hyperlipemic New Zealand White; and n = 3 New Zealand White control rabbits).
Interpretation and Analysis: Evaluation of 3D MRA data sets collected over a 5-day period following administration reveals an increasing signal within the aortic lumen. No vascular lesions can be detected at the time of initial imaging. The aortic wall of reference controls remains smooth and bright. Marked susceptibility effects become evident on day 4 within the aortic walls of hyperlipidemic rabbits. *Ex vivo* imaging of aortic specimens confirms such *in vivo* findings. Histopathology analysis can detect macrophage Fe uptake for cells embedded in atherosclerotic plaques in scenarios of hyperlipidemia. Electron microscopy analysis reveals multiple cytoplasmic Fe particles within macrophages. No such changes can be appreciated in reference controls or in conditions of hyperlipidemic not receiving Sinerem. In summary, USPIOs are phagocytosed by macrophages in atherosclerotic plaques of the aortic wall in chronic conditions of hyperlipidemia in a quantity sufficient to cause susceptibility effects that are detectable by MRI.

Magnetic resonance imaging of atherosclerotic plaque with ultrasmall superparamagnetic particles of iron oxide in hyperlipidemic rabbits. Ruehm SG, Corot C, Vogt P, Kolb S, Debatin JF. Circulation 2001 Jan 23; 103 (3), pp. 415–22.

Macrophages/Mononuclear/Neutrophil/ Phagocytic Function
Fluorescent Polymethacrylic Nanoparticles

Cellular/Molecular Mechanism of Action: Fluorescent polymethacrylic nanoparticles are recognized by and intracellularly assimilated by mononuclear cells and, to a lesser extent, neutrophils.
Application: Quantitative evaluation of phagocytosis by human mononuclear and polymorphonuclear cells. One possible application of fluorescent-labeled nanoparticles is the simultaneous characterization of cell surface antigens and cell phagocytic activity.
Route of Administration: Intravascular.
Semi-Synthetic Methods: Preparation of nanoparticles involves emulsion copolymerization of methacrylic monomers combined with delineation of their physicochemical properties.
Implementation: Evaluation of fluorescent polymethacrylic nanoparticles (0.3 micron) as a flow cytometric reagent in the quantitative evaluation of phagocytosis by human mononuclear and polymorphonuclear cells. In this context, nanoparticles coupled with a fluorescent agent (ethidium bromide) can be used in flow cytometric assays to study opsonin-independent phagocytosis by human neutrophils (PMNs) and human monocytes. Phagocytosis of nanospheres by monocytes is determined by flow cytometry based on their patterns of fluorescence distribution, while ingestion is visualized utilizing scanning and transmission electron microscopy.
Instrumentation: Flow cytometry and scanning and transmission electron microscopy.

Flow cytometric quantitative evaluation of phagocytosis by human mononuclear and polymorphonuclear cells using fluorescent nanoparticles. Rolland A, Merdrignac G, Gouranton J, Bourel D, Le Verge R, Genetet B. J Immunol Methods 1987 Feb 11; 96 (2), pp. 185–93.

Macrophage Function/Mononuclear Phagocytic System/Reticuloendothelial System
Nanoparticles: Copolymer Non-Ionic Surfactants/ Polyoxypropylene and Polyoxyethylene

Background Information: A frequently observed but ignored phenomenon following intravenous injection of polymer-modified long-circulating colloidal systems is their eventual recognition and clearance by macrophages (reticuloendothelial system, mononuclear phagocytic system).
Application: Nanoparticles composed of polyoxypropylene and polyoxyethylene copolymer non-ionic surfactants can be applied to detect and characterize the function of macrophage populations.
Route of Administration: Intravascular.
Implementation: Evaluation of the design, handling, and biological assessment of long-circulating nanoparticles composed of polyoxypropylene and polyoxyethylene copolymer non-ionic surfactants. Nanoparticles produced in this fashion are phagocytized by macrophage populations.
Interpretation and Analysis: The pharmacokinetics as well as the tissue distribution of such so-called "stealth" nanoparticles becomes altered after repeated intravenous injection in a time-dependent manner. An understanding of immunological and pathological factors that control the pharmacokinetic and biological behavior of long-circulating particles after single or repeated administration is therefore crucial for the design of a system possessing an optimal degree of diagnostic and/or therapeutic efficacy.

Capture of stealth nanoparticles by the body's defenses. Moghimi SM, Hunter AC. Crit Rev Ther Drug Carrier Syst 2001; 18 (6), pp. 527–50.

Neoplasia: Biomarker Detection/Cervical Cancer
[Probe Molecules]-Gold Nanoparticles

Cellular/Molecular Mechanism of Action: Physically bind to biomarker systems that are unique to specific neoplastic cell types.
Background Information: Recent developments in photonic technology have made it feasible to non-invasively image cells *in vivo,* and these diagnostic strategies therefore have the potential of dramatically improving the prevention, detection, and therapy of epithelial cancers. Endoscope-compatible microscopy, such as optical coherence tomography and reflectance confocal microscopy, image reflected light in a manner that can generate 3-dimensional pictures of tissue microanatomy with excellent spatial resolution (1–10 microm). However, their ability to image molecular biomarkers associated with cancer is limited.
Application: Vital *in vivo* imaging of precancerous conditions.
Route of Administration: Intravascular.
Semi-Synthetic Methods: Development of a class of molecule-specific contrast agents suitable for vital reflectance imaging that is based on attachment of gold nanoparticles to probe molecules with high affinity for specific (unique) cellular biomarkers.
Implementation: Evaluation of the effectiveness of vital reflectance imaging of precancerous conditions using gold bioconjugated nanoparticles containing probe molecules with high affinity for specific cellular biomarkers.
***Ex Vivo* Model:** Three-dimensional cancer cell suspensions in addition to normal and neoplastic fresh cervical biopsies.
Interpretation and Analysis: Gold conjugates can be delivered topically for imaging throughout the whole epithelial surface. Such contrast agents have the potential to extend the ability of vital reflectance microscopy for *in vivo* molecular imaging. In addition, they can potentially enable combined screening, detection, and therapy of diseases using inexpensive imaging systems. Such tools could allow mass screening of diseases such as cancer in resource-poor settings.

Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles. Sokolov K, Follen M, Aaron J, Pavlova I, Malpica A, Lotan R, Richards-Kortum R. Cancer Res 2003 May 1; 63 (9), pp. 1999–2004.

Neoplasia: Carcinoma (Mammary)/Tyrosine Kinase Her-2/neu Receptors
Streptavidin-Conjugated Iron Oxide Nanoparticles/ Biotinylated Monoclonal IgG (Anti Her-2/neu Receptor).

Cellular/Molecular Mechanism of Action: Monoclonal antibody fractions designed to selectively bind to Her-2/neu receptor complexes.
Background Information: One of the clinically relevant targets is the tyrosine kinase Her-2/neu receptor, which has importance in the staging and treatment of breast cancer.
Application: Creation of adjunct information directly relevant to the staging and treatment of breast cancer.
Route of Administration: Intravascular.
Implementation: Detection and characterization of Her-2/neu receptor expression in breast cancer cell populations that express the receptors on their exterior surface membranes at variable densities. Cell populations of interest are pretargeted with anti-Her-2/neu monoclonal antibody followed by exposure to streptavidin-conjugated superparamagnetic nanoparticles as a strategy for selective magnetic resonance (MR) imaging of HER-2/new receptor expression.

Interpretation and Analysis: Streptavidin-conjugated superparamagnetic nanoparticles, when used in combination with pretargeting strategies with biotinylated anti-Her-2/neu monoclonal IgG, can function as an effective strategy for detecting and characterizing the expression of these receptor complexes by magnetic resonance imaging analysis in conditions of mammary carcinoma. In this fashion, strong T_2 MR signals can be generated by Her-2/neu-expressing cells. Furthermore, the contrast observed in MR images is proportional to the expression level of Her-2/neu receptors determined independently by fluorescence-activated cell sorting (FACS) analysis. In this context, iron oxide nanoparticles are attached to the membrane surface of mammary carcinoma cell types, and they are not internalized into the cytosol, which is a major advantage for the *in vivo* applications of such diagnostic strategies.

MR molecular imaging of the Her-2/neu receptor in breast cancer cells using targeted iron oxide nanoparticles. Artemov D, Mori N, Okollie B, Bhujwalla ZM. Magn Reson Med 2003 Mar; 49 (3), pp. 403–8.

Neoplasia: Carcinoma (Pulmonary)/ Lewis Pulmonary Carcinoma *[Monoclonal IgG]-Bovine Serum Albumin Nanospheres (BSA-NS)*

Cellular/Molecular Mechanism of Action: Immunoglobulins (antibodies) can be either naturally or artificially designed to recognize and physically bind to specific molecules (e.g., biological proteins).
Application: Potentially can function as a selective delivery modality for neoplastic lesions, including Lewis lung carcinoma. Brief secondary (non-specific) accumulations also occur within the liver, lungs, and kidney.
Route of Administration: Intravascular.
Semi-Synthetic Methods: Bovine serum albumin nanospheres can be prepared using glutaraldehyde cross-linking and ultrasonication, followed by coating with antibodies utilizing covalent cross-linking techniques (Schiff's base formation) that involve cross-linking the aldehyde groups at the surface of the nanospheres with antibody (IgG) amino groups. Existence of the coating layer can be confirmed using fluorescein isothiocyanate-conjugated antibodies and also by assessment of antigen-antibody interactions using Sepharose beads. Rapid *in vitro* degradation of BSA-NS can initially be confirmed by incubating a radioactive nanosphere suspension at 37°C in sucrose solution (0.25 M) with liver or lung extracts (1%).
Implementation: The radioactive compound conjugated BSA-NS suspension can be evaluated to discover its *in vivo* biodistribution following intravenous injection. Organ/tissue distribution profiles for BSA-NS can be examined using whole body autoradiography.
Animal Model: Murine (mice bearing Lewis lung carcinoma).
Interpretation and Analysis: Preparations of BSA-NS localize mainly in the liver, the lungs, and the kidneys and by 4 hours and 24 hours after injection, almost all radioactivity has disappeared except for that in the kidneys. The *in vitro* binding ratio of monoclonal antibodies to tumor cells is 2–2.5 times greater than that of control antibodies (mouse IgG) based on gamma ray counter measurements. Binding tests performed have revealed that monoclonal antibodies might preferentially recognize the target organ (Lewis lung carcinoma). Based on the nature of these findings, BSA-NS coated with monoclonal antibodies have been found to be trapped in tumor masses of Lewis lung carcinoma-bearing mice more than in reference controls (BSA-NS coated with mouse IgG) at 24 hours after injection. Thus, BSA-NS preparations offer potential as useful drug carriers enabling the concentration (accumulation) of drugs at specific target sites. Furthermore, their rapid elimination from the body and their degradability suggest that side effects due to long-lasting accumulation in several organs may be avoided.

Preparation and evaluation of bovine serum albumin nanospheres coated with monoclonal antibodies. Akasaka Y, Ueda H, Takayama K, Machida Y, Nagai T. Drug Des Deliv 1988 Jul; 3 (1), pp. 85–97.

Neoplasia: Delivery (Selective)/Focal Vascular Extravasation/Example: Ovarian Carcinoma *Nanoparticles/Liposomes/Hyperthermia*

Background Information: The efficacy of novel cancer therapeutics has been hampered by the ability to deliver these agents to tumors at effective concentrations. Liposomes have been used as a method to overcome some delivery issues and, in combination with hyperthermia, have been shown to increase drug delivery to tumors. Particle size has been shown to affect the delivery of liposomes, but it is not known how hyperthermia affects size dependence.
Application: Potential strategy for selective delivery to neoplastic lesions.
Route of Administration: Intended for intravascular injection (selective or non-selective).
Dosage: Dependent upon temperature and size of delivery modality.
Implementation: Evaluation of the effects of hyperthermia (42°C) on the extravasation of different-sized nanoparticles (albumin; 100 nm, 200 nm, and 400 nm liposomes) from tumor microvasculature structures.
Animal Model: Murine (e.g., mice implanted with human SKOV-3 ovarian carcinoma xenografts grown in window chambers).
Interpretation and Analysis: At 34°C, no liposomes can be detected as having extravasated into the tumor interstitium. Alternatively, hyperthermia enables extravasation of liposomes of all sizes. The magnitude of hyperthermia-induced extravasation is inversely proportional to particle size. Thus, at normothermia (34°C), the pore cutoff size for this model is between 7 and 100 nm (e.g., liposomes do not extravasate). At 42°C, the pore cutoff size is increased to $>$ 400 nm, allowing all nanoparticles to be delivered to the tumor interstitium to some degree. With hyperthermia, 100 nm liposomes experience the largest relative increase in extravasation from the tumor vasculature. Hyperthermia does not enable extravasation of 100 nm liposomes from normal vasculature, potentially allowing for tumor-specific delivery. These experiments indicate that hyperthermia can enable and augment liposomal drug delivery to tumors and potentially help target liposomes specifically to tumors.

Hyperthermia enables tumor-specific nanoparticle delivery: Effect of particle size. Kong G, Braun RD, Dewhirst MW. Cancer Res 2000 Aug 15; 60 (16), pp. 4440–5.

Neoplasia/Delivery Delineation and Validation *Fluoroscein Isothiocyanate [FITC]-Glycol-Chitosan Nanoaggregates/Correlation between Biodistribution and Antineoplastic Properties/pH Sensitive Linker*

Application: Fluoroscein isothiocyanate can be applied as a fluorescent molecular marked to characterize a variety of physiological processes or for tracing the distribution of delivery modalities (e.g., [FITC]-glycol-chitosan nanoaggregates).
Route of Administration: Intravenous (e.g., rat tail vein).
Semi-Synthetic Methods: Conjugation of FITC to glycol-chitosan-doxorubicin (GC-DOX) results in the production of nanoaggregates with a diameter of about 250 nm in aqueous media. To increase the applicability of such preparations, GC-DOX nanoaggregates can be generated that contain an acid-sensitive spacer moiety.

Preparations of GC-DOX form micelle-like nanoaggregates spontaneously in aqueous media. GC-DOX nanoaggregates have a narrow and unimodal size distribution, and their hydrodynamic diameter as measured by dynamic light scattering ranges from between 250 to 300 nm. The extent that doxorubicin can be loaded into GC-DOX nanoaggregates is reflected by a pharmaceutical content level as high as 38%, with a loading efficiency as high as 97% using a physical entrapment method.

Implementation: Characterization of the *in vivo* biodistribution of GC-DOX nanoaggregates (doxorubicin-loaded GC-DOX nanoaggregates or DOX/GC-DOX) can be facilitated by conjugation to FITC. Distribution parameters can then be correlated with the observed antineoplastic properties exerted by conjugated preparations.

Animal Model: Rat (tumor-bearing animals developed by inoculating tumor cells into the region of the dorsal back).

Interpretation and Analysis: Nanoaggregates of FTC-GC distribute mainly to the kidney, tumor, and liver with very sparse amounts observed in other tissues/organ systems. They maintain high levels for 8 days and their distribution within neoplastic lesions increases gradually. Chitosan nanoaggregates appear to accumulate passively in tumor masses due to enhanced permeability characteristics and retention (EPR) effect. Tumor growth is suppressed over a 10-day period.

Biodistribution and anti-tumor efficacy of doxorubicin-loaded glycol-chitosan nanoaggregates by EPR effect. Son YJ, Jang JS, Cho YW, Chung H, Park RW, Kwon IC, Kim IS, Park JY, Seo SB, Park CR, Jeong SY. J Control Release 2003 Aug 28; 91 (1–2), pp. 135–45.

Neoplasia/Proliferation Rate of Tumors/Tumor-Associated Macrophages/Tumor-Associated Angiogenesis

Long-Circulating Dextran-Coated Iron Oxide Particles (LCDIO)/Rhodaminated LCDIO Green Fluorescent Protein (GFP)/Hoechst 33258

Cellular/Molecular Mechanism of Action: Hoechst 33258 dye can be applied as an intravital endothelial stain. Rhodaminated LCDIO particles can preferentially localize intracellularly within certain malignant neoplastic cell types (e.g., gliosarcoma).

Application: Detection and characterization of central nervous system neoplastic lesions (e.g., gliosarcoma).

Route of Administration: Intravascular.

Implementation: Determination of the distribution of LCDIO preparation in tumors. Accumulation of LCDIO in tissue sections is evaluated using multichannel fluorescence microscopy in combination with rhodaminated LCDIO, GFP functioning as a tumor marker, and Hoechst 33258 dye serving as an intravital endothelial stain. Uptake into tumor cells is corroborated with results from immunohistochemical and cell culture uptake analyses. The effect of intratumoral LCDIO uptake on magnetic resonance imaging (MRI) signal intensity is evaluated with a 1.5-T superconducting magnet.

Animal Model: Rodent gliosarcoma.

Interpretation and Analysis: Tumoral accumulation of LCDIO is 0.11% $\pm$ 0.06 of the injected dose per gram of tissue in brain tumors and is sufficient for detection using MRI. In tumor sections, LCDIO preferentially localizes in tumor cells (49.0% $\pm$ 4.6) but is also taken up by macrophages in tumors (21.0% $\pm$ 3.1) and by endothelial cells in the areas of active angiogenesis (6.5% $\pm$ 1.4). In cell culture, LCDIO uptake strongly correlates with the growth rate of tumor cell lines. Tumoral LCDIO accumulation is not negligible and helps explain MRI signal intensity changes observed in clinical trials. Microscopically, LCDIO accumulates predominantly in tumor cells and tumor-associated macrophages. Uptake into tumor cells appears to be directly proportional to cellular proliferation rate.

Instrumentation: Magnetic resonance imaging signal intensity evaluated with a 1.5-T superconducting magnet.

Tumoral distribution of long-circulating dextran-coated iron oxide nanoparticles in a rodent model. Moore A, Marecos E, Bogdanov A Jr, Weissleder R. Radiology 2000 Feb; 214 (2), pp. 568–74.

Neoplasia: Vascular and Lymphatic Endothelium (Tumor-Associated)

Nanocrystals/Semiconductor Quantum Dots (qdots)/Tumor Vasculature-Targeting Peptides/Peptide-Coated ZnS-Capped CdSe qdots/[Nanoparticles]

Background Information: Inorganic nanostructures that interface with biological systems have recently attracted widespread interest in biology and medicine. Nanoparticles are thought to have potential as novel intravascular probes and for the delivery of both diagnostic (e.g., imaging) and therapeutic (e.g., chemotherapy) purposes. Critical issues for successful nanoparticle delivery include the ability to target specific tissues and cell types and the discovery of approaches for escaping the biological particulate filter known as the reticuloendothelial system.

Application: Detection and characterization of vascular and lymphatic structures associated with neoplastic lesions.

Route of Administration: Intravenous.

Implementation: Explore the feasibility of *in vivo* targeting by using semiconductor qdots. Qdots are small ($<$ 10 nm) inorganic nanocrystals that possess unique luminescent properties because their fluorescence emission is stable and tuned by varying the particle size or composition.

Animal Model: Murine.

Interpretation and Analysis: ZnS-capped CdSe qdots coated with a lung-targeting peptide accumulate in the lungs of mice after intravenous injection, whereas 2 other peptides specifically direct qdots to blood vessels or lymphatic vessels in tumors. Addition of poly(ethylene glycol) to the qdot coating prevents non-selective accumulation of qdots in reticuloendothelial tissues. These results encourage the construction of more complex nanostructures with capabilities such as disease sensing and drug delivery.

Nanocrystal targeting *in vivo.* Akerman ME, Chan WC, Laakkonen P, Bhatia SN, Ruoslahti E. Proc Natl Acad Sci U S A 2002 Oct 1; 99 (20), pp. 12617–21.

Ophthalmic Delivery/Corneal and Aqueous Humor

Polyacrylic Acid/Subcolloidal Nanoparticulate/[Example: Brimonidine]

Background Information: In ocular drug delivery, a major problem is providing an adequate concentration of a therapeutic agent in the precorneal area. Mucoadhesive carriers such as polyacrylic acid in subcolloidal, nanoparticulate form have great potential as candidates for ophthalmic drug delivery.

Application: Localized delivery of diagnostic or therapeutic agents to ophthalmic structures.

Route of Administration: Topical.

Semi-Synthetic Methods: A formulation of brimonidine loaded in polyacrylic acid nanoparticles can be prepared as a delivery format for ophthalmic structures. Particles are prepared by a reverse microemulsion polymerization technique and their sizes are in the range of 50 nm.

Implementation: Evaluation of cellular viability following incubation with polyacrylic acid nanoparticles over varying time periods.

***Ex Vivo* Cellular Model:** Caco-2 cells (human primary colonic tumor adenocarcinoma) and human corneal epithelial cells.
Interpretation and Analysis: In preliminary biocompatibility tests, Caco-2 and corneal epithelial cells incubated with polyacrylic acid nanoparticles retain their viability over extended time periods. The loading efficiency of the drug brimonidine into the particles is between 80 and 85% and is pH dependent. Bioadhesive polyacrylic hydrogel nanoparticles can exhibit superior loading properties for brimonidine, and such formulations are stable for more than 5 weeks. The drug is slowly released over an interval of several hours when drug-loaded nanoparticles are dispersed in phosphate buffer saline (pH = 7.4). Two-photon laser scanning microscopic studies of dye-conjugated polyacrylic acid nanoparticles can be used to demonstrate the accumulation of particles on the surface and intercellular spaces of Caco-2 cells.

Brimonidine formulation in polyacrylic acid nanoparticles for ophthalmic delivery. De TK, Rodman DJ, Holm BA, Prasad PN, Bergey EJ. J Microencapsul. 2003 May–Jun; 20 (3), pp. 361–74.

Ophthalmic Delivery/Corneal and Aqueous Humor *Nanosphere Colloidal Suspensions/[PEGylated 1,2-Distearoyl-3-Phosphatidylethanolamine]*

Cellular/Molecular Mechanism of Action: Nanospheres can function as a platform for active or passive selected targeted delivery, in addition to providing a means for delaying premature elimination and biological degradation.
Background Information: The influence of polymer molecular weight and type and concentration of various surfactants on nanosphere properties has previously been investigated.
Application: Localized delivery of diagnostic or therapeutic agents to ophthalmic structures.
Route of Administration: Topical (ophthalmic).
Semi-Synthetic Methods: Colloidal suspensions of poly-*d*,*l*-lactic acid (PLA) nanospheres can be produced by a nanoprecipitation process. The surface properties of PLA nanospheres can be changed by the incorporation of PEGylated 1,2-distearoyl-3-phosphatidylethanolamine. The mean size and zeta potential of the nanospheres can be determined by light scattering analysis.
Implementation: Evaluation of nanosphere colloidal suspensions containing acyclovir as potential ophthalmic drug delivery systems. One approach to achieving this objective can involve delineation of the *in vivo* ocular pharmacokinetics of acyclovir-loaded PLA nanoparticles compared with aqueous suspension of free drug. The loading and release of acyclovir can easily be determined (e.g., *in vitro/in vivo*).

The ocular tolerability of PLA nanospheres can be assessed applying a modified Draize test. The aqueous humor acyclovir levels are monitored for 6 hours to determine the drug's ocular bioavailability for various formulations.

Animal Model: Rabbit (New Zealand White males).
Interpretation and Analysis: A reduction in the mean size and a decrease of the absolute zeta potential of PLA nanospheres results from increasing the surfactant concentration. The higher the polymer molecular weight, the smaller the nanosphere mean size. Poly(ethylene glycol) (PEG)-coated and uncoated PLA nanospheres show sustained acyclovir release and are highly tolerated by the eye. Both types of PLA nanospheres are able to increase the aqueous levels of acyclovir and to improve pharmacokinetics profiles, but the efficacy of PEG-coated nanospheres is significantly higher than that of simple PLA nanospheres. In summary, PEG-coated PLA nanospheres can potentially function as an ophthalmic delivery system for the treatment of ocular viral infections.

Influence of preparation conditions on acyclovir-loaded poly-*d,l*-lactic acid nanospheres and effect of PEG coating on ocular drug bioavailability. Giannavola C, Bucolo C, Maltese A, Paolino D, Vandelli MA, Puglisi G, Lee VH, Fresta M. Pharm Res 2003 Apr; 20 (4), pp. 584–90.

Optical Coherence Tomography/Reflectance Confocal Microscopy/Light Microscopy *Encapsulating Microspheres with Shell or Core Nanoparticles/[Carbon-/Gold-/ Melanin-Shelled Contrast Agents]*

Background Information: Contrast agents are utilized in virtually every imaging modality to enhance diagnostic capabilities.
Application: Reflection- or scattering-based techniques such as optical coherence tomography, light microscopy, and reflectance confocal microscopy.
Route of Administration: Intravenous.
Semi-Synthetic Methods: Encapsulating microspheres that are based on nanoparticles located within the shell or core.
Implementation: Evaluation of microspheres that are based not on fluorescence but on scattering nanoparticles incorporated within the shell or core.

Animal Model: Murine.
Interpretation and Analysis: Gold-, melanin-, and carbon-shelled contrast agents demonstrate an ability to enhance optical coherence tomography images.

Engineered microsphere contrast agents for optical coherence tomography. Lee TM, Oldenburg AL, Sitafalwalla S, Marks DL, Luo W, Toublan FJ, Suslick KS, Boppart SA. Opt Lett 2003 Sep 1; 28 (17), pp. 1546–8.

Optical Detection of Neoplastic Disease/ Biomarker Recognition/Cervical Cancer *[Probe Molecules]-Gold Nanoparticles*

Cellular/Molecular Mechanism of Action: Physically bind to biomarker systems that are unique to specific neoplastic cell types.
Background Information: Recent developments in photonic technology have made it feasible to non-invasively image cells *in vivo,* and these diagnostic strategies therefore have the potential of dramatically improving the prevention, detection, and therapy of epithelial cancers. Endoscope-compatible microscopy, such as optical coherence tomography and reflectance confocal microscopy, image-reflected light in a manner that can generate 3-dimensional pictures of tissue microanatomy with excellent spatial resolution (1–10 microm). However, their ability to image molecular biomarkers associated with cancer is limited.
Application: Vital *in vivo* imaging of precancerous conditions.
Route of Administration: Intravascular.
Semi-Synthetic Methods: Development of a class of molecule-specific contrast agents suitable for vital reflectance imaging that is based on attachment of gold nanoparticles to probe molecules with high affinity for specific (unique) cellular biomarkers.
Implementation: Evaluation of the effectiveness of vital reflectance imaging of precancerous conditions using gold bioconjugated nanoparticles containing probe molecules with high affinity for specific cellular biomarkers.

***Ex Vivo* Model:** Three-dimensional cancer cell suspensions in addition to normal and neoplastic fresh cervical biopsies.
Interpretation and Analysis: Gold conjugates can be delivered topically for imaging throughout the whole epithelial surface. Such contrast agents have the potential to extend the ability of vital reflectance microscopy for *in vivo* molecular imaging. In addition, they can potentially enable combined screening, detection, and therapy of

diseases using inexpensive imaging systems. Such tools could allow mass screening of diseases such as cancer in resource-poor settings.

Real-time vital optical imaging of precancer using antiepidermal growth factor receptor antibodies conjugated to gold nanoparticles. Sokolov K, Follen M, Aaron J, Pavlova I, Malpica A, Lotan R, Richards-Kortum R. Cancer Res 2003 May 1; 63 (9), pp. 1999–2004.

Optical Fluorescent Probe Delivery
Nanospheres: Fluorescent Probe Protected Delivery/ PEBBLES: Probes Encapsulated by Biologically Localized Embedding

Cellular/Molecular Mechanism of Action: Nanospheres can influence the pharmacodynamics of a compound by delaying elimination, slowing biological degradation, and facilitating selective delivery.
Route of Administration: Intravascular injection.
Semi-Synthetic Methods: Spherical optical nanosensors, or PEBBLEs, can be produced in various sizes, including ranges between 20 nm and 200 nm (diameter). The sensors are fabricated in a microemulsion and consist of fluorescent indicators entrapped in a polyacrylamide matrix. A generalized polymerization method has been developed that permits production of sensors containing any hydrophilic dye or combination of dyes within the matrix.
Interpretation and Analysis: The PEBBLE matrix protects the fluorescent dye from interference by proteins, allowing reliable *in vivo* dye calibration. Sensor response times are less than 1 ms. Cell viability assays indicate that PEBBLEs are biocompatible and exert negligible biological effects compared to reference controls. Several sensor delivery methods have been studied, including liposomal delivery, gene gun bombardment, and *pico*-injection into single living cells.

Optical nanosensors for chemical analysis inside single living cells. 1. Fabrication, characterization, and methods for intracellular delivery of PEBBLE sensors. Clark HA, Hoyer M, Philbert MA, Kopelman R. Anal Chem 1999 Nov 1; 71 (21), pp. 4831–6.

Pharmaceutical Controlled Release
Nanoencapsulation

Cellular/Molecular Mechanism of Action: Lambda-carrageenan is a sulfated polymer isolated from algae that can function as hydrophilic matrices.
Application: Controlled release of basic pharmaceuticals.
Considerations: Atomic force microscopy is a suitable form of technology for specific on-time monitoring of interaction processes that occur in pharmaceutical systems.
Interpretation and Analysis: Because hydrophilic matrices have been proposed as a modality for controlled drug delivery, many polymeric excipients have been studied to make drug release fit desired profiles. Furthermore, the relevance of hydrophobic interactions in drug-polymer aqueous systems has been demonstrated, although no references to morphological features or to the kinetics of the formation of interaction complexes have been extensively published. Three-dimensional morphological imaging of lambda-carrageenan sulfated polymer and dexchlorpheniramine maleate (e.g., formulated at very low concentrations as a combined mixture) acquired over a 24-hour period can be achieved by the use of atomic force microscopy analysis (nanometric scale). In this fashion, it is possible to monitor topographical interactions between lambda-carrageenan and dexchlorpheniramine maleate, in order to determine how release profiles can be modified and controlled. Such analysis has demonstrated that lambda-carrageenan can nanoencapsulate spontaneously dexchlorpheniramine maleate molecules, which offers the possibility of controlling the release rate of pharmaceutical agents with no need of complex technological processes.

Self-assembly of drug-polymer complexes: A spontaneous nanoencapsulation process monitored by atomic force microscopy. Oliva M, Diez-Perez I, Gorostiza P, Lastra CF, Oliva I, Caramella C, Marino EL. J Pharm Sci 2003 Jan; 92 (1), pp. 77–83.

Radiographic Imaging/Contrast Agents
Niosomes (Iobitridol Carrier)/Polyoxyethylene Glycol 4000 Stearate/D-Alpha Tocopheryl Polyethylene Glycol 1000 Succinate/Dicetylphosphate/Cholesterol

Cellular/Molecular Mechanism of Action: Non-ionic surfactant vesicles (niosomes) can function as carriers for various types of pharmaceuticals (e.g., iobitridol).
Application: Preparation functions as a carrier modality (non-ionic surfactant vesicle). Iobitridol is a diagnostic radiographic imaging preparation.
Considerations: Niosomes (150 and 175 nm diameter) can be prepared using the film-hydration method followed by sonication. Vesicle preparations of this type can be produced with appropriate mixtures of *D*-alpha tocopheryl polyethylene glycol 1000 succinate, polyoxyethylene glycol 4000 stearate, polyoxyethylene glycol 4000 stearate, cholesterol, and dicetylphosphate. The methodology can be modified to optimize/increase the rate of encapsulation and vesicle stability. Evaluation of vesicle formulation, vesicles, physicochemical aspects, and their morphological characteristics all aid in facilitating the design of preparations that possess the most desirable functional properties.

Niosomes as carriers of radiopaque contrast agents for x-ray imaging. Muller D, Foulon M, Bonnemain B, Vandamme TF. J Microencapsul 2000 Mar–Apr; 17 (2), pp. 227–43.

Targeted Delivery/Selective Site-Specific Drug Delivery System
Polymethacrylic Nanospheres/[^{111}Indium]/[^{125}Iodine] ([^{111}In/^{125}I])-Radiolabeled Nanospheres/Nanoparticles

Cellular/Molecular Mechanism of Action: Polymethacrylic nanospheres are presumably removed from the intravascular compartment by Kupffer cells lining the hepatic sinousoids. It is uncertain if radioactivity found in the kidney during a later time frame is actually intact radiolabeled nanospheres or dissociated (“free”) radioisotopes.
Application: Site-specific drug delivery system primarily relevant to the liver with secondary excretory-based delivery to the kidney.
Route of Administration: Intravascular.
Semi-Synthetic Methods: Polymethacrylic nanospheres (mean diameter 0.25–0.30 microns) can be prepared by aqueous emulsion copolymerization. Nanoparticles can then be radiolabeled either with [^{125}I] or [^{125}I].
Implementation: Evaluation of the organ-specific distribution of polymethacrylic nanospheres.
Animal Model: Mice (single IV injection of labeled particles) for assessment of blood clearance and organ biodistribution.
Interpretation and Analysis: A rapid clearance of [^{111}In]-labeled nanoparticles from the intravascular compartment to the mononuclear phagocyte system (MPS) can be visualized using external scintigraphic imaging. From 10 to 60 minutes postinjection, radioactivity measurements in blood and organs (liver, spleen, kid-

neys, lungs, heart) reveal that [^{125}I]-labeled nanospheres are rapidly removed from the bloodstream (distribution half-life approximately 3–5 minutes) and are mainly deposited within the liver (60% of the administered dose, 10 minutes after administration). Up to 1 hour postinjection, radioactivity in heart and lungs remains insignificant, while in the kidneys, radioactivity levels increase from 8% to 11%.

Blood clearance and organ distribution of intravenously administered polymethacrylic nanoparticles in mice. Rolland A, Collet B, Le Verge R, Toujas L. J Pharm Sci 1989 Jun; 78 (6), pp. 481–4.

Targeting Strategy/Selective
Avidin-Gelatin Nanoparticles/[Biotinylated Probes]

Cellular/Molecular Mechanism of Action: Biotin has a very strong avidity for avidin that is on a magnitude that exceeds that observed for antigen/antibody complexes.
Application: Selective targeting/detection of genes, biochemical enzymes, membrane-associated receptors/antigens, and other biological entities.
Route of Administration: Intravascular.
Semi-Synthetic Methods: Gelatin nanoparticles can be prepared by a 2-step desolvation process. Functional groups at the surface of the particulate system can be quantified with site-specific reagents. The surface of the nanoparticles can then be thiolated, followed by covalent conjugation of nanoparticles to avidin utilizing a bifunctional spacer.
Implementation: Classically, a biological marker system specific for a given disease state would be "pretargeted" with a biotinylated probe or delivery modality (e.g., oligonucleotides or monoclonal antibody), followed by the administration of an avidin-conjugated nanoparticle preparation incorporated with some indicator molecule that can be detected for the various diagnostic purposes.
Interpretation and Analysis: The method allows for high levels of avidin labeling. Biotinylated peptide nucleic acid (PNA) effectively complexes with avidin-conjugated nanoparticles. Avidin-conjugated protein nanoparticles should prove effective as a potential carrier system for biotinylated derivatives for the detection of *in vivo* gene expression or for use in antisense-based gene therapy strategies.

Preparation of avidin-labeled gelatin nanoparticles as carriers for biotinylated peptide nucleic acid (PNA). Coester C, Kreuter J, von Briesen H, Langer K. Int J Pharm 2000 Mar 10; 196 (2), pp. 147–9.

MOLECULAR PLATFORMS

MICRO-SYNTHETIC AGENTS

Cardiovascular: Chronic Heart Failure (CHF)/ Adrenergic Receptor Complexes/Pharmaceutical Efficacy Evaluation
[^{123}I]-Metaiodobenzylguanidine (MIBG)

Cellular/Molecular Mechanism of Action: [^{123}I]-MIBG functions as a norepinephrine analog.
Application: Previous studies have not investigated the efficacy of angiotensin II (AII) receptor antagonists against cardiac sympathetic overactivity in cases with CHF using MIBG myocardial imaging.
Route of Administration: Intravascular.
Implementation: Evaluation of CHF in the presence of an administered AII receptor antagonist (losartan or candesartan). Image evaluation is performed with MIBG and echocardiograms before and 6 months after administration in concert with neurohumoral factors.
Human Model: Cases of CHF (n = 34) with fractional shortening of the left ventricular (LV) diameter $< 25\%$ or LV ejection fraction $< 45\%$ in echocardiogram images.
Interpretation and Analysis: MIBG imaging reveals that the antagonist does not significantly change the heart-to-mediastinum ratio. However, the washout rate falls significantly (from 32.6% + 7.6% to 28.2% + 7.5%; $p < 0.001$). No significant changes occur in LV diameter, fractional shortening, or LV ejection fraction. Circulating atrial (ANP) and brain natriuretic peptides (BNP) and aldosterone fall significantly. Changes in MIBG washout rate correlate positively with changes in BNP ($r = 0.35$, $p < 0.05$). In cases also being treated with angiotensin-converting enzyme (ACE) inhibitors (e.g., n = 19), the MIBG washout rate also falls significantly with AII antagonists, as does BNP and aldosterone. Decreases in MIBG washout and BNP in cases with CHF induced by the AII receptor antagonists suggest the efficacy of these agents in modifying cardiac sympathetic function and neurohumoral factors, even with ACE inhibition. Combination therapy with AII receptor antagonists and ACE inhibitors appears effective for CHF.

Effects of angiotensin II receptor antagonists on [^{123}I]-metaiodobenzylguanidine myocardial imaging findings and neurohumoral factors in chronic heart failure. Shinohara H, Fukuda N, Soeki T, Sakabe K, Onose Y, Tamura Y. Heart Vessels 2002 Dec; 17 (2), pp. 47–52.

Cardiovascular: Endothelin Receptor Expression: Antagonist Receptor Occupancy/Individualization of Endothelin Receptor Antagonist Dosage/Renal Failure: Acute/Cardiac Failure: Congestive
BQ-788/L-749,329/[^{125}I]ET-1/BQ-123

Cellular/Molecular Mechanism of Action: The agents BQ-123 and BQ-788 bind to and block ETA and ETB type endothelin receptor complexes (receptor antagonists). Alternatively, L-749,329 (non-peptide antagonist) has a relatively balanced affinity for each of the 2 endothelin receptor subtypes.
Physiological Mechanism of Action: Endothelin (ET) is 1 of the most potent vasoconstrictors ever identified.
Background Information: Endothelin has been implicated in various disease states, including acute renal failure and congestive heart failure. Such observations imply that these conditions can possibly be managed with endothelin receptor antagonists. However, establishing the dosages for these antagonists may be difficult because no convenient physiologic indicator of action exists and because of complexities associated with receptor function. Two receptor subtypes have been identified for which selective antagonists have been described (e.g., BQ-123 for the ETA receptor and BQ-788 for the ETB receptor). Of the 3 natural peptide hormones (ET-1, ET-2, and ET-3), ET-1 exhibits high affinity for both subtypes of the receptor.
Application: Characterization of the relative expression of 2 endothelium receptor subtypes in a manner that provides information for establishing individualized dosage schedules for endothelin receptor antagonists relevant to the management of various disease states, including acute renal failure and congestive heart failure.
Implementation: Evaluation of the *in vivo* biodistribution of [^{125}I]ET-1 with and without the presence of selective peptide antagonists and a non-peptide antagonist (L-749,329). The agent [^{125}I]ET-1 possesses binding avidity for endothelin receptor complexes.
Animal Model: Rat.
Animal Model: Primate (rhesus monkey).
Interpretation and Analysis: The agents BQ-123, BQ-788, and L-749,329 inhibit binding consistent with avidity for a single

receptor site. However, the sum of inhibition by the selective antagonist is greater than 100% (as defined by inhibition with L-749,329), which suggests (i) lower *in vivo* selectivity than determined *in vitro,* and/or (ii) receptor subtype interactions. The latter explanation is supported, in part, by *in vitro* autoradiographic studies as well as studies in isolated tissues and cells. Synthesized ET-1 labeled with [^{123}I] facilitates the acquisition of images reflecting receptor distribution in 2 *in vivo* animal models. Therefore, it is possible to visualize, via planar, non-invasive imaging, the occupancy of endothelin receptor by antagonists in both the kidney and lung. [^{123}I]ET-1 can therefore be used to determine clinical dosages of antagonist needed for receptor saturation.

Radioiodinated endothelin-1: A radiotracer for imaging endothelin receptor distribution and occupancy. Gibson RE, Fioravanti C, Francis B, Burns HD. Nucl Med Biol 1999 Feb; 26 (2), pp. 193–9.

Cardiovascular: Muscarinic Receptors/Selectivity for Cardiac M2 Muscarinic Receptor Complexes/ Minimal Affinity for Gastrointestinal Muscarinic Receptor Complexes
5H-Dibenz[b,f]Azepine Derivatives

Cellular/Molecular Mechanism of Action: 5H-Dibenz[b,f] azepine derivatives possess binding avidity for membrane-associated parasympathetic muscarinic receptor complexes.
Application: Potential diagnostic probe for evaluation of cardiac physiology parameters without exerting any major effect on gastrointestinal function.
Route of Administration: Intravascular or intraduodenal (rat, mouse, canine).
Semi-Synthetic Methods: Preparation of a series of 5H-dibenz[b,f]azepine derivatives.
Implementation: Evaluation of the relative *in vitro* selective binding affinities of 5H-dibenz[b,f]azepine derivatives for muscarinic receptors (e.g., human recombinant M1, M2, M3, and M4 muscarinic receptors). Results from *in vitro* investigations can be validated *in vivo* to determine selective effects on bradycardia (rat), analgesia (mouse), and nocturnal bradycardia (canine). Ideally, it is desired to identify compounds that have relatively higher binding avidity for cardiac muscarinc receptor complexes and significantly lower binding avidity for gastrointestinal receptor complexes.

Animal Model: Rat (acethylcholine-induced bradycardia), mouse (analgesic), and canine (nocturnal bradycardia).
Interpretation and Analysis: One compound (e.g., n = 1/8) has been identified that has high binding affinity for human recombinant M2 receptors (K_i = 2.6 nm), a low affinity for M4 receptors (39-fold less than for M2 receptors), and a very low affinity for M1 and M3 receptors (119-fold and 112-fold less than for M2 receptors, respectively). The high M2 selectivity of compound 8 may be attributed to the olefinic bond of the azepine ring. Functional experiments reveal that the 1 desirable candidate derivative can function as a competitive antagonist with high affinity for cardiac (pA2 = 7.1) and low affinity for intestinal muscarinic receptors (IC_{50} = 0.54 μM).

The selectivity of compound 8 in studies performed *in vivo* confirm parameters observed *in vitro.* Acetylcholine-induced bradycardia is dose-dependently antagonized after both intravenous and intraduodenal administration of compound 8 (rat). Cholinergic functions mediated by M1 or M3 receptors (salivary secretion, pupil diameter, gastric emptying, intestinal transit time) are not affected by the oral administration of compound 8 even at doses as high as 30 times the antibradycardic effective dose. Furthermore, compound 8 has no analgesic activity (mice), indicating poor central nervous system penetration. In dogs, nocturnal bradycardia is dose-dependently inhibited following the oral administration of compound 8 and it exerts physiological effect for 24 hours. Compound 8 appears to be a promising cardioselective antimuscarinic agent for the treatment of dysfunctions of the cardiac conduction system such as sinus or nodal bradycardia ("sick-sinus syndrome") and atrioventricular block. Based on its biological properties, the candidate compounds also has potential as a diagnostic imaging ligand .

Synthesis of new cardioselective M2 muscarinic receptor antagonists. Mandelli GR, Maiorana S, Terni P, Lamperti G, Colibretti ML, Imbimbo BP. Chem Pharm Bull (Tokyo) 2000 Nov; 48 (11), pp. 1611–22.

Cardiovascular: Sodium-Potassium ATPase (Na/K-ATPase): Myocardial
[^{125}I]-Digoxin/[^{125}I]-Digoxin-Iodohistamine (bis(O-Carboxymethyloxime))

Cellular/Molecular Mechanism of Action: Cardiac glycosides recognize and physically bind to membrane-associated myocardial Na/K-ATPase complexes.
Background Information: Cardiac glycosides have a high binding avidity for myocardial Na/K-ATPase complexes.
Implementation: Survey of radioiodinated digoxin (cardiac glycoside) derivatives as potential candidates for myocardial imaging with particular emphasis on non-invasive monitoring of cardiac glycoside therapy.

Animal Model: Canine (dog myocardial imaging analysis).
Interpretation and Analysis: The radioiodinated digoxin derivative [^{125}I]-digoxin-iodohistamine(bis(O-carboxymethyloxime)) shows a relatively high level of accumulation within the myocardium and a degree of binding avidity for Na/K-ATPase similar to digoxin itself, and resistance against ouabain displacement, as indicated by *in vivo* and *in vitro* studies. Based on these results, [^{123}I] labeling of digoxin-histamine(bis(O-carboxymethyloxime)) and imaging analysis demonstrates uptake within the myocardium.

High myocardial accumulation of radioiodinated digoxin derivative: A possible Na,K-ATPase imaging agent. Fujibayashi Y, Takemura Y, Matsumoto K, Wada K, Yonekura Y, Konishi J, Yokoyama A. J Nucl Med 1992 Apr; 33 (4), pp. 545–9.

Cardiovascular Thrombosis/Platelet GPIIb/ IIIa Receptor/Deep-Vein Thrombosis/Pulmonary Embolism/Arterial Thromboembolic Disorders/ Cerebral Vascular Stroke/Coronary Artery Thrombotic Disorders/Peripheral Artery Thromboses
Platelet GPIIb/IIIa Receptor Antagonist/ [^{99m}Technetium] ([^{99m}Tc])-DMP728/[^{3}H]-DMP728

Cellular/Molecular Mechanism of Action: The agent DMP728 functions as a GPIIb/IIIa receptor antagonist on the exterior surface membrane of platelets.
Physiological Mechanism of Action: The composition of venous thrombi is predominantly polymerized fibrin monomer in contrast to arterial thrombi that have a significantly higher platelet content.
Background Information: Either venous or arterial thrombosis is a potentially life-threatening event and existing diagnostic modalities are inadequate for detecting such lesions or determining morphological alterations that occur during evolving thrombus development. Thus development of a non-invasive imaging agent that can detect clot location remains a critical and unmet need in the field of diagnostic nuclear medicine.

Application: Detection and characterization of intravascular venous and arterial thromboses. Preparations can also function as a means of assessing the efficacy of thrombin inhibitors and other pharmaceutical candidates.
Implementation: Evaluation of artificially (*in vitro*) created venous and arterial type thrombi using the potent radiolabeled GPIIb/IIIa receptor antagonist [^{3}H]-DMP728 as detection agent. Complementary investigations can involve the *in vivo* application of [^{99m}Tc]-DMP728 to determine clot/blood biodistribution ratios (e.g., target:background ratio), as well as the application of non-invasive single photon emission computed tomography (SPECT) of venous clots in deep-vein thrombosis.
Animal Model: Canine (dogs with mixed arterial/venous or venous clots in arteriovenous shunt, in venous clot foci, and deep-vein thrombi).
Interpretation and Analysis: Radiolabeled preparations of the GPIIb/IIIa receptor antagonist DMP728 in the form of [^{3}H]-DMP728 and [^{99m}Tc]-DMP728 retain marked binding avidity for platelet GPIIb/IIIa receptor complexes. DMP728 demonstrates a relatively greater affinity for activated in contrast to non-activated human platelets, which may be essential for attaining an optimal thrombus/blood (target/background) distribution ratio and the optimal detection of small clots (i.e., greater sensitivity). Therefore, [^{99m}Tc]-GPIIb/IIIa receptor antagonists have potential utility in the diagnosis of venous clots in deep-vein thrombosis, pulmonary embolism, and arterial thromboembolic disorders including stroke and coronary and peripheral artery thrombotic disorders. Thrombin inhibitors do not appear to be as effective in this same context.

Novel technetium-99m-labeled platelet GPIIb/IIIa receptor antagonists as potential imaging agents for venous and arterial thrombosis. Mousa SA, Bozarth JM, Edwards S, Carroll T, Barrett J. Coron Artery Dis 1998; 9 (2–3), pp. 131–41.

Cardiovascular Thrombosis
[Lys-Gla-Ala-Gly-Asp-Val]-Aerosomes-Entrapped Microbubbles

Cellular/Molecular Mechanism of Action: Microbubbles can function as a means for delaying excretion, suppressing biological degradation, and enhancing site-specific accumulation of diagnostic and therapeutic pharmaceutical agents.
Application: Ultrasound can be used as a primary diagnostic technique for the detection of deep venous thrombosis. Microbubble preparations can potentially enhance the detection and characterization of intravascular thrombi.
Route of Administration: Intravascular.
Semi-Synthetic Methods: In the 1st phase of production, synthetic linear hexapeptide (lysine-glutamine-alanine-glycine-aspartate-valine) can be coupled to a lipid moiety. The targeted lipid moiety is then incorporated into a lipid blend, followed by entrapment of Aerosomes contrast agent (ImaRx) yielding perfluorobutane-entrapped microbubbles. Prior to administration microbubble preparations are then sized for uniformity.
Implementation: Evaluation of the *in vitro* capacity of sized perfluorobutane-entrapped microbubbles to (i) retain high levels of acoustic stability; (ii) recognize (bind) to blood clots; and (iii) enhance ultrasound image analysis of intravascular thromboses (blood clots).
Interpretation and Analysis: Experimental results reveal that the mean size of the specific ultrasound contrast agent (MRX-408) is about 2.0 microm. Microbubbles appear as smooth spherical structures. Microscopic analysis reveals that targeted bubbles bind to blood clots, whereas control, non-targeted bubbles do not bind to blood clots. *In vitro* acoustic studies reveal similar levels of stability for modified microbubbles compared with control microbubbles. Targeted microbubbles enhance *in vitro* blood clot visualization, whereas non-targeted microbubbles do not increase clot detection. Therefore, this new thrombus-specific ultrasound contrast agent could potentially improve the detection of thrombosis by ultrasound and may be useful for distinguishing between new and old thrombosis. Investigations performed *in vivo* have been initiated to further characterize the applicability of these preparations.

In vitro studies of a new thrombus-specific ultrasound contrast agent. Unger EC, McCreery TP, Sweitzer RH, Shen D, Wu G. Am J Cardiol 1998 Jun 18; 81 (12A), pp. 58G–61G.

Central Nervous System (CNS)/Alpha 1-Adrenoceptor
N-[3-(^{1}H-Imidazol-4-Ylmethyl)-Phenyl] Ethanesulfonamide

Cellular/Molecular Mechanism of Action: *N*-[3-(^{1}H-imidazol-4-ylmethyl)-phenyl]-ethanesulfonamide (ABT-866, 1) is a novel alpha 1-adrenoceptor ligand.
Physiological Mechanism of Action: *N*-[3-(^{1}H-imidazol-4-ylmethyl)-phenyl]ethanesulfonamide (ABT-866, 1) possesses enhanced *in vitro* and *in vivo* binding avidity profiles relative to phenylpropanolamine and midodrine. The compound has a unique profile of α_{1A} (rabbit urethra, EC_{50} = 0.60 μM) agonism with α_{1B} (rat spleen, pA_2 = 5.4) and α_{1D} (rat aorta, pA_2 = 6.2) antagonism.
Application: Evaluation of urethral function.
Route of Administration: Intravascular.
Interpretation and Analysis: In an *in vivo* canine model, compound (1) is more selective for the urethra and interacts less with the vasculature compared to A-61603 (2), ST-1059 (3), the active metabolites of midodrine, and phenylpropanolamine (4).

N-[3-(1H-imidazol-4-ylmethyl)-phenyl]ethanesulfonamide (ABT-866, 1), a novel alpha 1-adrenoceptor ligand with an enhanced *in vitro* and *in vivo* profile relative to phenylpropanolamine and midodrine. Altenbach RJ, Khilevich A, Meyer MD, Buckner SA, Milicic I, Daza AV, Brune ME, O'Neill AB, Gauvin DM, Cain JC, Nakane M, Holladay MW, Williams M, Brioni JD, Sullivan JP. J Med Chem 2002; 45(20), pp. 4395–7.

Central Nervous System (CNS)/Amyloidosis ($Beta_2$)/Microglobulin: Example Amyloidosis
[^{111}Indium] ([^{111}In])-Recombinant Human $Beta_2$Microglobulin ($Rhbeta_2$mH5)

Cellular/Molecular Mechanism of Action: $Beta_2$-microglobulin recognizes and physically binds to amyloid of $beta_2$-microglobulin ($beta_2$m) origin.
Background Information: Amyloid of $beta_2$m origin can be detected using [^{131}I]-radiolabeled-$beta_2$m scintigraphy in cases with uremia and managed with hemodialysis treatment. As the tracer $beta_2$m is isolated from another affected case, it carries the common risks, including viral infections such as hepatitis B and C and human immunodeficiency virus (HIV) that are associated with human plasma products.
Application: The *in vitro* production of recombinant human $beta_2$-microglobulin yields a protein fraction that is devoid of any potential risk of exposing recipients to various viral pathogens. Purified protein fractions can then be radiolabeled for the purpose of detecting and characterizing various conditions of amyloidosis.
Route of Administration: [^{111}In]-$rhbeta_2$mH5 (intravenous).

Semi-Synthetic Methods: Beta$_2$m-type amyloid can be produced *in vitro* as rhbeta$_2$m in *Escherichia coli* cultures. The expression vector pASK40DeltaLbeta$_2$m(His)5 can be used for such purposes due to it containing a C-terminal (His)5-tag that facilitates purification via immobilized metal ion affinity chromatography (IMAC). Size exclusion chromatography on a Superose 12 column can be used as a 2nd purification step. The isolated rhbeta$_2$mH5 reacts in an immunochemically identical manner compared to native human beta$_2$m, and can be identified as a single band of approximately 11.8 kDa utilizing Western blot immunodetection methodologies. Additionally, fractions can be visualized as a single spot in 2-dimensional gel electrophoresis (2D-PAGE: expected molecular mass = 12,415.8 Da). Uniform purity can further be proven by crystallization and N-terminal amino acid sequence analysis.
Implementation: Evaluation of beta$_2$m-amyloidosis conditions applying sterile preparations of rhbeta$_2$mH5 radiolabeled with [^{111}In] to produce [^{111}In]-rhbeta$_2$mH5.

Human Model: Cases managed with hemodialysis (± known beta$_2$m-amyloidosis).
Interpretation and Analysis: The [^{111}In]-rhbeta$_2$mH5 tracer localizes specifically within areas known to contain amyloid deposits. Recombinant rhbeta$_2$mH5 preparations therefore are suitable for the *in vivo* detection of deposits of beta$_2$m-type amyloid contained within organs, and fulfill the requirements necessary for tracer agents to be applied in clinical environments. Finally, the use of [^{111}In] instead of [^{131}I] reduces the radioactive dose and results in higher levels of lesion resolution.

Production of recombinant human beta$_2$-microglobulin for scintigraphic diagnosis of amyloidosis in uremia and hemodialysis. Linke RP, Schäeffer J, Gielow P, Lindner P, Lottspeich F, Plückthun A, Weiss EH. Eur J Biochem 2000 Feb; 267 (3), pp. 627–33.

Central Nervous System (CNS)/Gliomas
p-[^{123}I]-Iodo-L-Phenylalanine (IPA)

Cellular/Molecular Mechanism of Action: The agent IPA functions as an analogue of phenylalanine and is preferentially assimulated by certain cell types that perceive it as an amino acid mimic.
Background Information: The radiopharmaceutical IPA is highly accumulated within gliomas.
Application: Detection and characterization of gliomas within the CNS.
Dosage: *p*-[^{123}I-iodo-L-phenylalanine (average effective dose for adults is estimated to be 0.0152mSv*MBq^{-1}, leading to an effective dose of 3.8mSv in a typical brain single photo emission computed tomography [SPECT] investigation with 250 MBq IPA).
Implementation: Evaluate the feasibility of IPA to image brain tumors under routine clinical conditions using SPECT. In this fashion, it is possible to delineate whole-body kinetic profiles and brain SPECT images, in addition to plasma, urinary, and dosimetric analysis in cases of glioma following intravenous IPA injection. Results obtained by IPA SPECT can then be compared with histopathology, magnetic resonance imaging, and positron emission tomography (PET) analysis with [^{18}F]-fluorodeoxyglucose.

Human Model: Cases of glioma (n = 4).
Interpretation and Analysis: Tumor lesions can clearly be demonstrated by IPA SPECT at 30 minutes, 1 hour, and 4.5 hours postinjection, even in cases with low-grade gliomas. In glioblastoma cases, excellent visualization of the tumor is possible even at 7 hours postinjection, which is indicative of high retention of the radiopharmaceutical within cerebral gliomas. Analysis of the radioactivity in plasma and urine validate the high *in vivo* IPA stability. Blood clearance of IPA is rapid (> 65% after 10 minutes), and elimination is predominantly facilitated by renal excretion with the urinary radioactivity excretion ranging from 27% at 1 hour to 54% of injected doses at 5 hours postinjection. The IPA agent therefore appears to be a potentially valuable brain tumor–imaging agent for widespread SPECT clinical studies. Its high tumor-specific uptake and retention even in low-grade gliomas represents a major advantage compared to presently available SPECT radiopharmaceuticals. Moreover, the radiation dose estimates indicate that clinical use of IPA will result in acceptable radiation dose levels in humans.
Instrumentation: Dual- and a triple-headed SPECT camera.

Initial evaluation of the feasibility of single photon emission tomography with *p*-[^{123}I]iodo-L-phenylalanine for routine brain tumor–imaging. Samnick S, Hellwig D, Bader JB, Romeike BF, Moringlane JR, Feiden W, Kirsch CM. Nucl Med Commun 2002 Feb; 23 (2), pp. 121–30.

Central Nervous System (CNS)/Glioma and Mammary Carcinoma
T3,4BCPC

Cellular/Molecular Mechanism of Action: The exact mechanism that facilitates the selective uptake of T3,4BCPC by certain neoplastic cell types has not been precisely established.
Application: Potential application as a detectable delivery modality that selectively recognizes certain neoplastic cell types, including glioma and mammary carcinoma.
Route of Administration: T3,4BCPC (intravenous).
Dosage: T3,4BCPC (5.0 mg/kg body weight; murine fibrosarcoma model).
Semi-Synthetic Methods: Preparation of the water-soluble photosensitizer 5,10,15,20-tetrakis[3,4-bis(carboxymethyleneoxy)-phenyl]chlorin (T3,4BCPC) followed by *in vitro* characterization and then [^{99m}Tc] radiolabeled.
Implementation: Evaluation of biodistribution studies in mouse and rat tumor models for determining the comparative properties of the tumor-seeking radiopharmaceuticals [^{99m}Tc^{V}]-dimercaptosuccinic acid (DMSA), [^{201}Thallous]-chloride (TlCl), and [^{99m}Tc]-citrate using a gamma camera computer-integrated system. In the 2nd phase of evalution, *in vivo* efficacy of the compound can be determined by establishing its potential as photodynamic therapeutic agent achieved by the conduction of a preclinical photodynamic therapy (PDT) study performed in fibrosarcoma-bearing mice (postinjection of T3,4BCPC).

Animal Model: Murine (C$_3$H/J virus-induced spontaneous mammary tumors).

Animal Model: Murine (fibrosarcoma-bearing).

Animal Model: Rats (Wistar strain).

Animal Model: Rat (N-nitrosomethylurea [NMU]-induced mammary tumors).

Animal Model: Rat (transplanted C$_6$-glioma).
Interpretation and Analysis: Accumulation of imaging agent activities within the liver, kidney, bladder, and large intestine at 4 hours postinjection indicate that the labeled ligand is largely eliminated by renal excretion and partly through the hepatobiliary system. In NMU-induced rat mammary tumors, the labeled ligand shows a 5-fold tumor to muscle (T/M) ratio compared to [^{99m}Tc^{V}]-DMSA (3-fold) and [^{201}Tl]Cl (3-fold). In the case of C$_3$H/J virus-induced spontaneous mammary tumors, differences are not marked. However, in the transplanted rat C$_6$-glioma, the T/M ratio of the labeled compound is appreciably higher (4-fold) than that noted with [^{99m}Tc^{V}]-DMSA (2-fold), [^{201}Tl]Cl (3-fold), and [^{99m}Tc]-citrate (more than 3-fold). Therefore, radiolabeled T3,4BCPC may have potential utility

for detection of neoplastic disease states. Photodynamic therapy applications of T3,4BCPC in fibrosarcoma-bearing mice following delivery of a laser dose of 20 mW for 60 seconds produces an 80% level of destruction within tumor masses. The molecule may therefore be useful for PDT of cancer. The labeled agent could also be useful in monitoring the progression/regression of tumors before, during, and after chemotherapy, radiation therapy, or PDT.

Preparation and biological evaluation of the new chlorin photosensitizer T3,4BCPC for detection and treatment of tumors. Murugesan S, Shetty SJ, Srivastava TS, Samuel AM, Noronha OP. J Photochem Photobiol B 2002 Aug; 68 (1), pp. 33–8.

Central Nervous System (CNS)/Glioma/Mammary Tumors: Carcinogen-Induced
[^{99m}Technetium] ([^{99m}Tc])Field Bean Protease Inhibitor/[^{99m}Tc]-(V)Dimercaptosuccinic Acid (DMSA)/ [^{201}Thallous] Chloride (TlCl)/[$^{99m}TcO_4^-$]-Citrate

Application: Detection and characterization of glioma and possibly other neoplastic disease states.
Route of Administration: [$^{99m}TcO_4^-$]-labeled purified field bean protease inhibitor (intravenous).
Semi-Synthetic Methods: Purified field bean protease inhibitor (FBPI) can be labeled with [$^{99m}TcO_4^-$] as a tracer molecule. Labeling is done in the presence of Sn^{2+} as a reducing agent and the resulting approximately a 95% product yield. At room temperature, the product is stable for at least a 2-hour period.
Implementation: Evaluation of the biodistribution of [^{99m}Tc]-labeled purified FBPI to ascertain its ability to localize within neoplastic lesions. Biodistribution analysis can be determined at various time intervals following administration.

Animal Model: Rat (Wistar strain bearing carcinogen-induced mammary tumors or transplanted C6 glioma).
Interpretation and Analysis: Biodistribution analysis of [^{99m}Tc]-FBPI reveals that it undergoes rapid blood clearance from the systemic circulation (approximately 5 hours). The complex is predominantly eliminated through renal and hepatobiliary excretion. The tumor-to-muscle (T/M) ratios obtained with [^{99m}Tc]-FBPI in C6 glioma explants are nearly 2- to 5-fold higher than observed with several conventional tumor-seeking radiopharmaceuticals such as [$^{99m}Tc^{V}$]-DMSA, TlCl, and [^{99m}Tc]-citrate. Similarly, the T/M ratio obtained with [^{99m}Tc]-FBPI in a mammary tumor model, on the other hand, appears to be 2-fold to 3-fold higher than observed with [$^{99m}Tc^{V}$]-DMSA and TlCl. The ratio is, however, comparable with that obtained with [^{99m}Tc]-citrate. The study indicated that [^{99m}Tc]-FBPI can potentially function as a specific imaging agent for gliomas and possibly other tumor types as well.

[^{99m}Tc]-labeled field bean protease inhibitor can function as an efficient tumor-detecting agent. Murugesan S, Banerji AP, Noronha OP, Samuel AM, Fernandes AO. Indian J Exp Biol 2001 Aug; 39 (8), pp. 742–7.

Central Nervous System (CNS)/Opiate Receptor Expression
[^{18}F]-Fluorophen/Phenazocine Derivative/ Benzomorphan Opiate Agonist

Cellular/Molecular Mechanism of Action: (±)-5,9 Alpha-dimethyl-2-[2-(4-fluorophenyl)ethyl]-2′-hydroxy-6, 7-benzomorphan (fluorophen) is a fluorinated derivative of the benzomorphan opiate agonist phenazocine, which physically recognizes and binds to opiate receptor complexes (selectivity μ congruent to δ greater than κ).
Application: Detection of opiate receptor complexes. Labeled with [^{18}F], fluorophen should be sufficiently potent to be useful as a non-invasive *in vivo* probe for visualizing opiate receptors complexes.
Semi-Synthetic Methods: (±)-5,9 Alpha-dimethyl-2′-hydroxybenzomorphan can be subjected to N-acylation utilizing (*p*-fluorophenyl)-acetyl chloride, followed by diborane reduction of the resulting amide.
Interpretation and Analysis: Fluorination produces only a 2-fold opiate receptor affinity loss when measured either by bioassay or receptor binding analysis (selectivity μ congruent to delta is greater than kappa). Labeled with [^{18}F], fluorophen should be sufficiently potent to be useful as an *in vivo* probe for visualizing opiate receptors by positron emission transaxial tomography (PETT).
Instrumentation: Positron emission transaxial tomography.

Synthesis and pharmacological characterization of (±)-5,9 alpha-dimethyl-2-[2-(4-fluorophenyl)ethyl]-2′-hydroxy-6,7-benzomorphan (fluorophen), a ligand suitable for visualization of opiate receptors *in vivo*. Rice KC, Konicki PE, Quirion R, Burke TR Jr, Pert CB. J Med Chem 1983 Nov; 26 (11), pp. 1643–5.

Central Nervous System (CNS)/Parasympathetic Autonomic System/Cardiac Muscarinic Acetylcholinergic Receptors/CNS Muscarinic Acetylcholinergic Receptors/Carcinoma of the Colon
[^{123}I]-PIP/[^{125}I]-PIP

Cellular/Molecular Mechanism of Action: The agent IPIP functions as a ligand for muscarinic acetylcholinergic complexes (mAChR).
Background Information: Prior to approval for *in vivo* use in humans, organ doses for radioiodinated IPIP ligands must 1st be extensively evaluated applying *ex vivo* and *in vivo* models.
Application: Radioiodinated IPIP ligands can be applied *in vivo* for imaging mAChR using modalities like single photon emission computed tomography (SPECT).
Route of Administration: Intravascular.
Implementation: Identify the most optimum mAChR ligand-binding properties of 4 different IPIP sterioisomers facilitated by the evalatuion of their biodistribution patterns. A biokinetic model can be developed for the Z-(S)-IPIP stereoisomer to estimate the internal absorbed dose in humans based on extrapolation from an appropriate rat model.

Animal Model: Rat (IPIP biokinetic model).
Interpretation and Analysis: The thyroid is the critical organ at risk for this radiopharmaceutical, with an absorbed dose estimate of 2.4 mGy/MBq for both males and females, when labeled with [^{123}I]. Even when blocked, the thyroid is still the critical risk organ, yet with a 90% dose reduction. The heart and brain receive the next highest doses in both males and females. Effective dose estimates for the use of pure [^{123}I]-PIP in humans are 0.16 mSv/MBq for males and 0.14 mSv/MBq for females. The biodistribution studies of the Z-(S)-IPIP stereoisomer show it has the most promise as a successful agent for imaging muscarinic receptor sites in the heart and brain. IPIP can potentially be applied as a therapeutic radiopharmaceutical for certain colon carcinomas where muscarinic receptor sites are expressed in the tumor cells. These results provide preliminary data for use of IPIP in clinical studies on humans.

In vivo biodistribution of [^{125}I]-PIP and internal dosimetry of [^{123}I]-PIP radioiodinated agents selective to the muscarinic acetylcholinergic receptor complex. Breeden WK 3rd, Hamby DM, Carey JE Jr, Eckerman KF, McPherson DW, Knapp FF Jr. Med Phys 2000 Apr; 27 (4), pp. 778–86.

Central Nervous System (CNS)/Parasympathetic/ Acethylcholine Nictotinic $\alpha_4\beta_2$ Receptor Complexes/ Neurodegenerative Disease/Tobacco Dependency
S-5-[^{123}I]Iodo-3-(2-Azetidinylmethoxy)Pyridine

Cellular/Molecular Mechanism of Action: *S*-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine selectively binds to $\alpha_4\beta_2$ subtype nicotinic acetylcholine receptor complexes.
Application: The iodinated analogue *S*-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine of A-85380 can be used for the specific detection and characterization of $\alpha_4\beta_2$ subtype nicotinic acetylcholine receptor complexes in neurodegenerative disease states in conditions of tobacco dependence.
Route of Administration: *S*-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine (intravenous).
Implementation: Accurate measurement of *S*-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine plasma concentrations in order to determine their capacity to function as a "tracer" molecule for *in vivo* quantification of $\alpha_4\beta_2$ subtype nicotinic acetylcholine receptor complexes.

Human Model: Non-human and human primates.
Interpretation and Analysis: In human or non-human primate whole blood as well as in plasma, the parent compound is only stable for approximately 5 minutes, after which it decomposes. Alternatively, the radioligand is stable in the injection solution and in protein-free (> 30 KMW) plasma ultrafiltrate for at least 18 hours. To preserve the parent compound in plasma the radioactive plasma must be mixed with equal volumes of acetonitrile within 5 minutes after its collection or, alternatively, radioactive blood should be collected and mixed with sodium azide (3 mg/ml blood). The *in vivo* metabolism of [^{123}I]5-IA generates 2 distinct components including (i) a radiometabolite that is less lipophilic than the parent compound; and (ii) a polar radiometabolite that is not free radioiodide because of the absence of radioactivity accumulation within the thyroid.

Measurement of plasma metabolites of *S*-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine (5-IA-85380), a nicotinic acetylcholine receptor imaging agent, in non-human primates. Zoghbi SS, Tamagnan G, Baldwin MF, Al-Tikriti MS, Amici L, Seibyl JP, Innis RB. Nucl Med Biol 2001 Jan; 28 (1), pp. 91–6.

Central Nervous System (CNS)/Parasympathetic: Nicotinic Receptors: Neurodegenerative Disease
TC-2559 [(E)-N-Methyl-4-[3-(5-Ethoxypyridin)yl]-3-Buten-1-Amine]

Cellular/Molecular Mechanism of Action: TC-2559 [(E)-*N*-methyl-4-[3-(5-ethoxypyridin)yl]-3-buten-1-amine] is a parasympathetic agonist at membrane-associated nicotinic receptor complexes.
Application: Related analogues may be capable of detecting and characterizing neurodegenerative disorders.
Implementation: Evaluation of the relative selective binding avidity of TC-2559 [(E)-*N*-methyl-4-[3-(5-ethoxypyridin)yl]-3-buten-1-amine], [^{3}H]-nicotine, and [^{125}I]-bungarotoxin for CNS nicotinic receptor complexes. Validation of laboratory results can entail detection of dopamine release from striatal synaptosomes, ion flux from thalamic synaptosomes, and ability to reduce *in vitro* glutamate-induced neurotoxicity. Evaluation of properties *in vivo* can involve characterization of behavioral changes in animal models.

Animal Model: Rat (behavioral studies).
Interpretation and Analysis: TC-2559 [(E)-*N*-methyl-4-[3-(5-ethoxypyridin)yl]-3-buten-1-amine] is markedly more selective than recently reported novel nicotinic receptor ligands (selectivity ratio for CNS to peripheral nervous system (PNS) > 4,000). In this context, TC-2559 competes effectively with [^{3}H]-nicotine binding (K_i = 5 nM) but not with [^{125}I]-bungarotoxin (> 50,000 nM). Dopamine release from striatal synaptosomes and ion flux from thalamic synaptosomes indicates that TC-2559 is potent and efficacious in the activation of CNS receptors and significantly reduces glutamate-induced *in vitro* neurotoxicity. TC-2559 has no detectable effects on muscle and ganglion-type nicotinic acetylcholine receptors at concentrations up to 1 mM.

TC-2559 significantly attenuates scopolamine-induced cognitive deficits in a step-through passive avoidance task. Acute and repeated oral dosing of TC-2559 enhances performance in a radial arm maze task. In contrast to the effects of equimolar concentrations of (-) nicotine, TC-2559 does not induce hypothermia and locomotor activity is not enhanced following repeated daily administration of 14 days. TC-2559 has a markedly enhanced CNS-PNS selectivity ratio and an intra-CNS selectivity as demonstrated by improved cognition without increased locomotor activity. Both *in vitro* and *in vivo* investigation studies suggest that TC-2559 has a desirable profile that warrants further evaluation as a potential diagnostic and therapeutic agent relevant to neurodegenerative disease processes.

TC-2559: A novel orally active ligand selective at neuronal acetylcholine receptors. Bencherif M, Bane AJ, Miller CH, Dull GM, Gatto GJ. Eur J Pharmacol 2000 Dec 1; 409 (1), pp. 45–55.

Central Nervous System (CNS)/Serotonin (5-HT_{1A}) Autoreceptors: Dorsal Raphe Nuclei (DRN)/ Pandolol Receptor Occupancy
[^{11}C]WAY 100635

Cellular/Molecular Mechanism of Action: Pandolol and [^{11}C]WAY 100635 possess binding avidity for membrane-associated serotonin (5-HT_{1A}) receptor complexes expressed by certain cell types within the CNS.
Background Information: Augmentation of selective serotonin reuptake inhibitor (SSRI) therapy by the 5-HT_{1A} receptor agent pindolol may reduce the delay between initiation of antidepressant treatment and clinical response. The hypothesis is based on the ability of pindolol to block 5-HT_{1A} autoreceptors in the DRN and to potentiate the increase in 5-HT transmission induced by SSRIs. However, placebo-controlled clinical studies of pindolol augmentation of antidepressant therapy have reported inconsistent results.
Application: The agent [^{11}C]WAY 100635 can be applied to evaluate the differential occupancy of membrane-associated somatodendritic and postsynaptic 5-HT_{1A} receptor complexes by established or prototype pharmaceutical agents (e.g., pindolol).
Route of Administration: Intravascular.
Implementation: Evaluate the occupancy of 5-HT_{1A} receptors during treatment with pindolol controlled release (CR) using [^{11}C] WAY 100635 and positron emission tomography (PET) imaging. Cases are then studied 4 times: at baseline, following 1 week of pindolol CR 7.5 mg/day (4 and 10 hours postdose), and following 1 dose of pindolol CR 30 mg (4 hours postdose).

Human Model: Healthy subjects (n = 9).
Interpretation and Analysis: Occupancy of DRN is approximately 40 ± 29%, 38 ± 26%, and 64 ± 15% (scans 2, 3, and 4, respectively). The average occupancy in all other regions is significantly lower at each dose (18 ± 5%, 12 ± 3%, and 42 ± 4%; scans 2, 3, and 4, respectively). Blockade within the DRN achieved under clinical conditions (7.5 mg/day) may be too low and variable to consistently augment the therapeutic effect of SSRIs. However, these

data indicate that pindolol exhibits *in vivo* selectivity for the DRN 5-HT_{1A} autoreceptors. As DRN selectivity is desirable for potentiation of 5-HT function, this observation represents an important proof of concept for the development of 5-HT_{1A} agents in this application.

Differential occupancy of somatodendritic and postsynaptic 5-HT_{1A} receptors by pindolol: A dose-occupancy study with [^{11}C]WAY 100635 and positron emission tomography in humans. Martinez D, Hwang D, Mawlawi O, Slifstein M, Kent J, Simpson N, Parsey RV, Hashimoto T, Huang Y, Shinn A, Van Heertum R, Abi-Dargham A, Caltabiano S, Malizia A, Cowley H, Mann JJ, Laruelle M. Neuropsychopharmacology 2001 Mar; 24 (3), pp. 209–29.

Central Nervous System (CNS)/Serotonin: 5-HT_{1A} Autoreceptor/Occupancy of Serotonin 5-HT_{1A} Autoreceptor: Dorsal Raphe Nuclei (DRN)/Dose Optimization for Serotonin 5-HT_{1A} Autoreceptor Agents

[Carbonyl-^{11}C]WAY-100635

Cellular/Molecular Mechanism of Action: The agent [carbonyl-^{11}C]WAY-100635 or [carbonyl-^{11}C] *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl)cyclohexa necarboxamide, abbreviated as [^{11}C]WAY-100635, possesses binding avidity for 5-hydroxytryptamine(1A) (5-HT_{1A} or serotonin) receptor complexes.

Background Information: Preclinical studies in rodents suggest that augmentation of serotonin reuptake inhibitor (SSRI) therapy by the 5-HT_{1A} receptor agent pindolol might reduce the delay between initiation of treatment and antidepressant response. Such a hypothesis is based on the ability of pindolol to potentiate increases in serotonin (5-HT) transmission induced by SSRIs, an effect achieved by blockade of the 5-HT_{1A} autoreceptors in the DRN. However, placebo-controlled clinical studies of pindolol augmentation of antidepressant therapy have reported inconsistent results.

Application: The binding avidity of [^{11}C]WAY-100635 for 5-HT_{1A} receptor complexes can be utilized to evaluate occupancy of these membrane-associated sites. In this context, [^{11}C]WAY-100635 can effectively assess new prototype pharmaceuticals through PET imaging modalities to determine their selectivity for DRN. In this context, this strategy can be used as an investigative probe to establish appropriate doses for clinical trials.

Implementation: Evaluation of the occupancy of 5-HT_{1A} receptors following treatment with controlled release pindolol in 9 healthy volunteers with positron emission tomography (PET). Each subject is then studied 4 times, at (i) baseline (scan 1) following 1 week of orally administered pindolol CR (7.5 mg/day) at peak levels; (ii) 4 hours after the dose (scan 2); (iii) 10 hours following the dose (scan 3); and (iv) following 1 dose of pindolol CR (30 mg) at 4 hours when peak levels occur (scan 4). Pindolol occupancy of 5-HT_{1A} receptors is then evaluated in the DRN and within cortical regions as the decrease in binding potential (BP) of the selective radiolabeled 5-HT_{1A} antagonist [^{11}C]WAY-100635).

Human Model: Healthy cases (n = 9).

Interpretation and Analysis: Pindolol dose-dependently decreases [^{11}C]WAY-100635 BP. Combining all regions-of-interest, occupancy is 20 ± 8%; 14 ± 8%; and 44 ± 8% (scan 2, 3, 4 respectively). Clinical studies designed to assess the augmentation of SSRI effects exerted by pindolol at doses of 2.5 mg t.i.d. reveal that the occupancy of 5-HT_{1A} receptors is moderate but highly variable between subjects. This factor may explain the variable results obtained in clinical studies. On the other hand, at each dose tested, pindolol occupancy of 5-HT_{1A} receptors one higher in the DRN compared to cortical regions, demonstrating a significant degree of *in vivo* selectivity for DRN 5-HT_{1A} autoreceptors relative to corticolimbic postsynaptic receptors. Selectivity of this magnitude is necessary for the potentiation of 5-HT transmission, and this finding represents an important proof of concept in the development of 5-HT_{1A} agents for this application.

Positron emission tomography study of pindolol occupancy of 5-HT_{1A} receptors in humans: Preliminary analyses. Martinez D, Mawlawi O, Hwang DR, Kent J, Simpson N, Parsey RV, Hashimoto T, Slifstein M, Huang Y, Van Heertum R, Abi-Dargham A, Caltabiano S, Malizia A, Cowley H, Mann JJ, Laruelle M. Nucl Med Biol 2000 Jul; 27 (5), pp. 523–7.

Central Nervous System (CNS)/Serotonin Transporters (SERT)/Cerebral Cortex/ Hypothalamus/Hippocampus/Striatum/ [Parkinson's Disease]

[^{125}I]-ADAM/[^{123}I]-ADAM/2-((2-((Dimethylamino) Methyl)- Phenyl)Thio)-5-Iodophenylamine

Cellular/Molecular Mechanism of Action: The substances 2-((2-((dimethylamino)methyl)-phenyl)thio)-5-iodophenylamine paroxetine and (+)McN5652 all function as ligands that selectively recognize and physically bind to serotonin transporter complexes.

Application: The iodinated tracer ADAM (2-((2-((dimethylamino)methyl)-phenyl)thio)-5-iodophenylamine) can be applied to detect and characterize SERT within the CNS.

Route of Administration: [^{125}I]-ADAM (intravenous).

Implementation: Detection of SERT within the CNS using single photon emission computerized tomography (SPECT) imaging modalities utilizing [^{125}I]2-((2-((dimethylamino)methyl)-phenyl) thio)-5-iodophenylamine paroxetine and (+)McN5652.

Animal Model: Rat.

Interpretation and Analysis: Scatchard analysis of saturation binding of [^{125}I]ADAM to frontal cortical membrane homogenates (rat) produces K_d values of 0.15 ± 0.03 nM and B_{MAX} values of 194 ± 65 fmol/mg protein. Biodistribution analysis of [^{125}I]-ADAM within the brain postadministration reveals high specific binding within regions of the hypothalamus, cortex, striatum, and hippocampus, where SERT are concentrated and specific binding phenomenon peaks at 120 to 240 minutes postinjection [(hypothalamus-cerebellum)/ cerebellum = 4.3 at 120 minutes post-IV injection]. Moreover, specific hypothalamic uptake is blocked by pretreatment with SERT selective competing drugs, such as paroxetine and (+)McN5652, while other non-competing drugs, such as ketanserin, raclopride, and methylphenidate, exert no effect. Radioactive material recovered from rat brain homogenates at 120 minutes after [^{125}I]-ADAM injection is primarily the original compound (> 90%) and is a good indication of the *in vivo* stability of the agent within brain tissues. Both male and female *in vivo* models show similar and comparable patterns of organ distribution and regional brain uptake. Autoradiograms (*ex vivo*) of brain sections (120 minutes after IV injection of [^{125}I]-ADAM) detect intense signal increases in several regions (olfactory tubercle, lateral septal nucleus, hypothalamic and thalamic nuclei, globus pallidus, central gray, superior colliculus, substantia nigra, interpeduncular nucleus, dorsal and median raphes, and locus coerulus), which parallel known SERT density profiles. Such findings strongly suggest that the novel tracer ADAM is superior to the congers (i.e., IDAM) reported previously. Labeling of ADAM with [^{123}I] to produce [^{123}I]-ADAM should produce an improved and useful SPECT imaging agent for SERT within the brain.

Selective *in vitro* and *in vivo* binding of [^{125}I]-ADAM to serotonin transporters in rat brain. Choi SR, Hou C, Oya S, Mu M, Kung MP, Siciliano M, Acton PD, Kung HF. Synapse 2000 Dec 15; 38 (4), pp. 403–12.

Central Nervous System (CNS)/Serotonin Transporter (SERT)/Hypothalamus Region
[^{125}I]-ODAM/[^{125}I]-[5-iodo-2-(2-Dimethylaminomethylphenoxy)-Benzyl Alcohol]

Background Information: The SERT are the primary binding sites for selective serotonin reuptake inhibitors, which are commonly used as antidepressants (e.g., fluoxetine, sertraline, and paroxetine). Imaging of SERT with positron emission tomography (PET) and single photon emission computed tomography (SPECT) in humans would provide a useful tool for understanding how alterations of this system are related to the development of depressive illnesses and other psychiatric disorders.
Application: [^{125}I]-ODAM [(5-iodo-2-(2-dimethylaminomethylphenoxy)-benzyl alcohol, 9)] can be applied as an imaging agent for evaluating CNS SERT.
Route of Administration: [^{125}I]-ODAM (intravenous injection).
Semi-Synthetic Methods: Radioiodination and semi-synthesis of [^{125}I]-ODAM [(5-iodo-2-(2-dimethylaminomethylphenoxy)-benzyl alcohol]. A new reaction scheme has been developed for the preparation of [^{125}I]-ODAM and the corresponding tri-*n*-butyltin derivative (compound 10). Upon reacting 10 with hydrogen peroxide and sodium [^{125}I]iodide, the radiolabeling efficiency can be observed to be relatively high (95%).
Implementation: Characterization of the binding characteristics of ODAM in preparations of cortical membrane homogenates reveals a good level of affinity (K_i = 2.8 ± 0.88 nM). Using LLC-PK(1) cells specifically expressing the individual transporter (i.e., dopamine [DAT], norepinephrine [NET], and SERT, respectively), ODAM shows strong inhibition of SERT (K_i = 0.12 ± 0.02 nM). Inhibition constants for the other 2 transporters are lower (K_i = 3.9 ± 0.7 μM and 20.0 ± 1.9 nM for DAT and NET, respectively). Biodistribution following intravenous injection of [^{125}I]-ODAM (rat) shows a rapid brain uptake and washout (2.03, 1.49, 0.79, 0.27, and 0.07% dose/organ at 2, 30, 60, 120, and 240 minutes, respectively). The hypothalamus region where the serotonin neurons are located exhibits a high specific uptake. Ratios of hypothalamus-cerebellum/cerebellum based on percent dose per gram of these 2 regions reveals values of 0.35, 0.86, 0.86, 0.63, and 0.34 at 2, 30, 60, 120, and 240 minutes, post-IV injection, respectively. The specific uptake in hypothalamus can be effectively blocked by pretreatment of known SERT ligands and serves to validate the idea that this ligand displays desirable *in vitro* and *in vivo* properties as a potential SERT imaging agent.
Animal Model: Rat (cortical brain homogenates and biodistribution studies).

A novel serotonin transporter ligand: (5-iodo-2-(2-dimethylaminomethylphenoxy)-benzyl alcohol. Zhuang Z, Choi S, Hou C, Mu M, Kung M, Acton PD, Kung HF. Nucl Med Biol 2000 Feb; 27 (2), pp. 169–75.

Central Nervous System (CNS)/Stem Cell Migration: Brain/[Parkinson's Disease Research]
Gadolinium Rhodamine Dextran (GRID)/ Ex Vivo Stem Cell Labeling

Background Information: The ability to track stem cell transplants in the brain by *in vivo* neuroimaging will improve current understanding about how these cells mediate functional recovery after neural transplantation. One major challenge for the development and refinement of stem cell transplantation is to map the spatial distribution and rate of *in situ* migration.
Application: Tracking transplanted stem cells in the ischemia-damaged rat hippocampus by magnetic resonance imaging (MRI).
Route of Administration: Intravascular.
Implementation: Assessment of stem cell migration applying *in vitro* labeling of populations with the novel bifunctional contrast agent GRID prior to transplantation. Detection is achieved applying both MRI and fluorescence microscopy, or with PKH26, which can be visualized exclusively under fluorescence microscopy. At different time points following engraftment, brains are evaluated by both histology and *ex vivo* MRI.
Interpretation and Analysis: Transplanted stem cells can be identified by MRI only if prelabeled with GRID, whereas fluorescence microscopy can detect transplanted cells using either labeling reagent. Distribution of GRID-labeled stem cells established through the application of MRI corresponds with results from fluorescence microscopy analysis. Stem cells and their migration can therefore be reliably identified applying GRID-enhanced MRI methodologies.

Tracking transplanted stem cell migration using bifunctional, contrast agent-enhanced, magnetic resonance imaging. Modo M, Cash D, Mellodew K, Williams SC, Fraser SE, Meade TJ, Price J, Hodges H. Neuroimage 2002 Oct; 17 (2), pp. 803–11.

Central Nervous System (CNS)/Trauma-Induced Neurochemical Changes
[^{3}H]Glutamate/[^{3}H]MK801

Background Information: Trauma is the leading cause of death in individuals between the ages of 1 and 44 years. In the case of severe head injury, mortality can reach as high as 35–70%. Despite this fact, there has been little progress in the development of effective pharmacological agents that can provide protection in cases suffering from brain injury. To date, little is known about the mechanisms involved in neuronal cellular insult after severe head injury, especially in humans. Glutamate acts both as a primary excitatory neurotransmitter and a potential neurotoxin within the mammalian brain. Evidence indicates that hyperactivity of the glutamate system contributes to neuronal death in brain trauma. Also, in animal models of neurotrauma, such forms of neural injury are followed by gliosis, which has been linked to the severity of brain injury.
Route of Administration: Intravascular.
Implementation: Evaluate the glutamate system in conditions of brain trauma, utilizing [^{3}H]glutamate and [^{3}H]MK801 (non-competitive N-methyl-D-aspartate [NMDA]-receptor antagonist) binding and [^{3}H]glutamate uptake assays in human cerebral cortex preparations obtained from severely brain-injured and reference control victims. Additionally, Grb-2-related adaptor protein (GRAP) immunohistochemistry can be peformed to investigate gliosis following brain injury.
Interpretation and Analysis: There are no significant differences in [^{3}H]glutamate binding (affinity or density of sites) between control and head-injured subjects. In contrast, cerebral cortical [^{3}H]MK801 binding reveals both a significant increase in the density of sites (B_{MAX}) and a decrease in the dissociation constant (K_d) in the head-injured group when compared to reference controls. There are no significant differences in [^{3}H]glutamate uptake between groups. The injured brains present with an increased number of GFAP-positive astrocytes and display a more intense GFAP reaction relative to control brains. In the context of traumatic brain injury, such findings encourage further investigation into compounds capable of selectively modulating NMDA receptor subtypes in humans while also therapeutically manipulating glial cell responses following brain trauma.

Neurochemical characterization of traumatic brain injury in humans. Regner A, Alves LB, Chemale I, Costa MS, Friedman G, Achaval M, Leal L, Emanuelli T. J Neurotrauma 2001 Aug; 18 (8), pp. 783–92.

Gastrointestinal/Colon-Specific Delivery
Chitosan Powder in Hydrophobic Polymer/ Aminoalkyl Methacrylate Copolymer RS

Cellular/Molecular Mechanism of Action: A chitosan dispersed system (CDS) composed of an active ingredient reservoir and an outer drug release-regulating layer (dispersing chitosan powder in hydrophobic polymer) can collectively serve as a means of physically sequestering and protecting orally administered pharmaceutical agents until they reach the colon.
Application: Colon-specific delivery.
Implementation: An aminoalkyl methacrylate copolymer RS (Eudragit RS) is a hydrophobic polymer that has very limited solubility in acidic mediums in contrast to chitosan, which is highly soluble in low pH environments. In this context, capsules coated with chitosan dispersed hydrophobic polymer facilitate the delivery of pharmaceutical agents in a time dependent and site-specific manner. (e.g., bifunctional release characteristics).
Animal Model: Canine (e.g., beagles).
Interpretation and Analysis: The release rate of pharmaceuticals can be controlled by changing the capsule thickness. Furthermore, for colon-specific drug delivery, an additional outer enteric coating is necessary to prevent drug release from CDS capsules within the stomach, because chitosan dispersed in the layer dissolves easily under acidic conditions. The enteric-coated preparation ultimately reaches the large intestine within 1 to 3 hours after oral administration and degrades by the time of analysis at the level of the colon.

Chitosan dispersed system for colon-specific drug delivery. Shimono N, Takatori T, Ueda M, Mori M, Higashi Y, Nakamura Y. Int J Pharm 2002 Oct 1; 245 (1–2), pp. 45–54.

Gastrointestinal/Colon: Delivery
Amylose-Ethylcellulose-Coated Hydroxypropylmethylcellulose

Application: The compound 4-aminosalicylic acid can potentially be used in the treatment of diseases of the colon.
Implementation: Amylose-ethylcellulose-coated hydroxypropylmethylcellulose capsules containing 4-aminosalicylic acid Na (135 mg) and radiolabeled with [^{99m}Tc] can be utilized to delineate time release parameters when analyzed by gamma camera imaging techniques in concert with the collection of plasma and urine samples.
Considerations: The compound 4-aminosalicylic acid can potentially be used for the treatment of diseases affecting the colon. The use of amylose-ethylcellulose-coated hydroxypropylmethylcellulose capsules protects the drug until the capsule reaches the colon, where 4-aminosalicylic acid is slowly released and absorbed. Thus, such a formulation has the potential for use in the treatment of inflammatory bowel disease.
Interpretation and Analysis: Uncoated capsules break down within 10 minutes in the stomach, allowing rapid and complete absorption of the drug. In contrast, amylose-ethylcellulose-coated hydroxypropylmethylcellulose capsules remain intact in the upper gastrointestinal tract, and have a median gastric emptying time of 61 minutes (interquartile range, 77 minutes) and a median colon arrival time of 363 minutes (interquartile range, 185 minutes). In coated capsules, only the metabolite is detected in the plasma and/or urine after the capsules reaches the colon.

Colonic delivery of 4-aminosalicylic acid using amylose-ethylcellulose-coated hydroxypropylmethylcellulose capsules. Tuleu C, Basit AW, Waddington WA, Ell PJ, Newton JM. Aliment Pharmacol Ther 2002 Oct; 16 (10), pp. 1771–9.

Gastrointestinal/Colon-Selective Oral Pro-Drug Delivery/Metabolically Trapped Prodrugs/Carrier Mediated and Local Delivery
[^{125}I]UdR

Cellular/Molecular Mechanism of Action: Represents a radiopharmaceutical agent that is usually modified biochemically by a specific enzyme fraction of interest. Biochemical transformation of the parent radiopharmaceutical agent directly corresponds to local levels of the "enzyme" indicator molecule of interest. Alternatively, pro-drug forms of radiopharmaceuticals can serve as a means of delaying elimination or protecting a parent pharmaceutical compound from premature biochemical degradation prior to interaction with a desired biological target. In this context, prodrug forms of radiopharmaceuticals can accumulate or become retained within a given organ or tissue type following initial "processing." Prodrugs of several diagnostic agents can function as imaging agents, whereby their primary "targeting" strategy involves mechanisms of metabolic trapping.
Application: Selective targeting of specific cell types or tissue structures.
Synthetic Chemistry and Analysis: Design and synthesize of site-selective prodrugs for cancer radiotherapy can be derived from 5-[^{125}I]iodo-2′-deoxyuridine ([^{125}I]UdR). The prodrugs of [^{125}I]UdR designed for the purpose of targeted delivery include several derivatives with altered permeability: (i) 3′,5′-dioctanoyl; (ii) 3′,5′-dioleoyl; (iii) 3′- and 5′-*N*-alkyl-dihydropyridyl; (iv) 3′- and 5′-*N*-alkyl-dihydroisoquinolyl; (v) 3′- and 5′-*N*-alkyl-dihydroacridinyl esters of [^{125}I]-UdR; (vi) polymeric and macromolecular prodrugs of [^{125}I]-UdR for carrier-mediated or selective local delivery; (vii) metabolically trapped [^{125}I]UdR prodrugs; and (viii) glycoconjugate prodrugs for oral colon-specific delivery of [^{125}I]-UDR, [^{125}I]-UDR-5′-β-d-cellobioside, [^{125}I]-UDR-5′-β-D-glucopyranoside, [^{125}I]-UDR-5′-β-D-galactopyranoside, and [^{125}I]-UDR-5′-beta-D-glucuronide.
Considerations: A basic set of rules exist that must be utilized for the molecular design of site-selective prodrugs and various modes of their activation. Examples include the application of prodrugs and targeted delivery of radiopharmaceutical agents. Site-specific prodrugs of diagnostic radiopharmaceuticals are routinely applied in diagnostic nuclear medicine, but the instances of targeting of radiotherapeutic prodrugs are surprisingly rare.

Prodrugs in site-selective delivery of radiopharmaceuticals. Baranowska-Kortylewicz J, Kortylewicz ZP, Hoffman D, Dalrymple GV. Q J Nucl Med 1997 Jun; 41 (2), pp. 127–39.

Hematology/Macrophages: Mannosyl-Fucosyl Receptors: Macrophages
Mannosylated Liposomes

Background Information: Despite the rapid development in medicinal and pharmaceutical technology, the targeting of drugs to phagocytic cells in macrophage-related diseases still remains a major unsolved problem.

Application: Selective liposome mediated delivery to macrophage populations.
Route of Administration: Mannosylated liposomes (intravascular).
Implementation: Evaluation of liposome modalities for their ability to selectively target mannosyl-fucosyl receptors on macrophages as a potential model for targeting antileishmanial drugs encapsulated within mannosylated or fucosylated liposomes to treat leishmaniasis.
Animal Model: Hamster (experimental leishmaniasis).
Interpretation and Analysis: Mannosylated liposomes are more effective in delivering antileishmanial drugs to phagocytic cells. Liposomes loaded with an indigenous drug, andrographolide, a labdane diterpenoid isolated from Indian medicinal plant *Andrographis paniculata,* can be prepared and tested against experimental leishmaniasis in a hamster model. Mannosylated liposomes loaded with the drug are the most potent in reducing parasitic burden within the spleen as well as reducing hepatic and renal toxicity. In addition, mannosylated drug-loaded liposome-treated animals show a normal blood picture and splenic tissue histoarchitecture when compared with those treated with free drug or regular liposomal drug. Such a drug-vehicle formulation should be considered for clinical trials.

Targeting of liposomal andrographolide to L. donovani-infected macrophages *in vivo.* Sinha J, Mukhopadhyay S, Das N, Basu MK. Drug Deliv 2000 Oct–Dec; 7 (4), pp. 209–13.

Neoplasia/Carbonic Anhydrase (CA) Isoenzyme Inhibitors
Aromatic/Heterocyclic Sulfonamides

Cellular/Molecular Mechanism of Action: Carbonic anhydrase isoenzyme inhibitor.
Application: Selective inhibition of CA isoenzyme CA I.
Synthetic Chemistry and Analysis: Reaction of 20 aromatic/heterocyclic sulfonamides containing a free amino, imino, hydrazine, or hydroxyl group, with tosyl isocyanate or 3,4-dichlorophenyl isocyanate afforded 2 series of derivatives containing arylsulfonylureido or diarylureido moieties in their molecular structure, respectively.
Implementation: Molecular derivatives are assayed as inhibitors of 3 CA isozymes: CA I, II (cytosolic forms), and IV (membrane-bound form). Potent inhibition can be appreciated against all 3 isozymes but especially against CA I, which is generally 10–75 times less susceptible to inhibition by the classical sulfonamides in clinical use in contrast to the other major red cell isozyme, CA II, or membrane-bound CA IV fractions. The derivatives obtained from tosyl isocyanate are generally more potent than the corresponding ones obtained from 3,4-dichlorophenyl isocyanate.
Interpretation and Analysis: Selective inhibition of CA I can potentially lead to more selective drugs/diagnostic agents from this class of pharmacologically relevant compounds.

Carbonic anhydrase inhibitors. Inhibition of cytosolic isozymes I and II and trans-membrane, cancer-associated isozyme IX with lipophilic sulfonamides. Franchi M, Vullo D, Gallori E, Pastorek J, Russo A, Scozzafava A, Pastorekova S, Supuran CT. J Enzyme Inhib Med Chem 2003 Aug; 18 (4), pp. 333–8.

Neoplasia: Carcinoma/Pulmonary Carcinoma/Murine Lewis Lung Carcinoma
N-[2-(hydroxyethoxy)methyl]-5-[^{3}H]methyluracil (^{3}H-1)

Application: Unsuitable agent for diagnostic imaging of neoplastic disease states.
Dosage: (specific activity 188 MBq mmol-1).
Semi-Synthetic Methods: The production of tritium-labeled acyclonucleoside, *N*-[2-(hydroxyethoxy)methyl]-5-[^{3}H]methyluracil (^{3}H-3), involves synthesis from the precursor, 5-[^{3}H]-methyluracil, [^{3}H]-1, which is subsequently converted to a 2,4-bis-trimethylsilyl intermediate and then coupled with 2-acetoxyethoxymethyl bromide to yield 1-[(2-acetoxyethoxy)methyl-5-[^{3}H]methyluracil (^{3}H-2). Treatment of [^{3}H]-2 with sodium methoxide in methanol produces [^{3}H]-3 (specific activity 188 MBq/mmol).
Implementation: Evaluation of the tissue distribution of [^{3}H]-3.
Animal Model: Murine (male BDF1 mice bearing Lewis lung carcinoma).
Interpretation and Analysis: The tissue distribution of [^{3}H]-3 reveals that long bones exhibit the highest tumor-to-tissue ratios. The kidney contains the highest radioactivity level relative to tumor masses, suggesting the kidney is the major route of excretion. The primary radioactive blood component (89.21%) has a biological half-life of approximately 0.19 minutes. The title compound is unsuitable for use as a diagnostic agent for LL carcinoma because of low tumor uptake and rapid urinary elimination of injected radioactivity from the body.

Tumor uptake of radiolabeled pyrimidine bases and pyrimidine nucleosides in animal models-VIII. Synthesis and tissue distribution of *N*-[2-(hydroxyethoxy) methyl]-5-[^{3}H]methyluracil. Lee YW, Iwashina T, Gati WP, Knaus EE, Wiebe LI. Int J Appl Radiat Isot 1985 May; 36 (5), pp. 395–8.

Neoplasia/Estrogen Receptors: Mammary Carcinoma
[^{99m}Tc-N_2S_2]-Z-Aminotamoxifen/[^{99m}Tc-N_2S_2]-E-Aminotamoxifen/[Rhenium-N_2S_2]-Z-Aminotamoxifen/[Rhenium- N_2S_2]-E-Aminotamoxifen

Cellular/Molecular Mechanism of Action: Aminotamoxifen recognizes and binds to membrane-associated estrogen receptor complexes.
Application: Imaging agent for the detection and characterization of breast cancer lesions.
Route of Administration: Intravascular.
Semi-Synthetic Methods: The bifunctional chelator agent N_2S_2 is conjugated to Z- and E-aminotamoxifens through an amide linker. Both [^{99m}technetium] ([^{99m}Tc]) and rhenium can be bound to N_2S_2 through mechanisms of simple chelation.
Implementation: Detection and characterization of mammary carcinoma lesions (*in vivo*).
Interpretation and Analysis: For the Z-isomer, chelation with rhenium in the presence of sodium acetate yields a mixture of 2 isomers, anti and syn, in a 1:1 ratio. In the presence of hydroxide only the anti isomer is produced. Both the Z- and E-tamoxifen conjugates can be chelated with [^{99m}Tc] at tracer levels, yielding a single isomer product, which is assigned as anti based on chromatographic comparison to the rhenium complexes. Radiochemical yields are consistently greater than 80%, with Sep-Pak column purification yielding a final product with $>$ 99% radiochemical purity and no residual starting material. Both *in vitro* and *in vivo* biological evaluation of the tamoxifen chelates indicate very limited estrogen receptor binding.

Single isomer technetium-99m tamoxifen conjugates. Hunter DH, Luyt LG. Bioconjug Chem 2000 Mar–Apr; 11 (2), pp. 175–81.

Neoplasia/Estrogen Receptor Alpha (ERalpha): Mammary Carcinoma
4-Para-Phenylvinyl Estradiol Analogues

Cellular/Molecular Mechanism of Action: Synthetic 17α-E-(4-substituted phenyl)vinyl estradiols represent a class of ligands

that retain significant binding affinity for the ERalpha-hormone binding domain (HBD).

Application: Detection and characterization of neoplastic disease states that overexpress ERalpha-HBD applying biodistribution diagnostic procedures.

Semi-Synthetic Methods: Production of an array of 4-para-substituted phenylvinyl estradiol derivatives that can be fabricated using a combination of reagent formulations in concert with solid-phase Palladium (Pd^0)-catalyzed methods.

Interpretation and Analysis: A spectrum of compounds (e.g., n = 1 through 10) have been evaluated for binding affinity characteristics using the Eralpha-HDB isolated from transfected BL21 cell lines. Although these compounds are somewhat lower in relative binding affinity (RBA at 25°C is 1–60%) than estradiol (100%), most have higher affinity than the unsubstituted parent phenylvinyl estradiol (RBA = 9%).

Because the substituents do not generate a structure-activity relationship directly based on physicochemical properties, evaluations can be performed using molecular modeling and molecular dynamics to establish key interactions between the ligand (e.g., para substituent especially), and the protein receptor molecule. The observed relative binding affinities are directly related to the calculated binding energies. Amino acids juxtaposed to the para position play a significant but not a dominant role in binding. In particular, 4-substitution tends to increase receptor binding affinity compared to unsubstituted analogues , as exemplified by semi-synthetic product 5 (e.g., 4-$COCH_3$), which has the highest RBA value (60%) of the series of compounds. Palladium-catalyzed coupling reactions on solid support or in solution using suitably substituted iodo arenes and 17α-E-tributylstannylvinyl estradiols offer a flexible approach to preparation.

Molecular modeling studies of the receptor suggest that there exists additional ligand accessible regions within the ERalpha-HBD that can foster interactions that may enhance receptor affinity or modify efficacy in the development of new therapeutic agents. Studies to undertake modification in the properties and/or position of the aryl substituents in subsequent series to further define that role are warranted.

Synthesis and evaluation of 17alpha-20E-21-(4-substituted phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17beta-diols as probes for the estrogen receptor alpha hormone binding domain. Hanson RN, Lee CY, Friel CJ, Dilis R, Hughes A, DeSombre ER. J Med Chem 2003 Jul 3; 46 (14), pp. 2865–76.

Neoplasia/Folate Receptor Expression
Folate Liposomes

The folate receptor is a cell surface protein complex that has been identified as a tumor marker, due to its differential overexpression in several malignancies. Current research indicates that folate can be covalently attached to the surface of liposomes to mediate their selective internalization by tumor cells through the folate receptor-mediated endocytic pathway. Optimized liposome formulations, characterized by improvements in drug loading, extended residence times in the circulation, and improved drug release, have been developed to improve the biodistribution of therapeutic molecules. Theoretically, folate receptor-targeting can be combined with liposome encapsulation to synergistically affect disease outcome by enhancing the delivery of chemotherapeutic agents to neoplastic cells, while reducing systemic toxicities to normal tissues.

Folate receptor-targeted liposomes as vectors for therapeutic agents. Gosselin MA, Lee RJ. Biotechnol Annu Rev 2002; 8, pp. 103–31.

Neoplasia/Folate Receptor Expression
[^{67}Ga]Folate-DF-Folate-α/[^{67}Ga]Folate-DF-Folate-γ

Cellular/Molecular Mechanism of Action: Folate binds to its respective folate receptor complex, which is overexpressed by several neoplastic cell types. Deferoxamine functions as a chelating agent for certain radioisotopes (e.g., [$^{67}Ga^{3+}$]).

Application: Identification of folate receptor positive neoplastic lesions applying nuclear medicine imaging. In this context, folate can be used as a carrier for radioisotopes in a manner that produces an agent that can be applied as a diagnostic radiopharmaceutical. In this context, folate receptors are expressed at relatively high membrane densities by certain neoplastic cell types compared to normal cell types. Deferoxamine functions as a chelating agent for certain radioisotopes (e.g., $^{67}Ga^{3+}$).

Route of Administration: Intravascular.

Semi-Synthetic Methods: The vitamin folic acid can be covalently linked to the chelating agent deferoxamine (DF) via an amide bond using a simple carbodiimide coupling reaction. A mixture of 2 isomers, DF-folate-α and DF-folate-γ, is produced pertaining to the α- and γ-carboxyl group of folic acid, respectively. The isomers can be separated by anion-exchange chromatography and the application of NH_4HCO_3 buffer gradients.

Implementation: Evaluation of DF-folate conjugates radiolabeled with the gamma-emitting radionuclide [$^{67}Ga^{3+}$] for their ability to be internalized by cultured KB cells overexpressing folate receptor complexes.

Interpretation and Analysis: Competitive binding studies reveal that only the DF-folate-γ is recognized by the folate receptor on KB cells, which interacts with an affinity comparable to unconjugated folic acid. The cellular accumulation of [^{67}Ga]-DF-folate-γ tracer exhibits rapid uptake kinetics in cell culture with a $T_{1/2}$ of approximately 3 minutes. The KB cell association of [^{67}Ga]-DF-folate-γ is competitively blocked by free folic acid, indicating that uptake of [^{67}Ga]-DF-folate-γ is specifically mediated by the folate receptor. Because the folate receptor is overexpressed on the surfaces of many neoplastic cells, these results suggest that [^{67}Ga]-DF-folate-γ preparations might be useful as a diagnostic agent for non-invasive imaging of folate receptor-expressing tumors.

Instrumentation: Computer-integrated nuclear medicine scintigraphy imaging camera.

Preparation of ^{66}Ga- and ^{68}Ga-labeled Ga(III)-deferoxamine-folate as potential folate-receptor-targeted PET radiopharmaceuticals. Mathias CJ, Lewis MR, Reichert DE, Laforest R, Sharp TL, Lewis JS, Yang ZF, Waters DJ, Snyder PW, Low PS, Welch MJ, Green MA. Nucl Med Biol 2003 Oct; 30 (7), pp. 725–31.

Synthesis, purification, and tumor cell uptake of 67Ga-deferoxamine-folate, a potential radiopharmaceutical for tumor imaging. Wang S, Lee RJ, Mathias CJ, Green MA, Low PS. Bioconjug Chem 1996 Jan–Feb; 7 (1), pp. 56–62.

Neoplasia/Folic Acid Receptor Expression
[^{99m}Technetium] ([^{99m}Tc])-Labeled Folic Acid

Cellular/Molecular Mechanism of Action: In the context of diagnostic pharmacology, folic acid binds to folic acid receptor complexes that are variably overexpressed on the surface membrane of different neoplastic cell types.

Background Information: It is known that membrane folic acid receptors are responsible for cellular accumulation of folate and folate analogues such as methotrexate. Certain neoplastic cell types are known to overexpress folate receptors to a variable degree. In normal differentiated tissues, folate receptor expression is highly

restricted. Results of limited *in vitro* and *in vivo* animal studies suggest that folate receptors can potentially function as a targeting platform for tumor diagnostic imaging.

Application: Detection and characterization of neoplastic lesions.

Route of Administration: [^{99m}Tc]-labeled folic acid (intravenous).

Dosage: [^{99m}Tc]-labeled folic (370 KBq/rat).

Semi-Synthetic Methods: Use of ethylenedicysteine (EC) as a chelating agent for the production of [^{99m}Tc]-EC-folate.

Implementation: Evaluate the labeling efficiency of [^{99m}Tc]-labeled folic acid and its potential use as a tumor-seeking agent. Tissue distribution of [^{99m}Tc]-EC-folate can be determined by imaging at 20 minutes and 1, 2, and 4 hours (n = 3/time interval). Validation of receptor-mediated processes can be established with blocking studies utilizing the co-administration of "cold" folic acid (50 and 150 μmol/kg) in concert with [^{99m}Tc]-EC-folate in tumor-bearing *in vivo* models. Planar imaging and whole-body autoradiograms are then performed and findings compared to [^{99m}Tc]-EC (control).

Animal Model: Rats (tumor-bearing).

Interpretation and Analysis: In animal studies, tumor/blood count density ratios at 20 minutes to 4 hours increase from 0.81 ± 0.09 to 1.23 ± 0.13 with [^{99m}Tc]-EC-folate. Conversely, these values show a time-dependent decrease from 0.77 ± 0.32 to 0.65 ± 0.01 with [^{99m}Tc]-EC within the same time period. Tumor/muscle and tumor/blood count density ratios significantly decrease with folic acid co-administrations. Planar images and autoradiograms confirm that tumors can be visualized clearly with [^{99m}Tc]-EC-folate.

[^{99m}Tc]-ethylenedicysteine-folate: A new tumor-imaging agent. Synthesis, labeling, and evaluation in animals. Ilgan S, Yang DJ, Higuchi T, Zareneyrizi F, Bayhan H, Yu D, Kim EE, Podoloff DA. Cancer Biother Radiopharm 1998 Dec; 13 (6), pp. 427–35.

Neoplasia/Folic Acid Receptors/Ovarian Cancer/ Sensitivity and Specificity of Detection *[^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)-Folate*

Cellular/Molecular Mechanism of Action: [^{111}In]-DTPA-folate is a radioactive chelation analogue of folic acid that binds to folate receptors.

Background Information: Folate receptor complexes are overexpressed at relatively high membrane densities by certain neoplastic cell types. The cell-membrane folate receptor is a potential molecular target for tumor-selective diagnostic and therapeutic pharmaceutical delivery.

Route of Administration: [^{111}In]-DTPA-folate (intravenous bolus).

Implementation: Evaluation of the effectiveness of [^{111}In]-DTPA-folate to selectively detect conditions of ovarian and endometrial neoplastic disease.

Applied as a diagnostic agent, whole-body images are obtained at 30 minutes, 4 hours, and (e.g., for the 1st 19 patients) 24 hours after injection; single photon emission computed tomography (SPECT) can also be performed at the delayed imaging time schedules as a validation procedure. To determine the influence of folate loading on tracer biodistribution, unlabeled "free" folic acid can be administered before injection of [^{111}In]-DTPA-folate (e.g., n = 19 cases).

Human Model: Female cases of ovarian cancer (e.g., n = 35) enrolled in a phase I/II clinical study (e.g., n = 33 completing the surgical follow-up required by the study protocol for definition of disease status). Individual cases either have a pathologically proven malignancy or are scheduled for surgery for suspected new ovarian cancer (e.g., n = 26), recurrent ovarian cancer (e.g., n = 5), or endometrial cancer (e.g., n = 2).

Interpretation and Analysis: In cases subjected to surgical intervention, slightly less than half have new or recurrent malignant tumors that can be detected (e.g., n = 14/33). A large percentage of newly diagnosed ovarian carcinomas can be identified blindly by multiple trained professionals (e.g., n = 7/7 or 100% sensitivity). Sensitivity for the detection of recurrent malignancies is approximately 38% (e.g., n = 3/7) for masked readings, compared to 85% for unmasked readings (e.g., n = 6/7), indicating that correlation with anatomic imaging studies (computed tomography) is highly important in the diagnosis of such lesions. A number of cases will be determined to have benign masses (e.g., n = 18). In the context of a limited population size, the specificity of [^{111}In]-DTPA-folate scintigraphy has been found to be approximately 76% and 82% for the masked and unmasked analyses, respectively. [^{111}In]-DTPA-folate is safe, and possibly effective, for scintigraphy differentiating between malignant and benign ovarian masses.

Evaluation of [^{111}In]-DTPA-folate as a receptor-targeted diagnostic agent for ovarian cancer: Initial clinical results. Siegel BA, Dehdashti F, Mutch DG, Podoloff DA, Wendt R, Sutton GP, Burt RW, Ellis PR, Mathias CJ, Green MA, Gershenson DM. J Nucl Med 2003 May; 44 (5), pp. 700–7.

Neoplasia/Multidrug Resistance-Associated Protein (MRP)/[Leukemia] *[^{99m}Technetium] ([^{99m}Tc])-Tetrofosmin (TF)/Verapamil*

Cellular/Molecular Mechanism of Action: Tetrofosmin is a substrate molecule for MRP expressed by certain neoplastic cell types.

Application: Detection and characterization of neoplastic cell types (e.g., leukemia) that possess resistance to chemotherapeutic intervention due to overexpression of MRP. Identification of such characteristics can provide prognostic information and the basis for the design of alternative therapeutic intervention strategies.

Route of Administration: [^{99m}Tc]-TF (intravenous).

Implementation: Evaluation of the kinetics of cellular accumulation and retention of [^{99m}Tc]-TF in leukemia wild-type cell lines and its corresponding chemotherapeutic-resistant cell line to determine if [^{99m}Tc]-TF is a substrate for MRP with and without the presence of verapamil.

Tissue Culture Model: Leukemia wild-type HL60/WT cell line and its corresponding doxorubicin-resistant HL60/DOX cell line that overexpresses MRP but without P-glycoprotein (Pgp) overexpression (37°C).

Interpretation and Analysis: [^{99m}Tc]-TF net accumulation is significantly lower in HL60/DOX (1.35 ± 0.23%) than in HL60/WT (12.79 ± 0.47%) at 60 minutes ($p < 0.001$). Three minutes after exchanging the incubation solution to the tracer-free medium, only 18.20 ± 0.34% of [^{99m}Tc]-TF remains in HL60/DOX, whereas 84.74 ± 0.65% is retained in HL60/WT ($p < 0.001$). In the presence of verapamil (10 μM), [^{99m}Tc]-TF net accumulation in HL60/DOX is 302% of the control and washout is significantly delayed. [^{99m}Tc]-TF therefore appears to be a substrate for MRP and [^{99m}Tc]-TF may be used as a functional *in vivo* imaging agent of profiling MRP expression.

Technetium-99m-tetrofosmin would be a substrate for multidrug resistance-associated protein (MRP): Comparison between a leukemia cell line with high MRP gene expression and its parental cell line. Li XF, Kinuya S, Yokoyama K, Konishi S, Ma YY, Watanabe N, Shuke N, Bunko H, Michigishi T, Tonami N. Cancer Biother Radiopharm 2001 Feb; 16 (1), pp. 17–23.

Neoplasia/Multidrug Resistance Protein (MRP)/ Predicted Chemotherapeutic Response Rate/ Osteosarcoma
[^{99m}Technetium] ([^{99m}Tc])-Methoxyisobutylisonitrile (MIBI)/[^{99m}Tc]-MIBI Washout Rate

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-MIBI is a pharmaceutical substrate transported by the multidrug resistance (MDR)-related proteins.

Background Information: *In vitro* studies have demonstrated that [^{99m}Tc]-MIBI is a transport substrate of MDR-related proteins. Minimal information is available about the potential of [^{99m}Tc]-MIBI scintigraphy as a functional imaging tool for the *in vivo* detection of MRP expression in osteosarcoma, or the role of MRP and [^{99m}Tc]-MIBI imaging for the prediction of clinical outcomes.

Application: Detection and characterization of MRP expression in neoplastic disease states (e.g., osteosarcoma) in a manner that can assist in predicting response to therapeutic intervention.

Implementation: Evaluation of osteosarcoma conditions before and after initiation of chemotherapy. Tumor-to-background ratios of both early (10 minutes) and delayed (1 hour) images and the percentage washout rate (WR%) of [^{99m}Tc]-MIBI can subsequently be calculated. Immunohistochemical analysis of MRP and P-glycoprotein (Pgp) can be performed on biopsy specimens, and the response to preoperative chemotherapy assessed by histopathologic examination. In this manner, it is possible to determine if scintigraphic parameters are of any benefit in distinguishing the functional capacity of Pgp and MRP.

Human Model: Cases of osteosarcoma (n = 24).

Interpretation and Analysis: Approximately 62.5% of osteosarcomas display significant MRP expression levels (e.g., n = 15/24).

The level of MRP expression significantly correlates with WR% of [^{99m}Tc]-MIBI (r = 0.58, p = 0.003), and the WR% of [^{99m}Tc]-MIBI is significantly faster in cases with high MRP expression than in those with a low MRP score (p = 0.007). The clearance rate of [^{99m}Tc]-MIBI is significantly slower in tumor tissue samples with negative or low expression of both Pgp and MRP (16% ± 6.2%) when compared with osteosarcomas that express high levels of both proteins (31.7% ± 8.7%) (p = 0.001). There appears not to be a significant difference between the WR% of [^{99m}Tc]-MIBI in tumors with co-expression of both proteins and in tumors with high expression of either Pgp or MRP. Both the rate of MRP expression and the WR% of [^{99m}Tc]-MIBI significantly correlate with response rate. Therefore, the WR% of [^{99m}Tc]-MIBI correlates with MRP expression levels. Both the WR% of [^{99m}Tc]-MIBI and MRP expression correlate with response to therapeutic intervention. [^{99m}Tc]-MIBI, therefore, is valuable as a general probe for functional imaging of both Pgp and MRP; however, it is not capable of differentiating the functional status of either MDR-related glycoprotein.

^{99m}Tc-MIBI imaging as a predictor of therapy response in osteosarcoma compared with multidrug resistance-associated protein and P-glycoprotein expression. Burak Z, Moretti JL, Ersoy O, Sanli U, Kantar M, Tamgac F, Basdemir G. J Nucl Med 2003 Sep; 44 (9), pp. 1394–401.

Neoplasia/Multidrug Resistance Protein (MDR)/ P-Glycoprotein (PgP) Transport Activity/[Mammary Carcinoma]/[Lung Tumor]
[^{99m}Tc(CO)$_3$(MIBI)$_3$] + (Tc-CO-MIBI)/LY335979 (MDR Modulator)

Cellular/Molecular Mechanism of Action: [^{99m}Tc(CO)$_3$(MIBI)$_3$]+ (Tc-CO-MIBI) can function as a transport substrate for MDR 1 (Pgp).

Background Information: Multidrug resistance mediated by the overexpression of MDR1 Pgp is 1 of the best characterized barriers limiting chemotherapeutic intervention in conditions of neoplastic disease. Furthermore, the protective function of Pgp-mediated efflux of xenobiotics in various organs has a profound effect on the bioavailability of drugs in general. Thus, there is an expanding requirement to non-invasively interrogate Pgp transport activity *in vivo*.

Application: Detection and characterization of MDR/Pgp transport function and genetic expression. Secondary applicability includes delineation of their capacity to evaluate the efficacy of inhibitors of MDR/Pgp transport biological function and genetic expression.

Implementation: Evaluation of the recognition properties (binding avidity) of [^{99m}Tc](I)-tricarbonyl complexes in the form of [^{99m}Tc(CO)$_3$(MIBI)$_3$] + (Tc-CO-MIBI) for Pgp fractions.

***Ex Vivo* Model:** Drug sensitivity KB 3-1 cell lines; colchicine-selected drug-resistant KB 8-5 cell types; chemotherapeutic-sensitive MCF-7 mammary carcinoma cell lines; H69 lung tumor cell lines.

Animal Model: Murine (mdr1a/1b(-/-) gene deleted mice and wild-type mice.

Interpretation and Analysis: Tc-CO-MIBI has a 60-fold higher accumulation in drug-sensitive KB 3-1 cells compared to colchicine-selected drug-resistant KB 8-5 cells. In KB 8-5 cells, tracer enhancement can be achieved with the potent MDR modulator LY335979 (EC50 = 62 nM). Similar behavior can be observed using drug-sensitive MCF-7 breast adenocarcinoma cells and MCF-7/MDR1 stable transfectants, thereby confirming that Tc-CO-MIBI is specifically excluded by MDR1 Pgp overexpression. By comparison, net accumulation in control H69 lung tumor cells is 9-fold higher than in MDR-associated protein (MRP1)-expressing H69AR cells, indicating only modest transport by MRP1. Biodistribution analysis following tail vein injection of Tc-CO-MIBI shows delayed liver clearance as well as enhanced brain uptake and retention in MDR-gene-deleted mice vs wild-type mice, directly demonstrating that Tc-CO-MIBI is a functional *in vivo* probe for detecting Pgp transport activity.

Characterization of a novel [^{99m}Tc]-carbonyl complex as a functional probe of MDR1 P-glycoprotein transport activity. Dyszlewski M, Blake HM, Dahlheimer JL, Pica CM, Piwnica-Worms D. Mol Imaging 2002 Jan–Mar; 1 (1), pp. 24–35.

Neoplasia/Sigma Receptors/Mammary Carcinoma
N-(N-Benzylpiperidin-4-yl)-2-[^{18}F]-Fluorobenzamide/ Haldol

Cellular/Molecular Mechanism of Action: *N*-(*N*-benzylpiperidin-4-yl)-2-[^{18}F]-fluorobenzamide physically binds to membrane-associated sigma receptor complexes.

Background Information: Sigma receptor complexes are expressed in a relatively selective manner by human mammary carcinoma cell types.

Application: *N*-(*N*-benzylpiperidin-4-yl)-2-[^{18}F]-fluorobenzamide can potentially function as a diagnostic ligand for PET imaging of breast cancer lesions that express sigma receptor complexes at relatively high membrane densities.

Route of Administration: *N*-(*N*-benzylpiperidin-4-yl)-2-[^{18}F]-fluorobenzamide (most frequently by intravascular injection).

Implementation: Detection of mammary carcinoma neoplastic lesions *in vivo* applying *N*-(*N*-benzylpiperidin-4-yl)-2-[^{18}F]-fluorobenzamide and positron emission tomography (PET) imaging modalities.

Animal Model: Mice (severe combined immunodeficient [SCID] mice bearing MDA-MB231 tumor and native wild-type explants of human mammary carcinoma).

Interpretation and Analysis: *In vivo* studies in SCID mice bearing MDA-MB231 tumors reveal a (i) high uptake of compound 2 in neoplastic lesions (3.8%/g); and (ii) ratios of tumor/muscle and tumor/blood of approximately 6.2 and 7.0, respectively, at 1 hour postinjection. Pretreatment of SCID mice with haldol increases the uptake of compound 2 in blood, muscle, and other well-perfused organs, while decreasing its uptake by tumors. The ratios of tumor/muscle and tumor/blood decrease from 6.2 and 7.0 to 1.3 and 1.1, respectively, at 1 hour postinjection. At 2 hours postinjection, the ratios of tumor/muscle and tumor/blood decrease from 4.9 and 7.8 to 1.4 and 1.4, respectively. The tumor uptake of compound 2 in SCID mice bearing primary tumor explants from human cases of breast cancer is lower than observed with MDA-MB231 tumors (1.66%/g vs 3.78%/g), and the ratios of tumor/muscle and tumor/blood are 3.5 and 3.7, respectively, at 1 hour postinjection. These results suggest that compound 2 may be a potential ligand for PET imaging of breast cancer.

N-(*N*-benzylpiperidin-4-yl)-2-[^{18}F]fluorobenzamide: A potential ligand for PET imaging of breast cancer. Shiue C, Shiue GG, Benard F, Visonneau S, Santoli D, Alavi AA. Nucl Med Biol 2000 Nov; 27 (8), pp. 763–7.

Neoplasia/Sigma-2 Receptor Complexes (σ2-Opioid)/Mammary Adenocarcinoma *[^{99m}Technetium] ([^{99m}Tc])-Sigma-2 Receptor Ligand*

Cellular/Molecular Mechanism of Action: [^{99m}Tc]-labeled [(*N*-[2-((3′-N′-propyl-[3,3,1]aza-bicyclononan-3α yl)(2′′-methoxy-5-methyl-phenylcarbamate)(2-mercaptoethyl)amino)acetyl]-2-aminoethanethiolato] technetium(V) oxide), [^{99m}Tc]$_2$, displays specific binding avidity toward sigma-2 receptor complexes (σ2-opioid).

Note: Pentazocine is an agonist at κ-opioid receptors and a weak agonist at μ-opioid receptors and is classified as a mixed opioid because it functions as an agonist at some opioid receptors and an antagonist at other.

Application: Detection and characterization of neoplastic disease states associated with the expression of σ2-opioid receptor complexes (e.g., human mammary carcinoma).

Route of Administration: Intravenous.

Semi-Synthetic Methods: Preparation of [^{99m}Tc]$_2$ can be achieved by heating the S-protected starting material, "1," in the presence of acid, a reducing agent (stannous glucoheptonate) and sodium [^{99m}Tc]pertechnetate. The lipophilic racemic mixture can be successfully prepared at a yield of 10–50% and with a radiochemical purity of > approximately 98%. Separation of the isomers, recognized as peak A and peak B, can be successfully achieved by using a chiralpak AD column eluted with an isocratic solvent (n-hexane/isopropanol; 3:1; v/v). The peak A and peak B appear to co-elute with the isomers of the surrogate, Re-2, under the same HPLC condition.

Implementation: Conduction of *in vitro* binding assays for the rhenium surrogate of [^{99m}Tc]$_2$, determination of Re-2 binding affinity, and delineation of selectivity toward sigma-2 receptors. Biodistribution studies in conditions of mammary carcinoma.

Animal Model: Murine (mouse mammary adenocarcinoma, cell line 66 that overexpresses sigma-2 receptor complexes).

Interpretation and Analysis: *In vitro* binding assays show that rhenium (Re-2) displays excellent binding affinity and selectivity toward sigma-2 receptors (K_i = 2,723 and 22 nM for sigma-1 and sigma-2 receptor, respectively). Biodistribution studies in tumor-bearing mice (mammary adenocarcinoma) reveal that racemic [^{99m}Tc]$_2$ localizes within neoplastic lesions. Uptake in the tumor is 2.11, 1.30, and 1.11 %dose/gram at 1, 4, and 8 hours postinjection, respectively. Such findings suggest good uptake and retention within tumor cells. Tumor uptake is significantly, but incompletely, blocked (about 25–30% blockage) by co-injection of "cold" (+)pentazocine or haloperidol (1 mg/Kg). A majority of the radioactivity localizes within the tumor tissue can be extracted (> 60%), and HPLC analysis shows that it is the original parent compound (racemic [^{99m}Tc]$_2$ purity ≥ 98%). Distribution of purified HPLC peak A and HPLC peak B can be detected in the same tumor-bearing mice at 4 hours postinjection. Tumor uptake is similar for both isomers, but the blood and peripheral tissue content for the isomer in peak B is higher than that for the isomer in peak A. It is evident that the isomer in peak A possesses significantly better tumor/blood and tumor/muscle ratios. The higher rate of *in vivo* metabolism can be confirmed by the higher thyroid uptake values for the peak B isomer when compared to the peak A isomer. Therefore, the [^{99m}Tc]-labeled sigma receptor imaging agent, [^{99m}Tc]$_2$, has demonstrated the feasibility of using a [^{99m}Tc]-labeled agent for recognizing sigma receptor complexes as a means of detecting certain forms of neoplastic disease.

Development of a Tc-99m labeled sigma-2 receptor-specific ligand as a potential breast tumor–imaging agent. Choi SR, Yang B, Plossl K, Chumpradit S, Wey SP, Acton PD, Wheeler K, Mach RH, Kung HF. Nucl Med Biol 2001 Aug; 28 (6), pp. 657–66.

Neoplasia/Topoisomerase I & II/Adenocarcinoma (HT29)/Gliobastoma (U87MG)/Melanoma (A375M Antigen Positive) *[^{11}C]-9-Methoxyphenazine-1-Carboxamide/ [^{11}C]2-(4-Pyridyl)Quinoline-8-Carboxamide/ [^{11}C]DACA/[Topoisomerases I & II]*

Cellular/Molecular Mechanism of Action: The tricyclic carboxamide *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA) is a DNA-intercalating agent capable of inhibiting both topoisomerases I and II enzyme fractions.

Background Information: The tricyclic carboxamide DACA is currently in phase II clinical trials. Many related analogues have been developed, but despite their potent *in vitro* cytotoxicities, they exhibit poor extravascular distribution.

Application: Delineation of rational therapeutic drug selection.

Semi-Synthetic Methods: Each candidate compound shares a common structural feature (e.g., *N*-dimethyl side chain), which is radiolabeled with the positron-emitting radioisotope, (e.g., carbon-11).

Implementation: Evaluate the biodistribution and metabolite profiles of the prototype compound, DACA, with 3 analogues to aid rational drug selection in order to identify agents that are "minimal intercalators" with lower DNA association constants. In this fashion, promising candidates will be identified in emerging preclinical studies in animals that can ultimately be evaluated in humans using positron emission tomography (PET).

Animal Model: Murine (mice bearing human tumor xenografts).

Interpretation and Analysis: Acridine DACA, phenazine SN 23490, pyridoquinoline SN 23719, and dibenzodioxin SN 23935 are cytotoxic when evaluated applying *in vitro* assays utilizing HT29, U87MG, and A375M cell lines (IC_{50} of 1.4 of 1.8 μM, 0.4 of 0.6 μM, 1.3 of 1.6 μM, and 24 of 36 μM, respectively). *Ex vivo* biodistribution studies with [^{11}C]- radiolabeled compounds in mice bearing human tumor xenografts show rapid clearance of [^{11}C] radioactivity (parent drug and metabolites) from blood and major organs. Rapid hepatobiliary clearance and renal excretion can also be observed. There is low [< 5% of injected dose/gram (%ID/g)] and variable uptake of [^{11}C] radioactivity in 3 tumor types for all of the com-

pounds. Tumor (U87MG) to blood [^{11}C]-radioactivity ratios for [^{11}C]DACA, [^{11}C]-(9-methoxyphenazine-1-carboxamide (SN 23490), [^{11}C]2-(4-pyridyl)quinoline-8-carboxamide (SN 23719), and [^{11}C]dibenzo[1,4]dioxin-1-carboxamide (SN 23935) at 30 minutes are approximately 2.9 ± 1.1, 2.3 ± 0.6, 2.6 ± 0.6, and 0.7 ± 0.2, respectively. For SN 23719, the distribution of [^{11}C]-radioactivity in normal tissues and tumors determined *ex vivo* is in broad agreement with findings determined *in vivo* by whole-body PET scanning. [^{11}C]DACA is rapidly and extensively metabolized to several plasma metabolites and a major tumor metabolite. Alternatively, [^{11}C]SN 23935, [^{11}C]SN 23490, and [^{11}C]SN 23719 show less extensive metabolism. In tumor samples, parent [^{11}C]DACA and [^{11}C]SN 23935 is present at between 0.3 and 1.5 %ID/g, whereas [^{11}C]SN 23490 and [^{11}C]SN 23719 is present at between 1.5 and 2.8 %ID/g. Therefore, by using a strategy employing [^{11}C]-labeling, it is possible to determine the tissue distribution and metabolic stability of novel tricyclic carboxamides.

Given the objective of identifying analogues with potentially improved *in vivo* activity against solid tumors, the agents SN 23490 and SN 23719 have more favorable distribution and metabolic stability compared with DACA and SN 23935. Such observations suggest that further development and evaluation are warranted. The radiolabeling strategy used allows *ex vivo* and *in vivo* evaluation of promising anticancer agents in animals and offers the potential of rapid translation to studies in humans using PET.

Comparative biodistribution and metabolism of carbon-11-labeled *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide and DNA-intercalating analogues. Osman S, Rowlinson-Busza G, Luthra SK, Aboagye EO, Brown GD, Brady F, Myers R, Gamage SA, Denny WA, Baguley BC, Price PM. Cancer Res 2001 Apr 1; 61 (7), pp. 2935–44.

PEPTIDES, PROTEINS AND IMMUNOGLOBULINS

General Concepts: Immunoglobulin-Based Delivery Modalities/Monoclonal Antibody Production for Specific Antigens

Immunotherapy for reversal of toxicity due to poisons and drugs is not new. However, refinements in antibody isolation and purification, as well as the advancement of hybridoma technology and recombinant DNA biotechnology, has led to a new generation of immunotherapeutic and diagnostic agents. The advent of monoclonal antibody technology in 1975 heralded the new age of immunopharmacology and immunotoxicology. Monoclonal antibodies designed for a specific antigen have resolved the problem of polyclonality and cross-reactivity of traditional antibodies. Along with the production and isolation of active antibody fragments from both polyclonal and human monoclonal sources, as well as the ability to tailor-make chimeric antibodies by recombinant biotechnology, the development of novel immunotherapeutic agents has taken place. Two immunotherapeutic modalities, digoxin-specific antibody fragments (Fab) and snake antivenin, have been available for the clinician's armamentarium for years. Along the same lines of antidigoxin Fab development, application of newer antibody isolation technology has led to a purified IgG(T) antibody for snake venom poisoning, which is still in the developmental stages. Potential future developments in immunotherapeutics must overcome the clinical problems of immunogenicity and adverse reactions to the antibodies. Human monoclonal sources, active antibody fragments, and chimeric antibodies from transfectomas are all potential resolutions to these problems.

Immunotherapy in the poisoned patient. Overview of present applications and future trends. Sullivan JB Jr. Med Toxicol 1986 Jan–Feb; 1 (1), pp. 47–60.

General Concepts/Immunoglobulin: Monoclonal Antibody Delivery Platforms

Antibodies, specifically monoclonal antibodies, are potentially very useful and powerful carriers of therapeutic agents and diagnostic detection modalities to "target" tissues or lesions. The loading or charging of antibodies with agents, especially radiotracers, has been extensively described in the literature. The choice of radioisotope for immunodetection and/or immunotherapy is based on its availability, half-life, nature of the radiation emitted, and the metabolic pathways of the radionuclide in the body. Most important of all are the derivatization techniques available for labeling antibody with a given radionuclide. Isotopes of iodine and divalent metal ions are the most commonly used radionuclides. Antibodies labeled with iodine at tyrosine residues are metabolized *in vivo* rather rapidly. Such events lead to the incorporation of metabolized radioactive iodine into various tissues, mainly the thyroid gland and stomach, and to the accumulation of high levels of circulating iodine in the blood, which masks tumor uptake considerably. To overcome these limitations, the use of iodohippurate as an iodine-anchoring molecule to the protein has been considered. When divalent or multivalent metal ions are used as the preferred radionuclide, bifunctional chelating reagents such as ethylenediaminepentaacetic acid (EDTA) or diethylenetriamine pentaacetic acid (DTPA) are 1st coupled to the protein or antibody. These chelating molecules are attached to the protein by the formation of an isopeptide linkage between the carboxylate of the chelating reagent and the amino group of the protein. Several procedures are available to generate the isopeptide linkage. When the anchoring of the chelating agent through isopeptide linkage results in the inactivation of the antibody, periodate oxidation of the carbohydrate moiety of the antibody, followed by reductive coupling of chelator, could be considered as an alternative. There is still a need for better, simpler, and more direct methods for labeling antibodies with radionuclides.

Labeling of monoclonal antibodies with radionuclides. Bhargava KK, Acharya SA. Semin Nucl Med 1989 Jul; 19 (3), pp. 187–201.

Amyloidosis of Beta$_2$ (Microglobulin) *[^{111}Indium] ([^{111}In])-Rhbeta$_2$mH5*

Cellular/Molecular Mechanism of Action: Beta$_2$-microglobulin (beta$_2$m) recognizes and physically (chemically) binds to amyloid of beta$_2$m origin.

Background Information: Amyloid of beta$_2$m origin can be diagnosed using [^{131}I]-radiolabeled-beta$_2$m scintigraphy in cases with uremia and managed with hemodialysis treatment. As the tracer beta$_2$m molecule is isolated from another affected case, it carries the common risks, including viral infections such as hepatitis B and C and human immunodeficiency virus (HIV), that are known to be associated with human plasma products.

Application: The *in vitro* production of recombinant human beta$_2$m yields a protein fraction that is devoid of any potential risk of exposing recipients to various viral pathogens. Purified protein fractions can then be radiolabeled for the purpose of detecting and characterizing various conditions of amyloidosis.

Route of Administration: [^{111}In]-rhbeta$_2$mH5 (intravenous).

Semi-Synthetic Methods: Beta$_2$m-type amyloid can be produced *in vitro* as recombinant human beta$_2$m (rhbeta$_2$m) in *Escherichia coli* populations. The expression vector pASK40Delta Lbeta$_2$m(His)5 can be used due to it containing a C-terminal (His)5-tag for purification via immobilized metal ion affinity chromatography (IMAC). Size exclusion chromatography on a Superose 12 column can be used as a 2nd purification step. The isolated rhbeta$_2$mH5

reacts in an immunochemically identical manner to native human $beta_2$m, and can be identified as a single band of approximately 11.8 kDa when analyzed by Western blot immunodetection methodologies. Additionally, fractions can be visualized as a single spot in 2-dimensional gel electrophoresis (2D-PAGE: expected molecular mass = 12,415.8 Da). Uniformity can be further established by crystallization and N-terminal amino acid sequence analysis.

Implementation: Evaluation of $beta_2$m-amyloidosis conditions applying sterile preparations of rhbeta$_2$mH5 radiolabeled with [^{111}In] to produce [^{111}In]-rhbeta$_2$mH5.

Human Model: Cases managed with hemodialysis (± known $beta_2$m-amyloidosis).

Interpretation and Analysis: The [^{111}In]-rhbeta$_2$mH5 tracer localizes specifically within particular areas known to contain amyloid. Recombinant rhbeta$_2$mH5 preparations therefore are suitable for the *in vivo* detection of deposits of $beta_2$m-type amyloid contained within organs and fulfills the requirements of a tracer for common use. Finally, the use of [^{111}In] instead of [^{131}I] reduces the radioactive load and results in higher lesion resolution.

Production of recombinant human $beta_2$-microglobulin for scintigraphic diagnosis of amyloidosis in uremia and hemodialysis. Linke RP, Schäeffer J, Gielow P, Lindner P, Lottspeich F, Plückthun A, Weiss EH. Eur J Biochem 2000 Feb; 267 (3), pp. 627–33.

Cardiovascular Endothelium/E-Selectin Complexes
Liposomes: Anti-(E-Selectin) Directed/ [Immunoliposomes]/Dithionite/Pyranine

Cellular/Molecular Mechanism of Action: E-selectin is expressed on the exterior surface membrane of endothelial cell populations.

Background Information: Previous *in vitro* experiments have demonstrated that the targeting of immunoliposomes to vascular selectins is a potential strategy for selective drug delivery to sites of inflammation.

Application: Detection and characterization of vascular endothelium.

Route of Administration: Intravenous.

Implementation: Evaluation of the cellular uptake of targeted immunoliposomes by interleukin-1 activated human endothelial cells detected by utilizing spectroscopy and microscopic fluorescence techniques. In order to follow the fate of liposomes after binding to target sites, the route and degree of liposome internalization is established with the analytical focus directed toward the analysis of cell activation state or various liposomal parameters (e.g., sterical stabilization, type of antibody, or antibody coupling strategy).

Interpretation and Analysis: In attempts to further adapt the targeting strategies for physiological conditions, it has been established that E-selectin-directed immunoliposomes can cumulatively bind to target cells under simulated shear force conditions of capillary blood flow for up to 18 hours. The use of nitrobenzoxadiazole (NBD)-labeled liposomes and subsequent fluorescence quenching outside the cells with dithionite show that circa 25% of the targeted immunoliposomes become internalized. According to inhibition experiments with agents that interfere with the endocytotic pathway, it has been determined that the major mechanism of liposome entry is endocytic. Cellular entry involves, at least in part, receptor-mediated endocytosis via E-selectin, with liposome accumulation occurring within the endosomes, and their acidification has been determined using pyranine spectroscopy. Furthermore, microscopy has demonstrated that a fusion of liposomes with the cell membrane occurs, followed by a release of entrapped calcein into the cytoplasm. Such observations have provided greater insight into the behaviour of E-selectin-targeted immunoliposomes and indicate that these immunoliposomes have great potential for use as drug carriers for intracellular drug delivery at inflammatory sites.

Investigation of the cellular uptake of E-selectin-targeted immunoliposomes by activated human endothelial cells. Kessner S, Krause A, Rothe U, Bendas G. Biochim Biophys Acta 2001 Oct 1; 1514 (2), pp. 177–90.

Cardiovascular Endothelial Growth Factor (VEGF) Receptor
Vascular Endothelial Growth Factor

Cellular/Molecular Mechanism of Action: Angiogenesis is stimulated by VEGF acting via endothelial cell-specific receptors, such as VEGFR-2, that are overexpressed at sites of angiogenesis.

Background Information: Fusion protein VEGF with a large N-terminal extension retains biological activity and can facilitate the development of VEGF-based vehicles for receptor-mediated delivery modalities pertaining to both therapeutic and diagnostic agents.

Application: Selective "targeting" of vascular endothelial cell populations as a delivery modality.

Semi-Synthetic Methods: The generation of VEGF fusion proteins can be constructed in *Escherichia coli*. Purified VEGF fusion proteins containing a 158-amino acid N-terminal extension fused to human VEGF(121), VEGF(165), and VEGF(189) have been produced in this fashion.

Interpretation and Analysis: In living systems, VEGF fusion proteins induce tyrosine autophosphorylation of VEGFR-2 and its downstream targets, as well as cell contraction in cells overexpressing VEGFR-2. Although N-terminal extensions decrease the affinity of VEGF fusion proteins to VEGFR-2, at saturating concentrations these proteins are as efficient as correct size VEGF(165). It has been proposed that VEGF fusion proteins may be employed for targeting endothelial cells at the sites of angiogenesis.

Functionally active VEGF fusion proteins. Backer MV, Backer JM. Protein Expr Purif 2001 Oct; 23 (1), pp. 1–7.

Cardiovascular: Endothelium/Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1)/Pulmonary Vascular Endothelium: Selective Targeting/Cardiac Endothelium Targeting: Selective Catheter Delivery
Anti-PECAM-[Beta-Galactosidase]

Cellular/Molecular Mechanism of Action: Anti-PECAM-1 IgG or IgG fragments recognize and physically bind to membrane-associated PECAM-1 complexes expressed on the exterior surface membrane of vascular endothelial cell populations.

Background Information: Therapeutic molecules conjugated with antibodies directed against PECAM-1 accumulate in the pulmonary endothelium after IV injection (e.g., mice).

Application: Modality for selective delivery to vascular endothelium.

Route of Administration: Anti-PECAM (intravascular including via selective catheter placement within the right pulmonary artery).

Implementation: Characterization of PECAM-directed targeting of the lung and heart after local injection compared to systemic intravascular administration. Complementary investigations can entail the estimation of the effectiveness of targeting facilitated by utilization of the active reporter enzyme, beta-galatosidase in the form

of streptavidin-conjugated beta-galactosidase (beta-gal), which can subsequently be coupled to anti-PECAM or IgG (anti-PECAM/beta-gal and IgG/beta-gal). Utilization for *in vivo* investigations entails direct injection into the right coronary artery (RCA).

Animal Model: Swine.

Interpretation and Analysis: Radiolabel tracing shows that 1 hour postintravenous injection, 35% of anti-PECAM vs 2.5% of control IgG accumulates within the lung. Infusion of anti-PECAM via a catheter placed in the right pulmonary artery (RPA) results in a 3-fold elevation in uptake within the right lower lobe and 2-fold reduction of uptake in the left lobes in the lung. Cardiac uptake of anti-PECAM is negligible after intravenous and right pulmonary artery infusion. Alternatively, delivery with a catheter placed in the RCA results in a 4-fold elevation of cardiac uptake of anti-PECAM, but not IgG, compared with intravenous injection. Beta-gal activity is markedly elevated within the heart and lungs (5- and 25-fold increase, respectively) after injection of anti-PECAM/beta-gal, but not IgG/beta-gal. Endothelial targeting of anti-PECAM/beta-gal in the heart and lungs can be confirmed by imaging analysis. In summary, anti-PECAM antibody conjugates can deliver agents to the pulmonary endothelium regardless of injection route, whereas local arterial infusion permits targeting of the cardiac vasculature. This paradigm may be useful for drug targeting to endothelium in lungs, heart, and possibly other organs.

Platelet-endothelial cell adhesion molecule-1-directed immunotargeting to cardiopulmonary vasculature. Scherpereel A, Rome JJ, Wiewrodt R, Watkins SC, Harshaw DW, Alder S, Christofidou-Solomidou M, Haut E, Murciano JC, Nakada M, Albelda SM, Muzykantov VR. J Pharmacol Exp Ther 2002 Mar; 300 (3), pp. 777–86.

Cardiovascular Endothelium/Intercellular Adhesion Molecule (ICAM) Expression/Microvascular (Endothelial) Leukocyte Receptor Complex Expression
[Anti-ICAM]-Paramagnetic Liposomes

Application: The *in vivo* targeting of molecules expressed on vascular endothelium, while providing sufficient signal enhancement at these sites for detection by MRI.

Route of Administration: Intravascular.

Implementation: [Anti-ICAM]-paramagnetic liposomes (ACPL) targeted for ICAM-1 complexes administered *in vivo* for the purpose of detecting endothelial leukocyte receptor upregulation by the cerebral microvasculature during autoimmune encephalitis.

Animal Model: Mouse (autoimmune encephalitis).

Interpretation and Analysis: Fluorescence microscopy analysis reveals that fluorescently tagged ACPLs are localized within the central nervous system (CNS) microvasculature in a pattern consistent with ICAM-1 upregulation. Such findings closely correlate with immunohistochemical analyses. High-resolution magnetic resonance imaging (MRI) analysis of brains *ex vivo* demonstrates that ACPL binding patterns confer significant enhancement of signal intensity compared to reference control images. Preparations of ACPLs, therefore, appear to be a useful MRI contrast agent for visualizing specific molecules expressed on vascular endothelium during various disease states.

Instrumentation: Magnetic resonance imaging instrumentation.

ICAM-1 expression in autoimmune encephalitis visualized using magnetic resonance imaging. Sipkins DA, Gijbels K, Tropper FD, Bednarski M, Li KC, Steinman L. J Neuroimmunol 2000 Apr 3; 104 (1), pp. 1–9.

Cardiovascular Endothelium/$\alpha_V\beta_3$ Integrin Complexes
[^{99m}Technetium] ([^{99m}Tc])-(HYNIC)-Arg-Gly-Asp/ [^{99m}Tc]-(HYNIC)-RGD-4C

Cellular/Molecular Mechanism of Action: The peptides amino acid residues, Arg-Gly-Asp (RGD), in vitronectin and fibronectin possess binding affinity for cell membrane-associated $\alpha_V\beta_3$ integrin complexes.

Background Information: Three amino acid residues, Arg-Gly-Asp (RGD), in vitronectin and fibronectin show affinity for $\alpha_V\beta_3$ integrins expressed in vascular endothelial cells. That tumor growth can upregulate the expression of these integrins on tumor cells for invasion and metastasis and in tissue neovasculature suggests the potential of developing radiolabeled RGD peptides as antagonists of $\alpha_V\beta_3$ integrins for broad spectrum tumor-specific imaging. The polypeptide RGD-4C, which contains 4 cysteine residues for cyclization, has shown preferential localization on integrins at sites of tumor angiogenesis.

Application: Detection and characterization of neoplastic cell types/tumors that express relatively high $\alpha_V\beta_3$ integrin D membrane densities.

Route of Administration: Polypeptides RGD-4C and RGE (Arg-Gly-Glu)-4C (intravascular).

Semi-Synthetic Methods: The polypeptide RGD-4C and control RGE (Arg-Gly-Glu)-4C are conjugated with 6-hydrazinopyridine-3-carboxylic acid (HYNIC) to facilitate [^{99m}Tc] radiolabeling. Separation of conjugated products from residual unreacted molecular components can be achieved by C18 chromatography (e.g., Sep-Pak cartridge) applied in combination with 20% methanol solvent. Each of the peptides can be radiolabeled using tricine. For cell binding studies, both [^{99m}Tc] peptides can be further purified by SE HPLC. High specific radioactivity of labeled cyclized RGD/E of about 20 Ci/μmol can be achieved. Both [^{99m}Tc] complexes are stable in the labeling solution for over 24 hours at room temperature.

Implementation: Evaluation of the relative binding avidities of RGD-4C and RGE (Arg-Gly-Glu)-4C peptides for endothelial cell membrane preparations. Determination of such properties can be established by exposure of umbilical vein endothelial (HUVE) to RGD-4C or RGE over an incubation period of 1-hour duration (4°C) at concentrations in the picomolar to nanomolar range. Specificity can be approximated by determining at what concentration native cyclized RGD blocks 50% of the [^{99m}Tc]-labeled RGD binding to cell preparations.

***Ex Vivo* Model:** Human (umbilical vein endothelium, or HUVE).

Interpretation and Analysis: In the HUVE cell binding-avidity analyses, cell accumulation of [^{99m}Tc] in the case of RGD can be as much as 16 times greater than with RGE control peptide. Specificity analyses reveal that 7 nM of native cyclized RGD blocks 50% binding of [^{99m}Tc]-labeled RGD to cells. The binding percentage of [^{99m}Tc]-labeled RGD to purified $\alpha_V\beta_3$ integrin protein, as determined by size exclusion high-performance liquid chromatography 9HPLC), increases with integrin concentration, while [^{99m}Tc]-labeled RGE displays no binding. The association constant for [^{99m}Tc]-RGD is rather modest at $7 \times 10^6\ M^{-1}$. In both human renal adenocarcinoma (ACHN) and human colon cancer cell line (LS174T) nude mouse tumor models, the accumulation of [^{99m}Tc]-labeled RGD/E exhibits no statistical difference. In conclusion, possibly because of limited numbers of $\alpha_V\beta_3$ integrin receptors per tumor cell and low binding affinity, radiolabeled RGD peptides may have limitations as tumor-imaging agents.

In vitro and *in vivo* evaluation of a technetium-99m-labeled cyclic RGD peptide as a specific marker of $\alpha_V\beta_3$ integrin for tumor imaging. Su ZF, Liu G, Gupta S, Zhu Z, Rusckowski M, Hnatowich DJ. Bioconjug Chem 2002 May–Jun; 13 (3), pp. 561–70.

Cardiovascular/Endothelial Integrity and Lesions (Balloon Denudation)/Atherosclerotic Arterial Wall Lesions
[^{99m}Technetium] ([^{99m}Tc])-Labeled Endothelin Derivative

Implementation: Neointima of different cellularity and severity of stenosis can be induced by balloon denudation in concert with the implementation of specific dietary regimens and drug applications. Angiograms and scintigrams can be acquired after injection of a [^{99m}Tc]-labeled endothelin derivative. Validation can be achieved by dissection of the aorta for autoradiography analysis, sudan-III-staining, morphometry, and immunohistology.

Animal Model: Rabbits (e.g., n = 32) with induction of neointima injury of different cellularity and severity of stenosis using balloon denudation.

Interpretation and Analysis: Induced lesions can be detected *in vivo* (whole-body scintigram) in the vast majority of *in vivo* models 15 minutes after the injection of a [^{99m}Tc]-labeled endothelin derivative. Autoradiography analysis reveals a strong relationship between tracer accumulation and sudan-III positive staining lesions. Sites of accumulation for the endothelin derivative correlate with the number of neointimal smooth muscle cells (SMC), but not with the number of medial SMC, neointimal macrophages, and neointimal area. The *in vivo* imaging of atherosclerosis with an endothelin derivative appears to be a feasible method of detecting and characterizing atherosclerotic arterial wall lesions at early stages.

Tc-99m-labeled endothelin derivative for imaging of experimentally induced atherosclerosis. Tepe G, Duda SH, Meding J, Brehme U, Ritter J, Hanke H, Hilger CS, Claussen CD, Dinkelborg LM. Atherosclerosis 2001 Aug; 157 (2), pp. 383–92.

Cardiovascular Endothelium/Vascular Endothelial Growth Factor Receptor (VEGFR-2)
Fusion Proteins

Cellular/Molecular Mechanism of Action: Selective recognition of molecular "targets" through the design of proteins with specific binding avidities. Applying this experimental approach, a targeting molecule is expressed as a fusion protein containing a recognition tag.

Application: Function as molecular vehicles for target-mediated delivery of diagnostic agents. An example preparation is VEGF-RNase A fusion protein, designed to interact with or recognize VEGFR-2 receptors overexpressed on the exterior surface membrane of vascular endothelial cells, in a manner that can facilitate the delivery of a genetic determinant.

Route of Administration: Intravenous.

Dosage: Designed to directly establish a correlation between the specific biological activity (efficacy) of aptamer preparations and the corresponding pathological condition.

Chemical Synthesis and Analysis: Polypeptide protein in the form of a fusion protein that can non-covalently bind another peptide possessing biological activity (e.g., adapter segment). In turn, the adapter is chemically conjugated to a carrier possessing biological (diagnostic) properties. The assembled molecular delivery vehicle contains a carrier-adapter conjugate bound non-covalently to a recognition tag fused to the targeting protein. An example of a prototype fusion protein can be illustrated by VEGF fusion proteins that contain a 15-aa S-peptide fragment of RNase A as a recognition tag, using the S-protein fragment of RNase A as an adapter and polyethylenimine as a DNA carrier that can achieve selective gene delivery to cells overexpressing VEGFR-2.

Considerations: Selective targeting of therapeutic and diagnostic agents improves their efficacy and minimizes potentially adverse side effects. Existing methods for selective targeting are based on chemical conjugation of therapeutics and diagnostics, or their carriers, to cell-specific targeting molecules (e.g., growth factors, antibodies). These methods are limited by potential damage to targeting molecules that can be inflicted by the conjugation procedure. In addition, conjugation procedures have to be developed on a case-by-case basis.

Interpretation and Analysis: The advantages of this technology are (i) no chemical modification of targeting molecules, and (ii) universal, "off-the-shelf" carrier-adapter constructs that can be combined with different fusion targeting proteins. In this fashion, sensor molecules (e.g., radioisotopes, chemiluminescent moieties) can be used in a diagnostic manner to recognize various types of biological targets of interest.

Molecular vehicle for target-mediated delivery of therapeutics and diagnostics. Gaidamakova EK, Backer MV, Backer JM. J Control Release 2001 Jul 6; 74 (1–3), pp. 341–7.

Cardiovascular Thromboses: Arterial
RGD-USPIO

Cellular/Molecular Mechanism of Action: The peptide RGD recognizes and physically binds to the exterior surface of activated platelet populations. The physical orientation of iron ions becomes altered when subjected to magnetic energy fields.

Application: Detection of platelet thromboses, especially those formed with arterial vasculature that have a relatively higher platelet composition than do venous thromboses.

Semi-Synthetic Methods: Preparation of ultrasmall superparamagnetic particles of iron oxide (USPIOs) coupled to an RGD peptide (RGD-USPIO) that selectively recognizes activated platelet populations.

Implementation: Evaluation of thrombus lesions applying USPIOs coupled to an RGD peptide (RGD-USPIO) targeted for activated platelets, applied in combination with magnetic resonance (MR) imaging modalities. Thrombus visualization *ex vivo* is compared using RGD-USPIO and a non-targeted UPSIO. The influence of thrombus visualization on thrombus exposure time to RGD-USPIO (*ex vivo*) and on the spatial resolution of the MR image (*ex vivo* and *in vivo*) is then assessed.

***Ex Vivo* Model:** Thrombus (*ex vivo* and *in vivo* preparations).

Interpretation and Analysis: RGD-USPIO provides superior thrombus visualization compared to non-targeted USPIO *ex vivo*, and maximum enhancement can be achieved after approximately 1 hour exposure time of the thrombus to RGD-USPIO preparations. The ability to visualize clots is highly dependent on the spatial resolution of the image. *In vivo*, an in-plane resolution of less than 0.2×0.2 mm^2 is required for good clot visualization after contrast enhancement. Achievable resolution and sensitivity is a potential limitation to the usefulness of active vascular targeting in MRI.

A targeted contrast agent for magnetic resonance imaging of thrombus: Implications of spatial resolution. Johansson LO, Bjornerud A, Ahlstrom HK, Ladd DL, Fujii DK. J Magn Reson Imaging 2001 Apr; 13 (4), pp. 615–8.

Cardiovascular Thrombosis/ Arterial and Venous/ Deep-Vein Thrombosis/Pulmonary Embolism/ Arterial Thromboembolic Disorders/Cerebral Vascular Stroke/Coronary Artery Thrombotic Disorders/Peripheral Artery Thromboses

Platelet GPIIb/IIIa Receptor Antagonist/[^{99m}Technetium] ([^{99m}Tc])-DMP728/ [^{3}H]-DMP728

Cellular/Molecular Mechanism of Action: The agent DMP728 functions as a GPIIb/IIIa receptor antagonist on the exterior surface membrane of platelets.

Physiological Mechanism of Action: The composition of venous thrombi is predominantly polymerized fibrin polymer compared to arterial thrombi that have a higher platelet content.

Background Information: Either venous or arterial thrombosis is a potentially life-threatening event. Many existing diagnostic modalities are inadequate for establishing a definitive diagnoses or for determining the morphology of an evolving thrombus. Thus development of a non-invasive imaging agent that can detect the location of clots remains a critical and unmet need in the field of diagnostic nuclear medicine.

Application: Detection and characterization of intravascular venous and arterial thromboses.

Route of Administration: Intravascular.

Implementation: Evaluation of artificially (*in vitro*) created venous and arterial type thrombi using the potent radiolabeled GPIIb/IIIa receptor antagonist [^{3}H]-DMP728. Complementary investigation can involve the *in vivo* application of [^{99m}Tc]-DMP728 to determine the clot/blood distribution ratio (e.g., target:background ratio). In addition, non-invasive single photon emission computed tomography (SPECT) of venous clots in deep-vein thrombosis can be performed. The efficacy of thrombin inhibitors can also be evaluated applying similar strategies.

Animal Model: Canine (dogs with mixed arterial/venous clots, arteriovenous shunts with venous clot foci, and deep-vein thrombi).

Interpretation and Analysis: Radiolabeled preparations of the GPIIb/IIIa receptor antagonist DMP728 in the form of [^{3}H]-DMP728 and [^{99m}Tc]-DMP728 retain marked binding avidity for platelet GPIIb/IIIa receptor complexes. DMP728 demonstrates a relatively greater affinity for activated compared to unactivated human platelets, which may be essential for attaining an optimal thrombus/blood (target/background) biodistribution ratio to facilitate optimal detection of small clots (i.e., greater sensitivity). Therefore, [^{99m}Tc]-GPIIb/IIIa receptor antagonists have potential utility in the diagnosis of venous clots in deep-vein thrombosis, pulmonary embolism, arterial thromboembolic disorders including stroke, and coronary and peripheral artery thrombotic disorders. Thrombin inhibitors do not appear to be as effective in this same context.

Novel technetium-99m-labeled platelet GPIIb/IIIa receptor antagonists as potential imaging agents for venous and arterial thrombosis. Mousa SA, Bozarth JM, Edwards S, Carroll T, Barrett J. Coron Artery Dis 1998; 9 (2–3), pp. 131–41.

Cardiovascular/Thrombosis: Platelet Populations

RGD-USPIO (RGD Peptide)

Cellular/Molecular Mechanism of Action: The peptide RGD recognizes and physically binds to the exterior surface of activated platelet populations. The physical orientation of iron ions becomes altered when subjected to magnetic energy fields.

Application: Detection of platelet thromboses, especially those formed with arterial vascular structures, which have a relatively higher platelet composition than do venous thromboses.

Semi-Synthetic Methods: Preparation of ultrasmall superparamagnetic particles of iron oxide (USPIOs) coupled to an RGD peptide (RGD-USPIO) that selectively recognizes activated platelet populations.

Implementation: Evaluation of thrombus lesions applying USPIOs coupled to an RGD peptide (RGD-USPIO) that "targets" activated platelets. Detection *in vivo* can be established by the application of magnetic resonance (MR) imaging modalities. Thrombus visualization *ex vivo* can be compared using RGD-USPIO and a non-targeted UPSIO. The influence of thrombus visualization on thrombus exposure time to RGD-USPIO (*ex vivo*) and on the spatial resolution of the MR image (*ex vivo* and *in vivo*) can subsequently be assessed.

***Ex Vivo* Model:** Thrombus (*ex vivo* and *in vivo* preparations).

Interpretation and Analysis: RGD-USPIO provides superior thrombus visualization compared to non-targeted USPIO *ex vivo*, and maximum enhancement can be achieved after approximately a 1-hour exposure of thrombus lesions to RGD-USPIO preparations. The ability to visualize clots is highly dependent on the spatial resolution of images. *In vivo*, an in-plane resolution of less than 0.2×0.2 mm^2 is required for good clot visualization after contrast enhancement. Achievable resolution and sensitivity is a potential limitation to the usefulness of active vascular targeting in MRI.

A targeted contrast agent for magnetic resonance imaging of thrombus: Implications of spatial resolution. Johansson LO, Bjornerud A, Ahlstrom HK, Ladd DL, Fujii DK. J Magn Reson Imaging 2001 Apr; 13 (4), pp. 615–8.

Central Nervous System (CNS)/Huntington's Disease (HD)/Huntingtin RNA Genetic Targeting/ Antisense Gene Targeting: *In Vivo* Gene Expression Quantification

Antitransferrin Receptor IgG/[^{125}I]-Peptide Nucleic Acid (PNA)-[Antitransferrin Receptor IgG]

Cellular/Molecular Mechanism of Action: The PNA agent is a 16-mer PNA that is the antisense strand that corresponds to the segment around the methionine initiation codon of the huntingtin gene associated with HD. In this context, it recognizes and physically binds to huntingtin RNA (HD exon-1 messenger RNA).

Background Information: Disease-specific genes of unknown function can be imaged *in vivo* with antisense radiopharmaceuticals, providing the trans-cellular transport of these molecules is enabled with drug-targeting technology.

Application: Central nervous system targeting.

Route of Administration: [^{125}I]-PNA/8D3 conjugate (intravenous).

Semi-Synthetic Methods: Biotinylation of PNA. Covalent cross-linking strepavidin and rat 8D3 monoclonal antibody (MAb) to the mouse transferrin receptor (TfR). The latter moiety contains a tyrosine residue that can facilitate [^{125}I] radioiodination. The reformulated PNA antisense radiopharmaceutical conjugated to 8D3 MAb is designated [^{125}I]-PNA/8D3.

Implementation: Evaluation of biotinylated PNA that can be rapidly captured by conjugates of covalently cross-linked strepavidin-[antimouse transferrin receptor IgG]. Complementary *in vivo* studies in mice can be used to determine the biodistribution of the PNA analogue [^{125}I]-PNA/8D3 through harvest of tissue/organ systems 6 hours postadministration. Preparations of the brain can be frozen sectioned and quantitated applying autoradiography.

Animal Model: Murine (littermate control mice or R6/2 transgenic mice that express the exon 1 of the human HD gene).
Interpretation and Analysis: Despite covalent cross-linking of the PNA analogue [^{125}I]-PNA/8D3 to monoclonal antibody, it can gain access to endogenous transferrin transport pathways at the level of both the blood-brain barrier and the brain cell membrane, resulting in import from the blood to the brain and export from the brain into the blood mediated by TfRs. In this manner, products are able to hybridize with "target" huntingtin RNA validated by analysis from both cell-free translation assays and ribonuclease protection assays.

Approximately a 3-fold *in vivo* increase in sequestration can be appreciated for antisense [^{125}I]-PNA/8D3 radiopharmaceutical conjugates within the brains of HD transgenic *in vivo* models. Such observations are consistent with the selective *in vivo* expression of the HD exon-1 messenger RNA sequence. Therefore, gene expression *in vivo* can be quantitated with antisense radiopharmaceuticals, provided these molecules are reformulated in concert with drug-targeting technology. Drug targeting enables access of the antisense agent to endogenous transport pathways, which in turn permits passage across cellular barriers that separate blood and intracellular compartments of target tissues.

Imaging gene expression in the brain *in vivo* in a transgenic mouse model of Huntington's disease with an antisense radiopharmaceutical and drug-targeting technology. Lee HJ, Boado RJ, Braasch DA, Corey DR, Pardridge WM. J Nucl Med 2002 Jul; 43 (7), pp. 948–56.

Central Nervous System (CNS)/Opioid Receptors/ Delta-OR Receptors/μ-Opioid Receptors
Fluo-Deltorphin/d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH_2/Fentanyl Citrate

Cellular/Molecular Mechanism of Action: Fluo-deltorphin is a fluorescent compound that physically binds to deltaOR in a highly selective manner. Similarly, d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH_2 selectively binds to μ-opioid receptor complexes. Fentanyl citrate similarly binds to μ-receptors.
Physiological Mechanism of Action: Fluo-deltorphin is a highly selective deltaOR agonist. The peptide d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH_2 is a selective μ-opioid receptor antagonist. Alternatively, fentanyl citrate functions as a μ-receptor agonist.
Background Information: Opioid receptors are known to undergo complex regulatory changes in response to ligand exposure.
Application: Examination of the effect of morphine on the *in vitro* and *in vivo expression* density and trafficking of δ-opioid receptors (deltaORs).
Route of Administration: Intravascular (traditionally).
Implementation: Prolonged exposure of cortical neurons in culture to morphine (10 μm) for a period of 48 hours with and without the presence of d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH_2 or fentanyl citrate. Alterations in the expression density of membrane-associated deltaORs can then be detected applying immunogold electron microscopy.
Interpretation and Analysis: Prolonged exposure of cortical neurons to morphine results in a profound increase in the internalization of Fluo-deltorphin. Such effects are μ-mediated because it is entirely blocked by d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH_2 and can be reproduced using the selective μ agonist, fentanyl citrate. Based on results from immunogold electron microscopy analysis, marked increases in the expression density of surface membrane-associated deltaORs occur in neurons exposed to morphine and indicates that increases in Fluo-deltorphin internalization is caused by elevations in receptor availability. Prolonged morphine exposure has no effect on deltaOR protein levels, as assessed by immunocytochemistry and Western blot analysis, suggesting that an increase in bioavailable deltaORs is caused by recruitment of reserve receptors from intracellular stores and not from receptor neosynthesis. Chronic treatment of adult rats with morphine (5–15 mg/kg, subcutaneous, every 12 hours) similarly augments targeting of deltaORs to neuronal plasma membranes in the dorsal horn of the spinal cord. Such treatment markedly potentiates intrathecal d-[Ala_2]deltorphin II-induced antinociception. Therefore, prolonged *in vitro* and *in vivo* stimulation of neurons with morphine markedly increases recruitment of intracellular deltaORs on the cell surface membrane. Receptor subtype cross-mobilization of this type may widen the transduction repertoire of G-protein-coupled receptors and offer new therapeutic strategies.

Prolonged morphine treatment targets δ-opioid receptors to neuronal plasma membranes and enhances δ-mediated antinociception. Cahill CM, Morinville A, Lee MC, Vincent JP, Collier B, Beaudet A. J Neurosci 2001 Oct 1; 21 (19), pp. 7598–607.

Central Nervous System (CNS)/Opioid Receptors: Microligands/Microreceptor Peptide-Based Affinity Labels
Dermorphin/[Phe_5(p-NH_2),Lys_7]Dermorphin

Cellular/Molecular Mechanism of Action: Selective micro-opioid receptor ligands originating from amphibian skin that have been modified with various electrophiles in either the "message" or "address" sequences so they can function as potential peptide-based affinity labels for microreceptors.
Application: Opioid receptor detection/recognition.
Route of Administration: Intravascular.
Synthesis/Biosynthesis: Introduction of the electrophilic isothiocyanate and bromoacetamide groups at the para position of Phe_3 and Phe_5 can be accomplished by incorporating Fmoc-Phe(p-NHAlloc) into the peptide, followed by selective deprotection and modification. The corresponding amine-containing peptides can also be prepared.
Implementation: Evaluation of binding avidity of pure peptides for membrane-associated micro-opioid and δ-opioid receptor complexes applying radioligand binding analyses.

Tissue Culture Model: Chinese hamster ovary cells (CHO) expressing micro-opioid and d-opioid receptors.
Interpretation and Analysis: In dermorphin, introduction of the electrophilic groups in the "message" domain lowers the binding affinity by $>$ 1,000-fold; only [Phe_3(*p*-NH_2)]dermorphin retains nanomolar affinity for microreceptors. Modifications in the "address" region of both dermorphin and [Lys_7]dermorphin produces a product that is relatively well tolerated. In particular, [Phe_5(*p*-NH_2),Lys_7]dermorphin has a level of affinity similar to dermorphin and has almost a 2-fold higher selectivity for microreceptors. [Phe_5(*p*-$NHCOCH_2Br$)]- and [Phe_5(*p*-$NHCOCH_2Br$),Lys_7]dermorphin exhibit relatively high affinity (IC_{50} = 27.7 and 15.1 nm, respectively) for micro-opioid receptors. However, neither of these peptides inhibit [^{3}H]DAMGO binding in a wash-resistant manner.

Dermorphin-based potential affinity labels for μ-opioid receptors. Choi H, Murray TF, Aldrich JV. J Pept Res 2003 Jan; 61 (1), pp. 40–5.

Central Nervous System (CNS)/Opioid Receptors: Kappa Selective
Arodyn

Cellular/Molecular Mechanism of Action: The aromatic dynorphin arodyn is a unique analogue of the opioid peptide dynor-

phin-A and possesses relatively selective κ-opioid receptor ligand binding affinity (K_i = 10 nM: κ/μ/δ = 1/174/583).

Physiological Mechanism of Action: Arodyn completely reverses the agonism of dynorphin $A^{1\text{-}13}NH_2$ in a concentration-dependent manner in adenylyl cyclase assays.

Application: Arodyn is a novel κ-opioid receptor selective antagonist that will be useful in the study of this receptor type.

Identification of arodyn, a novel acetylated dynorphin $A^{1\text{-}11}$ analogue, as a κ-opioid receptor antagonist. Bennett MA, Murray TF, Aldrich JV. J Med Chem 2002 Dec 19; 45 (26), pp. 5617–9.

Immune System/Antigen Delivery: Selective and Site-Specific
Monoclonal Antibody

Cellular/Molecular Mechanism of Action: Detects and physically binds to specific epitomes corresponding to distinct segments of macromolecules (e.g., polypeptides, glycoproteins, lipoproteins, pharmaceuticals, oligosaccharides, nucleotides).

Considerations: Most monoclonal antibody preparations that have the potential of being developed into *in vivo* diagnostic and therapeutic agents are of murine or rat origin. Ideally, monoclonal antibodies intended for *in vivo* diagnostic and therapeutic purposes need to be created so that they are homologous in nature to avoid rejection or induction of aberrant immune responses in the recipient host. In this context, genetic engineering techniques have made it possible to produce immunoglobulin isotypes that are compatible with domestic animal species and man. Despite these advances, there is still an insufficient level of understanding about their functional activities or how to determine which isotype may be the most effective for a given application. Similar in concept to the alteration of the biochemical function of a given enzyme through minor changes in amino acid sequence, similar alterations in monoclonal antibody induced by site-directed mutagenesis of a single amino acid substitution can substantially modify ligand-binding properties of an immunoglobulin preparation. Additionally, immunoglobulin glycosylation is critical for full functional activity because there is a subtle interplay between the polypeptide and oligosaccharide structures in the creation of viable ligand-binding sites. Taken together, these findings suggest that it will be possible to generate "customized" antibody molecules having a predetermined profile of effector functions.

Interpretation and Analysis: It is possible to generate customized monoclonal antibodies based on the evalution and molecular characterization of the desired predetermined effector function.

Structure-function relationships in human immunoglobulins. Jefferis R. Neth J Med 1991 Oct; 39 (3–4), pp. 188–98.

Immune System/Leukocytes: *In Vivo* Labeling of Neutrophil and Macrophage Tuftsin Complexes/ Inflammation: Septic and Non-Septic/Central Nervous System (CNS): Multiple Sclerosis (MS)/ Glucocorticoid Efficacy Evaluation/Selective Delivery to Leukocytes or Sites of Inflammation/ Experimental Allergic Encephalomyelitis (EAE)
[^{99m}Technetium] ([^{99m}Tc])-RP128

Cellular/Molecular Mechanism of Action: The agent RP128 functions as a chelation agent for [^{99m}Tc] in addition to recognizing and binding to tuftsin receptors expressed by neutrophils and monocyte-macrophages.

Application: The agent [^{99m}Tc]-RP128 can be applied for the *in vivo* labeling of neutrophil and macrophage tuftsin receptor complexes for the purpose of detecting and characterizing focal sites of septic inflammation. Preparations can also potentially be applied for the detection and characterization of human MS conditions, and potentially could be applied to selectively deliver agents to macrophage and neutrophil populations.

Route of Administration: Intravascular.

Implementation: Administration of [^{99m}Tc]-RP128 for the *in vivo* detection of CNS inflammation (e.g., EAE).

Animal Model: Murine EAE (EAE as a model for human MS).

Interpretation and Analysis: The radiopharmaceutical [^{99m}Tc]-RP128 can be detected at significantly increased levels within CNS tissues in conditions of EAE at 0.5, 1.0, and 3.0 hours post-injection using a dual radioisotope technique utilized to correct for non-extravasated tracer ($p < 0.05$). Moreover, extravascular accumulation of [^{99m}Tc]-RP128 can be clearly demonstrated in inflammatory tissues with minimal loss of sensitivity when the secondary isotopic correction for blood volume is omitted. In addition, [^{99m}Tc]-RP128 can successfully monitor glucocorticoid suppression of inflammation ($p < 0.05$), detected as a typical dose response to increasing steroid concentration. Clearly, [^{99m}Tc]-RP128 can quantitatively detect CNS inflammation and assess responses to therapy, indicating potential value as an imaging agent for both clinical and research purposes. Furthermore, the rapid *in vivo* labeling by [^{99m}Tc]-RP128 of specific inflammatory cells combined with the ability to monitor the progress of anti-inflammatory therapeutics may validate the use of this cellular radiolabeling agent for detecting a variety of inflammatory conditions.

The detection and quantitation of inflammation in the central nervous system during experimental allergic encephalomyelitis using the radiopharmaceutical [^{99m}Tc]-RP128. Paul C, Peers SH, Woodhouse LE, Thornback JR, Goodbody AE, Bolton C. J Neurosci Methods 2000 May 15; 98 (1), pp. 83–90.

Immune System/Mononuclear Cell Binding: Decreased Non-Specific Mononuclear Cell Binding
IgG Fv Fragments/Modified VH/VL

Synthetic Production and Analysis: Molecular modeling technology has made it possible to identify 2 positions in conserved framework regions of antibody Fv fragments (Fvs) that are distant from complementarity-determining regions (CDRs) and potentially can be used to make recombinant Fv fragments in which the unstable VH and VL heterodimer is stabilized by an interchain disulfide bond inserted between structurally conserved regions. A disulfide bond can be introduced at 1 of these positions, VH44-VL105, and has been shown to stabilize various Fvs that retain full binding and specificity. Recombinant immunotoxins (e.g., B3(dsFv)-PE38KDEL), in which this disulfide-stabilized Fv moiety is connected to a truncated form of *Pseudomonas* exotoxin (PE; PE38KDEL) that contains translocation and adenosine diphosphate (ADP) ribosylation domains, are indistinguishable in binding and specificity from single-chain immunotoxin counterparts. Analysis of the alternative position (VH111-VL48) by predictive modeling techniques for the purpose of disulfide stabilization of monoclonal antibody (MAb) B3(Fv) can be used as a guide for the production of recombinant immunotoxin with such disulfide-stabilized (ds) Fv immunoglobulin fragments.

Interpretation and Analysis: The immunotoxin preparation is very active and retains full specificity for B3 antigen-positive cells. However, it is 2- to 3-fold less active than the VH44-VL105 dsFv-molecule. Comparison of various biochemical features of VH44-VL105 and VH111-VL48 dsFv immunotoxins with the corresponding single-chain immunotoxin reveals that dsFv immunotoxins are more stable in human serum and more resistant to thermal and

chemical denaturation than the single-chain (sc) Fv immunotoxin. Because dsFv immunotoxins and dsFvs have full activity and specificity and improved stability, they may be more useful than scFv immunotoxins as therapeutic and diagnostic agents.

Engineering interchain disulfide bonds into conserved framework regions of Fv fragments: Improved biochemical characteristics of recombinant immunotoxins containing disulfide-stabilized Fv. Reiter Y, Brinkmann U, Webber KO, Jung SH, Lee B, Pastan I. Protein Eng 1994 May; 7 (5), pp. 697–704.

Immune System/Neutrophil Leukotriene LTB_4 Receptors/Induced Myocardial Ishchemia
[^{99m}Technetium] ([^{99m}Tc])-RP517/[F]-RP517

Cellular/Molecular Mechanism of Action: In whole blood, [F]-RP517 (500 nmol/L) preferentially labels neutrophil LTB_4 receptor complexes.
Application: [^{99m}Tc]-RP517 is an LTB_4 receptor antagonist that has been developed for imaging acute inflammation or infection. A unique property of [^{99m}Tc]-RP517 is its ability to label white blood cells *in vivo* after intravenous injection.
Route of Administration: [^{99m}Tc]-RP517 (intravenous).
Implementation: Evaluate the relative [^{99m}Tc]-RP517 binding to human leukocyte subtypes and the [^{99m}Tc]-RP517 uptake pattern in myocardium with induced inflammatory lesions. One approach to assessing such conditions can involve delineation of RP517 binding to neutrophil populations *in vitro* within whole blood, and *in vivo* within the intravascular compartment. This can be achieved through the use of fluorescence-activated cell sorter analysis performed on whole human blood samples (n = 2) and isolated neutrophils (n = 4) with a fluorescent analogue of [^{99m}Tc]-RP517 referred to as [F]-RP517. Evaluation approaches *in vivo* can entail administration of [^{99m}Tc]-RP517 to determine its biodistribution within the myocardium before and after coronary occlusion. To facilitate *ex vivo* imaging of the myocardium, the left ventricle can be divided into 72 segments for flow and [^{99m}Tc]-RP517 uptake analyses.

Animal Model: Canine (n = 9 dogs; anesthetized, open-chest surgical exposure before coronary occlusion [90 minutes] and reperfusion [120 minutes]; or n = 3 dogs, before intramyocardial tumor necrosis factor-alpha (TNF-α) administration). In this context, myocardium inflammation can be induced by either coronary occlusion and reperfusion or administration by injection of TNF-α preparations.
Interpretation and Analysis: In whole blood, [F]-RP517 (500 nmol/L) preferentially labels neutrophils. In isolated neutrophil populations, [F]-RP517 (10 nmol/L) binding is inhibited by 44% in the presence of LTB_4 (400 nmol/L). In the analysis of *ex vivo* cardiac samples, an inverse exponential relationship can be detected between [^{99m}Tc]-RP517 uptake and occlusion flow (r = 0.73). In the same 15 segments, [^{99m}Tc]-RP517 uptake is highly correlated with neutrophil myeloperoxidase enzyme levels (r = 0.91). *Ex vivo* images reveal tracer uptake in the reperfused area (ischemic to normal count ratio = 2.7 ± 0.2). Therefore, it can be concluded that RP517 binds to the neutrophil LTB_4 receptor complex after intravenous injection. After reperfusion, [^{99m}Tc]-RP517 uptake correlates with myeloperoxidase levels and can be observed on *ex vivo* images, indicating that this tracer may have potential as an imaging agent for detecting inflammatory conditions.

Assessment of myocardial inflammation produced by experimental coronary occlusion and reperfusion with [^{99m}Tc]-RP517, a new leukotriene B_4 receptor antagonist that preferentially labels neutrophils *in vivo*. Riou LM, Ruiz M, Sullivan GW, Linden J, Leong-Poi H, Lindner JR, Harris TD, Beller GA, Glover DK. Circulation 2002 Jul 30; 106 (5), pp. 592–8.

Neoplasia
[^{67}Ga] Ga-2-(p-SCN-Bz)-NOTA Anti-IL-2 IgG

Cellular/Molecular Mechanism of Action: Immunoglobulin-based recognition and binding to IL-2 receptor complexes.
Application: The low molecular weight protein (LMWP) has been proposed as a candidate diagnostic agent for the detection of certain neoplastic disease states.
Semi-Synthetic Methods: The antibody fragment disulfide-linked (dsFv) to the alpha subunit of the IL-2 receptor can be radiolabeled with a [^{67}Ga] Ga-2-(p-SCN-Bz)-NOTA derivative, which is linked through an isothiocyanato group to either the epsilon-amino group of lysine or the alpha-amino group to the N-terminal amino acid.
Considerations: Because different amino acids in the dsFv are radiolabeled applying the method described, it is possible to study the relative residence times of the various catabolites.
Implementation: Evaluation of the residence time for methionine and lysine conjugates within the same kidney.
Animal Model: Murine.
Interpretation and Analysis: Approximately > 60% of the injected dose localizes within the kidney. The major catabolite (> 95%) in the kidney is the Ga-2-(p-SCN-Bz)-NOTA conjugate with either lysine or methionine, with no evidence of transchelation of [^{67}Ga]. The methionine conjugate has a significantly shorter residence time than the lysine conjugate in the same kidney. Labeling the appropriate amino acid in an LMWP may lead to reduced residence times and increased diagnostic or therapeutic ratios. Appropriately synthesized radiobiological agents of this design would preferentially accumulate within neoplastic tissues, producing a high "target-to-background" ratio that could be detected by nuclear medicine scintographic imaging.
Instrumentation: Computer-integrated nuclear medicine scintigraphy instrumentation.

Biodistribution and catabolism of Ga-67-labeled anti-Tac dsFv fragment. Wu C, Jagoda E, Brechbiel M, Webber KO, Pastan I, Gansow O, Eckelman WC. Bioconjug Chem 1997 May–Jun; 8 (3), pp. 365–9.

Neoplastic Disease/Antigen Targeting/Membrane-Associated Cancer Antigens/[^{99m}Technetium] ([^{99m}Tc])-Anti-Carcinoembryonic Antigen (CEA) Monoclonal Antibody (MAb)/Colorectal Cancer/Bladder Cancer/Thyroid Cancer
Anti-Neoplastic Antigen Monoclonal IgG/ [^{99m}Tc]-Anti-CEA MAb

Cellular/Molecular Mechanism of Action: Monoclonal antibody is used as a targeting modality for unique antigens expressed on the external surface membrane of neoplastic cell types.
Background Information: Monoclonal antibody can be produced with binding avidity for almost any protein, glycoprotein, or lipoprotein antigen greater than 10 kDA in mass. Examples of preparations other than radiolabeled anti-CEA include (i) [^{111}In]-labeled anti-MUC1 mucin for invasive bladder cancer; and (ii) [^{131}I]-MN-14 $F(ab)_2$ anti-CEA MAb for medullary thyroid cancer. Although this allows flexibility in the design of new agents, binding avidities and specificity of binding can vary between different MAb preparations.
Diagnostic Application: Detection of neoplastic disease states that express membrane-associated CEA at relatively high densities.
Route of Administration: Intravascular.
Dosage: [^{99m}Tc]-anti-CEA MAb (925 MBq).

Considerations: Intact radiolabeled IgG immunoglobulin will accumulate within the spleen and liver due to binding to Fc-receptor sites expressed on the exterior surface membrane of cell populations comprising the reticuloendothelial (e.g., mononuclear phagocytic) system. Enzymatic cleavage of the Fc fragment with either pepsin or papain results in the generation of Fab′ or Fab'_2. Such IgG molecular fragments display a significantly higher target-to-background ratio than intact IgG. Examples of radiolabeled MAbs applied for either diagnostic or therapeutic purposes include those targeted against p57 protein fractions expressed by malignant melanoma cell types.

Implementation: Evaluate the usefulness of immunoscintigraphy with an anti-CEA MAb fragment labeled with [^{99m}Tc] for early detection of colorectal cancer recurrence in patients with rising serum CEA levels. Cases are then subjected to immunoscintigraphy imaging studies (e.g., n = 8 cases; n = 59 acquired images). Immunoscintigraphy is performed after injection of anti-CEA MAb. Planar images of the thorax, abdomen, and pelvis, as well as single photon emission computed tomography (SPECT) of the abdomen and pelvis, are obtained at 4 and 24 hours after injection. Abdominal CT scans can additionally be performed prior to scintigraphic imaging. Findings are subsequently validated by histopathological analysis (e.g., n = 28 cases) or by imaging and clinical follow-up of at least 6 months following the immunoscintigraphy (e.g., n = 31 cases).

Human Model: Cases of colorectal cancer (e.g., n = 51 consecutive patients; n = 27 women, n = 24 men; population mean age 68.9 ± 10.2 years) with rising CEA levels (e.g., 16.2 ± 18.2 ng/ml).

Interpretation and Analysis: A considerable number of cases will not show recurrence during the follow-up period (e.g., n = 41). Conversely, slightly less than two-fifths of all cases will have a confirmed diagnosis of extrahepatic abdominal or pelvic disease (e.g., n = 18/51); approximately one-fifth of cases will have liver metastases (e.g., n = 11/51); slightly less than one-fifth of cases can have thoracic involvment (e.g., n = 9/51); while a relatively small number will have bone metastasis (e.g., n = 2/51). In cases with pelvic and extrahepatic abdominal disease, immunoscintigraphy will be true-positive in most instances (e.g., n = 14/18 true-positive vs n = 4/18 false-positive). Of the cases classified as true-positive, only about half are detectable by CT imaging (e.g., n = 7/14).

In the remaining cases with no detectable lesions on immunoscintigraphy images, most of these findings can be classified as true-negatives (e.g., n = 37/41 true-negative, n = 4/41 false-negative). Therefore, the sensitivity and specificity for immunoscintigraphy in extrahepatic abdominal and pelvic disease is approximately 78% and 90%, respectively. Computed tomography results show a lower sensitivity of 61% ($p < 0.05$) and specificity of 83%. Liver metastases may not be detected by CT (e.g., n = 9 cases), but such lesions may alternatively only be detected in a small number of cases utilizing immunoscintigraphy (e.g., n = 2/9). Therefore, scintigraphy with anti-CEA MAb fragment labeled with [^{99m}Tc] is superior to CT for the detection of pelvic and extrahepatic abdominal recurrence of colorectal cancer, while CT is more sensitive in the detection of liver and lung metastases. Immunoscintigraphy has a limited usefulness in the detection of distant metastases, but it may be helpful in the diagnosis of suspected colorectal recurrence in patients with non-conclusive CT findings, and when FDG-PET is not available.

Is there a role for [^{99m}Tc]-anti-CEA monoclonal antibody imaging in the diagnosis of recurrent colorectal carcinoma? Fuster D, Maurel J, Muxí A, Setoain X, Ayuso C, Martín F, Ortega ML, Fuertes S, Pons F. Q J Nucl Med 2003 Jun; 47 (2), pp. 109–15.

Neoplasia/Carcinoma of the Breast/Mammary Carcinoma
Single-Chain Anti-HER-2 Monoclonal Antibody (MAb)

Cellular/Molecular Mechanism of Action: Monoclonal antibody fragment designed to recognize and physically bind to HER-2 antigen expressed on the exterior surface membrane of (human) mammary carcinoma cell types.

Application: Selective, non-invasive *in vivo* detection, characterization, monitoring, and therapy of mammary carcinoma. Single-chain variable fragment MAb preparations that recognize and bind to HER-2 antigen also potentially have the capacity to function as a selective *in vivo* delivery format for diagnostic and therapeutic agents. Several monoclonal antibody preparations that recognize mammary carcinoma HER-2 antigen expression also inhibit the proliferation of this neoplastic cell type.

Route of Administration: Intravenous.

Semi-Synthetic Methods: To obtain a smaller immunoglobulin-based reagent possessing improved *in vivo* properties, a single-chain variable fragment (scFv) of antigen-specific monoclonal antibody (e.g., harvested from MGR6-producing hybridoma cell) can be generated applying phage display technology. Rapid purification of biologically active monoclonal IgG antibody can be obtained by applying metal affinity chromatography. Identification of epitopes on the HER-2 extracellular domain that are shared by single-chain variable fragment anti-HER-2 and intact MAb can be identified utilizing competition fluorescence-activated cell sorting (FACS) and ELISA methodologies. Based on BlAcore analysis, single-chain variable fragment anti-HER-2 can be produced that possesses characteristics very similar to the properties of intact monoclonal antibody (e.g., Koff of 9.3×10^{-4}/s).

Interpretation and Analysis: Monoclonal antibody preparations with binding avidity for HER-2 antigen have the advantage of recognizing a protein molecule expressed at relatively low levels in normal tissues and a homogeneous distribution within masses of mammary carcinoma. Relatively fast distribution and elimination half-lives of single-chain variable fragment anti-HER-2 measured following *in vivo* administration preclinical evaluations (13 minutes and 6.2 hours, respectively) represent a theoretical improvement in pharmacokinetic properties compared to intact murine MAb. Such preparations of immunoglobulin fragments, therefore, have the potential for being utilized for selectively delivering diagnostic imaging agents and therapeutics to different human carcinoma cell types overexpressing HER-2 complexes.

Production and validation of the pharmacokinetics of a single-chain Fv fragment of the MGR6 antibody for targeting of tumors expressing HER-2. Turatti F, Mezzanzanica D, Nardini E, Luison E, Maffioli L, Bambardieri E, de Lalla C, Canevari S, Figini M. Cancer Immunol Immunother 2001 Feb; 49 (12), pp. 679–86.

Neoplasia: Carcinoma of the Breast/Mammary Carcinoma/Tyrosine Kinase Her-2/neu Receptors
Streptavidin-Conjugated Iron Oxide Nanoparticles/ Biotinylated Monoclonal IgG (Anti Her-2/ neu-Receptor).

Cellular/Molecular Mechanism of Action: Monoclonal antibody designed to selectively bind to Her-2/neu-receptor complexes.

Background Information: One of the clinically relevant targets is the tyrosine kinase Her-2/neu receptor, which has importance in the staging and treatment of breast cancer.

Application: Creation of adjunct information directly relevant to the staging and treatment of breast cancer.
Route of Administration: Intravascular.
Implementation: Detection and characterization of Her-2/neu receptor expression in breast cancer cell populations that express the receptors on their exterior surface membranes at variable densities. Cell populations of interest are pre-targeted with anti-Her-2/neu monoclonal antibody followed by exposure to streptavidin-conjugated superparamagnetic nanoparticles as a strategy for selective magnetic resonance imaging of HER-2/new receptor expression.
Interpretation and Analysis: Streptavidin-conjugated superparamagnetic nanoparticles when used in combination with pretargeting strategies with biotinylated anti-Her-2/neu monoclonal IgG can function as a effective approach for detecting and characterizing the expression of these receptor complexes by magnetic resonance imaging analysis in conditions of mammary carcinoma. In this fashion, strong T_2 magnetic resonance signals can be generated by Her-2/neu-expressing cells. Furthermore, the contrast observed in MR images is proportional to the expression level of Her-2/neu receptors determined independently by fluorescence-activated cell sorting (FACS) analysis. In this context, iron oxide nanoparticles become attached to the membrane surface of mammary carcinoma cell types and they are not internalized into the cytosol, which is a major advantage for application *in vivo* as a diagnostic strategy.

MR molecular imaging of the Her-2/neu receptor in breast cancer cells using targeted iron oxide nanoparticles. Artemov D, Mori N, Okollie B, Bhujwalla ZM. Magn Reson Med. 2003 Mar; 49 (3), pp. 403–8.

Neoplasia/Carcinomas of the Breast, Colon, and Lung
Antifibroblast Activation Protein Monoclonal Antibody (MAb)

Cellular/Molecular Mechanism of Action: Represents an MAb that recognizes and physically binds to fibroblast activation protein (FAP), which is selectively expressed on activated fibroblasts residing within the tumor stroma of more than 90% of lung, breast, and colon carcinomas.
Application: Fibroblast activation protein is selectively expressed by activated fibroblasts of the tumor stroma on more than 90% of lung, breast, and colon carcinomas. The high prevalence and abundance of FAP in the stroma of these carcinoma cell types make it a promising molecule that can be used as an *in vivo* "target" for a variety of diagnostic and therapeutic agents. Specialized histological analyses (staining procedures) could be applied in the evaluation of biopsy specimens as a means of prescreening carcinomas vulnerable to such tumor stroma targeting strategies.
Route of Administration: Intravenous.
Considerations: Preparations of immunoglobulin that are completely of human origin are superior to murine or humanized antibody derivatives when applied in clinical hospital environments.
Semi-Synthetic Methods: Highly diverse IgG, IgM, and IgD isotypes comprised of heavy-chain variable domain libraries can be generated using cDNAs derived from diverse lymphoid organs of various donors types. Human single-chain antibody fragment can be converted into bivalent minibodies and expressed in eukaryotic cells to facilitate additional functional characterization.
Interpretation and Analysis: Binding-competition studies and analysis by fluorescence-activated cell sorting show that preparations have a high binding affinity (10–20 nM) for 2 clones and recognize the same epitope as the murine reference antibody. Minibodies can be successfully used for immunohistopathology analysis of a variety of human carcinoma biopsies, revealing specific staining of stromal fibroblasts. Based on the nature of these findings, immunoglobulin preparations should be suitable as a vehicle for selective *in vivo* delivery of diagnostic and therapeutic agents to neoplastic cell types.

Generation of human high-affinity antibodies specific for the fibroblast activation protein by guided selection. Schmidt A, Müller D, Mersmann M, Wüest T, Gerlach E, Garin-Chesa P, Rettig WJ, Pfizenmaier K, Moosmayer D. Eur J Biochem 2001 Mar; 268 (6), pp. 1730–8.

Neoplasia/Carcinoma of the Breast, Colon and Lung
Antifibroblast Activation Protein Monoclonal Antibody (MAb)

Cellular/Molecular Mechanism of Action: Represents an MAb that recognizes and binds to fibroblast activation protein (FAP), which is selectively expressed on activated fibroblasts residing within the tumor stroma of more than 90% of lung, breast, and colon carcinomas.
Application: The high prevalence and abundance of FAP in the stroma of carcinomas of the lung, breast, colon, and other tissue structures make it a promising molecule that can be used as an *in vivo* "target" for a variety of diagnostic and therapeutic agents. Specialized histological analyses (staining procedures) can be applied in the evaluation of biopsy specimens as a means of prescreening carcinomas that would be vulnerable to such tumor stroma targeting strategies.
Route of Administration: Intravenous.
Considerations: Investigations have described the humanization of the murine FAP-specific MAb (e.g., F19). Such preparations have been applied clinically for *in vivo* diagnostic purposes.
Semi-Synthetic Methods: Using phage-display technology and human V-repertoires, VL and VH regions of antigen-specific MAb can be replaced by analogous human V-regions while retaining the original 15-amino acid HCDR3 sequence in a manner that maintains F19 epitope specificity.
Interpretation and Analysis: The resulting human single-chain fragments of immunoglobulin variable regions (scFv 34, scFv 18) have binding affinities of 6 nM for cell membrane-bound FAP. Bivalent scFv 34 is expressed as a minibody (Mb 34). The antigen-binding characteristics of Mb 34 are comparable to the parental and a complementarity-determining region-grafted type of F19. Such characteristics can be validated by competitive binding studies, fluorescence-activated cell sorting (FACS) analyses, and immunohistochemistry on various tumor samples, including carcinomas of the breast, colon, and lung. Compared to the complementarity-determining region-grafted humanized scFv version of F19, the V-regions of selected human scFv 34 possess sequence homology with the parental antibody (Ab) only over a short, 15-amino acid long HCDR3. Thus, a largely reduced xenoantigenic potential is expected.

Human antibody derivatives against the fibroblast activation protein for tumor stroma targeting of carcinomas. Mersmann M, Schmidt A, Rippmann JF, Wüest T, Brocks B, Rettig WJ, Garin-Chesa P, Pfizenmaier K, Moosmayer D. Int J Cancer 2001 Apr 15; 92 (2), pp. 240–8.

Neoplasia/Carcinomas of the Breast, Lung (Small Cell Type), Prostate and Pancrease
[^{99m}Technetium] ([^{99m}Tc])-MAG3-Coupled Bombesin (BN) Peptide

Background Information: Several human cancers, including small cell lung, prostate, breast, gastric, colon, and pancreatic cancers, express receptors for BN-like peptides. Bombesin peptides that bind specifically to these receptors are useful for the *in vivo* detection of BN receptor-expressing cancers.

Application: Detection and characterization of neoplastic disease states, including small cell lung, prostate, breast, gastric, colon, and pancreatic cancers that express receptors for BN-like peptides at relatively high membrane densities.

Semi-Synthetic Methods: Through the use of solid-phase synthesis techniques, MAG3-coupled BN peptide (MAG3-BN) can be prepared and subsequently radiolabeled with [^{99m}Tc] using a chemical exchange methodology. The resulting product is referred to as [^{99m}Tc]-labeled-BN peptide.

Implementation: Evaluation of the *in vitro* binding avidity of [^{99m}Tc]-MAG3-BN for MDA-MB-231 and MCF-7 human breast cancer cell lines. Complementary investigations can entail determining *in vivo* radioactivity biodistribution profiles.

Animal Model: Murine (normal and nude mice bearing BN receptor-positive tumors including MCF-7 cell lines).

Interpretation and Analysis: Radiolabeling of MAG3-BN with [^{99m}Tc] produces a single radioactive species ($>$ 95%) referred to as [^{99m}Tc]-MAG3-BN peptide. *In vitro* cell-binding analyses validate the affinity and specificity of [^{99m}Tc]-MAG3-BN towards BN receptor complexes. *In vivo* biodistribution in mice reveals that [^{99m}Tc]-MAG3-BN clears rapidly from the blood and most nontargeted tissues and is excreted mainly via the kidneys. Uptake in BN receptor-positive tissues and in the tumor is low to moderate. [^{99m}Tc]-MAG3-BN displays good radiolabeling together with certain favorable biological characteristics and might be a useful peptide radiopharmaceutical in the *in vivo* detection of BN receptor-expressing cancers.

Synthesis, radiolabeling, and biological characteristics of a bombesin peptide analogue as a tumor-imaging agent. Okarvi SM, al-Jammaz I. Anticancer Res 2003 May–Jun; 23 (3B), pp. 2745–50.

Neoplasia: Carcinoma/Colon Carcinoma Cell Adhesion to Endothelium/Intravital Observation of Cancer Cell/Microcirculation Interactions
CalceinAM

Background Information: Organ-specific tumor cell adhesion within the microcirculation of host organs is an important step in the metastatic cascade. Circulating tumor cells adhere to the luminal surface of microcirculatory vessels, then quickly stabilize these adhesion interactions. Ultimately, they leave the circulation to avoid the toxic effects of hydrodynamic shear forces exerted by circulating blood.

Application: Intravital observation of colon carcinoma cell adhesion within the hepatic microcirculation.

Route of Administration: Intravascular.

Implementation: CalceinAM can be used to label colon carcinoma cell populations (e.g., HT-29 [human] and CC531 [rat]) in order to track their biodistribution following intra-arterial injection as a single-cell suspension. Intravital fluorescence microscopy can then be applied to detect the development of adhesive interactions between circulating tumor cells and vascular endothelial surfaces within the hepatic microcirculation. These interactions are then analyzed regarding their time course and site localization within the vascular tree.

Animal Model: Rats (Sprague-Dawley).

Interpretation and Analysis: Autofluorescence of liver parenchyma is sufficient to distinguish hepatic sinusoids. Intravital microscopy is capable of differentiating early events in adhesion formation within hepatic sinosoids, adhesion stabilization, and extravasation of tumor cells into the liver parenchyma. Tumor cell adhesion occurs almost exclusively within sinusoidal capillaries; however, the diameter of these vessels is usually larger than that of tumor cells, leaving remaining area of perfused capillary lumen. Colon carcinoma cells rapidly migrate into the liver parenchyma after development of adhesion interactions within the sinusoids. In contrast to common end point assays of the metastatic cascade, this particular *in vivo* model allows investigation of metastatic colon carcinoma cell adhesion phenomenon within the liver microcirculation at specific steps during the formation of hematogenous metastasis and their underlying mechanisms.

Instrumentation: Intravital fluorescence microscopy.

An intravital model to monitor steps of metastatic tumor cell adhesion within the hepatic microcirculation. Haier J, Korb T, Hotz B, Spiegel HU, Senninger N. J Gastrointest Surg 2003 May–Jun; 7 (4), pp. 507–14; discussion 514–5.

Neoplasia/Carcinoma of the Colon (LS-180 Cell Type)/Guanyl Cyclase-C Receptor
[^{125}I]-Tyr(5)-6-Ahx-Phe(19)-ST[1-19]/ [^{111}In]-DOTA-NCS-6-Ahx-Phe(19)-ST[1-19]

Cellular/Molecular Mechanism of Action: [^{111}In]-DOTA-NCS-6-Ahx-Phe$_{19}$-ST[1-19] and [^{125}I]-Tyr$_5$-6-Ahx-Phe$_{19}$-ST [1-19] both recognize and bind to guanylate cyclase-C (GC-C) receptor complexes.

Application: Detection and characterization of human colon cancer (e.g., LS-180 cell strains).

Route of Administration: [^{111}In]-DOTA-NCS-6-Ahx-Phe$_{19}$-ST[1-19] and [^{125}I]-Tyr$_5$-6-Ahx-Phe$_{19}$-ST[1-19] (intravenous).

Implementation: Evaluation of the relative *in vitro* competitive binding of [^{111}In]-DOTA-NCS-6-Ahx-Phe$_{19}$-ST[1-19] to GC-C receptor complexes expressed by human colon cancer LS-180 cells compared to [^{125}I]-Tyr$_5$-6-Ahx-Phe$_{19}$-ST[1-19].

Animal Model: Murine (tumor-bearing severe combined immunodeficient [SCID] mice [LS-180 colon cancer cells]).

Interpretation and Analysis: Competitive *in vitro* binding studies of [^{111}In]-DOTA-NCS-6-Ahx-Phe$_{19}$-ST[1-19] and [^{125}I]-Tyr$_5$-6-Ahx-Phe$_{19}$-ST[1-19] for GC-C receptors expressed by human colon cancer LS-180 cell lines reveal an IC_{50} value of 7.7 $\pm$ 0.1.6 nM. In cellular residualization studies performed *in vitro*, binding of [^{111}In]-DOTA-NCS-ST to GC-C receptors stimulates cGMP production in LS-180 cell populations and demonstrates that this peptide selectively binds to LS-180 cells in an agonistic fashion. *In vivo* biodistribution studies in mice bearing LS-180 tumors reveal that the [^{111}In]-DOTA-NCS-ST peptide targets tumors with a specific uptake of 0.94 $\pm$ 0.31 percent injected dose/gram (%ID/g) at 1 hour postinjection and approximately 23% is retained by the tumor at 4 hours postinjection. Radioactivity clears rapidly from the bloodstream with 84.5 $\pm$ 3.4 %ID at 1 hour postinjection found in the urine. High activity in urine and kidney, and minimal activity in liver and intestines, demonstrates preferential clearance of the radioactivity through renal/urinary mechanisms. The specific *in vitro* and *in vivo* accumulation of radioactivity within LS-180 human colonic cancer cells highlights the potential of radiometallated-DOTA-ST analogues as candidate diagnostic and therapeutic radiopharmaceuticals.

In vivo evaluation of a [^{111}In]-labeled ST-peptide analogue for specific targeting of human colon cancers. Gali H, Sieckman GL, Hoffman TJ, Owen NK, Chin DT, Forte LR, Volkert WA. Nucl Med Biol 2001 Nov; 28 (8), pp. 903–9.

Neoplasia: Carcinoma/Colorectal Cancer
Antiphage Protein Combinatorial Fab

Cellular/Molecular Mechanism of Action: Immunoglobulin fractions that recognize membrane-associated antigens uniquely expressed by human colorectal cancer cell types.

Application: Immuno-detection of human colorectal cancer.
Route of Administration: Intravenous.
Semi-Synthetic Methods: A combinatorial Fab phage display library can be generated from antibody variable-region genes of each of 2 BALB/c mice immunized with the human colorectal cancer cell lines SW480, SW948, and SW837. Such libraries have been shown to have diverse nucleotide sequencing and diagnostic restriction enzyme digestion fragments (fingerprinting) of individual members.
Implementation: Utilization of 2 libraries (combined) selected for binding to a suspension of formaldehyde-fixed human colorectal cancer cells in 2 successive rounds of selection and phage amplification facilitated by infection of bacteria.
Interpretation and Analysis: Analysis of the selected libraries as well as individual library clones by ELISA reveals binding to cancer cell lines in both formaldehyde-fixed and native forms of tissue sections. Fifty-five percent and 94% of library clones are positive for colorectal cancer cell binding properties after the 1st and 2nd rounds of selection, respectively. Fingerprinting of individual clones shows that the 1st selected library is very diverse, while the 2nd selected library has a more limited level of diversity. After absorption with normal human cell types, these anticancer selected libraries could be used to develop alternative therapeutic and/or diagnostic agents.

Generation of anticolorectal cancer Fab phage display libraries with a high percentage of diverse antigen-reactive clones. Williams BR, Sompuram SR, Sharon J. Comb Chem High Throughput Screen 2002 Sep; 5 (6), pp. 489–99.

Neoplasia: Carcinoma/Colorectal Carcinoma/ Human Colorectal Carcinoma Xenographs (SW1222) in Mice
Pretargeting Monoclonal Antibody (MAb) Medicated/ [^{64}Cu]-DOTA-Biotin/[^{64}Cu]-DOTA-NR-LU-10

Background Information: Pretargeting involves administration of a tumor-targeting MAb covalently linked to a molecule having a high-affinity binding site for a rapidly distributed radiolabeled effector molecule.
Application: Pretargeted [^{64}Cu]-DOTA-biotin should afford markedly superior positron emission tomography (PET) imaging contrast and therapeutic efficacy due to its extremely rapid tumor uptake and blood clearance.
Semi-Synthetic Methods: DOTA-biotin (biotinylated 1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid) and the intact immunoconjugate DOTA-NR-LU-10 can be labeled to high specific activities with [^{64}Cu], and the serum stabilities and target binding capabilities of each agent can be determined *in vitro* prior to application.
Implementation: Compare pretargeting strategies and conventionally labeled antibody for tumor antigens for their capcity to target neoplastic lesions following labeling with the intermediate-lived radionuclide [^{64}Cu], which has shown promise for PET imaging and radioimmunotherapy of cancer. One approach to characterizing "pretargeting" strategies can involve the evaluation of preparations in nude mice bearing SW1222 human colorectal carcinoma xenografts. Semi-synthetic preparations of [^{64}Cu]-DOTA-biotin are administered, with and without MAb-streptavidin conjugate NR-LU-10/SA pretreatement and the synthetic clearing agent Biotin-GalNAc$_{16}$, or injected with [^{64}Cu]-DOTA-NR-LU-10. Biodistributions of both agents are obtained from 5 minutes to 48 hours after injection.
Interpretation and Analysis: Both [^{64}Cu]-DOTA-biotin and [^{64}Cu]-DOTA-NR-LU-10 are 100% stable *in vitro* in serum. [^{64}Cu]-DOTA-biotin exhibits $>$ 98% specific binding to immobilized streptavidin, whereas the immunoreactivity of [^{64}Cu]-DOTA-NR-LU-10 averages nearly 80%. Biodistribution analysis in SW1222-bearing mice shows that NR-LU-10/SA-pretargeted [^{64}Cu]-DOTA-biotin attains a peak tumor uptake of 18.9% of the injected dose per gram (%ID/g) at 1 hour, with concomitant rapid disappearance from blood and renal excretion. In the absence of pretargeting, [^{64}Cu]-DOTA-biotin has very similar biodistribution and clearance properties, except with extremely low non-specific tumor uptake. Alternatively, [^{64}Cu]-DOTA-NR-LU-10 reaches 80.3 %ID/g in tumor tissue after 48 hours, whereas blood clearance is considerably slower than pretargeted [^{64}Cu]-DOTA-biotin. Comparison of the time-activity curves for tumor uptake and blood clearance of pretargeted [^{64}Cu] and the [^{64}Cu]-labeled antibody reveal that the maximum tumor accumulations of radioactivity are similar for each agent (e.g., 17.9% of injected activity per gram [%IA/g] and 20.7%IA/g, respectively). However, the tumor-to-blood ratio of areas-under-the-curve is 14 times higher for pretargeted [^{64}Cu]-DOTA-biotin because of the substantial increase in blood clearance of the small effector molecule. The extremely rapid tumor uptake and blood clearance of pretargeted [^{64}Cu]-DOTA-biotin should afford the development of a markedly superior PET imaging agent, compared to conventionally labeled [^{64}Cu]-DOTA-NR-LU-10. Further comparison of the therapeutic efficacy, toxicity, and dosimetry of these 2 agents is warranted.

In vivo evaluation of pretargeted ^{64}Cu for tumor imaging and therapy. Lewis MR, Wang M, Axworthy DB, Theodore LJ, Mallet RW, Fritzberg AR, Welch MJ, Anderson CJ. J Nucl Med 2003 Aug; 44 (8), pp. 1284–92.

Neoplasia: Carcinoma/Intestinal Microcarcinomas
Indocyanine Green (ICG)-Labeled Anti-MUC1 Mucin IgG

Cellular/Molecular Mechanism of Action: Anti-MUC1 mucin IgG possesses binding avidity for complexes expressed on the exterior surface membrane of human gastric carcinoma and colon carcinoma.
Application: Labeling of microcarcinomas to a degree sufficient for accurate and sensitive detection by videoendoscopy.
Considerations: Videoendoscopy has not significantly advanced diagnostic accuracy beyond that attainable by conventional fiberscopy, with respect to microcarcinomas of the digestive tract.
Semi-Synthetic Methods: Conjugation of ICG to anti-MUC1 mucin immunoglobulin to produce a conjugated product that has binding avidity for complexes expressed on the exterior surface membrane of intestinal carcinoma cell types.
Animal Model: Nude mice transplanted with human gastric cancer or colorectal cancer.
Interpretation and Analysis: Anti-MUC1 mucin antibody binds *in vivo* to human gastric cancer or colorectal cancer transplants in nude mice, which can be confirmed by performing immunohistochemistry analysis with a secondary antibody. The antibody labeled with an ICG derivative may therefore be clinically useful in detecting gastrointestinal microcarcinomas by videoendoscopy.
Instrumentation: Videoendoscopy.

Vital immunostaining of human gastric and colorectal cancers grafted into nude mice: A preclinical assessment of a potential adjunct to videoendoscopy. Kusaka Y, Ito S, Muguruma N, Tadatsu M, Bando T, Ii K, Irimura T, Shibamura S. J Gastroenterol 2000; 35 (10), pp. 748–52.

Neoplasia/Carcinomas of the Ovary and Breast
Anti-gp185HER-2 IgG Monoclonal Antibody (MAb)/ Tunicamicyn

Cellular/Molecular Mechanism of Action: Anti-gp185HER-2 IgG MAb recognizes and physically binds to the trans-membrane glycoprotein gp185HER-2 (185-kDa). The oncogene HER-2/neu encodes for this trans-membrane glycoprotein that is known to pos-

sess tyrosine-kinase activity. Gene amplification and high levels of gp185HER-2 expression have been found to correlate with poor clinical outcome in breast and ovarian carcinomas. (Note: Tunicamicyn functions as an inhibitor of N-linked glycosylation.)

Semi-Synthetic Methods: Given the objective of establishing efficient methods for the production of murine MAb with a high degree of antigen specificity, somatic cell hybrid fusion protocols that yield a high production frequency for hybridomas can be utilized to evaluate immune responses to the extracellular domain of gp185 glycoprotein fractions. In this manner, immunogen cell types (e.g., NIH 3T3) that express high levels of tranfected gene for (human) HER-2 can be utilized to generate monoclonal antibody preoparations that are primarily the IgG1 isotype and possess relatively high affinity for gp185 fractions (e.g., 10^7 to 10^{10} mol/L). Five distinct groups of spatially related epitopes have been identified through the application of epitope specificity analysis. Each of these segments provides different levels of immunodominancy, and all are resistant to formalin fixation. Immunoglobulins bind to epitopes localized in the protein core region of gp185HER-2 based on experimental findings observed with the application of tunicamicyn (inhibitor of N-linked glycosylation).

Interpretation and Analysis: In normal tissues, gp185HER-2 has a restricted expression and is homogenously detectable in metastatic foci of gp185 + primary tumors. Conservative speculation suggests that antibodies to this macromolecule glycoprotein potentially have *in vitro* as well as *in vivo* diagnostic, prognostic, and possibly therapeutic value.

Production and characterization of murine MAbs to the extracellular domain of human neu oncogene product GP185HER-2. Digiesi G, Giacomini P, Fraioli R, Mariani M, Nicotra MR, Segatto O, Natali PG. Hybridoma 1992 Aug; 11 (4), pp. 519–27.

Neoplasia/Carcinoma of the Ovary/HER-2/neu Overexpression
Anti-HER-2/neu Single-Chain Fv IgG

Cellular/Molecular Mechanism of Action: Anti-HER-2/neu single-chain Fv antibodies possess binding avidity for neoplastic cell HER-2/neu complexes.

Background Information: Single-chain Fv (scFv) molecules (MR 25,000) are rapidly eliminated from the circulation of immunodeficient mice, yielding highly specific retention of small quantities of scFv in human tumor xenografts.

Application: Detection and characterization of ovarian carcinoma lesions.

Route of Administration: Anti-HER-2/neu single-chain Fv antibodies (intravascular).

Implementation: Evaluation of the hypothesis that the specific retention of scFv in tumors can be enhanced by bioengineering to significantly increase the affinity of the scFv for target antigens. Affinity mutants of the human anti-HER-2/neu (c-erbB-2) scFv C6.5 can be generated by site-directed mutagenesis, which targets the same antigenic epitope with a 320-fold range in affinity (3.2×10^{-7} to 1.0×10^{-9} M).

***Ex Vivo* Model:** Human ovarian carcinoma (SK-OV-3 cell type overexpressing HER-2/neu).

Animal Model: Murine (severe combined immunodeficient [SCID] mice bearing established SK-OV-3 tumors).

Interpretation and Analysis: *In vitro*, the Kd of each scFv correlates closely with the duration of its retention on the surface of ovarian carcinoma cells. In biodistribution studies performed in tumor-bearing mice, the degree and specificity of tumor localization increases significantly with increasing affinity. At 24 hours after injection, tumor retention of the highest affinity scFv is 7-fold greater than that of a mutant with 320-fold lower affinity for HER-2/neu. Because the rapid renal clearance of scFv may blunt the impact of improved affinity on tumor targeting, the distributions can also be established in the absence of renal clearance (e.g., in mice rendered surgically anephric). In such a model, the peak tumor retentions of the 2 higher affinity scFv approximate that reported previously for IgG targeting the same SK-OV-3 tumors in SCID mice with intact kidneys. In contrast, mutants with the lowest affinity for HER-2/neu fail to accumulate within tumors, indicating the presence of an affinity threshold that must be exceeded for active *in vivo* tumor uptake to occur. These results indicate that affinity can significantly impact the *in vivo* tumor-specific retention of scFv molecules.

Increased affinity leads to improved selective tumor delivery of single-chain Fv antibodies. Adams GP, Schier R, Marshall K, Wolf EJ, McCall AM, Marks JD, Weiner LM. Cancer Res 1998 Feb 1; 58 (3), pp. 485–90.

Neoplasia/Carcinomas of the Prostate, Breast and Vascular Endothelium/Human Carcinoid Bon Cells/ Rat Pancreatic Tumor Cells (CA20948)
[^{111}In]-[c(Arg-Gly-Asp-D-Tyr-Lys)]/ [^{125}I]-[c(Arg-Gly-Asp-D-Tyr-Lys)]

Cellular/Molecular Mechanism of Action: Purified fractions of [c(Arg-Gly-Asp-D-Tyr-Lys)] appear to bind specifically and with high affinity to $\alpha_V\beta_3$ receptors on neovascular blood vessel sections of different major human cancers, including prostatic carcinoma and mammary carcinoma, that express this receptor type.

Background Information: Tumors depend on sufficient blood supply for their growth. They are able to promote new blood vessel formation (neoangiogenesis) via angiogenic factors. Inhibition of this process typically results in tumor involution or necrosis. RGD (Arg-Gly-Asp) peptides have been found to antagonize neoangiogenesis, for example, by binding to $\alpha_V\beta_3$ receptors on blood vessels.

Implementation: Evaluate the ability to visualize neoangiogenesis phenomenon in tumors *in vitro* and *in vivo*, with the RGD analogue [c(Arg-Gly-Asp-D-Tyr-Lys)], coupled to the chelator group diethyleletriame pentaacetic acid (DTPA). This analogue can subsequently be radiolabeled with both [^{111}In] and [^{125}I].

Interpretation and Analysis: In autoradiography and immunohistochemistry studies, [^{125}I]-labeled [c(Arg-Gly-Asp-D-Tyr-Lys)] appears to bind specifically and with high affinity to $\alpha_V\beta_3$ receptors on neovascular blood vessel sections of different major human cancers, including prostate and breast neoplastic conditions that express this receptor type. The radioiodinated pharmaceutical also binds to and becomes internalized in human carcinoid Bon cells and rat pancreatic CA20948 tumor cells. Internalization is receptor specific and appears to be time and temperature dependent. Administration of different peptide amounts (0.1, 0.5, and 100 μg) *in vivo* (e.g., rats) reveals that the optimum amount of the radiolabeled analogues appears to be 0.1 μg/rat, because uptake decreases with increasing peptide doses. The [^{111}In]-labeled analogue accumulates in transplantable CA20948 pancreatic tumors in a receptor-specific manner. The introduction of a DTPA group in this peptide results in renal clearance of the radiopharmaceutical, in contrast to the non-DTPA-conjugated compound that is cleared predominantly via the liver. [^{111}In] emits auger and conversion electrons besides gamma radiation, therefore this radiopharmaceutical is suitable not only for tumor scintigraphy but also has potential as a radionuclide for therapeutic purposes, including some of the major forms of human cancer. Moreover, after coupling to the chelator DOTA, the analogue can be radiolabeled in a stable manner with beta-emitters, for example, [^{90}Y] and [^{177}Lu], expanding its theoretical potential.

Evaluation of a radiolabeled cyclic DTPA-RGD analogue for tumor imaging and radionuclide therapy. van Hagen PM, Breeman WA, Bernard HF, Schaar M, Mooij CM, Srinivasan A, Schmidt MA, Krenning EP, de Jong M. Int J Cancer (Radiat. Oncol. Invest.) 2000 Aug 20; 90 (4), pp. 186–98.

Neoplasia: Carcinoma/Prostatic Carcinoma/Gene Expression Imaging
[Gd]-Anti-mRNA Oligonucleotide Strand/Trans-Membrane Carrier Peptide

Cellular/Molecular Mechanism of Action: The physical (special) orientation of gadolinium changes when placed within a magnetic field. Trans-membrane carrier proteins are transported across cell membrane structures by specific membrane-associated complexes. Oligonucleotides can be designed as complementary sequence to specific messenger RNA (mRNA) strands and the resulting cDNA/mRNA double strand effectively prevents translation of the mRNA strand. Diagnostically, complementary strands of cDNA theoretically accumulate intracellularly within cells that are actively transcribing the corresponding mRNA sequences that cDNA strands have been designed to recognize.

Background Information: The term "molecular imaging" can be broadly defined as the *in vivo* characterization and measurement of biological processes at the cellular and molecular level.

Application: Detection of *in vivo* gene expression, including neoplastic cell oncogenes, applying magnetic resonance imaging (MRI) modalities.

Route of Administration: Intravascular injection would be the most preferred method of delivery.

Semi-Synthetic Methods: Development of a novel intravital and intracellular MRI contrast agent composed of a gadolinium complex, an oligonucleotide sequence (peptide nucleic acid [PNA]), and a trans-membrane carrier peptide composed of a peptide sequence similar to that of the homeodomain of the Antennapedia protein.

Implementation: Evaluate the capacity for [Gd]-oligonucleotide complexes to accumulate in tumor cells *in vitro* and *in vivo,* and investigate whether the specificity of a PNA for the upregulated c-myc mRNA in the cell's cytoplasm has an effect on contrast agent retention within tumor cells.

Tissue Culture Models: HeLa cell lines.

Animal Model: Rat (Dunning R3327 AT1 rat prostate adenocarcinoma).

Interpretation and Analysis: Using the c-myc-specific and a c-myc-non-specific control PNA, an increase in signal intensity in tumor cells can be observed after 10 minutes *in vitro* and *in vivo* (maximum reached *in vitro* at 60 minutes in HeLa cells compared to 30 minutes *in vivo* in Dunning R3327 AT1 rat prostate adenocarcinoma cells). Such increases in signal intensity can be maintained *in vitro* in HeLa cells for only 4 hours and in Dunning R3327 AT1 rat prostate adenocarcinoma cells *in vivo* for at least for 5 hours by using the c-myc mRNA-specific PNA as a "retention" agent.

Intracellular visualization of prostate cancer using magnetic resonance imaging. Heckl S, Pipkorn R, Waldeck W, Spring H, Jenne J, von der Lieth CW, Corban-Wilhelm H, Debus J, Braun K. Cancer Res 2003 Aug 15; 63 (16), pp. 4766–72.

Neoplasia: Carcinoma/Pulmonary Small Cell Cancer
Anti-Vasopressin (VP)-Associated Neurophysin Monoclonal IgG

Cellular/Molecular Mechanism of Action: Monoclonal antibody (MAb) fractions that are designed to recognize and physically bind to the VP-associated glycoprotein segment of pro-VP polypeptide that is associated with neurophysin-related cell surface antigen.

Application: Detection of small cell lung cancer lesions based on the concept that these neoplastic cell populations have neurophysin-related cell surface antigen incorporated into their exterior surface membrane.

Route of Administration: Anti-VP-associated neurophysin MAb (intravascular).

Considerations: The VP gene is largely expressed in hypothalamic neurons, where the resultant pro-VP polypeptide is enzymatically cleaved into its peptide hormone components, including the neuropeptides VP, VP-associated neurophysin, and VP-associated glycopeptide. Small cell lung cancer tumors also express the VP gene, but the tumor pro-VP protein can remain intact and localize on the cell surface membrane.

Interpretation and Analysis: Detection of an increased uptake (binding) of radiolabeled anti-VP-associated neurophysin MAb by tissues residing within the thoracic cavity reflects positive detection of small cell lung cancer lesions.

Polyclonal antibodies directed against different regions of the pro-VP molecule bind specifically to the surface of cultured small cell lung cancer cells and recognize proteins of approximately 20-kDa in contrast to the 40-kDa antigen identified in cultured small cell lung cancer whole-cell lysates. By convention, these protein antigens have been designated neurophysin-related cell surface antigen (plasma membrane-associated complex). A MAb preparation (e.g., MAG-1) has been produced that is directed against a synthetic peptide representing the COOH-terminal sequence of VP-associated glycopeptide.

Monoclonal antibodies with these binding characteristics recognize neurophysin-related cell surface antigen expressed by small cell lung cancer cells and contained in tissue lysates based on Western blot analysis. Immunofluorescent cytometric and microscopic analyses indicate that these same MAb preparations react specifically with neurophysin-related cell surface antigen on the surface of viable small cell lung cancer cells (classical and variant subtypes). Selectivity of MAb preparations as determined by immunohistochemical analysis react with human small cell lung cancer cells, but not with normal pulmonary epithelial cells in lung tissue. Limited proteolytic Fab fragment products of MAb also possess binding avidity for neurophysin-related cell surface antigen.

Targeting the neurophysin-related cell surface antigen on small cell lung cancer cells using a monoclonal antibody against the glycopeptide region (MAG-1) of provasopressin. Keegan BP, Memoli VA, North WG. Mol Cancer Ther 2002 Nov; 1 (13), pp. 1153–9.

Neoplasia/CCK-B Receptor Expression/ Meningiomas/Gastroenteropancreatic Tumors/ Medullary Thyroid Carcinoma/Small Cell Lung Cancer/Astrocytomas/Gastrointestinal Tumors/ Stromal Ovarian Cancer
[^{111}In]-DTPA-[Nle$_{28,31}$]-CCK$_{26-33}$ (MP2286)/ DTPA-[d-Asp$_{26}$,Nle$_{28,31}$]-CCK$_{26-33}$ (MP2288)

Cellular/Molecular Mechanism of Action: [^{111}In]-labeled MP2288 and [^{111}In]-labeled MP2286 recognize and physically bind to CCK-B receptor complexes.

Background Information: Receptors for regulatory peptides such as somatostatin or vasoactive intestinal polypeptide are expressed by a number of human neoplasms and can be visualized *in vivo* with peptide receptor scintigraphy. The CCK-B receptor, which binds both gastrin and cholecystokinin with high affinity, has been found using *in vitro* methods to be overexpressed by a number of

human tumor cell types, including medullary thyroid carcinomas, small cell lung cancers, astrocytomas, gastrointestinal tumors, and stromal ovarian cancers.

Semi-Synthetic Methods: Design of a novel, unsulfated CCK octapeptide analogue linked to diethylenetriamine pentaacetic acid (DTPA) and DOTA metal chelating agents.

Implementation: Evaluation of the binding avidity of unsulfated CCK octapeptide analogues linked to the metal chelating groups DTPA and DOTA for CCK-B receptor-positive human tumor tissues.

Animal Model: Rat (biodistribution studies).

Interpretation and Analysis: Assays reveal that the most potent compounds are DTPA-[$Nle_{28,31}$]-CCK_{26-33} (MP2286) and DTPA-[d-Asp_{26},$Nle_{28,31}$]-CCK_{26-33} (MP2288) with an IC_{50} of 1.5 nM. For comparison, analogues with C-terminal DTPA, such as [$Nle_{28,31}$,$Aphe_{33}$(*p*-NH-DTPA)]-CCK_{26-33} and CCK_{26-33}-NH$(CH_2)_2$ NH-DTPA, have an IC_{50} of $>$ 100 nM. DOTA-[D-Asp_{26}, $Nle_{28,31}$]-CCK_{26-33} has an IC_{50} of 3.9 nM. The compounds are selective for CCK-B receptors, as they do not bind with high affinity to CCK-A receptors expressed in human tumors (meningiomas or gastroenteropancreatic tumors). Biodistribution studies with [^{111}In]-labeled MP2286 and MP2288 show that the primary mode of clearance is renal, and the primary sites of uptake (percent injected dose/gram [%ID/g] 24 hours postinjection) are the kidney (0.270 and 0.262, respectively) and gastrointestinal tract (rat). The CCK-B receptor-expressing gastric mucosa shows specific *in vivo* accumulation of [^{111}In]-labeled MP2288 that can be blocked by excess unlabeled MP2288. [^{111}In]-labeled MP2286 and MP2288 are also found to be stable in human plasma, whereas both compounds are degraded in urine ($>$ 40% after 3 hours at 37°C). The affinity, specificity, biodistribution, and stability of these 2 DTPA-CCK analogues indicate that these compounds hold substantial promise for use in the *in vivo* visualization of CCK-B receptor-expressing tumors.

Unsulfated DTPA- and DOTA-CCK analogues as specific high-affinity ligands for CCK-B receptor-expressing human and rat tissues *in vitro* and *in vivo*. Reubi JC, Waser B, Schaer JC, Laederach U, Erion J, Srinivasan A, Schmidt MA, Bugaj JE. Eur J Nucl Med 1998 May; 25 (5), pp. 481–90.

Neoplasia/Gastrin-Releasing Peptide (GRP) Receptors/Prostatic Carcinoma/[Mammary Carcinoma]/[Small Cell Lung Cancer]/[Pancreatic Cancer]
[^{111}In]-Bombesin/[^{111}In]-DOTA-X-BBN_{7-14}-NH_2/Beta-Ala, 5-Ava Spacer/8-Aoc Spacer

Cellular/Molecular Mechanism of Action: Bombesin has been known to bind to GRP receptors with high affinity and specificity.

Background Information: Gastrin-releasing peptide receptors have been shown to be expressed with high densities on several types of cancer cells, including prostate, breast, small cell lung, and pancreas cancers.

Application: Hydrocarbon spacer chains can effectively improve the specificity of detecting and characterizing human prostatic carcinoma cell types when employing [^{111}In]-DOTA-bombesin as a template molecule for the purpose of recognizing gastrin-releasing peptide receptor complexes.

Route of Administration: Intravascular.

Implementation: Evaluation of the relative tumor "uptake" and pharmacokinetic parameters for various [^{111}In]-labeled bombesin analogues as potential candidates for specific targeting of human prostate cancers.

***Ex Vivo* Model:** Human (PC-3 androgen-independent human prostate cancer cells).

Animal Model: Murine (PC-3 human prostate cancer-derived xenografts in CF-1 and severe combined immunodeficient [SCID] mice).

Interpretation and Analysis: *In vitro* competitive binding assays, using PC-3 androgen-independent human prostate cancer cells, reveal values $<$ 2.5 nmol/L for inhibitory concentration of 50% for analogues with beta-Ala, 5-Ava, and 8-Aoc spacers. *In vivo* biodistribution studies of [^{111}In]-DOTA-X-BBN_{7-14}-NH_2 conjugates performed on CF-1 mice at 1 hour after injection reveals that radioactivity uptake within the pancreas (Note: A GRP-receptor-expressing tissue) increases as a function of hydrocarbon spacer length (Data: 0.20 + 0.04 percentage injected dose [%ID] per gram for the analogue with no spacer to a maximum of 26.97 + 3.97 %ID/g for the analogue with 8-Aoc spacer). Radioactivity is cleared efficiently from the blood pool by excretion mainly through the renal/urinary mechanisms (e.g., 71.6 + 1.8 %ID at 1 hour postinjection for 8-Aoc spacer analog). *In vivo* pharmacokinetic studies of the [^{111}In]-DOTA-8-Aoc-BBN[7-14]NH_2 conjugate conducted with PC-3 human prostate cancer-derived xenografts in SCID mice show a specific uptake of radioactivity in tumors, with 3.63 + 1.11 %ID/g observed at 1 hour postinjection. High tumor-to-blood and tumor-to-muscle ratios of approximately 6:1 and 45:1, respectively, can be appreciated at 1 hour after injection. Relative to the radioactivity observed in the tumor at 1 hour after injection, 43%, 19%, and 9% of the radioactivity is retained respectively, at 24, 48, and 72 hours postinjection.

These studies show that radiometallated DOTA-X-$BBN_{7-14}NH_2$ constructs with hydrocarbon spacers ranging from 5 to 8 carbon atoms are feasible candidates for further development as diagnostic and therapeutic radiopharmaceuticals for GRP-positive neoplastic disease states.

Novel series of [^{111}In]-labeled bombesin analogues as potential radiopharmaceuticals for specific targeting of gastrin-releasing peptide receptors expressed on human prostate cancer cells. Hoffman TJ, Gali H, Smith CJ, Sieckman GL, Hayes DL, Owen NK, Volkert WA. J Nucl Med 2003 May; 44 (5), pp. 823–31.

Neoplasia/Gliomas/Mammary Tumors (Carcinogen-Induced)
[^{99m}Tc])Field Bean Protease Inhibitor (FBPI)/[^{99m}Tc]-(V)Dimercaptosuccinic Acid (DMSA)/[^{201}Thallous] Chloride (TlCl)/[$^{99m}TcO_4^-$]-Citrate

Application: Detection and characterization of glioma and potentially other forms of neoplastic disease.

Route of Administration: [$^{99m}TcO_4^-$]-labeled purified FBPI (intravenous).

Semi-Synthetic Methods: Purified field bean protease inhibitor can be labeled with [$^{99m}TcO_4^-$] as a tracer molecule for diagnostic purposes. Radiolabeling is done in the presence of [Sn^{2+}] functioning as a reducing agent and the resulting yield is 95% (approximately). At room temperature, the product is stable for at least a 2-hour incubation period.

Implementation: Evaluation of the biodistribution of [^{99m}Tc]-labeled purified FBPI to ascertain its ability to locate within neoplastic lesions. Biodistribution analysis can be determined at various time intervals following administration.

Animal Model: Rat (Wistar strain bearing carcinogen-induced mammary tumors or transplanted C6 glioma cell lines).

Interpretation and Analysis: Biodistribution analysis of [^{99m}Tc]-FBPI reveals that it undergoes rapid blood clearance from the systemic circulation (approximately 5 hours). The complex is predominantly eliminated through renal and hepatobiliary excretory mechanisms. The tumor to muscle (T/M) ratios obtained with

[^{99m}Tc]-FBPI in C6 glioma explants is nearly 2-fold to 5-fold higher than observed with several conventional tumor-seeking radiopharmaceuticals such as [$^{99m}Tc^{V}$]-DMSA, TlCl, or [^{99m}Tc]-citrate. Similarly, the T/M ratio obtained with [^{99m}Tc]-FBPI in an *in vivo* mammary tumor model appears to be 2-fold to 3-fold higher than observed with [$^{99m}Tc^{V}$]-DMSA and [^{201}TlCl]. The ratio is, however, comparable with that obtained with [^{99m}Tc]-citrate. Such findings indicate that [^{99m}Tc]-FBPI has the specific potential for effectively imaging gliomas and possibly other neoplastic conditions as well.

[^{99m}Tc]-labeled field bean protease inhibitor can function as an efficient tumor-detecting agent. Murugesan S, Banerji AP, Noronha OP, Samuel AM, Fernandes AO. Indian J Exp Biol 2001 Aug; 39 (8), pp. 742–7.

Neoplasia/Insulin-Like Growth Factor (IGF) Binding Proteins
[^{125}I]-Labeled-IGF

Cellular/Molecular Mechanism of Action: Insulin-like growth factors recognize and bind to membrane-associated receptor complexes (IGF binding proteins).
Application: Detection and characterization of certain neoplastic disease types.
Considerations: [^{125}I]-labeled IGF-I is degraded quite rapidly within the cytosol of some neoplastic cell types.
Semi-Synthetic Methods: Production of [^{125}I]-labeled-IGF-I by the radioiodination of IGF-I using chloramines-T reagent.
Implementation: Evaluation of the biodistribution and tumor targeting ability of radiolabeled IGFs in the form of [^{125}I]-labeled-IGF-I. Because IGF binding proteins (IGFBPs) play a critical role in modulating IGF activity, the binding properties of [^{125}I]-labeled IGF-I to IGFBPs can be investigated in both *in vitro* and *in vivo* environments. Validation of the relative specificity of uptake can in part be established by co-administration of unlabeled peptide. Delineation of the catabolism of IGF-I by tumor cells *in vitro* can also be performed because a large amount of IGF-I is catabolized *in vivo* following intravascular injection. In summary, chromatography analysis can be performed on *in vitro* and *in vivo* biological samples in order to determine the binding profile to IGFBPs and measure serum/urine catabolite concentrations.

Animal Model: Rat and mouse (tumor-bearing nude mice).
Interpretation and Analysis: Binding of radiolabeled IGF-I to IGFBPs *in vitro* occurs in a dose-dependent manner. However, there is a difference in complex formation between serum and heparinized plasma. In heparinized plasma, radioactivity shifts from a 30- to 50-kDa complex to a 150-kDa complex and to a free ligand, because the binding of heparin with IGFBPs decreases its affinity for IGF-I. In plasma prepared with acid citrate dextrose a binding pattern identical to that of serum can be observed. Moreover, there is a binding difference between mouse and rat. The [^{125}I]-labeled IGF-I is catabolized very quickly when incubated at 37°C but not at all at 4°C. In tumor-bearing nude mice, the uptake of radioactivity in normal tissues decreases quickly, particularly in the kidneys. In mice coinjected with unlabeled carrier, the radioactivity in most normal tissues is lower and the tumor uptake higher than in mice without carrier administration. [^{125}I]-labeled IGF-I is avidly bound to IGFBPs, both *in vitro* and *in vivo*. By partially saturating this binding site with unlabeled peptides, a favorable biodistribution can be achieved, including faster clearance from normal tissue and higher tumor uptake, which results in better tumor-to-nontumor ratios. Nevertheless, the rapid catabolism and release of the radiolabel from tumor tissue result in a suboptimal targeting agent.

Effects of insulin-like growth factor binding proteins on insulin-like growth factor-I biodistribution in tumor-bearing nude mice. Sun BF, Kobayashi H, Le N, Yoo TM, Drumm D, Paik CH, McAfee JG, Carrasquillo JA. J Nucl Med 2000 Feb; 41 (2), pp. 318–26.

Neoplasia/Leukemia/Jurkat Leukemia Cells (Human)
[^{99m}Tc]-Tat-Peptide 4/[Fluorescein-5-Maleimide]-Tat-Peptide

Cellular/Molecular Mechanism of Action: [Tc/Re(V)] complexes can rapidly translocate across cell membranes into intracellular cytosol. Fluorescein-5-maleimide can be used to label biological peptides.
Background Information: Development of rapid and efficient modalities for delivery of radioactive metal complexes to the cell interior would facilitate novel applications in medical imaging and radiotherapy.
Application: [^{99m}Tc]-Tat-peptide 4 can potentially be applied for diagnostic imaging of certain neoplastic disease types.
Route of Administration: [^{99m}Tc]-Tat-peptide 4 (intravascular).
Semi-Synthetic Methods: A membrane permeant peptide conjugates incorporating HIV-1 Tat transactivation protein sequences (GRKKRRQRRR) can be conjugated to an appropriate peptide-based motif (epsilon-KGC) that provides an N_3S donor core for chelating technetium or rhenium. Oxotechnetium(V) and oxorhenium(V) Tat-peptide complexes can be prepared by facile transchelation reactions with permetalates, tin chloride ($Sn^{+2}CL_2$)and sodium glucoheptonate. Reverse-phase high-pressure liquid chromatography (RP-HPLC) can detect 2 major [^{99m}Tc]-Tat-peptide species that differ in retention time by approximately 2 minutes corresponding to 2 [Re]Tat-peptide species that have identical mass. Such findings are consistent with the formation of 2 isomers, likely to be oxo-metal diastereomers. [^{99m}Tc]-Tat-peptides are stable transchelates *in vitro*. To facilitate analysis using fluorescent microscopy, the C-terminus thiol of the prototypic Tat-peptide can be labeled with fluorescein-5-maleimide (yielding conjugate 8).
Implementation: Evaluation of binding avidity and stability of [^{99m}Tc]-Tat-peptide 4 in the presence of human Jurkat cell populations.

Tissue Culture Model: Human Jurkat KB 3-1 and KB 8-5 leukemia cell types.

Animal Model: Murine (*in vivo* investigations).
Interpretation and Analysis: In human Jurkat cells, [^{99m}Tc]-Tat-peptide 4 primarily accumulates within cell populations (30-fold greater than extracellular concentrations) and is associated with rapid uptake kinetics ($t_{1/2} < 2$ min) in a diastereomeric-comparable manner. Paradoxically, uptake is enhanced at 4ºC in buffer compared to 37ºC, while depolarization of membrane potential as well as inhibition of microtubule function and vesicular trafficking show no inhibitory effect. Cells preloaded with [^{99m}Tc]-Tat-peptide species (conjugate 4) show rapid washout kinetics into peptide-free solutions. Modification of [^{99m}Tc]-Tat-peptide by the deletion of the N-terminus Gly with or without biotinylation minimally impacts net-cell uptake.

Fluorescence microscopy reveals that the [fluorescein-5-maleimide]-Tat-peptide complex (conjugate 8) can directly localize within the cytosol and nuclei (possibly nucleolus) of human Jurkat, KB 3-1 and KB 8-5 tumor cells. Preliminary *in vivo* imaging studies in mice following intravenous administration of prototypic [^{99m}Tc]-Tat-peptide (conjugate 4) reveals an initial whole-body distribution and rapid clearance by both renal and hepatobiliary excretion. Analysis of murine blood *in vivo* and human serum *ex vivo* reveals > 95% of the complex in the intact parent product, while murine urine *in vivo* contains 65% parent complex. Thus, these novel Tat-peptide chelate conjugates, capable of forming stable [Tc/Re(V)]complexes, rapidly translocate across cell membranes into intracellular compart-

ments and can be readily derivatized for further targeting applications in molecular imaging and radiotherapy.

Novel Tat-peptide chelates for direct transduction of technetium-99m and rhenium into human cells for imaging and radiotherapy. Polyakov V, Sharma V, Dahlheimer JL, Pica CM, Luker GD, Piwnica-Worms D. Bioconjug Chem 2000 Nov–Dec; 11 (6), pp. 762–71.

Neoplasia/Lymphoma: Non-Hodgkin's Lymphoma *[^{99m}Technetium] ([^{99m}Tc])-Antilymphoma IgG Fragment*

Cellular/Molecular Mechanism of Action: Antilymphoma IgG fragment detects and physically binds to membrane-associated antigens uniquely expressed on the exterior surface of neoplastic populations of this histiological cell type.

Application: Detection and characterization of non-Hodgkin's lymphoma. Administration of IgG fragments has the advantage of decreased non-specific binding to Fc receptor complexes expressed at a high density on the exterior surface membrane of the mononuclear phagocytic system (reticuloendothelial cell types) found in high concentrations within the spleen, liver, and lung.

Route of Administration: Intravenous.

Implementation: Staging of recurrent or newly diagnosed non-Hodgkin's lymphoma (NHL) applying [^{99m}Tc]-labeled antilymphoma immunoglobulin fragment, bectumomab [LymphoScan]. Assessment can be performed applying computed x-ray tomography (CT), radiogallium imaging, FDG-PET, and bone marrow biopsy (e.g., n = 117 defined sites).

Human Model: Non-Hodgkin's lymphoma (recurrent and at initial diagnosis; n = 7 cases and n = 14 cases prior to radioimmunotherapy; n = 7 low-grade, n = 11 intermediate-grade, and n = 3 high-grade histiological cell subtypes).

Interpretation and Analysis: Bectumomab reveals 56% of these sites. In some cases (e.g., n = 4) bectumomab can discover sites not evident applying conventional imaging modalities (e.g., n = 5 sites). In addition, it can detect lesions within the brain (e.g., n = 1), which is an anatomical location not addressed in the standard work-up of asymptomatic cases. Bectumomab imaging most often fails in central abdominal and thoracic locations, but excells in revealing disease affecting the head and neck. Relative to [^{67}Ga]-citrate imaging, the performance of bectumomab is variable, with no clear relation to anatomic location. However, it is capable of better targeting low and intermediate grade NHL. Radiogallium outperforms bectumomab imaging in a relatively large number of cases (e.g., n = 23), some of which are high or intermediate-grade disease states (e.g., n = 19/23). Bectumomab is superior to radiogallium at a number of sites (e.g., n = 6), some of which are associated with low-grade tumors (e.g., n = 5/6). Bectumomab shows promise as a pre-RIT probe for targeting of B-cell NHL. It excels at defining small volume, low-grade disease. However, as a purely diagnostic agent, its performance is variable.

Radioimmunoscintigraphy (RIS) with bectumomab (Tc99m labeled IMMU-LL2, Lymphoscan) in the assessment of recurrent non-Hodgkin's lymphoma (NHL). Lamonica D, Czuczman M, Nabi H, Klippenstein D, Grossman Z. Cancer Biother Radiopharm 2002 Dec; 17 (6), pp. 689–97.

Neoplasia/Melanoma (Metastatic)/Melanocyte-Stimulating Hormone Receptor Expression (MSH Receptor) *[^{111}In])-DOTA-NDP-MSH/[^{111}In]-DOTA-MSH(OCT)*

Cellular/Molecular Mechanism of Action: The peptide [Nle$_4$, D-Phe$_7$]-alpha-MSH is a very potent alpha-MSH analogue that recognizes and physically binds to melanocyte stimulating hormone receptor complexes. Similarly, [betaAla$_3$, Nle$_4$, Asp$_5$, D-Phe$_7$, Lys$_{10}$]-alpha-MSH$_{3\text{-}10}$ is an alpha-MSH octapeptide analog.

Background Information: Scintigraphic imaging of metastatic melanoma lesions requires highly tumor-specific radiolabeled compounds. Because both melanotic and amelanotic melanomas overexpress receptors for alpha-melanocyte-stimulating hormone (alpha-MSH; receptor name: melanocortin type 1 receptor, or MC1R), radiolabeled alpha-MSH analogues are potential candidate diagnostic agents for the detection and characterization of melanoma lesions.

Application: Detection and characterization of metastatic melanoma lesions including melanotic and amelanotic cell types.

Route of Administration: [^{111}In]-DOTA-NDP-MSH and [^{111}In]-DOTA-MSH(OCT) most frequently would be administered intravascularly in a clinical environment.

Semi-Synthetic Methods: Covalent conjugation of [Nle$_4$, D-Phe$_7$]-alpha-MSH (NDP-MSH) and the alpha-MSH octapeptide analog, [betaAla$_3$, Nle$_4$, Asp$_5$, D-Phe$_7$, Lys$_{10}$]-alpha-MSH$_{3\text{-}10}$ (MSH(OCT)) to the metal chelator group 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) to facilitate radiometal incorporation into conjugated complexes.

Implementation: Evaluation of the (i) potential *in vitro* binding avidity of [^{111}In]-DOTA-NDP-MSH and [^{111}In]-DOTA-MSH(OCT) for melanocortin type 1 receptor (MC1R); (ii) melanogenic activity of [^{111}In]-DOTA-NDP-MSH and [^{111}In]-DOTA-MSH(OCT) in isolated mouse B16F1 cell lines; and (iii) *in vivo* biodistribution of the hormone receptor ligands in mouse models of primary and metastatic melanoma.

Animal Model: Murine (melanoma B16F1-bearing mice).

Interpretation and Analysis: DOTA-MSH(oct) binds with high affinity (inhibitory concentration of 50% IC$_{50}$ = 9.21 nmol/L) to MC1R, although with lower potency than does DOTA-NDP-MSH (IC$_{50}$ = 0.25 nmol/L). In B16F1 melanoma-bearing mice, both [^{111}In]-DOTA-NDP-MSH and [^{111}In]-DOTA-MSH(OCT) exhibit high MC1R-mediated uptake by melanoma, which differs by a factor of only 1.5 at 4 hours postadministration. The main route of excretion for both radioconjugates is the kidneys, whereby [^{111}In]-DOTA-MSH(OCT) produces somewhat higher kidney values than does [^{111}In]-DOTA-NDP-MSH. In contrast, the latter conjugate is much more poorly cleared from other non-malignant tissues, including bone, the most radiosensitive organ. Therefore, [^{111}In]-DOTA-MSH(OCT) displays higher uptake ratios of tumor to non-target tissue (e.g., tumor-to-bone ratio at 4 hours postadministration is approximately 4.9 for [^{111}In]-DOTA-NDP-MSH and 53.9 for [^{111}In]-DOTA-MSH(OCT)). Lung and liver melanoma metastases can easily be visualized on tissue section autoradiographs after injection of [^{111}In]-DOTA-MSH(OCT). Radio-reversed-phase high-performance liquid chromatography analysis of urine samples reveals that most [^{111}In]-DOTA-MSH(OCT) is excreted intact 4 hours after injection, indicating good *in vivo* stability. Therefore, [^{111}In]-DOTA-MSH(OCT) appears to exhibit more favorable overall performance than does [^{111}In]-DOTA-NDP-MSH in murine models of primary and metastatic melanoma, making it a promising melanoma imaging agent.

A novel DOTA-alpha-melanocyte-stimulating hormone analogue for metastatic melanoma diagnosis. Froidevaux S, Calame-Christe M, Tanner H, Sumanovski L, Eberle AN. J Nucl Med 2002 Dec; 43 (12), pp. 1699–706.

Neoplasia/Melanoma/Melanoma-Stimulating Hormone Receptor Complexes (MSH Receptor) *[^{111}Indium] ([^{111}In])-DOTA-MSH(OCT)/ [^{111}In]-DOTA-NDP-MSH*

Cellular/Molecular Mechanism of Action: The octapeptide [betaAla$_3$, Nle$_4$, Asp$_5$, D-Phe$_7$, Lys$_{10}$]-alpha-MSH$_{3\text{-}10}$ referred to as

MSH(OCT) is an analogue of alpha-melanocyte-stimulating hormone.

Background Information: Scintigraphic imaging of metastatic melanoma lesions requires highly tumor-specific radiopharmaceuticals. Because both melanotic and amelanotic melanomas overexpress melanocortin-1 receptors (MC1R), radiolabeled analogues of alpha-melanocyte-stimulating hormone (alpha-MSH) are potential candidate diagnostic agents for the detection and characterization of melanoma lesions.

Application: Detection and characterization of malignant melanoma lesions.

Route of Administration: [^{111}In]-DOTA-MSH(OCT) and [^{111}In]-DOTA-NDP-MSH (intravascular).

Semi-Synthetic Methods: Molecular design of the octapeptide analogue [betaAla$_3$, Nle$_4$, Asp$_5$, D-Phe$_7$, Lys$_{10}$]-alpha-MSH$_{3\text{-}10}$ (MSH(OCT)) involves the implementation of conjugation reactions involving its N-terminal amino group to the metal chelator group 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) to enable incorporation of radiometals (e.g., ^{111}In) into peptide products.

Implementation: Evaluation of the *in vitro* ligand binding-avidity of non-radioactive DOTA-MSH(OCT) for melanocrotin-1 receptors (MC1R). Complementary investigations can involve determining the *in vivo* biodistribution of [^{111}In]-DOTA-MSH(OCT) in normal reference control and tumor-bearing mice.

Animal Model: Murine (B16-F1 melanoma tumor-bearing mice).

Interpretation and Analysis: The non-radioactive ligand DOTA-MSH(OCT) possesses a relatively high level of *in vitro* binding avidity for MC1R complexes (IC_{50} 9.21 nM). The peptide analog, [^{111}In]-DOTA-MSH(OCT) *in vivo* exhibits a favorable biodistribution profile after injection in tumor-bearing mice. The radiopeptide is rapidly cleared from the blood through the kidneys and, most importantly, accumulates preferentially within melanoma lesions. Lung and liver melanoma metastases can be clearly imaged on tissue section autoradiographs 4 hours after injection of [^{111}In]-DOTA-MSH(OCT). A comparative study evaluating the relative merits of [^{111}In]-DOTA-MSH(OCT) relative to [^{111}In]-DOTA-[Nle$_4$, D-Phe$_7$]-alpha-MSH (^{111}In-DOTA-NDP-MSH) reveals that DOTA-MSH (OCT) peptide is a superior ligand reagent, particularly with regard to the amount of radioactivity taken up by non-malignant organs, including bone, the most radiosensitive tissue exposed. Therefore, [^{111}In]-DOTA-MSH(OCT) appears to be a promising melanoma-imaging agent.

DOTA alpha-melanocyte-stimulating hormone analogues for imaging metastatic melanoma lesions. Froidevaux S, Calame-Christe M, Sumanovski L, Tanner H, Eberle AN. Ann N Y Acad Sci 2003 Jun; 994, pp. 378–83.

Neoplasia/Melanoma (Multiple Neoplasia Types)
Spermidine Analogues/N-Acetyldopamine/ Chlorambucil

Application: Selective recognition of neoplastic cell types in a manner suitable for the design of a diagnostic agent or as a delivery modality for chemotherapeutic agents.

Semi-Synthetic Methods: Spermidine can be conjugated or covalently linked to various molecular groups. Reference example spermadine analogs include the cytotoxic biopharmaceutal agents; (compound 1a) *N*-[3,4-bis(benzyloxy)phenethyl]-*N* α-(3-aminopropyl)-*L*-ornithinamide trihydrochloride; and (compound 1b) *N*-[4-]bis(2-chloroethyl)amino]phenethyl]-*N* α-(3-aminopropyl)-*L*-ornithinamide tetrahydrochloride. These compounds can be prepared from the fully protected spermidine molecule with a carboxyl group side chain (compound 8).

Implementation: Evaluation of the ability for polyamine cytotoxic agents to inhibit B16-BL6 melanoma cell growth in culture in addition to the influence of DMSO pretreatment has on the activity of the synthesized compounds.

Interpretation and Analysis: The IC_{50} values of compounds 1a and 1b are on the same order of magnitude as the control compounds, N-acetyldopamine and chlorambucil, respectively. The inhibitory activities of compounds 1a and 1b are not enhanced by pretreatment with DMSO, suggesting that depletion of intracellular polyamines does not enhance the activity of these compounds.

Synthesis and evaluation of novel spermidine derivatives as targeted cancer chemotherapeutic agents. Stark PA, Thrall BD, Meadows GG, Abdel-Monem MM. J Med Chem 1992 Nov 13; 35 (23), pp. 4264–9.

Neoplasia/Neuroectoderm-Derived Tumors
Monoclonal Antibody: Paclitaxel Conjugate

Background Information: Paclitaxel (Taxol) is a chemotherapeutic agent that prevents disassembly of microtubular polymers, thereby causing growth arrest in the G2-M phase of the cell cycle and subsequent development of apoptosis phenomenon. Paclitaxel has remarkable efficacy against fast-growing tumors but possesses major drawbacks, such as poor solubility and lack of tumor selectivity. Conversely, monoclonal antibodies usually have low therapeutic efficacy but are highly soluble and capable of selectively targeting unique tumor markers overexpressed by neoplastic cell types.

Application: Selective targeting of pharmaceutical agents for neoplastic lesions in a manner that also simultaneously reduces their potential systemic toxicity. Example: Improved therapeutic index of taxane chemotherapeutics while decreasing the high toxicity of paclitaxel following conjugation to highly selective and highly solubile monoclonal antibodies functioning as a targeting delivery platform.

Semi-Synthetic Methods: Covalent linkage of paclitaxel to monoclonal antibody fractions capable of selectively targeting neuroectoderm-derived tumors to produce paclitaxel-antibody conjugates.

Implementation: Evaluation of the relative antineoplastic activity of paclitaxel-antibody conjugates compared to "free" paclitaxel alone.

Animal Model: Mouse (xenograph tumors).

Interpretation and Analysis: Covalent cross-linking of paclitaxel to antibody to form paclitaxel-antibody conjugates provides selective toxicity toward neuroectodermal tumor cells expressing target markers for which the monoclonal IgG has been designed to recognize. Interestingly, *in vitro* cytotoxicity of preparations is greater than equimolar concentrations of free paclitaxel or free paclitaxel plus free antibody. Systemic (*in vivo*) administration of paclitaxel-antibody conjugates prevents tumor growth and prolongs survival superior to free drugs. In addition, paclitaxel-antibody conjugates are highly soluble in water and stable at −20°C for at least 3 months.

Taxane-antibody conjugates afford potent cytotoxicity, enhanced solubility, and tumor target selectivity. Guillemard V, Saragovi HU. Cancer Res 2001 Jan 15; 61 (2), pp. 694–9.

Neoplasia/Neurotensin: Adenocarcinoma/ Pancreatic Cancer
[^{111}Indium] ([^{111}In])-Diethylenetriamine Pentaacetic Acid (DTPA)-Neurotensin [Arg$_8$, Arg$_9$, and Ile$_{12}$]/Fluorescein-Neurotensin [Arg$_8$, Arg$_9$, and Ile$_{12}$]

Cellular/Molecular Mechanism of Action: Native neurotensin (NT) is a tridecapeptide that binds to neurotensin receptor (NTR) and induces tumor growth.

Background Information: The prevalence of NTR in several human tumor cell types makes it an attractive target for the delivery of cytotoxic drugs and the development of imaging agents. Unfortunately, NT has a short plasma half-life, which hinders its use for *in vivo* biomedical applications. Numerous reports suggest that Arg_8-Arg_9 and Tyr_{11}-Ile_{12} amide bonds are particularly susceptible to degradation by proteolytic enzymes.
Semi-Synthetic Methods: Substitution of Arg_8, Arg_9, and Ile_{12} amino acids with the corresponding commercially available mimics. Preparations can be conjugated to DTPA chelation groups for subsequent radiolabeling to produce [^{111}In]-DTPA or alternatively conjugated with fluorescein.

Animal Model: Mouse (severe combined immunodeficient [SCID] bearing NTR-positive human adenocarcinoma [HT29] xenografts).
Interpretation and Analysis: The surrogate amino acids are amenable to standard Fmoc peptide synthesis strategies, and the resulting compounds are stable in biological media for $>$ 4 hours and bind to NTR with a high level of affinity. Conjugation of DTPA to the synthetic peptides and subsequent labeling with [^{111}In] to produce [^{111}In]-DTPA-NTR for nuclear imaging or fluorescein-NTR for optical imaging does not diminish NTR binding avidity. Evalution of the *in vivo* biodistribution of a representative [^{111}In]-DTPA-NT peptide analogue in HT29-bearing SCID mice reveals that the conjugate is primarily retained in tumor tissue (2.2% ID/g) and the kidneys (4.8% ID/g) at 4 hours postinjection. Coinjection of cold NT and the radiolabeled NT peptide analogue inhibits tumor but not kidney uptake, and demonstrates that retention of the radiolabeled compound in tumor tissue is mediated by NTR specific uptake while it accumulates in the kidneys by a non-specific mechanism. Conjugated NT peptide analogues therefore are robust and can deliver imaging agents to NTR-positive tumors including pancreatic cancer.

Novel bioactive and stable neurotensin peptide analogues capable of delivering radiopharmaceuticals and molecular beacons to tumors. Achilefu S, Srinivasan A, Schmidt MA, Jimenez HN, Bugaj JE, Erion JL. J Med Chem 2003 Jul 17; 46 (15), pp. 3403–11.

Neoplasia/Oxytocin Receptor Complex Expression/ Endothelial Carcinoma/Mammary Carcinoma/ Glioblastoma/Neuroblastoma
[^{111}Indium] ([^{111}In])-DOTA-Lys$_8$-Vasotocin (LVT)

Cellular/Molecular Mechanism of Action: Lys_8-vasotocin is an analogue of oxytocin that possesses high binding affinity for oxytoxin receptor complexes.
Semi-Synthetic Methods: Covalent cross-linking the chelating agent 1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid (DOTA) to LVT.
Implementation: Evaluate the ability of the radioactive ligand [^{111}In]-DOTA-Lys_8-vasotocin to recognize and physically bind to oxytocin receptors expressed by mammary carcinoma and glioblastoma cell types.

***Ex Vivo* Model:** MCF-7 (mammary carcinoma); MOG-U-V-W (glioblastoma) and transiently transfected COS7 cell lines.

Animal Model: Murine (TS/A mouse mammary tumor).
Interpretation and Analysis: The agent DOTA-Lys_8-vasotocin (DOTA-LVT) retains high *in vitro* binding affinity for human oxytoxin receptor complexes endogenously expressed by cell lines such as MCF7 breast carcinoma and MOG-U-V-W glioblastoma cells lines, as well as to transiently transfected COS7 cells. In investigations performed *in vivo,* [^{111}In]-DOTA-Lys_8-vasotocin specifically binds to oxytoxin receptor-positive TS/A mouse mammary tumors. Based on the nature of these laboratory findings, DOTA-LVT could potentially function as a plateform for imaging and, possibly, the treatment of oxytoxin receptor-positive human tumors such as breast and endometrial carcinomas, neuroblastomas, and glioblastomas.

^{111}In-labeled 1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid-Lys_8-vasotocin: A new powerful radioligand for oxytocin receptor-expressing tumors. Bussolati G, Chinol M, Chini B, Nacca A, Cassoni P, Paganelli G. Cancer Res 2001 Jun 1; 61 (11), pp. 4393–7.

Neoplasia/Pheochromocytoma/[Elevated Glucagon-Receptor Expression]
Des-[His$_1$Glu$_9$]Glucagon Amide/[^{125}I-Thyr$_{10}$]Glucagon

Cellular/Molecular Mechanism of Action: [^{125}I-$Thyr_{10}$] glucagon is a radiolabeled analogue of glucagon. Des-[His_1Glu_9] glucagon amide is a glucagon receptor antagonist.
Background Information: Pheochromocytoma lesions express glucagon receptor complexes at elevated membrane densities.
Application: Acquisition of potential diagnostic parameters for detecting conditions of pheochromocytoma. Des[His_1Glu_9]glucagon amide (GR-A) abolishes the secretory response to glucagon by pheochromocytomas.
Route of Administration: Experimental (*in vitro*).
Dosage: Experimental (*in vitro*).
Implementation: Evaluation of glucagon mRNA and glucagons receptor expression in pheochromocytoma lesions.

Human *Ex Vivo* Model: Pheochromocytoma lesions (n = 3 and n = 4 normal adrenal medullas).
Interpretation and Analysis: Glucagon-receptor mRNA can be detected by reverse transcription-polymerase chain reaction in human pheochromocytomas, but not in normal adrenal medullas. Quantitative autoradiography demonstrates the presence of abundant [^{125}I-$Thyr_{10}$]glucagon binding sites in pheochromocytomas, that can be displaced by both cold glucagon and the glucagon receptor antagonist Des-[His_1Glu_9]glucagon amide (GR-A). The adrenal medulla is weakly labeled, and binding is not displaced by GR-A. Glucagon enhanced epinephrine and norepinephrine release can be detected in pheochromocytoma slices at minimal and maximal effective concentrations of 10^{-8} M and 10^{-6} M respectively. Adrenomedullary slices display a weak catecholamine response only with 10^{-5} M glucagon.

GR-A abolishes pheochromocytoma secretory responses to glucagon, but this effect is not appreciated in normal adrenal medullas. Collectively, these findings indicate that human pheochromocytomas, but not the normal adrenal medulla, express glucagon receptors and possess a marked secretory response to glucagon, thereby providing the rationale to explain the specificity of the glucagon provocative test in the diagnosis of pheochromocytoma.

Human pheochromocytomas, but not adrenal medulla, express glucagon-receptor gene and possess an *in vitro* secretory response to glucagon. Albertin G, Aragona F, Gottardo L, Malendowicz LK, Nussdorfer GG. Peptides 2001 Apr; 22 (4), pp. 597–600.

Neoplasia/Reticulosarcoma: Potential Delivery Format
Carboxymethylpullulan:Doxobrubicin

Application: Potential application for the selective delivery of diagnostic pharmaceutical agents to neoplastic cell populations.
Route of Administration: Doxorubicin:carboxymethylpullulan conjugate (CMPul-FG-DXR) is administered intravascularly.
Dosage: Doxorubicin:carboxymethylpullulan conjugate (doxorubicin dose equivalent of 10 mg/kg).
Semi-Synthetic Methods: Conjugation of doxorubicin (DXR) to carboxymethylpullulan (CMPul) using a Phe-Gly spacer (CMPul-FG-DXR)

Implementation: Evaluation of the antitumor efficacy of CMPul-FG-DXR using murine tumor models compared to DXR alone.

Animal Model: Rat (Lewis lung carcinoma).

Animal Model: Mouse (M5076 reticulosarcoma and non-solid tumor P388 leukemia).

Interpretation and Analysis: Conjugated doxorubicin:carboxymethylpullulan exhibits higher antitumor efficacy against Lewis lung carcinoma than DXR. Complete tumor regression followed by long-term tumor-free survival is frequently observed when CMPul-FG-DXR is administered intravenously 3 times at a dose equivalent to 10 mg/kg of DXR. Superior survival as well as antimetastatic effect of CMPul-FG-DXR in comparison with DXR is also evident in the M5076 murine reticulosarcoma model. Body weight loss in mice treated with the conjugate is less than that observed with doxorubicin treatment indicating lower systemic toxicity of CMPul-FG-DXR. Simply mixing CMPul with doxorubicin does not enhance the antitumor activity of doxorubicin, showing that the conjugation of DXR with CMPul improves antitumor activity. However, no enhanced antitumor efficacy of the conjugates can be appreciated against certain non-solid tumor models (e.g., P388 leukemia). The improved antitumor efficacy and reduced systemic toxicity of CMPul-FG-DXR has been effectively demonstrated. CMPul-FG-DXR may be a useful delivery platform for diagnostic pharmaceutical and biopharmaceutical agents in addition to chemotherapeutic agents with biological activity against solid tumors and metastases

Improved *in vivo* antitumor efficacy and reduced systemic toxicity of carboxymethylpullulan-peptide-doxorubicin conjugates. Nogusa H, Hamana H, Uchida N, Maekawa R, Yoshioka T. Jpn J Cancer Res 2000 Dec; 91 (12), pp. 1333–8.

Neoplasia/Somatostatin Receptors/Lymphoma and Mammary Carcinoma
Somatostatin Receptor-Avid Peptide-[Fluoroscein]/ Somatostatin Receptor-Avid Peptide-[Carbocyanine]/ Fluorescein-Bombesin/Carbocyanine-Bombesin

Cellular/Molecular Mechanism of Action: Somatostatin receptor avid peptides and bombesin peptide recognize and possess binding avidity for somatostatin receptor complexes.

Background Information: Site-specific delivery of drugs and contrast agents for tumors protects normal tissues from the cytotoxic effects of drugs and enhances the contrast between normal and pathologic tissues. One approach to achieving targeting selectivity is to direct delivery platforms at receptor complexes overexpressed on the exterior membranes of tumor cells to facilitate visualization by a non-invasive optical imaging methods.

Application: Detection and characterization of neoplastic lesions that express relatively high densities of membrane-associated somatostatin receptor complexes.

Route of Administration: Intravascular.

Implementation: Evaluation of the *in vitro* and *in vivo* characteristics of carbocyanine derivatives of somatostatin and bombesin analogues related to their binding avidity for their respective receptor complexes.

Animal Model: Rats (bearing somatostatin receptor positive and bombesin receptor positive tumors).

Semi-Synthetic Methods: The tracer dyes fluorescein and carbocyanine can be conjugated to somatostatin and bombesin receptor-avid peptides to examine their receptor binding affinities. Dual-imaging probes can also be synthesized consisting of a bioactive peptide for tumor targeting, a biocompatible dye for optical imaging, and a radioactive or paramagnetic metal chelator for scintigraphic or magnetic resonance imaging of tumors.

Interpretation and Analysis: Carbocyanine derivatives of somatostatin and bombesin analogues retain high binding avidity for their respective receptors. Further evaluation of representative molecules in rats bearing somatostatin receptor and bombesin receptor positive tumors show selective uptake of the agents by the tumor cells. Unlike carbocyanine derivatives, receptor binding of fluorescein-somatostatin peptide conjugates is highly sensitive to the type of linker and the site of fluorescein attachment on the non-receptor binding region of the peptide. In general, the presence of flexible linkers disrupts binding affinity, possibly due to the interaction of the linker's thiourea group with the peptide's cyclic disulfide bond. While the receptor binding affinity of the dual probe is not dependent on the type of chelating group utilized, it is affected by the relative positions of fluorescein and chelator on the lysine linker. For somatostatin compounds, best results are obtained when the chelator is on the alpha-amino lysine linker and fluorescein is on the epsilon-amino group. In contrast, conjugation of the chelator to epsilon- and fluorescein to the alpha-amino lysine linker of bombesin peptides results in high receptor binding. These findings indicate that despite their small size, conjugation of dyes to truncated somatostatin and bombesin peptide analogues results in the generation of promising diagnostic agents that retain high *in vitro* receptor binding activity. These contrast agents can selectively and specifically localize in receptor-positive tumors in rat models.

Synthesis, *in vitro* receptor binding, and *in vivo* evaluation of fluorescein and carbocyanine peptide-based optical contrast agents. Achilefu S, Jimenez HN, Dorshow RB, Bugaj JE, Webb EG, Wilhelm RR, Rajagopalan R, Johler J, Erion JL. J Med Chem 2002 May 9; 45 (10), pp. 2003–15.

Neoplasia/Somatostatin Receptors: Subtype 2 (sst2)/Pituitary/Pancreatic/Adrenal
[^{111}Indium] ([^{111}In])-(DTPA0,Tyr$_3$)-Octreotide/ [^{111}In]-(DOTA0,Tyr$_3$)-Octreotide/ [^{111}In]-(DTPA0)-Octreotide

Cellular/Molecular Mechanism of Action: Possess physical binding avidity for subtype 2 somatostatin receptor (sst2) complexes.

Application: Detection and characterization of pituitary, pancreatic, and adrenal neoplastic lesions that express an overabundance of sst2 complexes.

Implementation: Evaluate the potential usefulness of radiolabeled [DTPA0,Tyr$_3$]octreotide and [DOTA0,Tyr$_3$]octreotide as radiopharmaceuticals for somatostatin receptor-targeted scintigraphy and radiotherapy. Analyses involve the characterization of *in vitro* somatostatin receptor binding complemented by the determination of *in vivo* patterns of metabolism of agents compared to [^{111}In]-DTPA0-octreotide.

Animal Model: Murine (mouse AtT20 pituitary tumor membranes).

Animal Model: Rats (CA20948 tumor-bearing Lewis rats).

Interpretation and Analysis: Comparing the different peptide-chelator constructs reveals that [DTPA0,Tyr$_3$]-octreotide and [DOTA0,Tyr$_3$]octreotide have a higher affinity than [DTPA0] octreotide for subtype 2 somatostatin receptors (sst2) in preparations of mouse AtT20 pituitary tumor cell membranes (all IC_{50} values obtained are in the low nanomolar range). Studies *in vivo* in CA20948 tumor-bearing Lewis rats reveal a significantly higher uptake of both [^{111}In]-labeled [DOTA0,Tyr$_3$]octreotide and [DTPA0,Tyr$_3$]octreotide in sst2-expressing tissues than after the injection of [^{111}In]-(DTPA0)octreotide. Such observations suggest that substitution of Tyr for Phe at position 3 in octreotide results in

increased affinity for its receptor and in a higher target tissue uptake. Uptake of [^{111}In]-labeled [DTPA0]octreotide, [DTPA0,Tyr$_3$]octreotide, and [DOTA0,Tyr$_3$]octreotide in pituitary, pancreas, adrenals, and tumors is decreased to less than 7% of control by pretreatment with unlabeled octreotide (0.5 mg/rat), indicating specific binding to sst2 complexes. Comparing different radionuclides, [^{90}Y]-(DOTA0, Tyr$_3$)-octreotide has the highest uptake in sst2-positive organs, followed by [^{111}In]-(DOTA0,Tyr$_3$)-octreotide, whereas (DOTA0,[^{125}I]-Tyr$_3$)-octreotide uptake is low compared to that of the other radiobiopharmaceuticals, when measured 24 hours after injection. Renal uptake of [^{111}In]-labeled (DTPA0)-octreotide, (DTPA0,Tyr$_3$)-octreotide and (DOTA0,Tyr$_3$)-octreotide is reduced over 50% by intravenous injection of D-lysine (400 mg/kg), whereas radioactivity in blood, pancreas, and adrenals is not altered.

Pre-clinical comparison of [DTPA0] octreotide, [DTPA0,Tyr$_3$] octreotide and [DOTA0,Tyr$_3$] octreotide as carriers for somatostatin receptor-targeted scintigraphy and radionuclide therapy. De Jong M, Bakker WH, Breeman WA, Bernard BF, Hofland LJ, Visser TJ, Srinivasan A, Schmidt M, Béhé M, Mäcke HR, Krenning EP. Int J Cancer 1998 Jan 30; 75 (3), pp. 406–11.

Neoplasia/Thyroid Carcinoma/Thyroid Medullary Carcinoma/Lung Carcinoma: Small Cell/ Cholecystokinin B (CCK-B) Receptor Complexes
[^{131}I]-Labeled Gastrin-I

Cellular/Molecular Mechanism of Action: Radiolabeled [^{131}I]-human heptadecapeptide gastrin-I functions as CCK-B receptor binding ligand.
Background Information: The outstanding sensitivity of pentagastrin in detecting the presence of primary, recurrent or metastatic medullary thyroid cancer (MTC) suggests widespread expression of the corresponding receptor type in human MTC. Indeed, autoradiographic studies have demonstrated the presence of CCK-B or gastrin receptors not only in more than 90% of MTCs but also in a high percentage of small cell lung cancers, stromal ovarian cancers, astrocytomas, and several other tumor cell types.
Application: Detection and characterization of metastatic thyroid carcinoma lesions.
Route of Administration: [^{131}I]-labeled gastrin-I (intravenous).
Dosage: [^{131}I]-labeled human heptadecapeptide gastrin-I (1 μg).
Implementation: Evaluate the *in vivo* potential of radiolabeled [^{131}I]-gastrin I to target CCK-B receptor-expressing tumors assessed by determining the biodistribution profiles in conditions of metastatic MTC.

Animal Model: Murine (nude mice bearing subcutaneous xenografts of the human MTC cell line, TT).

Human Model: Cases of metastatic medullary thyroid carcinoma.
Interpretation and Analysis: At peptide amounts of approximately 1 μg, maximum tumor uptake (8.9 ± 2.9%ID/g) is observed in animals at 1 hour postinjection, with tumor-to-blood ratios as high as 6.3 ± 1.9. Physiological CCK-B receptors in the stomach, gallbladder, and pancreas of the mice are targeted as well. The major route of excretion is the kidney, but strong evidence for a biliary excretion pathway also exists. Pilot therapy studies with [^{131}I]-labeled gastrin show significant antitumor efficacy compared to untreated reference controls. In accordance with preclinical data, good receptor targeting is observed at tumor sites within the stomach, gallbladder, and pancreas of cases with metastatic MTC. Such observations suggest that gastrin and its analogues may represent a useful new class of receptor binding peptides for the diagnosis and therapy of a variety of tumor types, including MTC and small cell lung cancer. Related preclinical and clinical studies need to be performed to address in more detail the molecular features that render CCK-B receptor binding agents potentially useful candidates for *in vivo* scintigraphy and radionuclide therapy.

Targeting of cholecystokinin-B/gastrin receptors *in vivo*: Preclinical and initial clinical evaluation of the diagnostic and therapeutic potential of radiolabelled gastrin. Behr TM, Jenner N, Radetzky S, Béhe M, Gratz S, Yücekent S, Raue F, Becker W. Eur J Nucl Med 1998 Apr; 25 (4), pp. 424–30.

Neoplasia/Vascular Tumor Antigens/Prostatic Carcinoma/Prostate-Specific Membrane Antigen/ Glutamyl Carboxypeptidase/Endothelial Tumor Antigen
Prostate Specific Membrane Antigens (PSMA)/ PSMA Catalytic Site Inhibitor/Soluble Extracellular Tissue Factor (PSMA Catalytic Site)

Cellular/Molecular Mechanism of Action: Prostate-specific membrane antigen is a glutamyl-preferring carboxypeptidase.
Background Information: Prostate-specific membrane antigen is a glutamyl-preferring carboxypeptidase that is expressed by prostatic carcinoma and other carcinoma cell types present on both tumor cells and associated microvascular cells. It has been shown that the channel structures delineated by PSMA-expressing cells in human and rat prostate tumors are in functional continuity with the vasculature and thus form part of the tumor microvasculature network. The PSMA-positive cell-outlined channels are CD31 negative and mutually exclusive of CD31-positive cell-lined channels elsewhere in the tumor consistent with tumor cells adapted to a pseudoendothelial phenotype in vasculogenic mimicry.
Application: Molecular strategy for selectively targeting the microvasculature network of neoplastic masses (e.g., prostate specific membrane antigen catalytic site inhibitor).
Route of Administration: Intravascular.
Implementation: Evaluate the potential function attributes of (i) PSMA-lined microvasculature to serve as a selective *in vivo* target for diagnostic and therapeutic modalities; and (ii) selectively directed infarctive tumor therapy in the form of a soluble extracellular domain of tissue factor conjugated to a PSMA catalytic site inhibitor to create a PSMA-directed selective tumor vascular thrombogen (STVT).
Interpretation and Analysis: The semi-synthetic protein conjugate, PSMA-STVT induces selective local *in vivo* infarctive necrosis of the rat Mat Lu prostate tumor when administered intravenously. The combined administration of this STVT with low-dose doxorubicin produces a profound tumoricidal effect, resulting in complete eradication of some tumors. This is consistent with the therapeutic potential for a PSMA-directed STVT and expands the potential for selective infarctive ablation of tumors.

Prostate-specific membrane antigen directed selective thrombotic infarction of tumors. Liu C, Huang H, Doñate F, Dickinson C, Santucci R, El-Sheikh A, Vessella R, Edgington TS. Cancer Res 2002 Oct 1; 62 (19), pp. 5470–5.

Protein and Peptide Selective Delivery/ Example Application: Synovial Joint Structures
"Homing" Peptides

Cellular/Molecular Mechanism of Action: Selective "homing" peptide specific for synovial joint structures.
Application: Potentially can function as a "targeting" modality for selective delivery of agents to synovial joint structures.
Route of Administration: Intravenous (most frequently because they are protein/peptide compounds).

Dosage: Designed to directly establish a correlation between the specific biological activity (efficacy) of aptamer preparations and the corresponding pathological condition.
Biological Source and Production: Initial preparations entail the harvest of human synovium and skin samples that are then transplanted into severe combined immunodeficient (SCID) mice. A disulfide-constrained 7-amino acid peptide phage display library is then injected intravenously into the animals and synovial homing phage recovered from synovial grafts.
Considerations: Following 3–4 cycles of enrichment, DNA sequencing of homing phage clones allow the identification of specific peptides synthesized by a-fluorenylmethyloxycarbonyl chemistry and used in competitive *in vivo* assays and immunohistochemistry analyses.
Interpretation and Analysis: Isolated synovial homing phages can be produced that display specific peptides that bind to synovial but not skin or mouse microvascular endothelium (MVE). In an *in vivo* environment, they retained their tissue homing specificity independently from the phage component, the original pathology of the transplanted tissue, and the degree of human/murine graft vascularization. One such peptide (CKSTHDRLC) in capable of maintaining synovial homing specificity both when presented by the phage, and as a free synthetic peptide. The synthetic peptide also competes with and inhibits *in vivo* binding of the parent phage to the cognate synovial MVE ligand. Experimental findings of this nature represents a description of peptides with homing properties specific for human synovial MVE. The identification of such peptides opens the possibility of using these sequences to construct joint-specific drug delivery systems that may have considerable impact in the diagnosis and treatment of arthritic conditions.

Identification of synovium-specific homing *peptides by in vivo* phage display selection. Lee L, Buckley C, Blades MC, Panayi G, George AJ, Pitzalis C. Arthritis Rheum 2002 Aug; 46 (8), pp. 2109–20.

Renal/Proximal Tubule/Enhanced Proximal Renal Tubule Catabolization
Lysozyme Low Molecular Weight Protein (LMWP)/ Naproxen-Lysozyme Conjugate

Cellular/Molecular Mechanism of Action: Lysozyme is an LMWP that is mainly taken up and catabolized by the renal proximal tubule.
Background Information: A renal-specific controlled release of an active drug may enable a reduction of the required dose and serve to reduce extrarenal toxicity.
Semi-Synthetic Methods: Production of a conjugate with an average coupling degree of 2 mol naproxen per 1 mol lysozyme in which the drug is directly coupled to the protein via a peptide bond.
Implementation: Evaluate the potential to specifically target the kidney applying the LMWP lysozyme as a carrier (e.g., delivery of the non-steroidal anti-inflammatory drug [NSAID] naproxen) because it is mainly taken up and catabolized within the renal proximal tubule.
Interpretation and Analysis: Naproxen-lysozyme conjugates are predominantly and rapidly ($<$ 20 minutes) taken up by the kidney. Subsequent decreases in renal content reflect renal degradation of conjugated lysozyme and appear to be similar to that of native lysozyme with a half-life of 4 hours. Biodistribution analysis of naproxen-lysozyme reveals no detectable amounts of free naproxen present in the plasma after the administration of the conjugate in concert with the objective of reducing extrarenal side effects. Conjugation of naproxen to lysozyme results in a pronounced (70-fold) increase of naproxen accumulation within the kidney. In agreement with protein disposition studies, conjugates are rapidly taken up by the kidney and subsequently degraded. Renal selective targeting of the NSAID naproxen can be achieved by conjugation with the LMWP lysozyme. This strategy for renal drug delivery has the potential to improve drug efficacy and safety.

Drug-targeting to the kidney: Renal delivery and degradation of a naproxen-lysozyme conjugate *in vivo*. Haas M, Kluppel AC, Wartna ES, Moolenaar F, Meijer DK, de Jong PE, de Zeeuw D. Kidney Int 1997 Dec; 52 (6), pp. 1693–9.

MECHANICAL PLATFORMS

Ophthalmic Delivery: Mechanical/Ocular and Conjunctival Pharmaceutical Delivery
Ocular Inserts

Cellular/Molecular Mechanism of Action: Mechanical pharmaceutical delivery format.
Background Information: Reservoir-type ocular inserts can be fabricated using sodium alginate containing ciprofloxacin hydrochloride as the core (drug reservoir) sandwiched between Eudragit and/or polyvinylacetate films. Ocular inserts are packaged in aluminum foil and can be sterilized by gamma radiation.
Implementation: Ocular inserts can potentially function as a delivery modality for ciprofloxacin hydrochloride that is dependent upon *in vitro* rate of release, antimicrobial efficacy, *in vivo* release studies, efficacy against induced bacterial conjunctivitis, aqueous humor concentrations, and stability studies as per the International Conference on Harmonization (ICH) guidelines. Preparations can be tested for sterility according to British Pharmacopoeia (BP) guidelines.
Animal Model: Rabbit (bacterial conjunctivitis).
Interpretation and Analysis: Sterile ocular inserts display zero-order release of test pharmaceuticals in *in vitro* and *in vivo* release studies over a period of 120 hours. Pharmaceuticals are found to be active against selected microorganisms according to microbial efficacy trials. A high correlation coefficient exists between *in vitro* and *in vivo* pharmaceutical release rates. Significantly better improvement in artificially-induced bacterial conjunctivitis can be appreciated with ocular inserts compared to eye drops and reference control placebo preparations. Drug concentrations in the aqueous humor are above minimum inhibitory concentrations (MIC-90) against test microorganisms. Shelf life of the product is greater than 2 years.

Ophthalmic delivery of ciprofloxacin hydrochloride from different polymer formulations: *In vitro* and *in vivo* studies. Charoo NA, Kohli K, Ali A, Anwer A. Drug Dev Ind Pharm 2003 Feb; 29 (2), pp. 215–21.

Respiratory System/Aerosolizer
SmartMist

Cellular/Molecular Mechanism of Action: Device creates a fine mist that upon inhalation can be delivered directly into the farthest reaches of the pulmonary respiratory network (e.g., alveoli).
Application: Delivery of diagnostic or therapeutic pharmaceuticals to the respiratory tree.
Route of Administration: Pulmonary inhalation.
Dosage: Predetermined for each individual agent being administered by pulmonary inhalation.
Considerations: Non-compliance with prescribed medication is a major reason for poor therapeutic outcomes, leading to the generation of unnecessary health care costs. Poor technique in self-admin-

istration of inhalation therapy is a special type of non-compliance associated with this route of administration. However, pulmonary drug delivery has fundamental advantages for the therapeutic management of diseases affecting the respiratory tract because it is site directed. The lung is also a promising portal for drug delivery into the systemic circulation.

Interpretation and Analysis: The incorporation of microprocessors into pulmonary drug delivery systems can facilitate the implementation of sophisticated management schemes for patient compliance in conditions of chronic diseases including asthma and diabetes. Microprocessor-assisted systems afford control over administration technique during therapeutic inhalation, thus leading to efficient and reproducible regional deposition of inhaled diagnostic or therapeutic agents. SmartMist is a hand-held asthma management device that aids in optimizing the clinical use of metered dose inhalers. It also measures pulmonary lung function and provides a long-term downloadable electronic record of therapeutic and diagnostic events. The AERx pulmonary delivery system utilizes similar microprocessor capabilities; however, it employs a novel means of generating aqueous aerosols from unit dose packages, thus providing a broad inhalation technology base for delivery of a wide variety of therapeutic and diagnostic agents into the respiratory tract, and via the lung into the systemic circulation.

Inhalation delivery systems with compliance and disease management capabilities. Gonda I, Schuster JA, Rubsamen RM, Lloyd P, Cipolla D, Farr SJ. J Control Release 1998 Apr 30; 53 (1–3), pp. 269–74.

Respiratory System/Aerosol Delivery System
AERx

Background Information: AERx is a microprocessor-controlled, bolus inhalation device that is actuated by preprogrammed stimuli (e.g., inspiratory flow-rate and volume), resulting in the creation of a fine mist that upon inhalation is delivered directly into the farthest reaches of the pulmonary respiratory network (e.g., alveoli).

Application: Delivery of diagnostic or therapeutic pharmaceuticals within the respiratory tree. Example: Deposition of [^{99m}technetium]-diethylenetriamine penta-acetic acid ([^{99m}Tc]-DTPA) aqueous radioaerosols to faciliate *in vivo* (human) planar and single photon emission computed tomography (SPECT) imaging.

Route of Administration: Pulmonary inhalation.

Dosage: Predetermined for each indivudal agents being administered by pulmonary inhalation.

Considerations: AERx is a microprocessor-controlled, bolus inhalation device that is actuated at preprogrammed values of inspiratory flow rate and volume. Transmission-emission planar and tomographic images can be simultaneously acquired. Diffuse deposition of the aerosol in the lung can be appreciated using this delivery modality. The uniformity of radioisotope distributed throughout the lung is attributed to the fine particle size (mass median aerodynamic diameter of 2 μm) of the aerosol and the electronic control of aerosol inhalation by the device.

Interpretation and Analysis: Evaluation of 2 postural positions (supine vs sitting) and 2 inspiratory maneuvers (vital capacity [VC] vs "fixed volume" above functional residual capacity) on radioaerosol inhalation reveals that it is largely unaffected by inhaled volume and posture. The efficiency of the device can limit the total radiation exposure of patients and staff administering the radioaerosols, and can make it suitable for delivery of expensive drugs. Neither the breathing maneuver (inhaled respiratory volume), nor posture affects the distribution of the aerosol as measured by the ratio of the activity (counts per pixel) in the peripheral:central (penetration index, PI) or in the apex:base regions of the planar lung images ($p > 0.1$). A small, albeit statistically significant, difference in PI ($p < 0.03$) can be appreciated between VC and fixed volume sitting maneuvers with SPECT only. The PI values themselves indicate that the radioaerosol is well distributed in the lung, with the periphery having 45%-64% of the activity of that counted in the central region. Superposition of transmission SPECT lung outline on emission SPECT visually confirms the excellent peripheral deposition of the aerosol. The AERx system shows high efficiency of delivery, with approximately 50% of the extruded dose in the device deposited in the lung.

Comparison of *in vitro* and *in vivo* efficiencies of a novel unit-dose liquid aerosol generator and a pressurized metered dose inhaler. Farr SJ, Warren SJ, Lloyd P, Okikawa JK, Schuster JA, Rowe AM, Rubsamen RM, Taylor G. Int J Pharm 2000 Mar 30; 198 (1), pp. 63–70.

Respiratory System/Nebulization Strategies

Dosage: Development and use of inhalation aerosols requires an appreciation of the properties of these dynamic systems and the ways in which they interact with the anatomically and pathophysiologically complex human respiratory tract. The amounts of aerosol particles, or droplets, initially deposited at various sites in the respiratory tract will determine the intensity of local effects as well as the rate of clearance of these materials from the respiratory lumen. Activity can be modulated by suitable formulation modifications, such as presentation of the active agent in a slow releasing form carrier.

Considerations: Inhaled diagnostic aerosols are examples of mechanical targeting delivery systems that achieve high concentration in the respiratory tract (the usual target organ with these systems) while appearing in low concentrations in other parts of the human body.

Interpretation and Analysis: Extension of duration of action of inhaled drugs by such means could be possible. As more discriminating methods for location of receptors and for following the fate of inhaled drugs and their carriers are becoming available, the ultimate potential for improvement of selective toxicity of agents used in pulmonary therapy by direct delivery into the respiratory tract can be critically evaluated.

Note: If excess mucus is preventing delivery of depostion, nebulization of a mildly alkalotic aqueous solution helps dissipate mucus deposits thereby facilitating removal by the tracheal mucocilliary escalator and expectoration.

Index

A
A23187, 65–66, 254, 325
A-278637, 356
A-304121, 69–70
A-317920, 69–70
Abdominal aorta aneurysm (AAA), 19–20, 259
Abdominal imaging
  magnetic resonance imaging (MRI), 544–545
  ultrasound, 791–792
Abeta nanoparticles, 532–533
Abscess, 439–441, 611–614, 760–761, 791–792, 842
Aceclidine, 66
ACE inhibitors, 323
Acepromazine, 118
Acetaldehyde, 387, 396–397
Acetaminophen, 184–185, 187, 202, 327
Acetazolamide, 48, 113, 114, 344, 462, 705
Acetic acid, 351, 515
Acetylated low-density lipoprotein (AcLDL), 576
Acetylator polymorphism, 298–299
Acetylcholine (ACh)
  atherosclerotic plaque deformation, 51–52, 774
  Brugada syndrome, 8–9
  COPD, 381
  coronary artery disease, 8–9, 21–22, 23–24, 50–51
  left ventricular hypertrophy, 32
  vascular grafts, 44, 49
Acetylcholine receptors
  muscarinic, 66, 677–678, 685–686, 873
  nicotinic, 63–64, 874
Acetylcholinesterase, 260, 678
Acetylcysteine, 739
Acetylsalicylic acid (ASA), 258
Achlasia, 181
Achlorhydria, 217
Acipimox, 57, 158, 683, 684
Acivicin, 455
AcoHSA, 428–429, 828
Acoustic lability, 768
Acridine orange, 20, 125–126, 478, 481–482
Acromegaly, 150–151, 460–461
$ACTH_{1-24}$, 63, 142–143
Actinomycin-D, 397–398
Acute renal failure, 355, 363–364, 513
Acute respiratory distress syndrome (ARDS), 450–451, 670–671
Acylovir, 289
Addison′s disease, 134
Ademetionine, 70, 72, 79
Adenocarcinoma, 425, 505–506, 653, 727–728, 882–883, 900–901
Adenosine
  arterial resistance and dilation, 114
  atrial flutter, 5
  coronary artery function, 48, 775
  left ventricular hypertrophy, 32
  myocardial perfusion evaluation, 58, 681–682
  single photon emission computed tomography (SPECT), 55, 679
  stress echocardiography, 786
  tachycardia, 6–7, 14–15
  vascular perfusion evaluation, 44, 114
Adenosine A2A receptor complexes, 758
Adenosine diphosphate (ADP), 19–20, 256–259
Adenosine monophosphate (AMP), 387, 396
Adenosine receptor antagonist ligand, 61
Adenosine triphosphate (ATP), 5, 14–15, 32, 106, 356, 370, 470, 515
Adenoviral vector delivery, 430, 812, 820, 831
Adenovirus-expressing firefly luciferase, 18, 435
Adhesion molecule expression, 528
Adrenal gland
  cortex hypertrophy, 603–604
  function and structure, 604, 626, 677
  hypothalamus, pituitary, and adrenal axis, 134–150
  incidentaloma, 142–143, 147, 415–416
  insufficiency, 134–136
  neoplasia, 626–627, 661–662
Adrenergic β-blocker therapy response, 322, 578, 587
Adrenergic β-receptor downregulation, 588, 603
Adrenergic neurotransmitters, 246, 314–315
Adrenergic receptors, 579, 588, 600–602, 603, 680, 685, 869
Adrenocortical tumors, 136, 137, 143
Adrenocorticotropic hormone (ACTH), 89–90, 132, 134, 136–146, 166, 296
Adrenocorticotropic hormone (ACTH) stimulation test, 139, 144, 415–416
Adrenocorticotropin-releasing hormone, 132, 146
Adrenomedullary phenylethanolamin N-methyltransferase, 144
Adult respiratory distress syndrome (ARDS), 669
Adverse reactions
  computed tomography (CT) image-enhancing agents, 750–751
  magnetic resonance imaging (MRI) contrast agents, 518–521
  radiographic image-enhancing agents, 731–741, 766
Aerosolizer, 904–905
Aerosomes, 772, 847, 871
AERx, 905
AF0150, 773
Aflatoxin B1, 297
Agitated colloid, 788
Aglycemia, 455
AGT, 722
AIDS, 209, 446
Air microbubbles, 850
Ajmaline, 9
Akinesis, myocardial, 780–781, 784
Alanine, 275
Albumin
  air-filled microbubbles, 783
  BSA nanospheres, 865
  clearance, 360–361
  microspheres, 856
  octafluoropropane-filled microbubbles, 783
Albumin, labeled, 204–205, 219–220, 223, 360–361
  aconitylated, 428–429
  [^{125}I]-HSA, 442, 708
  indotricarbocyanine (ITCC)-human serum albumin, 219–220, 413, 500–501
  [In]-DTPA, 439, 609, 614
  intravascular compartment volume estimation, 39–40, 473–474
  kupffer cell phagocytic function, 793–794
  [^{99m}Tc]-diethylenetriamine pentaacetic acid (DTPA)-galactosyl human serum albumin, 609, 706–707
  [^{99m}Tc]-human serum albumin, 422, 442, 493, 589–590, 616–617, 640

Albumin, labeled, (*continued*)
[^{99m}Tc]-macroaggregated albumin (MAA), 593, 671–673, 756
[^{99m}Tc]-rat serum albumin, 450–451, 670–671
Albumin-bound [Gd]-based media, 525
Albumin-coated cationic lipoplexes, 428–429
Albumin/dextrose/deca-fluorobutane, 776
Albuterol, 382
AlCAM-1, 450–451
Alcohol dehydrogenase, 66
Alcoholic detoxification seizures, 65, 82, 100
Alcohol-induced cirrhosis, 279
Alcohol-induced hepatitis, 279–280
Alcoholism, 64–66, 325–326
Alcohol sensitivity, 325
Aldosterone-producing adenoma (APA), 135, 416
Alfentanil, 307–309
Alkaline phosphatase, heat-resistant isoenzyme, 139
Alkaloids, 23
Allergen challenge test, 228, 237–238, 395–396
Allergen sensitivity, 376–377
Allergic encephalitis, 621–622, 812–813, 889
Allergic rhinitis, 237, 264, 383, 385, 396–397
Allergy, 228–229, 233
All-trans-retinoic acid (ATRA), 838
Alpha 1-adrenoceptor, 871
α-ketoisocaproic acid (KIC), 467
α-ketoisovaleric acid (KIV), 183, 275, 276, 379, 399, 402, 459, 467
α-naphthoflavon, 305–306, 313
Alpha(V)beta$_3$ integrin, 246, 528, 529
Alprazolam, 306–307
Altitude, effect on cardiopulmonary function, 36
Altropane, 694–695
Aluminum body stores, 496, 609
Aluminum phthalocyanine, 123–124, 507
Alveolar macrophages, 833
Alzheimer's disease (AD), 66–70, 314, 468–469, 482–484, 532–533, 570, 595–597, 686–689, 758
Amantadine, 93
Amenorrhea, 164
Amino acids
[^{14}C]-labeled, 277
liposomes, 830–831
metabolism and neoplasia, 665, 728
Aminoalkyl methacrylate copolymer RS, 877
2-aminoanthracene (2-AA), 311–312
Aminohippurate sodium, 351, 514
5-aminolevulinic acid (5-ALA), 180, 370, 413–414, 423, 499–500, 500
Aminolevulinic acid methyl ester (AME), 370, 423, 500
Aminophylline, 240, 394
4-aminopyridine, 62–63, 347
Aminotamoxifen, 497
Amiodarone-induced pulmonary toxicity (AIPT), 669–670
Amitriptyline, 72, 104, 281–282, 313–314
Ammonium chloride, 341–342, 364–366
Amniotic fluid volume estimation, 351, 514
Amobarbital
epilepsy, 84–86, 597–598, 698
pharmacogenetics, 282
seizures, 101, 698
vascular malformation, 43, 112–113
AMP579, 28, 581
Amphetamine, 75, 96, 110, 126
Amylin, 184–185
Amyloid, 66–68, 468–469, 482–484
computed tomography, 758
delivery modalities and, 871–872, 883–884
magnetic resonance imaging (MRI), 532–533
nuclear planar scintigraphy and, 569–571, 595–597
positron emission tomography (PET), 687–689
Amyloid angiopathy, 596
Amyloid P component, [^{123}I]-labeled, 570–571, 597
Amylose-ethylcellulose-coated hydroxypropylmethylcellulose, 877
Amytal, 95–96, 122, 123
Anaphylaxis, 233–234, 481
Androgen receptor gene, 328–329
Anemia, 221–222, 260, 425
Aneurysm, 770
Angina/angina pectoris, 21–26, 29–30
acetylcholine (ACh), 8–9, 21–24
atropine, 29
cardiovascular response tests, 47–51
coronary artery spasm, 21–24
differential diagnosis with esophageal spasm, 22, 29–30
edrophonium chloride, 29
ergonovine maleate, 8–9, 21–25, 29–30, 50–51, 678
esophagitis/gastroesophageal reflux disease (GERD), 30
molsidomine, 23
nitroglycerin, 23–24
Prinzmental's variant angina, 23–26
procainamide, 9–10
scintigraphy, 575, 577, 583–584
single photon emission computed tomography (SPECT), 681
thoracic pain of non-cardiac origin, 29–30
ultrasound, 769
Angiogenesis, 36–37, 407, 473, 506
computed tomography, 752
delivery modalities, 858–860, 866
magnetic resonance imaging (MRI), 528–529, 543–544, 558–560
positron emission tomography (PET), 726
Angiography, 123–124, 507
adverse reactions, 739, 740
aortic stenosis, 51
choriocapillaris, 484
computed tomography, 752–757
fluoroscopy, 764
laser targeted, 825
magnetic resonance imaging (MRI), 523, 526, 529–530
positron emission tomography (PET), 678–679
radiography, 731, 742–747
ultrasound, 769–772, 788–789, 792, 794–795
vascular graft evaluation, 49
vascular malformations, 42–43
Angioplasty
magnetic resonance imaging (MRI), 527
percutaneous transluminal coronary angioplasty (PTCA) therapy, 581
Angiotensin-coverting enzyme (ACE), 322–323
Angiotensin II, 65, 575–576
Anhydrosis, 337
Anmmonia tolerance test, 277–278
Annexin V, 407–408, 456–457, 504, 507, 572–573, 627
Anoxia, 455, 470
ANS fluorescent probe, 493–494, 844
Anterior pituitary function, 130–131, 133
Anti-apoptotic transgenes, 818
Antiarrhythmics, 300
Anticarcinoembryonic antigen (anti-CEA), 717
Anti-CD15 IgM, 447
Anticoagulant properties of contrast agents, 734
Antidepressants, 70, 79, 285, 301
Antidisialoganglioside, 431, 838–839
Antidiuretic hormone, 363, 368
Anti-DTPA, 439
Anti-EGFR coated supermagnetic particles, 411–412, 557
Antifibrinogen-liposomes, 774, 822
Antifibroblast activation protein Mab, 892
Antiglioma Ki-67 antigen IgG validation, 630, 716
Anti ICAM-1, 428, 528, 534, 770–771, 828, 846
Anti-ICAM-1-liposomes, 774, 822, 825

[Anti ICAM]-paramagnetic liposomes (ACPL), 528, 534, 825, 885
Anti-IgE, 234–235, 390
Anti-Leu antibody, 243
Antimelanoma IgG, fluorescein isothiocyanate-labeled, 337, 422, 498
Anti-MUC1 mucin, 220, 413, 501, 502, 894
Antimyosin antibody, 26–27, 581
Antinoceptive morphine sensitivity, 326–327
Anti-PECAM, 884–885
Antiperistaltic agents, 759
Antiphage protein Fab, 893–894
Antiplatelet properties of contrast agents, 735
Antiplatelet therapy, 593–594, 814
Anti-P-selectin immunoglobulin, 125, 225–226
Antipyrine, 274–275, 278, 290–292, 314
Antisense cDNA, 333, 438
Antisense gene targeting, 435–436, 574–575, 598, 887–888
Antisense IGF-IR, 333, 438
Antisense nucleotide radiopharmaceuticals, 630
Antisense oligonucleotides, 97, 242, 420–421, 426, 428–430, 433, 436, 488, 510, 828, 838–839, 850
Antithrombin, 253
Antithrombotic properties of contrast agents, 735, 736
Anti-TNF-α, 316, 334–335, 343, 427–428
Antitransferrin receptor, 630, 887–888
Anti-vasopressin-associated neurophysin Mab, 896
Anuria, 355, 487–488
Aorta, ultrasound of, 771–773
Aortic stenosis, 51, 772–773
Aortic stent graft monitoring, 773
Aortic valve, 30–31, 772
Apomorphine, 75, 110
Apoptosis, 455–457, 504, 572–573, 582–583, 627, 818
Appendicitis, 446–447, 608–609, 616
Aprepitant, 689
Aptamers, 451
Arabian foals, 242
Arabino pyrimidine nucleosides, 649
Arachidonic acid, 382
Area X, 541–542
Arecoline, 66
Arginine, 152–154, 155–160
Arginine vasopressin (AVP), 94, 140–141, 230, 460
Arodyn, 888–889
Arrhythmia, 5–16, 576–577, 588, 679, 685, 772
Arterial phase computed tomography, 760
Arterial resistance and dilation, 114
Arteriography
  computed tomography, 753–754, 760
  magnetic resonance imaging (MRI), 530
  ultrasound, 772–777, 789, 792
Arteriovenous fistulas, 114
Arteriovenous malformations, 42–43, 70, 112–113, 743–744, 770
Arthrography, 552–553
Arthropathy, 569–570
Articular cartilage, 798
Arylamine N-acetyltransferase, 282
Aspirin, 224–225, 250–251
Aspirin-insensitive asthma, 234–235, 240–241, 384–386, 390–391
Asthma, 234–237, 240–241, 249, 264–265, 376–379, 383–397, 673
Astrocytoma, 629, 633–634, 713, 896–897
Atheroma, 774, 822
Atherosclerosis, 21, 342, 423, 466–467
  delivery modalities, 858, 863, 886
  magnetic resonance imaging (MRI), 528, 530–531
  scintigraphy, 576, 592
  ultrasound, 773, 774, 822
Atherosclerotic plaque deformation, 51–52, 774
Atopy, 247
Atopy patch test, 238–239, 337
Atrial flutter, 5
Atrioventricular (A-V) accessory conduction pathway, 5
Atrioventricular (A-V) block, 5–6
Atrioventricular nodal block induction, 6–7
Atrioventricular nodal reentrant tachycardia, 6–7
Atropine, 12–13, 29, 97, 122, 378
Attention deficit hyperactivity disorder (ADHD), 97, 326, 599
Autism, 70–71
Autoimmune disease, 229–233
  encephalitis, 528
  Graves' ophthalmology, 229
  inner ear disease, 705
  multiple sclerosis (MS), 229–230
  myasthenia gravis (MG), 230–232
  pancreatitis, 213
  rheumatoid arthritis, 232–233
  systemic lupus erythematosis (SLE), 233
  thrombocytopenia, 815
Autonomic nervous system, 61–62, 71, 600–603
Avidin, 842
Avidin-gelatin nanoparticles, 869
Axons, demyelinated, 533
Azathioprine, 94–95, 230, 282, 321

B
Balloon coronary occlusion, 586, 601–602
Barbiturate elimination, 282
Barium sulfate, 184, 518, 747–748
Basal cell carcinoma, 337, 421, 498–499
Basophil stimulation test, 234–235, 390
Bayesian methods, 281–282, 289, 313–314
Beclomethazone, 247, 249, 376–377
Bee-sting venom, 240
Behavior
  depression, 71–73
  modification, 73
  neuropsychological testing, 73
  opioid abstinence, 73–74
  panic disorder, 74
  pharmaco-electroencephalogram mapping, 74
  psychotropic side effects, 74
  schizophrenia, 74–75
  Tourette's syndrome, 75–76
Benzene, 293
Benzo-a-pyrene, 305–306
Benzoate, 356–357
Benzodiazepine receptor ligands, 76, 105
Benzomorphan, 873
Benzydamine, 312–313
Benzylpenicillin, 99, 329–330, 367
β-adrenergic blockers, 300, 322, 578, 587
Beta-amyloid precursor protein IgG, 110
Betadine, 343
β-galactosidase, 432–433, 534, 545, 818, 826, 830
11β-HSD2, 355, 459
11β-hydroxylase, 136, 142–143, 415–416
Beta-lactam antibiotics, 239
Beta-oxidation, 457–458, 707–708, 710–711
β_2-microglobulin, 358, 571
Bethanechol, 186, 189
Bicarbonate, 344, 365, 462
Bicuculline, 89, 105, 485
Bile acid transport, 268–269, 488–489
Biliary atresia, 267, 610
Biliary colic, 267, 792
Biliary dyskinesis, 267
Biliary excretion capacity, 489
Biomarker selective targeting, 37
Biotin, 630, 842, 864, 891–892

Bis (acetate), 522
Bisacodyl, 211
[bis-propanoic acid cyanine]-IgG, 408, 502–503
Bis-tetraazamacrocycle xylyl-bicyclam, 451–452
Bladder cancer, 370, 423, 500, 651, 890–891
Bladder contractions, 11–12, 370
Bladder contrast-enhanced voiding urosonography, 767
Bladder function, 76, 103–104, 370–372
Bleomycin, 615, 647–648, 649, 651, 670
Blood-brain barrier (BBB)
  diffusion, 533
  disruption, 629–630, 755
  penetration, 533, 858–859
  permeability, 533
Blood-brain cerebrospinal fluid barrier, 76
Blood flow, mismatch, 785
Blood oxygen level-dependent (BOLD), 533–534
Blood-pool agents, 526, 529–531, 539, 543, 558–559, 754–755, 822
Blood volume estimation, 473–474
BMIPP, 465, 581–582
Bolus tracking MRI, 541
Bombesin, 421, 423, 505–506, 604, 626–629, 636, 642, 677, 892–893, 897, 902
Bone
  erosion detection, 554
  growth, 341, 496
  scintigraphy, 622–625
Botulism, 62–63
Boyden chemotactic chamber, 253
BQ-123/BQ-788, 579–580, 667, 869–870
BR14, 786, 789–790
Brachial artery vasodilation, 774
Brain blood flow, 789
Brain parenchyma acoustic density, 789–790
Brain perfusion, 790–791
Brain reperfusion injury with ischemia, 531
Brain tumor, 533, 557–558, 629. *See also specific neoplasms*
Breath tests, 398–403
  hepatic system and, 276, 306, 308–310, 315, 379
  nuclear planar scintigraphy, 673–675
Brilliant blue microspheres, 495–496, 855
Brimonidine, 120, 866–867
Bromocriptine, 90, 94, 98, 133, 229–230, 233, 852
Bromophenol blue, 369, 513–514
Bromosulfophthalein (BSP), 269–272, 271, 277, 490
Bromothymol blue, 369, 513–514
Bronchial constriction response, 375–376
Bronchial hyperresponsiveness, 265
Bronchial provocation test, 389–390
Bronchospasm, 750–751
Brugada syndrome, 7–10, 576–577, 588, 678–679, 685
BSB (1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene), 68, 482–483
BT-PABA, 203, 212–216
Buccal mouthwash, 335
Budd-Chiari syndrome, 792
Budesonide, 389, 852
Bumetanide, 27, 355, 477, 523–524
Bupivicaine, 62, 342, 376
Bupropion, 91
Burst ultrasound, 789
Butyrylcholinesterase, 323

C
C5a, 234–235, 253–254, 390
[^{11}C]-acetate, 604, 626, 677
[^{13}C]-acetate breath test, 185, 191, 399–400
[^{11}C]-acryloyl chloride, 719
Cadmium hepatotoxic injury, 271, 490
Cadmium selenide-zinc sulfide quantum dots, 37, 474
Caffeine, 74, 77, 80
  cerebral perfusion, 533–534
  cytochrome 450 metabolism, 295–296, 305–306
  glomerular filtration, 360
  hepatic metabolism, 288
  malignant hyperthermia, 260–263, 330–332, 344–347, 426–427, 463
  microsomal enzyme metabolizing capacity, 291–292
  N-acetyltransferase, 316
  rhabdomyolysis, 348–349
Calcein, 843
CalceinAM, 16–17, 221, 407, 411, 476, 491, 501, 812, 893
Calcitonin, 170–172, 419
Calcium
  carcinoid syndrome, 410
  gastrinoma, 129, 412–413
  hepatic neoplasia, 280
  insulin (islet cell) adenoma, 130, 414, 464–465
  pancreatic disease, 216–218
  parathyroid hormone secretion, 177
  thyroid carcinoma, 171–172, 419
  VIPoma syndrome, 130, 415
Calcium analogs, MRI and, 541–542
Calcium binding peptides, 179
Calcium chloride, 65–66, 171–172, 325, 416–417
Calcium gluconate, 171–172, 176–177, 344, 414–417, 462
Calcium gluconolactate, 176
Calcium-induced calcium release (CICR), 260–261, 262, 332, 345
[^{14}C]-allantoin, 367
Calmodulin inhibitor, 15–16
[^{13}C]-aminopyrine, 276–277
CAMP, 177
$CaNa_2EDTA$, 569
Capillary bed tolerance of small particle or liquid plastic occlusion, 38
Capillary blood flow, 37, 41, 474, 479
Capillary permeability, 474
Captopril, 82, 89, 372–373, 459–460, 591–592, 668
[1-^{14}C]-arachidonate, 691, 710–711
Carbamazepine, 80–81, 87, 845
Carbamoyl-PROXYL, 531
Carbidopa, 716–717
Carbocyanine, 109–110, 421, 423, 505–506
Carbon breath tests, 315, 398–399
Carbon dioxide, 74
  breath tests, 315, 398–399, 458, 674–675
  [$^{13}CO_2$], exhaled, 279, 401–402
  [$^{14}CO_2$], exhaled, 276, 379, 399, 402
  malignant hyperthermia, 260, 330, 345
  microbubbles, 798, 848–849
  as radiographic image-enhancing agent, 742–747, 756–757, 764
Carbonic anhydrase deficiency, 458–459
Carbonic anhydrase isoenzyme inhibitors, 878
Carbon monoxide diffusing capacity, 378–379
Carborane [Gd]-DTPA2, 555
Carboxydextran-coated maghemite, 551–552
Carboxydextran iron-oxide nanoparticles, 551
Carboxyfluorescein diacetate succinimidyl ester, 16, 124, 125, 225–226, 476, 491–492
Carboxyfluorescein (encapsulated), 484, 825
Carboxylic acid metabolism, 183, 379, 399, 459
3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid, 367
Carboxymethylpullulan, 901–902
Carcinembryonic antigen (CEA), 569, 717–718
Carcinoids, 129, 216–218, 410, 628, 634, 716–717, 895–896
Cardiac catheterization, 519–520
Cardiac disease and physiology, 21–47
Cardiac failure, 52, 602, 778, 869–870
Cardiac filling and output, 35–36
Cardiac imaging, by MRI, 523–527

Cardiac output, 474, 778
Cardiac shunts, 778
Cardiac washout evaluation, 591
Cardiomyopathy, 52–54, 245–246, 577–578, 583–584, 587, 680, 683, 778–779
Cardiopulmonary physiology, 35–36
Cardiovascular response tests, 47–60
Cardiovascular system, 5–60
  cardiac arrhythmias and electropharmacology, 5–16
  cardiac disease and physiology, 21–47
  cardiovascular response tests, 47–60
  cell physiology, 16–20
  computed tomography (CT) image-enhancing agents, 752–758
  delivery modalities, 822–823, 846–847, 857–858, 869–870, 884–887
  ex vivo, 245–246
  fluorescent and pigment dye reagents, 473–481
  liposomes as delivery system, 822–823
  magnetic resonance imaging (MRI), 523–532, 589
  nuclear planar scintigraphy, 575–595
  pharmacogenetics, 322–325
  positron emission tomography (PET), 677–685
  radiographic image-enhancing agents, 742–747
  sepsis and inflammation, 444
  ultrasonography, 768–788
Carnitine, 458
Carotid stenosis, 113, 770
Cartilage integrity, 553
Cartilage volume and thickness estimation, 798
Carvedilol, 52, 282–283, 588–589, 778
Casein, 845, 847
CASPc, 507
Cataracts, 117, 508
Catecholamines, 8
Catechol O-methyltransferase (COMT), 246, 314–317
CATEEM, 79
Catheter placement validation, 780
[^{14}C]benzoate, 356–357
[^{11}C]-BS-DMPX, 758
C-Cell disease, 170
[^{14}C]-CG, 483
[^{11}C]-CPC-222, 700
CD3, fluorescein isothiocyanate-labeled, 499
[^{11}C]DACA, 425, 652–653, 882–883
[^{11}C]-DASB, 104–105
CD34 cells, 820
CDDP, 333–334
CD10 expression, 393
CD44 expression, 333
CD68 IgG, 110
[^{11}C]-dopa, 716–717
[^{14}C]D-xylose, 197
[^{11}C]-edrophonium, 678
Cefoperzone, 277
[^{13}C]-egg white breath test, 218–219, 402
Cell cycle phases, 222
Cell nuclei visualization, 351
Cell physiology, 16–20
Cell transfection, 817
Cellular-based delivery systems, 811–817
Cellular fluorescent and pigment dye reagents, 481–482
Cellular proliferation rate, 574
Central nervous system, 61–127
  angiography, 755
  autonomic nervous system, 61–62
  brainstem, spinal cord, and peripheral nervous system, 114–117
  cerebral cortex and cerebral hemispheres, 63–114
  computed tomography (CT) image-enhancing agents, 758–759
  delivery modalities, 818, 823–825, 858–860, 871–877, 887–889
  fluorescent and pigment dye reagents, 482–486
  gene delivery, 429–430, 432–433
  gene expression, 435–436
  liposomes as delivery system, 823–825
  magnetic resonance imaging (MRI), 532–544
  neoplasia, 95–96, 263–264, 409–410, 629–634, 713–716
  nuclear planar scintigraphy, 595–600
  peripheral nervous system and neuromuscular function, 62–63
  pharmacogenetics, 325–328
  positron emission tomography (PET), 685–705
  radiographic image-enhancing agents, 747
  sepsis and inflammation, 444–445
  special senses, 117–127
  ultrasonography, 788–791
Central precocious puberty (CCP), 168
Cerebral blood flow, 599, 703–705, 755
Cerebral cortex and cerebral hemispheres, 63–114
Cerebral cortex ischemia-reperfusion, 470
Cerebral perfusion, 533–534
Cerebral vascular stroke, 594–595, 685, 870–871, 887
Cerebrospinal fluid rhinorrhea, 77, 485
Cerivastatin, 262
Cervical cells, 515, 816, 867–868
Cevimeline, 180–181
C_3F_8 microbubbles, 768–769
CFT, 96, 540
CFTR, 434, 830
[$^{1\text{-}13}$C]glucose, 379–380, 674–675
[^{11}C]-glucose, 18
Chelate-labeled peptides, 569
Chelerythrine, 469
Chemiluminescent stain, 250
Chemotaxis, 253–254
Chemotherapeutic agents, pharmacogenetics of, 286
Chemotherapy-induced apoptosis, 572–573
1-[^{13}C]hexadecanol, 379–380
[^{14}C]hippurate, 356–357
Chitosan, 500, 509–510, 825, 853, 860–861, 865–866, 877
Chlorambucil, 452, 900
Chlordiazepoxide, 65, 82, 100
Chlorin e6, 123–124, 507
Chloropyramine HCl, 74, 79–80
Chlorpromazine, 83, 105–106
Chlorzoxazone, 301–304
Cholangiocellular carcinoma, 797
Cholangiopancreaticography, 748–749
Cholecystography, 267
Cholecystokinin (CCK), 74, 214–215, 604, 626, 677
Cholecystokinin (CCK) receptor expression, 633–634, 663–665, 896–897, 903
Cholera toxin, 109–110, 486
Cholestasis, 546
Cholesterol, 603–604, 824, 830, 831, 868
Cholesterol-lowering agents, 324–325
Chondroitin sulfate, 836
Chorea, 93
Chorea-acanthocytosis, 77, 131
Choreoathatosis, 697
Choriocapillaris, 484, 825
Choroidal microvasculature, 121, 507
Chronic obstructive pulmonary disease (COPD), 381–382, 565, 782–783
Chrysamine-G, 66–67, 483, 569–570, 595–596, 688
[^{11}C]-5-HTP, 716–717
Chylomicrons, 610, 761
CI2MBP-liposomes, 833
Cimetadine
  Cytochrome P450 metabolism capacity/activity, 273–274, 490–491
  duodenal ulceration, 208–209, 397
  excretion, 329–330, 367
  gastrinoma, 218

Cimetadine (*continued*)
immune hypersensitivity, 397
mixed function oxidases, 314
organic anion transporter, 364
organic anion transporter 3, 99
tubular absorption, 512
[^{11}C]-inositol, 692, 711
[^{14}C]-inulin, 359, 367–368, 741
[^{14}C]-iodoanitpyrine, 627
Ciprofloxacin, 441
Ciproxifan, 69
Cirazoline, 103
Cirrhosis, 192, 610, 708, 791–793
Cisapride, 104, 182, 210
[^{11}C]-IS-DMPX, 758
Cisternogram, 520–521
Citalopram, 306–307
Citric acid cough threshold, 383
[^{14}C]-KW-6002, 693–694
Clenbuterol, 378
Clodronate liposomes, 430, 812, 820, 823–824, 831
Clomiphene, 164
Clomipramine, 76, 103–104, 371–372, 700–701
Clonazepam, 80, 84
Clonidine, 98, 103, 146, 149–152, 155, 459–460
Clozapine, 300–301, 700–701
4-CmC (4-Chloro-m-cresol), 261, 263, 332, 345–346, 348–349
[^{11}C]-McN5652, 703
CMD-A2-Gd-DOTA, 525–526
[^{13}C]-methacetin breath test, 274, 401, 673–674
[^{11}C]-9-methoxyphenazine-1-carboxamide, 425, 652–653, 882–883
[^{11}C]-methoxy-X04, 688
[^{11}C]-methyl-choline, 726
CMPF, 329–330
C-myc gene, 426, 431, 556–557, 838–839
[$^{13}CO_2$], exhaled, 279, 401–402
[$^{14}CO_2$], exhaled, 276, 379, 399, 402
Coagulation alterations, 734–736
Cobalamin, 200–201
Cobalt-57-bleomycin, 649, 651
[^{57}Co]-BLM, 649, 651
Cocaine, 62
Cochlea, 126–127, 544
Cockcroft-Gault formula, 359
[^{57}Co]Cl_2, 221–222
1-[^{11}C]-octanoate, 706–707
[$^{1\text{-}13}$C]-octanoate, 401, 457–458
[^{13}C]-octanoic acid breath test, 185–186, 187, 400
[^{11}C]-octylamine, 671
Coded harmonic angiography, 794–795, 800
Codeine, 267, 268, 792
CoEDTA$^-$, 38
[^{58}Co]-labeled cyanocobalamine, 607, 615–616, 625
Colitis, 446, 614–615, 814
Collagen, 19–20, 256–259, 625
Collateral flow index (CFIp), 787
Colon carcinoma cell adhesion, 16–17, 221, 407, 411, 491, 893c
Colon (colorectal) cancer, 219–220, 241–242, 333, 410–411, 413, 420, 476, 500–501, 505, 600, 636–638, 651, 677–678, 685–686, 717–719, 795–796, 834, 873, 890–894
Colon motility, 210
Color blooming, 791
Coma, 90–91
Combined hormonal-stimulation test (CHST), 162–163
Combined immunodeficiency (CID), 242
Compound-S (CMP-S), 145
Computed tomography (CT)
angiography, 773
arterial portography (CTAP), 550
helical, 759, 801
isolated non-compaction of the left ventricular myocardium (INVM), 589
MR phantoms for, 518
multidetector row spiral, 756–757, 764
myocardial perfusion evaluation, 58
radiography image-enhancing agents, 750–764
x-ray micro-computed tomography, 764
Computed tomography (CT) image-enhancing agents, 750–764
adverse effects, 750–751
cardiovascular, 752–758
central nervous system, 758–759
gastrointestinal, 759
general concepts, 751–752
hepatic, 759–760
inflammation and sepsis, 760–761
lymph nodes and lymphatics, 761
neoplasia, 762–763
renal, 763
respiratory, 763–764
technique, 764
Concanavalin A, 242, 247–248
Congestive heart failure, 288, 577–580, 587–589, 600–601, 869–870
Congo red, 66–67, 68–69, 483, 569–570, 689
Conjugation, 279, 314–322
Constitution growth delay, 151, 165
Constitutive active receptor, 329
Continuous spiral computed tomography, 752–753
Contrast agents, 518–523, 765–768. *See also specific agents; specific applications*
Contrast-enhanced doppler ultrasound, 37
Contrast-enhanced power doppler sonography (CEPD), 797–798, 805
Coronary allograft vasculopathy, 59
Coronary artery
angiography, 577, 583–584, 678–679
angioplasty, 581
arteriography, 575, 775–776
balloon coronary occlusion, 586, 601–602
blood flow in athletes, 775
contrast echocardiography, 784
flow quantification, 786
ischemia, 775, 846
single photon emission computed tomography (SPECT), 680–681, 685
thrombosis, 594–595, 685, 870–871, 887
T wave alternans, 13
Coronary artery spasm
acetylcholine (ACh), 8–9, 21–22, 23–24, 50–51
angina, 8–9, 21–24, 50–51
ergonovine maleate, 8–9, 21–24, 50–53, 680–681
scintigraphy, 578–579
Coronary flow reserve, 48, 51, 772
Corticospinal neuron trophic dependencies, 112, 484–485
Corticosteroids, 293
adenocorticotropic hormone (ACTH) deficiency, 132
allergic rhinitis, 237
asthma, 236–237
glaucoma, 120
hepatitis, 244–245, 279–280
immune hypersensitivity, 387, 392–393
multiple sclerosis, 94–95, 230
rheumatoid arthritis, 232, 248, 342–343
systemic lupus erythematosis (SLE), 233
Corticotropin-releasing factor/hormone (CRF/CRH)
Addison's disease, 134
adenocorticotropic hormone (ACTH) deficiency, 132
adrenal insufficiency, 134–135
anterior pituitary function, 130–131, 133
Cushing's disease/hyperadrenalcorticism, 136–138, 140–141, 146
diabetes insipidus, 460
gastric motility, 186
pituitary tumors, 133–134, 149
synthetic human, 135

Cortisol, 134–135, 141–142, 329, 355, 459
Cotinine, 297
Cough reflex induction, 77–78
Coumarin, 296, 297
[$^{13}C_6$]-PABA, 203, 213
[^{13}C]-palmitic acid, 202–203, 683
C-peptide, 461
[^{13}C]-phenylalanine breath test, 674
[^{11}C]2-(4-pyridyl)quinoline-8-carboxamide, 425, 652–653, 882–883
[^{11}C]-raclopride, 75, 110
Cranial trauma, 131–132, 485
Craniopharyngioma, 599–600
[^{51}Cr]-chromate, 841
Creatine monohydrate, 83–84, 85–86
Creatinine clearance, 357–359, 668
Cresyl violet, 210, 487
Creutzfeldt-Jacob disease, 697
Crohn's disease, 209–210, 282, 445–446, 607, 615–616, 791–792
Cromoglycate, 384–385
Cromolyn sodium, 386–387
Cryprotection, 842–843
Cryptorchidism, 168–169, 353–354
[^{11}C]-SA4503, 692–693
[^{11}C]-SA5845, 693
[^{11}C] stilbene derivatives, 688
[^{11}C]-temozolomide, 714–715, 725
Ctenocephalides felis, 238
CTFO (circular triple helix-forming oligonucleotides), 435
[^{11}C]-thymidine, 726
[^{14}C]-triolein-[^{3}H]-oleic acid, 212
[^{64}Cu]-DOTA-anti-CEA minibody, 717
[^{64}Cu]-DOTA-biotin, 638, 718, 894
[^{64}Cu]-DOTA-NR-LU-10, 638, 718, 894
[Cu]-DTS, 464, 583
[^{13}C]-urea breath test, 188, 189, 400
Cushing's disease/syndrome, 136–145, 415–416, 417
[^{11}C]-WAY 100635, 699–702, 874–875
Cyanamide, 66
Cyanine fluorochromes, 494
Cyanoacrylate glue, 42–43
4-cyano-5, 5-bis(methoxyphenyl)-4-pentenoic acid, 814
Cyanocobalamine, labeled, 209, 445–446
Cyclic carbonate of ioxilan, 749
Cyclobenzaprine, 104
Cyclodextrins, 429, 829, 851–852, 861–862
Cyclohexamide, 397–398
Cyclooxygenase, 224, 265–266, 397–398
Cyclopentolate, 117–118
Cyclophosphamide, 94–95, 230, 456–457, 572–573, 627
Cycloplegia induction for retinoscopy, 117–118
Cyclosporin, 94–95, 230, 241, 265, 305, 321, 394–395, 693
Cy3-labeled oligonucleotides, 429–430, 827
Cypate, 408–409, 503
Cyproheptadine, 104
Cystatin C, 358
Cysteinyl-leukotriene, 386
Cystic fibrosis, 434, 830
Cystine oxidation, 455
Cystinosis, 169
Cytarabine, 539, 692
Cytochrome P450, 246–247, 292–312
  2A6, 2C9, 2C19, 2D6, and 2E1, 296–305
  3A, 305–311
  1A1 and 1A2, 294–296
  CY21B gene expression, 415–416
  CYP2E1, 296
  general, 292–294
  markers of activity, 490–491
  metabolism capacity, 273–274, 690–691
  4 microsomal enzymes, 311–312
  organophosphate metabolism, 280–281
  pharmacogenetics, 286–287
Cytokines, 245
Cytomegalovirus (CMV), 811, 819

D
8D3, 574–575
DAB-Am64-(1B4M-Gd)(64), 547
DACA, 425, 652–653, 726, 727–728, 882–883
D-alpha tocopheryl polyethylene glycol 1000 succinate, 868
D-amphetamine, 83, 105–106
Dantrolene, 262
Dapiprazole, 122–123
Debrisoquine, 282–283, 300, 317–318
Deep venous thrombosis, 584, 594–595, 685, 870–871, 887
Deferoxamine, 341, 496, 609
Definity, 765, 800–801
Delivery modalities, 811–905
  cellular-based delivery systems, 811–817
  liposomes, 820–845
  macro-biological platforms, 811–820
  macro-synthetic platforms, 820–869
  mechanical platforms, 904–905
  microbubbles and microspheres, 845–857
  micro-synthetic agents, 869–883
  molecular platforms, 869–904
  nanoparticles and nanospheres, 857–869
  peptides, proteins and immunoglobulins, 883–904
  vector-mediated gene (oligonucleotide) delivery systems, 817–820
Demethylation biochemical capacity, 176–277
Dental caries, 179–180
Deoxycorticosterone, 146
2-deoxy-2-[^{18}F]-fluoro-D-glucose, 687
3′-deoxy-3′[^{18}F]fluorothymidine, 408, 468
2′-deoxy-5-[^{18}F]fluorouridine, 467, 665
Deoxyglucose, 728–729
Depression, 71–73, 314
Dermal microvasculature, 45, 338, 475–476, 481
Dermal sweat-spot test, 381
Dermophin, 888
1-desamino-8-arginine vasopressin (DDAVP), 365, 368, 460
Desferrioxamine, 341, 496, 609
Des-His(1)[Glu_9]glucagon amide, 147, 901
Desipramine, 78–79, 105
Desmopressin, 138, 227–228
Detrusor contractions, 356
Devitalized tissue, 343
Dexame, 143
Dexamethasone
  Cushing's disease/hyperadrenalcorticism, 136–139, 141–145, 417
  cyclooxygenase synthesis, 265–266
  cytochrome 450 metabolism, 308–309
  glaucoma, 118
  immune hypersensitivity, 241, 264–265, 389, 394–395
  inflammatory responses, 397–398
  pituitary adenomas, 149, 417
  RANTES, 266
Dextran
  fluorescein isothiocyanate-labeled, 41, 479, 498
  [^{99m}Tc]-labeled, 493, 592–593, 607, 640–641
  Texas red-labeled, 337, 422
Dextran-coated iron oxide particles, 859–860, 866
Dextroamphetamine, 75, 110, 121, 126
Dextromethorphan, 300, 310
Dextrorphan, 300
D-fenfluramine, 83, 105–106
D-glucose, 192
DHEA (dehydroepiandrosterone), 318–319
Diabetes insipidus, 460

Diabetes mellitus
cardiomyopathy, 683
charge permselectivity of protein, 459–460
contrast agent, 737
erythrocyte anionic charge, 460
foot lesions, 338–339, 475–476
impaired glucose tolerance, 460
insulin resistant, 155, 460–461
lipoprotein physiology, 466–467
nephropathy, 460, 746, 749–750
osteomyelitis, 449–450, 617
retinopathy, 46–47, 125, 479
vascular permeability, 46–47, 125, 479
Diamine oxidase, 203–204
3,4-diaminopyridine, 62–63
Diamond-Blackfan anemia, 222
Diarrhea, 206–207, 208, 445
4Di-10ASP, 124, 508
Diatrizoate, 518–519, 732–734, 750–751
Diazepam, 74, 78, 161, 254–255
DIC (disseminated intravascular coagulation), 222, 815
Dicetylphosphate, 868
Dichloromethane, 845
Dichloromethylene diphosphonate, 833
Diclofenac, 297
Diepoxybutane (DEB), 221, 260, 425
Diethylcarbamazine, 452–453
Diethylenetriamine pentaacetic acid (DTPA), 569
Diethyl iminodiacetic analogues, 546, 611
Diffuse optical tomography (DOT), 514, 555, 722–723, 807–808
Diffusion-weighted imaging, 541
Digital subtraction angiography (DSA), 519, 530, 736, 743
Digital subtraction arteriography (DSA), 753–754, 773
Dihydroethidium, 19, 28, 469, 470, 477, 495
Dihydropteridine, 334
2,3-dihydroxyterephthalamide, 522
Diisopropylfluorophosphate (DFP), 280–281
Dilated cardiomyopathy, 52–54, 577–578, 587, 680
Diltiazam, 9, 272
Dimethylarginine dimethylaminohydrolase (DDAH) overexpression, 36–37, 407, 473, 506, 528, 543–544, 559–560
1,1′-dioctadecyl-3,3,3,′3′-tetramethylinodocarbocyanine, 109–110, 486
Dipetalonema perstans, 452–453
Diphenhydramine, 78
Diphenylhexatriene, 821, 844
Diphenyl sulfide derivatives, 104–105
Dipyridamole
angina, 47–49
aortic stenosis, 51, 772
myocardial ischemia, 19, 57, 776
myocardial perfusion assessment, 57–59, 684
stress echocardiography, 786
stress test, 575, 678–679, 776
ventricular wall motion, 59, 783
Discography, 554
2,9-disubstituted quinolino[2′,3′-5,4](3-pyrazolino)purin-2-ones, 76
2,9-disubstituted quinolino[2′,3′-5,4](3-pyrazolino)pyrimidin-2-ones, 76
Disulfiram, 296, 304
3,6-disunstituted piperidine derivatives, 97–98
Dithionite, 884
Dithiosemicarbazone derivatives, 464, 583
Ditrizoate, 744–745
Dityrosine, 469
Diverticulitis, 446–447
D-luciferin, 18, 435
D-lysine, 655
DMP444, 444, 579
DMP728, 594–595
DNA intercalation, 425
DNA synthesis, 425–426, 574, 657, 712
Dobutamine
aortic stenosis, 51
aortic valve dysfunction, 30–31
cardiac failure, 52, 778
cardiomyopathy, 53–54, 577, 680, 778–779
long Q-T interval syndromes, 10, 324
low-dose dobutamine echocardiography, 778–779, 780
myocardial function, 55, 781–782
myocardial infarction, 56
myocardial ischemia, 56–57
myocardial perfusion, 58
stress echocardiography, 54, 779, 781, 784–786
T wave lability, 13–14
ventricular (myocardial) function, 59
Dopamine
anuria/oligouria, 355
balance, 103
bupropion and, 91
gludopa, 368–369
presynaptic metabolism, 689
receptor, 301, 327, 534, 689–690, 694–696
transporter, 97–98, 326, 598–599, 694, 696–698
urinary excretion, 135, 416
DOPE, 430, 434, 827, 829–830
Doppler ultrasound, 41–42, 51
Dopram, 116, 380
Dorsal raphe nuclei, 874–875
DOTAP, 428–429, 430, 828, 829
Doxifluridine, 246–247, 292
Doxopram, 116, 380
Doxorubicin, 223, 245, 455, 573, 576, 811, 813, 837, 844, 856–857, 901–902
DPCPX, 61, 211
D-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH_2, 96
DPH fluorescent probe, 493–494
D-pro(2)-endomorphin-2, 83
Drug addiction, 286
D-sorbitol, 269
DSPC, 831
DSPE, 842
DST, 71
DTPA, 263–264, 358–359, 439, 497–498, 595, 668–672, 896–897
Dual thermal-dye double indicator technique, 475
Duodenal ulceration, 208–209
Dural arteriovenous fistulas, 114, 705
Dwarfism, pituitary, 150–151, 167–168, 460–461
D-xylose
enteropathy, 191, 399
hepatic vascular malformations, 281
intestinal absorption, 192–193, 196–199, 208, 445
malabsorption, 190–191, 674
portosystemic shunt, 43
Dy-EOB-DTPA, 762
Dynamic susceptibility MRI, 529, 537, 542, 558
Dynamic time-frequency analysis of autonomic nervous system, 61, 71
Dysphagia, 348
Dystonia, 78

E
Echistatin microbubbles, 801–802, 849
Echocardiography
cardiac failure, 52
cardiomyopathy, 52–54
coronary artery, 48, 775–776
flow-mediated dilation, 47
low-dose dobutamine, 778–779, 780
myocardial contrast echocardiography (MCE), 767, 775–776, 780–781, 787
myocardial dysfunction, 55–56
myocardial infarction, 56
stress, 54, 779, 781, 784–786

transthoracic assessment, 48
vasospastic angina, 50–51
ventricular (myocardial) function, 59, 589
Echo-contrast agents, 790
Echoscintigraphy, 791
Echovist 200/Echovist 300, 778
Eczema, 238–239, 337
EDDA (ethylenediaminediacetic acid), 263–264
Edrophonium, 29, 34, 108–109, 114–116, 182–183, 183, 211, 230–231, 347–348, 371
EDTA, 193, 197, 208, 445
Effective renal plasma flow (ERPF), 356–357, 362, 364
Efonidipine, 361
EGFP, 434, 820
Ehrlich solid tumor, 634
Eiksoneuropeptide, 254–255
Electrical stimulation mapping, 597–598, 698
Electroacupuncture, 144
Electrochochleography (EcochG), 127
Electroencephalography, 64, 65, 69, 71–74, 77–83, 87–89, 91, 100, 102, 105, 281, 325, 698–699
Electrolyte membrane transport complexes, 461
Electropharmacology, 784–785
Embolization for arteriovenous malformations, 112–113
Enalaprilat, 82, 89
Enamel proteins, 179
Encainide, 11
Encephalitis, 824, 889
Encephalopathy, hepatic, 281
Endocarditis, 444, 579
Endocrine system, 129–177
gastrointestinal, 129
growth hormone, 150–164
hypothalamus, pituitary, and adrenal axis, 134–150
hypothalamus, pituitary, and thyroid axis, 170–176
hypothalamus and pituitary, 130–134
neoplasia, 415–420
nuclear planar scintigraphy, 603–606
pancreatic, 129–130
parathyroid, 176–177
pharmacolgenetics, 328–329
reproduction, 164–170
Endolymphatic hydrops, 127
Endometriosis, 164, 351–352
Endomorphins, 83
Endophyte (Fescue) ingestion (sheep), 314
Endoscopy, 502
Endothelial adhesion, 16–17, 221, 476, 491–492, 501, 825
Endothelial carcinoma, 650–651, 901
Endothelial inflammation, 590, 615
Endothelial integrity, 17–18, 46, 480–481, 886
Endothelial intercellular adhesion molecule (ICAM), 528, 534
Endothelial permeability, 746
Endothelial tumor antigen, 836, 903
Endothelin blockers, 739
Endothelin (ET) receptor expression, 579–580, 869–870
Endothelium
delivery modalities, 857–858, 866, 884–886
gene delivery and, 428–429, 432–433
platelet interactions, 225–226, 257–258, 476, 491–492
scintigraphy and, 590–591, 628
Endotoxemia, 207
Enhanced optical coherence tomography, 792
Enteropathy, 191, 399
Enzyme prolidase stability, 854, 856
EOE-13, 593, 756
Ephedrine, 337
Epidepride, 695–696, 698
Epidermal growth factor, 183–184, 411–412, 557, 719–720, 850
Epidermoid carcinoma, 493, 501–502
Epilepsy, 83–89
electroencephalography, 80–82
fluorescent and pigment dye reagents, 89, 485
induction, 82
magnetic resonance imaging (MRI), 540
pharmacogenetics, 286
scintigraphy, 597–598
single photon emission computed tomography (SPECT), 698–699
Epinephrine, 10–11, 144, 256–258, 323–324, 337
Epstein-Barr virus, 433
Ergonovine maleate, 8–9, 21–25, 29–30, 50–53, 678
Erythema multiforme, 239–240
Erythromycin breath test, 305, 308–310
Erythromycin metabolism, 306, 310
Escherichia coli, 443, 445, 515–516
E-selectin, 822–823, 884
Esmolol, 13
Esophageal function screening test, 115–116, 231
Esophageal leakage, 747–748
Esophagitis, 30, 182–183
Esophagogastrectomy, 747–748
Esophagus
function and disorders, 181–183
spasm/motility, 22, 29–30, 181–182
squamous cell carcinoma, 411–412, 557
Estradiol, 165–166, 165–167, 292, 353, 722, 848, 854
Estrogen
cytochrome 450 metabolism, 304–305
receptors, 304–305, 328, 497, 643–644, 878–879
Ethanesulfonamide, 871
Ethanol, 64–66, 89, 276, 325–326, 402
Ethidium bromide, 19, 494–495
Ethyl ester of diatrizoic acid (EEDA), 760–761
Eticlopride, 695
EtNBA fluorescent dye, 95, 409, 504
Etorphine, 97
Eudragit, 853
Euthyroidism, 172
Evans blue dye
cutaneous anaphylaxis test, 233–234
endothelial integrity, 17, 225
intravascular compartment volume estimation, 40
labeled albumin, 223
myocardial infarction, 27, 477, 523
vascular extravasation, 46, 480–481
vascular permeability, 46, 480–481
Excretory urography, 740
Exercise intolerance, 625–626
Extracellular fluid space distribution parameters, 38–39, 476–477
Extracellular tissue space, volume estimation, 38
Extravasation, 46, 480–481
Extravascular volume, 475
Ex vivo living systems
cardiovascular, 245–246
hematopoietic: lymphocytes, 247–248
hematopoietic: macrophages and monocytes, 248–252
hematopoietic: neutrophils, 252–256
hematopoietic: platelets, 256–259
hematopoietic: red blood cells, 260–261
hepatic, 246–247
musculoskeletal, 261–263
neoplastic disease, 263–264
respiratory, 264–266
stem cell labeling, 566, 876

F

Fadrozole, 297
Fallypride, 695–696
[^{18}F]-altanserin, 700–701
Familial amyloid polyneuropathy, 597

Familial chromaffinomatosis, 170, 420
Familial medullary thyroid carcinoma, 170, 416–417
Famotidine, 397
Fanconi σ syndrome, 221, 260, 425
Fast blue, 112, 484–485
Fat malabsorption, 202–203
FDG, 57, 301, 442, 450, 604, 626, 641, 663, 677, 683, 684, 690–691, 693, 707–709, 712–716, 718–719, 721, 723, 726–729
[^{59}Fe], 341, 496, 609
[^{18}F]-EF5, 709, 710
Fenfluramine, 103
Fenoldopam, 739
Fenoterol, 387–388
Fentanyl citrate, 96, 888
[FeO]-BPA ultrasmall superparamagnetic iron oxide agent, 529–530
3-FEPB/4-FEPB, 686
Ferrimagnetic iron oxide nanoparticles, 532
Ferumoxides, 545–546, 550–551, 566, 816
Ferumoxtran, 545–546, 816
Fescue endophyte ingestion (sheep), 314
Fetal omphalomesenteric duct cysts, 741
[^{18}F]-FAU, 711, 712, 723–724
[^{18}F]-FAZA, 709–710
[^{18}F]-FBAU, 711, 723
[^{18}F]-FDDNP, 67, 482, 689, 697
[^{18}F]-FDM, 728
[^{18}F]-FEPB, 686
[^{18}F]-FES, 726
[^{18}F]-FHBG, 437–438, 705–706, 720
[^{18}F]-FIAU, 711, 723
[^{18}F]-FLT, 691–692, 711–712, 723–724, 726–727
[^{18}F]-fluorodeoxyglucose (FDG), 57, 301, 442, 450, 604, 626, 641, 663, 677, 683, 684, 690–691, 693, 707–709, 712–716, 718–719, 721, 723, 726–729
[^{18}F]-5-fluorodeoxyuridine, 467, 665, 728
[^{18}F]-fluorodopa, 694–697
[^{18}F]-fluoroethylspiperone (FESP), 701
[^{18}F]fluoro-L-proline, 625
4- [^{18}F]-fluorometaraminol, 245–246
[^{18}F]-fluorophen, 692, 873
[^{18}F]-5-fluorouracil (5-FU), 637, 717–718
[^{18}F]-FMAU, 711, 723
[^{18}F]-FMISO, 709–710
Fibrin, 466
Fibrinogen, 259
Fibrinolysis, 466
Fibrosarcoma, 501–502
Fibrovascular connective tissue proliferation, 555
Ficoll, 369
Fire ant venom, 239
Firefly luciferase, 18, 435, 498
FK352, 61, 211
Flavin monooxygenase metabolizing capacity, 312–313
Fleas, 238
Flecainide, 5, 7–9, 11–13
Flumazenil, 90–91, 105, 281
Fluo-deltorphin, 96, 888
Fluoratec, 97, 598–599
Fluorescein
  bladder rupture, 372
  cerebrospinal fluid rhinorrhea, 77, 485
  corneal trauma, 117
  dermafluorometry, 338
  intestinal injury, 203–204
  retinal vascular permeability, 46–47, 125, 479, 508–509
  vascular perfusion, 45, 338–339, 475–476, 481
Fluorescein isothiocyanate (FITC), 509
Fluorescein isothiocyanate-labeled
  antimelanoma IgG, 337, 422, 498
  CD3, 499
  dextran, 41, 479
  erythrocytes, 520
  glycol-chitosan nanoaggregates, 500, 509–510, 865–866
  GS I-A4, 410–411
  H-2K(b) CTL epitope, 241, 492
  hydroxyethyl starch, 40, 473–474
  liposomes, 832
Fluorescein-labeled phosphorothionate, 420–421, 426, 488
[fluorescein-5-maleimide]-tat-peptide, 639, 898–899
Fluorescent and pigment dye reagents, 473–516
  biochemical markers, 473
  cardiovascular system, 473–481
  cellular, 481–482
  central nervous system, 482–486
  gastrointestinal system, 487–488
  genetics, 488
  hematology, 491–492
  hepatic system, 488–491
  immune system, 492–493
  membrane characterization, 493–496
  musculoskeletal system, 496
  neoplasia, 497–507
  pathology, general, 509
  pharmaceutical delivery, 509–511
  renal system, 511–514
  reproductive system, 514–515
  respiratory system, 515
  sepsis, 515–516
  special senses, 507–509
Fluorescent bisphosphonate derivatives, 21, 342, 423, 496
Fluorescent imaging
  angiogenesis, 36–37
  capillary bed blood flow, 37
Fluorescent-lectin, 837–838
Fluorescent microspheres, 785
Fluorescent polymethacrylic nanoparticles, 254, 494, 864
Fluorescent probe delivery, 860, 868
Fluorocarbon microbubbles, 768–769
5-fluoro-2′-deoxyuridine (FUdR), 834
Fluorogold, 124, 508
Fluoroscopic imaging, 764
Fluoroscopic micturating cystourethrography (MCU), 807
5-fluorouracil (5-FU), 637, 717–718, 726, 728, 843, 850
Fluoxetine, 76, 78–79, 103–105, 306–307, 371–372
Flurbiprofen, 297
Fluticasone, 228, 238, 264, 395–396, 397
FMAU, 437–438, 705–706, 711, 720, 723
[^{18}F]-metafluoro-benzyl-guanidine, 604, 626, 677
fMLP, 253–254, 387–388, 390, 728–729
FMO-II, 671
Folate liposomes, 838, 879
Folate receptors, 501–502, 634–636, 654, 838, 879–880
Folic acid absorption, 201
Follicle-stimulating hormone (FSH), 164–166, 169, 170, 337–338, 353
Forced expiratory volume in 1 second (FEV1), 375–376, 382, 396
FP 736-03, 763
[^{18}F]-piperidyl benzilate, 686
[^{18}F]-pyrrolidyl benzilate, 686
Free radical formation, 468–469
[F]-RP517, 441, 580, 619, 813, 890
Frusemide, 341–342, 364–365
[^{18}F]-SPA-RQ, 689
FUdR, 834
Functional MRI (fMRI), 530, 533–534
Fungal infections, 243, 244
Furafyllin, 308
Furosemide, 127, 355, 373
Fusogenic di-peptide, 835–836

G
GABA receptors, 105, 106
Gabexate mesilate, 738
Gadobenate dimeglumine, 535–536, 544, 557–559, 564
Gadobutrol, 536, 564, 752–754
Gadodiamide
  adverse reactions, 518, 521, 731
  CNS imaging, 537–538
  computed tomography, 753–754
  magnetic resonance imaging (MRI), 518, 521, 537–538
Gadolinium
  adverse reactions, 518–520, 736
  angiography, 743, 746, 753–754, 757
  cardiac imaging, 526
  CNS imaging, 537, 538–540, 543
  discography, 554
  fluoroscopy, 764
  hepatic imaging, 547, 552
  hepatocyte uptake, 547
  intestinal imaging, 545
  magnetic resonance imaging (MRI), 518–520, 523, 526, 530, 537–538, 539–540, 543, 545, 552, 554–557, 559, 561–562, 629
  mammary gland imaging, 514, 555, 722–723, 807–808
  neoplasia, 555–557, 559
  pancreatitis, 561–562
  properties, 523
  vascular imaging, 530
Gadolinium-anti-mRNA oligonucleotide strand, 431, 556–557, 657–658
Gadolinium butylbenzyl diethylenetriaminopentaacetic acid, 546
Gadolinium-DTPA
  adverse reactions, 518–520, 732
  cardiac imaging, 524, 527
  cartilage integrity, 553
  CNS lesions, 535, 539–540
  computed tomography, 751, 753
  hepatocyte uptake, 547
  liposomes, 554
  magnetic resonance imaging (MRI), 518–520, 524, 527, 532, 535, 553–554, 562, 804
  radiography, 743
  renal abscesses, 562
  scintigraphy and, 569
  vascular thromboses, 532
Gadolinium ethoxybenzyl diethylenetriaminopentaacetic acid, 546
Gadolinium rhodamine dextran (GRID), 93, 226, 486, 566, 876
Gadopentetate dimeglumine
  adverse reactions, 520–521
  angiography, 745–746, 752
  arthrography, 552–553
  cardiac imaging, 525
  CNS imaging, 534–535, 537, 541
  hematology and, 545–546
  liposomes, 816
  magnetic resonance imaging (MRI), 520–521, 525, 534–535, 537, 541, 545–546, 552–553, 564–565
  pulmonary imaging, 564–565
Gadophrin-2, 524, 549–550, 552
Gadophrin-3, 525
Gadoteridol, 518, 521, 537–538, 564–565
Gadoversetamide, 521, 525
Gadoxetic acid, 546
Galactopyranose functional group, 534, 545
Galactose
  based air microbubbles, 850, 856
  based ultrasound contrast agents, 556, 767, 768, 789–790, 804
  elimination capacity, 269–270
  hepatic metabolism, 277
  intestinal absorption, 194
Gallamine, 38–39, 476–477
Gallbladder imaging, 547–548
Gallium-67 ([^{67}Ga]), 641, 650, 669–670, 721, 890
Gallium-67 ([^{67}Ga])-citrate, 447–448, 569, 617–618, 618, 623–624, 638
Gallium-67 ([^{67}Ga])-folate-DF-folate, 634–635, 720, 879
GalNAc, 410–411
γ-glutamyl transpeptidase, 455
Gamma-hydroxybutyric acid (GHB), 106
Ganglioneuroblastoma, 631
Ganglioneuroma, 631
Garlic, 259
Gastrectomy, 747–748
Gastric cancer, 190, 410, 413, 501–502
Gastric emptying, 184–186, 399–400
Gastric function and disorders, 183–190
Gastric motility, 186–187, 399–400, 606–607
Gastric reflux, 30
Gastric ulceration, 187–188
Gastric urease activity, 188
Gastrinoma, 129, 208–209, 212–219, 412–413, 488
Gastrin-releasing peptide (GRP), 627–628, 636, 897
Gastritis, 190
Gastroenteropancreatic tumors, 633–634, 896–897
Gastroesophageal reflux disease (GERD), 30, 182, 188–189, 606–607
Gastrografin, 759
Gastrointestinal permeability, 191–192, 204–208
Gastrointestinal system, 179–220
  computed tomography (CT) image-enhancing agents, 759
  delivery modalities, 825–826, 860, 877
  dentistry, oral cavity, and salivary glands, 179–181
  esophagus, 181–183
  fluorescent and pigment dye reagents, 487–488
  gastric function and disorders, 183–190
  intestinal absorption, 190–203
  intestinal inflammation, 209–210, 615–616
  liposomes as delivery system, 825–826
  magnetic resonance imaging (MRI), 544–545
  motility and transit time, 210–212
  neoplasia, 219–220, 241–242, 410–415, 633–634, 636–638, 894, 896–897
  nuclear planar scintigraphy, 606–609
  pancreas function and disease, 212–219
  permeability and ulceration, 191–192, 203–209
  radiographic image-enhancing agents, 747–748
  sepsis and inflammation, 445–447
  ultrasonography, 791–792
[Gd]-20790, 547
[Gd]-BOPTA, 544, 547, 550, 557–558
[Gd]-2,5-BPA-DO3A, 547–548
Gd-DOTA, 537
Gd-DTPA-BMA, 126–127, 544
[Gd]-EOB-DTPA, 547, 550
Gd-HP-DO3A, 538, 547–548
Gd-PCP2A(H_2O_2), 522–523
Gelatin microspheres, 847–848
Gene delivery, 428–434, 801, 860–862
  liposomes and, 826–830
Gene expression, 435–438
  delivery modalities, 817, 861, 896
  nuclear planar scintigraphy, 573–575, 657–658
  positron emission tomography (PET), 705–706
Gene expression imaging, 18, 435, 556–557, 657–658, 896
Gene therapy, 426, 488, 504–505, 510
Genetics, 425–438, 488, 545
Genipin-cross-linked gelatin, 847–848
Genomics, pharmacogenetics and, 288–290
GH-releasing peptides (GHRPs), 154–156, 162, 163
Ghrelin, 156
Giemsa stain, 481–482
Gilbert's syndrome, 268

Glaucoma, 118–120
Glibenclamide, 27, 477, 523–524
Glioblastoma, 425, 650–653, 727–728, 882–883, 901
Glioma/gliosarcoma, 95–96, 263–264, 409, 433, 456, 485, 503–504, 510–511, 542–544, 558–560, 629–631, 660–661, 714–716, 801–802, 827, 849, 872–873, 897–898
Gliomatosis cerebri, 534–535
Glomerular filtration, 357–358, 666
Glomerular filtration rate (GFR), 288, 358–360, 362–363, 668
Glomerular hydrostatic pressure, 361
Glomerulonephritis, 361, 511–512
Glucagon
  diabetes mellitus, 461
  growth hormone deficiency, 155, 163
  pancreatic function, 129
  pheochromocytoma, 147–149, 417–419, 627, 633
  receptor, 147, 633, 901
  seizures, 100–101
  thyroid carcinoma, 171, 419
  tolerance test, 144, 460, 461–462
Glucagon-like peptide-1, 167
Glucamine-substituted cyanine, 424, 497
Glucocorticoids, 172–173, 444–445
Gluconeogenesis, 461–462
Glucose
  acromegaly, 150–151
  beta-oxidation of fatty acids, 458
  binding avidity of amino acids, 830–831
  bladder rupture, 372
  1-[^{13}C]glucose, 379–380
  cytochrome 450 metabolism, 297–298
  diabetes mellitus, 460–461
  esophagitis, 182–183
  glycogen storage disease, 254
  growth hormone deficiency, 155
  6,6 [$^{2}H_2$]glucose, 274
  hyperkalemic periodic paralysis (HYPP), 344, 462, 463
  impaired glucose tolerance, 460
  intestinal absorption, 193–194, 195
  metabolism, myocardial, 18
  metabolism and disease alterations, 690
  metabolism and neoplasia, 665, 728
  pancreatic function in obesity, 129–130
  seizures, 100–101
Glucose infusion test, 43–44
Glucose-insulin-potassium infusion, 55, 782
Glucose-6-phosphate, 461–462
Glucose tolerance, 131
Glucose tolerance test (GTT), 150
Glucose transport protein (GluT), 462
Gludopa, 368–369
Gluosamine-substituted cyanine, 424, 497
Glutamate carboxypeptidase, 455
Glutamyl carboxypeptidase, 836, 903
Glutaric acidemia, 78
Glutathione-S-transferase, 314
Glycerin trinitrate. *See* Nitroglycerin
Glycerol, 127, 274
Glycogen storage disease, 254
Glycoprotein hormone marker, 333–334
Glycopyrolate, 36
Glycosaminoglycans, 460, 553
Glycosylated cationic liposomes, 826
G250 Mab, 658–659
Gold nanoparticles, 864, 867–868
Gonadorelin, 164
Gonadotropin insufficiency, 165
Gonadotropin-releasing hormone (GnRH)
  agonist (GnRH-a), 166–167
  anterior pituitary function, 130–131, 133
  cranial trauma, 110–111, 131–132
  Cushing's disease, 137–138
  delayed growth, 151, 165
  FSH, 165
  gastric ulceration, 187–188
  gonadotropin levels, basal, 169
  growth hormone deficiency, 162–163
  infertility, 166
  LH, 165
  puberty, 168
  scleroderma, 337–338
  sclerosis, 168
  varicoceles, 169–170
G-protein antisense oligonucleotide, 97
Grain dust bronchoprovocation test, 236, 391
Granulocyte-macrophage colony stimulating factor (GM-CSF), 264–265, 389, 394
Granulocytes, labeled, 446–448, 614–615, 617–618, 623
Graves' ophthalmology, 118–119, 229
Green fluorescent protein
  delivery modalities, 818–819, 859–860, 866
  gastric neoplasia, 190, 412, 485, 502
  gene delivery, 430–432, 828
  gliosarcoma, 95, 409, 543, 559
Growth delay, 151, 165
Growth hormone, 71, 131, 150–164, 293
  acromegaly, 460–461
  cytochrome 450 metabolism, 304–305
  deficiency, 151–163
  fatty acids influence on plasma levels, 163–164, 176
  infertility, 353
Growth hormone releasing hormone (GHRH), 98, 111–112
  acromegaly/short stature, 151
  anterior pituitary function, 130–131, 133
  Cushing's disease, 137–138
  growth hormone deficiency, 152, 155–162
  pituitary tumors, 133–134
GS I-A4, 410–411
GT-2331, 69
Guanethidine, 120–121
Guanidino-labeled L-arginine, 468
Guanyl cyclase-C receptor, 636, 893
Guerbet, 863

H
Haldol, 881–882
Hallerman-Streiff Syndrome (HSS), 132
Haloperidol, 75, 110, 263, 326, 327, 659, 692–693
Halothane, 260–263, 330–332, 344–347, 426–427, 463, 782
Hamilton score, 281–282, 313–314
Harmine, 716–717
HAT-DO1, 409, 502–503
Haundice, 610
HCl, 182–183
5H-dibenz[b,f]azepine derivatives, 870
[^{3}H]-DMP728, 594–595, 685, 870–871, 887
Headache pain, 89–90
Head and neck neoplastic disease, 638
Heart. *See* Cardiovascular system
Heart transplant, 783
Heat shock protein, 705
Heavy metal chelates, 764
Helical computed tomography, 759, 801
Helicobacter pylori, 188, 189, 298, 400
Helium, 375, 564–565
Hemagglutination virus of Japan, 432–433, 818, 824–827
Hemangioma, 795
Hemangiosarcoma, 795

Hematology
delivery modalities, 831, 877–878
fluorescent and pigment dye reagents, 491–492
magnetic resonance imaging (MRI), 545–546
nuclear planar scintigraphy, 609–611
Hematopoietic stem progenitor cells, 226–227, 492, 545–546, 816
Hematopoietic system, 221–266
ex vivo living systems, 245–266
hematopoiesis, hematology, and hemostasis, 221–228
immune system, 228–244
inflammation, 244–245
Hemizona assay, 353
Hemodialysis, 466–467, 519, 609
Hemoglobin, 469, 552
Hemorrhage, intracranial, 39, 90
Heparin, 203–204, 222–223, 466
Hepatic amyloidosis, 569–570
Hepatic artery, 792
Hepatic asialoglycoprotein, 428
Hepatic cirrhosis, 269, 271, 274–276, 279
Hepatic cytochrome P450 (15-α), 293
Hepatic disease, MRI, 517
Hepatic encephalopathy, 90–91, 281
Hepatic free radical production, 548
Hepatic metabolism, 273–278, 673–674
Hepatic necrosis, 549–550
Hepatic perfusion, 489
Hepatic posttransplant renal amyloid deposition, 571, 597
Hepatic system, 267–335
biliary and hepatic diagnostics, 267–281
biopsy, 305
computed tomography (CT) image-enhancing agents, 759–760
extrahepatic disease, effects of, 281
ex vivo, 246–247
fluorescent and pigment dye reagents, 488–491
liposomes as delivery system, 831
magnetic resonance imaging (MRI), 546–552
mixed function oxidases, 313–314
neoplasia, 275–276, 280, 547–548, 550–551, 638–639, 760, 762–763, 767–768, 776–777, 792–798
pathology and disease states, 279–281
pharmacogenetics, 281–290
pharmacogenetics: extrahepatic, 322–335
pharmacogenetics: phase II metabolizing enzyme systems, 314–322
pharmacogenetics: phase I metabolizing enzyme systems, 290–314
positron emission tomography (PET), 706–708
radiographic image-enhancing agents, 748–749
scintigraphy, 609–611, 673–674
ultrasonography, 792–798
Hepatitis, 244–245, 279–280
Hepatotropic diagnostic media, 278
HER-2, 864–865, 891–892, 894–895
Herpes simplex virus type 1 thymidine kinase, 311–312, 573–574, 705–706, 720
Hexabrix, 750
Hexarelin, 153–154, 160
[^{197}Hg]-bleomycin, 651
[^{3}H]glutamate, 603, 876–877
Hickey-Hare test, 363
High-dose dexamethasone suppression test (HDDT), 136–137, 139
[^{3}H]-indoxyl sulfate, 367
Hippocampus neurotransmitter function, 91–92
Hippuric acid/hippurate, 329–330, 356–357, 362, 367
Hirudin, 39, 90, 478
Histamine
bronchial response, 375–376, 380–381, 391–392
immune hypersensitivity, 383, 390, 391–393, 397
pheochromocytoma, 148
release, 744
thyroid carcinoma, 171, 419
Histamine H_3 receptors, 69–70, 92, 447
HIV, 209, 446, 451–452
H-2K(b) CTL epitope, fluorescein isothiocyanate-labeled, 241, 492
[^{3}H]-methoxy-inulin, 362–363
[$^{2}H_3$]-methyl-1-[^{13}C]methionine, 279, 315, 401–402
HMG, 353
HMG-CoA, 324–325
[^{3}H]MK801, 603, 876–877
HMPAO, 441
H_2[^{15}O], 18, 703–705
Hodgkin's disease, 641, 721
Hoechst 33258, 95, 409, 485, 543, 559, 859–860, 866
Hoechst 33342, 36–37, 222, 407, 473, 506, 528–529, 543–544, 559–560
[^{3}H]-oestrone-3-sulfate, 76
Homing peptides, 903–904
Homopurine-homopyrimidine, 426
Horner's syndrome, 120–121
[^{3}H]-pentazocine, 692
[^{3}H]-prednisolone liposomes, 823
[^{3}H]-quinuclidinyl benzilate, 64–65, 325–326
[2,3,3-$^{2}H_3$]-serine, 315
HSV1-tk reporter gene, 436, 437–438
[^{3}H]-thymidine, 442, 467, 665, 708–709, 728
5-HT(4) receptors, 210
Human chorionic gonadotropin (hCG), 168–169, 169, 353–354
Human genome project, pharmacogenetics and, 288
Human papilloma virus, 433
Human tumor gene index, 289
Humoral response, 242–244
Hunter's syndrome, 433, 811, 819
Huntington's disease, 92–93, 435–436, 574–575, 598, 887–888
Hyaluronan, 842–843
Hyaluronic acid, 273, 491
Hydrocortisone, 247, 249
Hydrogel, 817, 825, 861
Hydrogen breath test, 190–191, 674
Hydrogen peroxide, 260
Hydroxyamphetamine, 120–121
Hydroxyapatite deposition, 21, 342, 423, 496
Hydroxychlorzoxazone, 301–302
5-hydroxydecanoate, 38, 470, 477
Hydroxyethyl starch, 40, 473–474
2-hydroxyisophthalamide, 522
Hydroxylamines, 298–299
21-hydroxylase, 142–143, 415–416
Hydroxylation, 317–318, 330, 355–356
Hypercapnia, 599
Hyperemia, 585
Hyperhomocysteinemia, 315, 458
Hyperkalemic periodic paralysis (HYPP), 343–344, 462–463
Hyperlipidemia, 585
Hyperparathyroidism, 176–177
Hyperprolactinemia, 133, 351–352
Hypertension, 245–246, 667, 680
Hyperthermia, 260–263, 842, 865
Hyperthyroidism, 174
Hypertonic saline, 363
Hypogammaglobulinemia, 242–243
Hypoglycemia, 100–101, 157, 158–159
Hypogonadism, 151, 165, 328–329
Hypolipemic agents, 324
Hypoparathyroidism, 176–177
Hypopituitary conditions, 132
Hypothalamic-pituitary dysfunction, 70–71, 77
Hypothyroidism, 172–175
Hypothyroid obesity, 172–173
Hypoxia, 463–464, 709–710
Hysterosalpingography, 750, 807, 848

I
[^{123}I], 605–606, 662–663, 665
[^{131}I], 605–606, 662–663, 665
[^{125}I]A-312110, 11–12, 370
[^{123}I]-ADAM, 702, 875
[^{125}I]-ADAM, 702, 875
[^{124}I]-annexin V, 726
[^{123}I]-anti-human milk fat globulin (HMFG1), 642
[^{124}I]-anti-VEGF, 726
[^{125}I]-B72.3 anti-colorectal carcinoma monoclonal antibody, 411, 637
[^{123}I]β-CIT, 694, 696, 698
[^{131}I]-6-beta-iodomethyl-19-norcholesterol, 603–604, 626, 677
IBIS psychometry, 79
[^{123}I]-BMIPP, 581–582, 681–683
Ibopamine, 118, 120
Ibuprofen, 689
ICAM-1, 770–771, 775, 825, 846, 885
ICE catheter, 782
Ice test, 347
[^{123}I]-cholesterol iopanoate, 576
[^{125}I]-digoxin, 461, 585–586, 870
[^{125}I]-digoxin-iodohistamine (bis(O-carboxymethyloxime)), 461, 585–586
Idiopathic bile acid malabsorption (IBAM), 199–200
Idiopathic diffuse esophageal spasm (IDES), 181
Idiopathic dilated cardiomyopathy (IDCM), 53–54, 778–779
Idiopathic hypertrophic cranial pachymeningitis, 537
Iduronate-2-sulfatase (IDS), 433, 811, 819
[^{123}I]-estradiol derivates, 644
[^{125}I]ET-1, 579–580, 667, 869–870
I-fenfluramine, 83, 105–106
[^{123}I]-2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyluracil, 436–437, 573–574
[^{125}I]-2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyluracil, 436–437, 573–574
[^{123}I]-FP-β-CIT, 698
[^{123}I]-FP-CIT, 695
[^{131}I]-gastrin-I, 663–664, 903
[^{125}I]-glycoprotein liposomes conjugate, 834
[^{125}I]-HIPDM, 599
[^{131}I]-hippuran, 666
[^{131}I]-HPMA, 657
[^{125}I]-HSA, 442, 708
[^{125}I]-IBZM, 689–690, 694, 695
[^{125}I]-IGF, 639, 652, 898
[^{123}I/^{125}I]IMPY, 570, 587
[^{125}I]-IMSB, 687–688
[^{123}I]-interleukin-8, 440–441
[^{131}I]-interleukin-8, 449–450, 617
[^{123}I]-iodo-α-methyl tyrosine (IMT), 629
[^{123}I]-iodobenzamide, 698
[^{125}I]-iodocyanopindolol (ICYP), 27, 245, 586, 588, 602, 603, 680
[^{131}I]-iodohippurate, 356
[^{123}I]-iodophenylenanthic acid (IPEA), 457–458
5-[^{125}I]iodo-4′-thio-2′-deoxyuridine (ITdU), 574, 706
5-[^{125}I]iodo-4′-thio-2′-deoxyuridine (IUdR), 574, 706
[^{123}I]-iomazenil, 698
[^{131}I]-iothalamate, 359
[^{123}I]-IPA, 715
[^{123}I]-IPEA, 707
[^{123}I]-IPVA, 707
[^{125}I]-LDL, 361, 466–467
Ile8-AII, 575–576
Ileus, postoperative, 61, 116
Iliac vein, thrombosis of, 595
[^{125}I]-L-3-iodo-a-methyl tyrosine, 213–214, 607–608
Ilioprost, 750, 763
[^{123}I]-metaiodobenzylguanidine (MIBG), 50, 53, 576–579, 582, 586–589, 600–604, 626, 631–632, 677–681, 685, 724, 869
[^{124}I]-metaiodobenzylguanidine (MIBG), 604, 626, 677
[^{125}I]-metaiodobenzylguanidine (MIBG), 245–246, 680
[^{131}I]-metaiodobenzylguanidine (MIBG), 27, 146, 149, 418–419, 586, 588, 602, 604, 626–627, 632, 677
Imidapril, 323
Iminodiacetic acid-enhance scintigraphy, 795
Imipramine, 72, 78–79, 95, 105
Immune desensitization, 239–240
Immune hypersensitivity, 233–241, 264–265, 383–397
Immune system, 228–244
  delivery modalities, 831–833, 889–890
  fluorescent and pigment dye reagents, 492–493
  liposomes as delivery system, 831–833
  neoplasia, 420–421
  nuclear planar scintigraphy, 611–622
  positron emission tomography (PET), 708
  ultrasonography, 798
Immunoliposomes, 822–823, 834–836, 838–839, 884
Immunomagnetic absorbance, 515–516
Impotency, 352
[^{111}In]-anti-CEA Mab, 569, 636–637
[^{111}In]-anti-ICAM-1 Mab, 615, 669–671
[^{111}In]-antimyosin antibody, 455–456, 573, 576, 582–584
Inappropriate sinus tachycardia (IST), 14
[^{111}In]-bleomycin, 647–648, 651
[^{111}In]-bombesin, 627–628, 636, 897
[^{111}In]-CYT-103 monoclonal antibody, 637–638, 718–719
[^{111}In]-diethylenetriamine pentaacetic acid (DTPA), 666, 896–897
[^{111}In]-diethylenetriamine pentaacetic acid (DTPA)-antimyosin-Fab, 584
[^{111}In]-diethylenetriamine pentaacetic acid (DTPA)-folate, 635–636, 654, 880
[^{111}In]-diethylenetriamine pentaacetic acid (DTPA)-neurotensin, 653, 900–901
[^{111}In]-diethylenetriamine pentaacetic acid (DTPA)-octreotide, 604, 626, 641, 654–655, 660–662, 664–665, 677
Indocyanine dye Cy5.5, 522
Indocyanine green (ICG)
  biliary excretion capacity, 489
  Cytochrome P450 metabolism capacity/activity, 273–274, 490–491
  gastrointestinal cancer, 220, 413, 501, 502
  glomerular filtration, 360
  hepatic function, 269–273, 288, 489–491, 708
  hepatic perfusion, 489
  hepatic plasma flow, 269
  intraocular, 117, 508
  intravascular compartment volume estimation, 39, 475
  leukocyte visualization, 121, 507
  mammary gland disease differentiation, 514, 555, 722–723, 807
  neoplasia, 408–409, 424, 497, 503, 894
  stabilization of optical tracer agents, 511
  vascular blood flow, 41–42, 480, 771–772
  vascular permeability, 45–46, 474
  visual pathway staining, 121, 509
Indomethacin
  adverse effects, 750–751
  intestinal absorption, 202
  pain sensation/threshold, 327
[^{111}In]-DOTA-CCMSH, 645–646
[^{111}In]-DOTA-CMSH, 645–646
[^{111}In]-DOTA-Lys$_8$-vasotocin, 650–651, 901
[^{111}In]-DOTA-MSH(OCT), 646, 647, 899–900
[^{111}In]-DOTA-NCS-6-Ahx-Phe(19)-ST[1-19], 636, 893
[^{111}In]-DOTA-NDP-MSH, 645–647, 899–900
[^{111}In]-DOTA-octreotide, 604, 626, 677
[^{111}In]-DOTA-ReCCMSH, 645–646
[^{111}In]-DOTA-X-BBN, 627–628, 897
[^{111}In]-(DOTOA,Tyr$_3$)-octreotide, 902–903
Indotricarbocyanine (ITCC), 219–220, 413, 500–501, 636
Indotricarbocyanine (ITCC)-human serum albumin, 219–220, 413, 500–501
Indotricarbocyanine (ITCC)-octreotate, 409–410, 485–486
Indotricarbocyanine (ITCC)-transferrin, 219–220, 413, 500–501
Indoxyl sulfate, 329–330, 367
[^{111}In]-DPC11870, 450, 613
[^{111}In]-DTPA-bovine serum albumin, 439, 609, 614

[^{111}In]-DTPA-D-glu(1)-minigastrin, 664–665
[^{111}In]-(DTPAO,Tyr$_3$)-octreotide, 902–903
[^{125}I]-neuropeptide FF (NPFF), 599
Infant biliary atresia, 610
Infectious disease, 286, 439–453
Infertility, 165–166, 352–353, 807, 848
Inflammation, 244–245, 381–382, 439–453
  computed tomography (CT) image-enhancing agents, 760–761
  general abscess formation and sepsis, 439–443
  nuclear planar scintigraphy, 608, 611–622
  organ- and tissue-associated, 444–452
  parasitology, 452–453
  positron emission tomography (PET), 708–709
Inflammation bowel disease (IBD), 282, 608, 615
[^{111}In]-granulocytes, 617–618, 623
[^{111}In]-hCTMO1 mab, 654
Inhibin, 170
[^{111}In]-leukocytes, 209, 445–446, 607, 615–616, 625, 670–671
[^{111}In]-merc neutrophils, 813
[^{111}In]-merc platelets, 815
[^{111}In]-MP2288/[^{111}In]-MP2286, 633–634
Inner ear disease, 705
[^{111}In]-octreotide, 659
[^{111}In]-oxine neutrophils, 813
[^{111}In]-oxine platelets, 815
[^{111}In]-pentetreotide, 599–600, 629, 713
[^{111}In]-platelets, 223–224, 593–594, 814–815
[^{111}In]-rhbeta$_2$mH5, 571, 871–872, 883–884
Insecticides, 101
Insulin
  Addison's disease, 134
  delivery modalities, 855
  diabetes, 460–461
  endometriosis, 164, 351
  growth hormone deficiency, 152–153, 155, 156, 159, 162–163
  hyperkalemic periodic paralysis (HYPP), 344, 462, 463
  induced hypoglycemia, 157, 158–159
  polypeptide delivery, 511
Insulin (islet cell) adenoma, 130, 414, 464–465
Insulin-like growth factor, 151–152, 333, 353, 438, 639, 652, 898
Insulinoma, 129, 216–218
Insulin tolerance test, 143–144, 157, 159, 160, 162
Integrin complexes, 590–591, 828–829, 857–858, 885
Integumentary system, 337–339, 421–422
Interferon-alpha-2b (IFN-α-2b), 841
Interferon-beta (IFN-β), 94–95, 230
Interferon-gamma (IFN-γ)
  allergic reactions, 228–229
  cyclooxygenase synthesis, 265–266
  immune hypersensitivity, 240, 241, 264–265, 389, 393–394
  inflammatory responses, 397–398
  RANTES, 266
  systemic lupus erythematosis (SLE), 247–249, 333
Interleukin-1 (IL-1), 553, 855, 860–861
Interleukin-2 (IL-2), 650, 811–812
Interleukin-4 (IL-4), 840–842
Interleukin-6 (IL-6), 860–861
Interleukin-8 (IL-8), 440–441, 449–450, 617
Interleukin-10 (IL-10), 841–842, 860–861
Interleukins
  abscess formation, 439–441
  allergic reactions, 228–229
  allergic rhinitis, 237, 264
  cyclooxygenase synthesis, 265–266
  Graves' ophthalmology, 118–119, 229
  hepatitis, 244–245, 279–280
  immune hypersensitivity, 234–235, 240, 241, 264–265, 383–384, 389–391, 393–394, 397
  inflammatory responses, 245, 397–398
  multiple sclerosis, 230
  neutrophil function, 253–254
  osteomyelitis, 448–450
  RANTES, 266
  systemic lupus erythematosis (SLE), 233–249, 247
Interstitial fluid compartment, 38–39, 476–477
Intervertebral disc pathology, 554
Intestinal absorption, 190–203, 445, 674
Intestinal carcinoids, 129, 216–218
Intestinal inflammation, 209–210
Intestinal injury, 203–204
Intestinal motility and transit time, 210–212
Intestinal neoplasia, 219–220, 413, 502, 894
Intradermal testing, 243, 244
Intravascular compartment volume estimation, 39–40, 475
[^{111}In]-tropolone-platelets, 594
Inulin
  excretion/clearance, 357, 366–367, 512
  extracellular fluid space distribution parameters, 38–39, 476–477
  GFR, 362
In vitro fertilization, 166–167
In vivo detection of gene expression, 573–575, 598, 887–888
Iobitridol, 738, 741, 742, 744, 757
Iocarmate, 750–751
[^{125}I]-ODAM, 702–703, 876
Iodinated contrast-loaded agarose gel, 518
Iodipamide ethyl ester (IDE), 744
Iodixanol, 521, 563, 731–735, 738, 740–741, 742, 749, 750, 751–752
Iodocyanopindolol (ICYP), 27
6-iodo-2-(4′-dimethylamino-)phenyl-imidazo[1,2-a]pyridine, 570, 597
Iodophenyl-9-methyl pentadecanoic acid (9MPA), 681
5-iodo-4′-thio′2′-deoxyuridine (ITdU), 425–426, 467–468, 574, 657, 706, 712
5-iodo-4′-thio-2′-deoxyuridine (IUdR), 467–468, 574, 706, 877
Iohexol, 357–358, 593
  adverse reactions, 731–741, 750–751
  angiography, 744–745, 756
  glomerular filtration, 360
  lipid emulsion, 763
  liposomes, 531, 754–755, 822
  myelography, 747, 759
  portography, 759–760
  urography, 750
Iomeprol, 733–734, 736, 747, 750–751, 759
Iopamidol, 518–519, 732, 734, 736–738, 748–749, 750–751, 759
Iopentol, 733–734
Iopiperidol, 734, 735
Iopromide, 535–536, 732–738, 750, 752–754, 763
[^{123}I]-orthoiodohippurate (OIH), 364, 666
Iothalamate, 288, 359–360, 362–363
Iotrolan, 740, 747, 750–751, 759, 763
Ioversol, 733–734, 737–738
Ioxaglate, 733–735, 738–741, 750–751
Ioxilan nanoparticles/micoparticles, 749
IPA, 715, 872
IPEA, 457, 707
[^{125}I]-peptide nucleic acid (PNA), 435–436, 503–504, 574–575, 598, 630, 887–888
[^{131}I]-1-(2-phenoxy-ethyl)-2-nitroimidazole, 649–650
[^{123}I]-15-(p-iodophenyl)-3-R,S-methyl pentadecanoic acid, 578–579, 581–582
[^{123}I]-PIP, 600, 677–678, 685–686, 873
[^{125}I]-PIP, 600, 677–678, 685–686, 873
[^{125}I]-PNA-[antitransferrin receptor IgG], 574–575, 598
[^{125}I]-PNA-avid, 630
4-ipomeanol (4-IM), 311–312
Ipomiadol, 116–117
Ipratropium, 387–388
IPVA, 707
[^{125}I]-PYY, 642
IRDye78, 503

[^{125}I]-RFRP-3, 599
[^{123}I]-(rho-iodophenyl)-3-R,S-methylpentadecanoic acid, 465, 466
[^{123}I]-(ρ-iosophenyl)-3-R,S-methylpentadecanoic acid, 682–683
[^{123}I]-Ro 16-0154, 690
Iron deficiency, 221–222, 341, 496, 609
Iron fluorophore particle (IFP), 20, 478
Iron oxide-loaded myogenic precursor cells, 527
Iron oxide nanoparticle, 746
[^{131}I]-rose bengal, 267, 610
[^{123}I]Sar1, 575–576
Ischemia
  CNS, 465, 470, 535–536, 827–828
  magnetic resonance imaging (MRI), 535–536, 552
  myocardial, 19, 56–57, 464, 465, 466, 577, 580–584, 619, 682–683, 776, 785, 813
  tracking stem cell migration in CNS, 93
Isobutyl nitrite, 32
Isoflurane, 377–378
Isolated non-compaction of the left ventricular myocardium (INVM), 589
Isoprenaline, 12–13, 33–34, 74, 107–108, 385–386
Isoproterenol
  bronchodilatory response, 378
  Brugada syndrome, 8
  COPD, 382
  immune hypersensitivity, 385–386
  long Q-T interval syndromes, 8
  sick sinus syndrome, 12–13
  syncope, 32–35, 106–107, 109
Isoproterenol-induced oncosis, 573, 576
Isoremoxipride, 695–696
Isosorbide dinitrate, 33, 51–52, 107, 774
Isosulfan blue dye, 241–242, 420, 422, 493, 505, 640
ITdU, 425–426, 467–468, 574, 706, 712
[^{125}I-Thyr$_{10}$]glucagon, 147, 633, 901
[^{125}I]-tyr(5)-6-Ahx-Phe(19)-ST[1-19], 636, 893
[^{123}I]-tyr(4)-bombesin, 642
[^{123}I]-tyr-octreotide, 604, 626, 641–642, 661–662, 677
[^{125}I]-TZDM, 687–688
IUdR, 467–468, 574, 706, 877
[^{125}I]-vasoactive intestinal peptide (VIP), 642
Iversol, 746

J
Johnes disease, 243–244
Joint effusion, 555

K
Kainate-induced neurotoxicity, 539–540
KATP, 370, 477
Kawasaki disease, 58–59, 584–585, 684
Kazal, 334
Ketamine, 118
Ketanserin, 104, 280, 410
Ketoconazole, 308, 311
Ketogenic diet, 465
Ketogenic hypoglycemia, 467
17-ketogenic steroids (17-KGS), 143–144
Ketogenic test, 465
Ketoisocaproic acid (KIC), 467
Ketoisovaleric acid (KIV), 183, 275, 276, 379, 399, 402, 459, 467
Ketosis, 465
Kinevac, 267
Knee, rheumatoid arthritis and, 799
[^{81m}Kr], 672
Kupffer cell, 793–794, 812, 820, 831

L
L-749,329, 579–580, 667, 869–870
Lactose cryoprotectant, 843
Lactose-[^{13}C]-ureide breath test (LUBT), 211–212, 400–401
Lactose hydrogen breath test, 191, 399
Lactulose, 191–192, 194–199, 204–208
LacZ gene, 429
Lambert-Eaton myasthenic syndrome, 63
Langerhans cell histiocytosis (LCH), 204–205
Language area identification, 597–598, 698
Lanreotide, 150
Lansoprazole, 30
Lanthanice-based compounds, 751
Laryngectomy, 747–748
Laser capture microdisection (LCM), 289
Laser targeted angiography, 825
Laser-targeted angiography, 484
L-[^{11}C]-methionine, 708–709
L-dopa
  chorea-acanthocytosis, 77, 131
  depression, 73
  growth hormone deficiency, 158–159, 161–163
  Huntington's disease, 92
  Parkinson's disease, 70–71, 98–99, 695
  renal tubule excretion, 368–369
  serotonin and adrenergic system evaluation, 103
Lectins, 410–411
Left ventricular aortic outflow obstruction, 31
Left ventricular ejection fraction, 53, 577, 587–588, 600–601, 680
Left ventricular function/dysfunction
  computed tomography, 757–758
  halothane-induced, 782
  magnetic resonance imaging (MRI), 527
  scintigraphy, 589, 625–626
  stem cells, 817
  ultrasound, 782–783
Left ventricular hypertrophy, 32
Left ventricular opacification, 783
Lefty ventricular volume, 36
Lentiform nucleus, 791
Leucine kinetics, 348
Leucocyte chemotaxis, 19
Leukemia, 242, 250, 294–295, 334
  cytochrome 450 metabolism, 294–295
  delivery modalities and, 838, 880, 898–899
  gene delivery-transfer, 433
  gene therapy, 426, 510
  magnetic resonance imaging (MRI), 539
  pharmacogenetics, 287–288
  scintigraphy, 639, 648
  single photon emission computed tomography (SPECT), 692
  TPMT and, 320
Leukocyte adhesion molecules (LAMs), 770–771
Leukocyte rolling phenomenon, 20, 478
Leukocytes
  ICG-stained, 121, 507
  [^{111}In]-labeled, 209, 445–446, 607, 615–616, 625
  [^{99m}Tc]-labeled, 443, 446–447, 608, 620
Leukotriene B_4, 441, 450, 613–614, 744
Leukotriene receptor antagonist (LTRA), 384
Levamfetamine succinate, 75, 110
Levovist, 41–42, 480, 678–679, 767–769, 771–772, 775–777, 780, 788–802, 804, 806–808, 846, 848, 856–857
Lewis lung carcinoma, 456, 504
L-[guanidino $^{15}N_2$]-arginine monohydrochloride, 379, 402–403, 673
L-[^{3}H]-methionine, 708–709
L-5HTP (L-5-hydroxytryptophan), 71–72
L-5-hydroxy-tryptophan (L-5-HTP), 103
Lidocaine
  capillary bed tolerance of small particle or liquid plastic occlusion, 38
  cough reflex induction, 77–78
  Cytochrome P450 metabolism capacity, 273–274

hepaticexcretory function, 269–270
hepatic metabolism, 274–276, 490–491
long Q-T syndrome, 11
neurologic *versus* musculoskeletal differentiation, 62
ophthalmic artey dependent vision, 95–96, 122, 123
overactive bladder, 370–371
pain and lameness disorders, 342
reflex airway, 376
safety evaluation prior to vascular embolization, 113
seizures, 102
tachycardia, 15
ventricular arrhythmia, 15
xenobiotic metabolism, 461, 479–480
Lignocaine, 102
Lipemia, 222–223, 466
Lipid-based perfluorobutane-filled microbubble, 770–771
Lipid metabolism, 324, 466, 581–582, 691, 710–711
Lipid peroxidation, 250–251
Lipid-shelled microbubbles, 777–778
Lipofectamine 2000, 817, 861
Lipofection cell labeling, 566–567, 815–816, 818–819
Lipoplexes, 827–828
Lipopolysaccharide (LPS), 249, 264–265, 389, 394
Lipoprotein physiology, 466–467, 622
Liposomes
cardiovascular system, 822–823
central nervous system, 823–825
concepts and perspectives, 820–822
delivery modalities, 812, 818, 820–845
fluorescent and pigment dye reagents, 484, 492–494
gastrointestinal system, 825–826
gene delivery, 826–830
genetics and, 428–434
hepatic system, 831
immune system, 831–833
inflammation, infection and sepsis, 447–448, 841–842
iohexol, 754–755
iopromide-containing, 754
magnetic resonance imaging (MRI), 528, 531, 534, 545–546, 554
myocardial infarction, 26
neoplasia, 834–840
PEG, 447–448, 617–618, 623, 800, 823, 834–837, 839–840, 842, 849
peptide delivery, 840–841
pH sensitivity, 241
plasmid-complexed, 801, 818, 820, 824–826, 849
production, 842–845
small unilamellar vesicles (SUV)-PEG liposomes, 800, 834
[^{99m}Tc]-hexamethylpropylene amine oxime (HMPAO)-[SSL-VIP], 644–645
[^{99m}Tc]-liposome, 615, 825–826, 833
[^{99m}Tc]-PEG liposomes, 447–448, 617–618, 623
ultrasound, 774, 800
Lisinopril, 323, 361
Lithium, 75, 315, 327, 474–475
Lithium dilution cardiac output (LiDCO), 35–36, 474–475
Liver. *See* Hepatic system
Liver transplant, 792
Living cells, vitality staining, 481–482
L-methyl-[^{14}C]methionine, 665, 728
LNGFR, 434, 820
Loderix, 74, 79–80
Long-chain fat loading, 458
Long Q-T interval syndromes, 7–8, 10–11, 286, 323–324
Lorcainide, 11
Low-density lipoprotein (LDL), 361, 466–467, 839
Low-density lipoprotein (LDL) receptors, 604, 626, 677
Low-dose dexamethasone suppression test (LDDT), 136–137
L-[ring-$^{13}C_6$]phenylalanine, 348
L-selectin, 244–245
L2SN, 811, 819
Luciferase, 18, 435, 498, 503–504, 630, 830
LumenHance, 562
Lung cancer, 263–264, 437, 627–629, 633–634, 636, 649, 658, 660–661, 663–664, 726–727, 763, 828, 865, 878, 881, 892–893, 896–897, 903
Luteinizing hormone (LH), 164–166, 169, 170, 337–338
Luteinizing hormone-releasing hormone (LHRH), 133–134, 164
Lymphangietasia, 607
Lymphatic system delivery, 862–863
Lymph nodes, 241–242, 420, 422, 492–493, 505, 557, 640–641, 798, 863
computed tomography (CT) image-enhancing agents, 761
Lymphocytes, 248–249
as delivery system, 811–812, 819, 848–849
ex vivo, 247–248
function test, 242
gene delivery-transfer and, 433
positron emission tomography (PET), 708
stimulation test, 247–248
Lymphography, 761
Lymphoma, 242, 420–421, 423, 505–506, 599–600, 641–642, 721, 899, 902
Lymphotrophis superparamagnetic nanoparticles, 557
Lysine, 460
Lysine acetylsalicylic acid (L-ASA), 235, 240–241, 276, 327, 384–386, 390–391, 750–751
Lysine aspirin bronchoprovocation (L-ASA BPT), 234, 385
Lysine vasopressin (LVP), 133–134, 143–144
Lysozyme, 904
Lysyl fluorescein conjugated bile acid analogues (LFCBAA), 268–269, 488–489

M
Macro-biological delivery platforms, 811–820
Macromolecular polyamidoamine dendrimer cores, 548–549
Macrophage inflammatory protein 1alpha (MIP-1_), 264–265, 389, 394
Macrophages
delivery modalities, 812–813, 823–824, 832–833, 863–864, 889
ex vivo, 248–252
gene delivery and, 430
hepatic, 549
mannosyl-fucosyl receptors, 877–878
phagocytosis, 251, 254, 255, 494, 863–864
radiographic image-enhancing agents, 749
respiratory burst potential, 251–252, 255–256
in vivo labeling, 444–445, 621–622, 889
Macro-synthetic delivery platforms, 820–869
Magnesium-based contrast media, 525
Magnesium sulfate, 15
Magnetic bead-linked oligonucleotide probes, 438, 861
Magnetic microspheres, 850–851
Magnetic polysaccharide nanoparticles, 545–546, 816
Magnetic resonance imaging (MRI), 517–567
cardiovascular, 523–532, 589
central nervous system, 532–544
contrast agent profiles and properties, 521–523
experimental imaging contrast-enhancing agents, 565–566
gastronintestinal, 544–545
general concepts and principles, 517–518
genetic, 545
hematology, 545–546
hepatic, 546–552
ischemia and infarction, 552
musculoskeletal system, 552–555
myocardial ischemia, 56–57
neoplasia, 555–560
pancreas, 560–562
reactions to contrast agents, 518–521
renal, 562–563
respiratory, 563–565

Magnetic resonance imaging (MRI), (*continued*)
special senses, 544
stem cells, 566–567
Magnetite dextrane nanoparticles, 530, 542
Magnetite nanospheres, PEG-coated, 798
Malabsorption, 190–191, 193–203, 674
Maldigestion, 213
Malignant hyperthermia, 260–263, 286, 330–332, 344–347, 426–427, 463
Malnutrition, infantile, 161
Malondialdehyde, 224–225, 250–251
Maltose, 199
Mammary carcinoma, 242, 263–264, 333–334, 421, 423–424, 437–438, 497, 505–506, 555–556, 599–600, 627–629, 642–645, 649–652, 659–661, 722, 800–804, 835, 864–865, 872–873, 878–879, 881–882, 891–898
Mammary gland disease differentiation, 514, 555, 722–723, 807–808
Mammography, 506
Mangafodipir trisodium, 546, 548, 560–561
Manganese chloride, 540–541, 547, 554
Manganese-dependent contrast MRI, 540–541
Manganese dipyridoxal diphosphate [Mn]-DPDP, 546, 547, 550
Manganese (Mn^{+2}), 541–542, 547
Mannitol, 192, 194–199, 205–208, 629–630, 843
Mannosylated liposomes, 832–833, 877–878
Mannosyl-fucosyl receptors, 877–878
Markovian analysis, 361, 511–512
Mass spectrometry, 289, 290
Mast cells, 391
Matrix metalloprotease, 232, 248, 334, 342–343
Maximal mid-expiratory flow (MMEF), 382
Mazzotti test, 452
MBEC4 cell transfection, 818
MCI-028, 135
MCLA, 470
MC-PROXYL, 531
MDMA (3,4-methylenedioxymethamphetamine), 103
Mebrofenin, 546, 611
Mechanical delivery platforms, 904–905
Mechanical ventilation, 782–783
Mediastinal tumors, 723
Mediastinum ratio, 577–578, 582, 601
Medullary carcinoma of thyroid, 170, 416–417, 419–420, 633–634, 663–665, 896–897, 903
Medulloblastoma, 543, 559
Megakaryocyte, 224
Meglumine iothalamate, 755
MEGX (monoethylglycinexylidide), 461
Melanocyte-stimulating hormone (MSH) receptor, 645–647, 899–900
Melanoma, 263–264, 337, 422, 425, 429, 431, 493, 498, 505, 640, 645–647, 660–661, 727–728, 811–812, 828–829, 837–839, 842, 882–883, 899–900
Melatonin, 105
Membrane characterization, 493–496
Membrane pore size, 494
Membrane protein-lipid interactions, 495
Ménière's disease, 127
Meningioma, 122, 599–600, 633–634, 896–897
Meningitis, 537
MEN-2 (multiple endocrine neoplasia type 2), 148, 171–172, 416–417
Mephenytoin, 282–283
Mepivicaine, 62, 342
6-mercaptopurine, 282, 320
Mesenchymal stem cells, 20, 566
Messenger RNA (mRNA), 428–430, 437–438
Metabolism, 455–470, 622, 709–712
Metaclopramide
aldosterone-producing adenoma, 135
Diamond-Blackfan anemia, 222
gastric motility, 186
gastroesophageal reflux disease (GERD), 189
hyperprolactemia, 167
infertility, 165–166
multiple sclerosis, 94, 230, 233
pheochromocytoma, 147, 148, 149, 418, 626–627
Metaiodobenzylguanidine (MIBG), 27, 50, 53, 149, 245–246, 576–579, 582, 586–589, 600–604, 626, 631–632, 677–681, 680, 685, 724, 869
Metalloproteases, 860–861
Metaproterenol, 391
Metformin, 749–750
Methacholine
asthma, 236–237, 381, 383, 386–389, 391–393, 396
bronchial response, 375–378, 391–392
immune hypersensitivity, 383, 386–389, 391–393, 396
pheochromocytoma, 148
Methimazole, 312
Methionine, 279, 315, 398–399, 401–402, 442, 458, 467
Methohexital, 42, 70, 85, 87–88, 101, 198–199
Methotrexate
blood-brain barrier disruption, 755
MTHFR and, 315–316, 334–335
rheumatoid arthritis, 232–233, 343, 427–428
TPMT and, 319–320
3-methoxymorphinan, 300
Methoxypoly(ethylene glycol)2000, 199
Methoxy-X04, 66–67, 483
Methylation, 314–316, 398–399
Methylation of RNA, 427
Methylene blue, 39, 45–46, 219, 412–413, 474, 475, 488
Methylene diphosphate, 645
Methylenetetrahydrofolate, 458
Methylergonovine maleate, 25–26
Methylglucose, 191–192, 204–208
3-0 methylglucose (3-0 MG), 194, 196, 198–199, 208
Methylphenidate, 73, 95, 326
Methylprednisolone, 118–119, 229
4-methylpyrazole, 66
Methyl 2-tetradecylglycidate, 691, 710–711
Methyl thymol blue, 552–553
Metimazol, 313
1MeTIQ (1-methyl-1,2,3,4-tetrahydroisoquinoline), 102
Metoclopramide, 351–352, 353
Metoprolol, 52, 778
Metrizamide, 116–117, 755
Metylenetetrahydrofolate (MTHFR), 315–316, 334–335
Metyrapone, 132, 136, 140–141, 143–145, 146, 604, 626, 677
MFG (milk fat globulin), 820
MGDNF gene, 818
MIBG, 27, 50, 53, 149, 245–246, 576–579, 582, 586–589, 600–604, 626, 631–632, 677–681, 680, 685, 724, 869
MIBI (methoxy-isobutyl-isonitrile), 577, 583–584, 626, 630, 648–649, 649, 662–663, 665, 716, 719, 727, 881
Microalbulimuria, 460
Microarrays, pharmacogenetics and, 288, 289
Microbubbles, 40–42, 41–42, 480, 765–773, 775–778, 780–781, 783, 785–805, 807, 845–857
Microcapsules, 845, 847
Microencapsulation, 852–853
Microglial cells, 558, 823–824
Microglobulin, 871–872, 883–884
Microparticles, 852, 854
Microsomal enzyme metabolizing capacity, 290–292
Microspheres, 44, 429, 466, 495–496, 511, 581–582, 775, 829, 840, 845–857, 851–856, 861–862
Micro-synthetic delivery agents, 869–883
Microvascular leukocyte receptor complex expression, 534
Microvascular permeability/integrity, 526
Micturating cystourethrography (MCU), 807
Midazolam, 261, 305–309, 311, 344–345, 463
Milacemide, 81–82, 88–89
MION-46L, 566, 816–817

Mitochondrial disorders, 458
Mitochondrial K/ATP channels, 477
Mitochondrial respiration uncoupling, 276, 402
Mitosis, 408, 467–468, 691–692, 711–712, 723–724
Mitotane (o,p′DDD), 153
Mitral regurgitation, 788
Mivacurium, 323
MK-0869, 689
425 MoAb-PEG-PE, 835–836
Molecular delivery platforms, 869–904
Molsidomine, 23
Mometazone, 247, 249
Monoclonal antibody, as delivery system, 889, 900. *See also specific antibodies*
Monocrystalline iron oxide nanoparticles, 539, 563
Monocytes, 245, 248–252, 749, 813
Monoethylglycinexylidide (MEGX), 269–270, 274–276, 479
Mononuclear cell function, 254, 494
Mood disorders, 285
Morphine withdrawal, 73–74, 97
Mosapride, 104, 182, 185, 210
Motion sickness, 93–94
MP2269, 543, 549–550, 552, 558–559
MP2286, 896–897
MRNA/cDNA microarrays, 249–250
MS-325, 543, 558–559
Mucosal integrity and permeability, 487
Mu-lac transducing phage, 435
Multidetector row spiral computed tomography, 756–757, 764
Multidrug resistance, 648–649, 880–881
Multiphoton *in vivo* imaging, 474
Multiple sclerosis (MS), 94–95, 133, 229–230, 533, 537–538, 889
Multistate isoproterenol tilt-table test, 35
Multitest CMI, 242, 248
Murmers, 32
Muscarinic acetylcholinergic receptors, 66, 600, 677–678, 685–686, 870, 873
Muscimol-[Alexa Fluor 532], 91–92
Musculoskeletal system, 341–349
  ex vivo, 261–263
  fluorescent and pigment dye reagents, 496
  magnetic resonance imaging (MRI), 552–555
  neoplasia, 423
  nuclear planar scintigraphy, 622–626
  pharmacogenetics, 330–332
  positron emission tomography (PET), 712–713
  sepsis and inflammation, 447–450
  ultrasonography, 798–800
Myasthenia gravis (MG), 61, 114–116, 230–232, 347–348
Mycobacterium spp., 243–244
Myelography, 520–521, 539, 747, 759
Myeloperoxidase, 252–253, 786
Myocardial akinesis, 780–781, 784
Myocardial contrast echocardiography (MCE), 767, 775–776, 780–781, 787
Myocardial dysfunction, 55–56
Myocardial function, 55–56, 683, 781–782
Myocardial hypoxia, 580–581
Myocardial infarction, 26–28, 56, 477
  echocardiography, 56, 784–785
  magnetic resonance imaging (MRI), 523–525
  nuclear scintigraphy, 26–28, 581–583, 586, 602
  prognosis, 27–28, 56
  stem cells, 817
Myocardial injury, 582–583
Myocardial ischemia, 19, 56–57, 464, 465, 466, 577, 580–584, 619, 682–683, 776, 785, 813
Myocardial Na/K ATPase, 461, 585–586, 870
Myocardial necrosis, 584
Myocardial perfusion, 57–59, 525–526, 584–585, 684, 776, 785–787
Myocardial sarcoidosis, 527
Myocyte apoptosis, 582–583
Myocyte labeling, 525
Myogenic precursor cells, 817
Myo-inositol, 367–368
Myopathy, 263, 348–349, 626
Myotonic dystrophy, 275, 379, 399

N
N-acetylcysteine, 470, 477, 519–520
N-acetyldopamine, 900
N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide, 113–114
N-acetyltransferase, 316
NAD-229, 701–702
Nafamostate mesilate, 738
Nafarelin, 151, 165
Na/K ATPase, 461, 585–586, 870
Nalipride, 695
Nalorphine, 73–74
Naloxone, 73–74, 149
Naltrexone, 164–165, 166
Nanocrystals, 556
Nanoencapsulation, 868
Nanoparticles, 251, 254, 429, 473, 494, 556, 761, 787, 817, 829, 851–852, 857–869
Nanospheres, 857–869
Naphthoflavone, 291–292
Naproxen, 689, 904
Narcolepsy, 95
NAT2 inborn errors, 298–299
Natural killer cells, 811–812
NC100100, 785
NC100150, 36–37, 407, 473, 506, 528–529, 543–544, 559–560
N-[^{11}C]methylamino-4′-hydroxystilbene, 68
Near-infrared (NIF) probes, 21, 342, 407–408, 423–424, 473, 477, 494, 496, 501–503, 507
  diffuse optical tomography (DOT), 514, 555, 722–723, 807–808
  magnetic resonance imaging (MRI), 522, 555
  mammary gland differentiation, 514, 555, 722–723
Nebulization, 905
Necrosis-avid contract agents, 552
Necrotic tissue, 555
Nedocromil, 384–385
Neoplasia, 407–424. *See also specific forms*
  adrenal gland, 626–627, 661–662
  angiogenic neovascularization, 558
  apoptosis, 455–457
  bladder, 370, 423, 500, 651, 890–891
  central nervous system, 95–96, 263–264, 409–410, 542–544, 629–634, 660–661, 713–716, 872–873
  colon (colorectal), 219–220, 241–242, 410–411, 413, 420, 476, 500–501, 505, 600, 636–638, 651, 677–678, 685–686, 717–719219–220, 795–796, 834, 873, 890–894
  computed tomography (CT) image-enhancing agents, 762–763
  delivery modalities, 812, 820, 849–850, 864–866, 878–883, 890–903
  DNA intercalation, 425
  endocrine system, 415–420
  ex vivo, 263–264
  fluorescent and pigment dye reagents, 497–507
  gastrointestinal system, 190, 219–220, 241–242, 410–411, 410–415, 896–897
  gene delivery and, 429–435
  gene expression, 436–437
  glioma/gliosarcoma, 95–96, 263–264, 409, 433, 456, 485, 503–504, 510–511, 542–544, 558–560, 629–631, 660–661, 714–716, 801–802, 827, 849, 872–873, 897–898
  hepatic system, 275–276, 280, 547–548, 550–551, 638–639, 760, 762–763, 767–768, 776–777, 792–798
  immune system, 242, 250, 420–421
  integumentary, 337, 421–422
  intestine and colon, 219–220

Neoplasia, (*continued*)
liposomes as delivery system, 834–840
lung, 627–629, 633–634, 636, 649, 658, 660–661, 663–664, 726–727, 763, 828, 865, 878, 881, 892–893, 896–897, 903
lymphoma, 242, 420–421, 423, 505–506, 599–600, 641–642, 721, 899, 902
magnetic resonance imaging (MRI), 555–560
mammary, 242, 263–264, 333–334, 421, 423–424, 437–438, 497, 505–506, 555–556, 599–600, 627–629, 636, 642–645, 649–652, 659–661, 722, 800–804, 835, 864–865, 872–873, 878–879, 881–882, 891–898
melanoma, 263–264, 337, 422, 425, 429, 431, 493, 498, 505, 640, 645–647, 660–661, 727–728, 811–812, 828–829, 837–839, 842, 882–883, 899–900
metastatic, 647–648, 663–665, 713–714, 762–763, 899
multidrug resistance, 648–649
musculoskeletal system, 423
neuroendocrine, 409–410, 485–486, 632–633, 660–661, 724
nuclear planar scintigraphy, 626–665
oral, 180, 413–414, 499–500
osteosarcoma, 342, 423, 496, 625, 648–649, 881
ovarian, 430–431, 633–636, 654, 803, 829, 835–836, 865, 880, 894–897
pancreatic, 130, 212–219, 414–415, 627–629, 634, 636, 653, 661–662, 895–897, 900–903
pharmacogenetics, 333–334
pituitary tumors, 133–134, 661–662
positron emission tomography (PET), 709–710, 713–729
prostatic, 431, 556–557, 627–628, 634, 636, 657–658, 777, 803–806, 835–836, 850, 895–896, 903
renal system, 423, 562, 658–659
reproductive system, 423–424
thyroid gland, 170–172, 173, 416–417, 419–420, 633–634, 651, 662–665, 890–891, 896–897, 903
ultrasonography, 767–768, 792–798, 800–804
Neopterin, 334
Neostigmine, 12–13, 61, 63, 115, 116, 231
Nephropathy, 362, 460, 746, 749–750
Nephrostogram, 731
Nephrostolithotomy, 731
Nephrotoxicity, 736–739, 745–746
Nephroureteral stent, 731
Nerve agents, 101
Neural crest tumors, 631
Neu receptors, 864–865, 891–892, 895
Neuroblastoma, 631–632, 650–651, 724, 901
Neuroblastoma disialoganglioside, 431
Neuroectoderm-derived tumors, 900
Neuroendocrine neoplasia, 409–410, 485–486, 632–633, 660–661, 724
Neuroendopeptidase, 393
Neurofibrillary tangles, 482–483, 688–689
Neurokinin-1, 689
Neurolepsy, 326
Neuromuscular disorders, 62–63
Neuromuscular spasticity, 104
Neuropeptide receptor distribution, 599
Neurophysin, 896
Neurosonography, 791
Neurotensin, 130, 415, 497–498, 653, 900–901
Neurotoxicity, 539–540, 692, 737–738
Neurotransmitter dynamics, 96, 540
Neurotrophic factor, 818
Neutrophil chemotactic activity (NCA), 234
Neutrophil leukotriene B_4 receptors, 580, 619, 813
Neutrophils
chemotaxis, 253–254
delivery modalities, 812–814, 864, 889
elastase expression, 391
ex vivo, 252–256
[^{111}In]-labeled, 223, 245, 450–451, 670–671
margination, 223
metabolism, 728–729
phagocytosis, 251, 254, 494, 864
respiratory burst activity, 251–256
[^{99m}Tc]-labeled, 446–447, 666, 814
in vivo labeling, 444–445, 621–622, 889
New methylene blue, 481–482
N-[3-(1H-imidazol-4-ylmethyl)-phenyl]ethanesulfonamide, 871
NHS-A-biotin, 443
NHS-LC-biotin, 443
N-[2-(hydroxyethoxy)methyl]-5-[^{3}H]methyluracil, 658
Niacin, 74–75
Nicotinamide, 278
Nicotinamide N-methyltransferase (NNMT), 278
Nicotine, 280–281, 297, 326
Nicotinic acetylcholine receptors, 63–64, 874
Nicotinic ligands, 63–64
Nifedipine, 23, 25–26, 750, 763
Nile blue dye, 504
Niosomes, 868
NIR96010, 506
Nitric oxide
asthma, 237, 381, 396
immune hypersensitivity, 387, 394, 396
production potential, 468
release and contrast media, 732–733
Nitric oxide synthase, 394, 548
Nitroblue tetrazolium (NBT), 251–252, 255–256
Nitrogen/dye laser, 421–422, 498–499
Nitrogen mustard (NTM), 221, 260, 425
Nitroglycerin
angina pectoris, 23–24, 50–51
brachial artery vasodilation, 774
syncope, 33–25, 106–108
vascular grafts, 44, 49, 106–107
vasodilation response, 47, 59–60
ventricular (myocardial) function, 59, 781–782
Nitroimidazole, 463–464
2-nitroimidazoles, 709
Nitroprusside, 750, 763
Nitrosamines, 293, 297
NMDA receptors, 93, 465, 710
N-2-mercaptopropionyl glycine (2-MPG), 470, 477
[^{15}N]-methacetin breath test, 673–674
N-methylation, 278
N-methyl-D-glucamine dithiocarbamate $(MGD)_2$-Fe^{+2}-NO complex, 548
N_2 microbubbles, 769
N-(1-naphthyl)-N′-(3′-[^{125}I]-iodophenyl)-N′-methylguanidine, 465, 710
N-(N-benzylpiperidin-4-yl)-2-[^{18}F]-fluorobenzamide, 643, 722, 881–882
N,N-dimethyl-2-(2-amino-4-[^{18}F]-fluorophenylthio)benzamide, 702
N-nitrosamines, 293
N-nitrosodiethylamine, 293–294
N-nitrosomethylurea (NMU), 644
Noble gases, hyperpolarized, 564–565
[^{15}NO] exhalation, 379, 402
Non-Hodgkin's lymphoma, 641–642, 721, 899
Non-steroidal anti-inflammatory drugs (NSAIDs), 42, 209–210, 327, 445–446, 607, 615–616, 625
Noonan's syndrome, 163
Norepinephrine, 91, 144
Nortriptyline, 78–79, 105, 301
N-tert-butyl-alpha-phenylnitrone, 67, 468–469, 484
N-1-tritylsulfenyl histamine, 458–459
Nuclear scintigraphy, 569–675
amyloid and amyloidosis, 569–571
angina, 47–48, 50
apoptosis, 572–573
autonomic nervous system, 600–603
breath tests, 673–675
cardiovascular, 575–595
central nervous system, 595–600
endocrine, 603–606

gastrointestinal, 606–609
gene expression, *in vivo* detection of, 573–575
general, 569
hematology, 609–611
immune system and inflammatory responses, 611–622
metabolic and metabolism, 622
musculoskeletal, 622–626
myocardial infarction, 26–28
myocardial ischemia, 57
myocardial perfusion assessment, 57–59
neoplasia, 626–665
pancreas, 666
renal, 666–669
respiratory, 669–673
thrombosis, 40
Nucleic acids
gene delivery, 826–830, 850–851
metabolism and neoplasia, 665, 718
Nucleus robustus archistriatalis, 541–542
[^{15}N]urea, 189
Nutrient drink test, 187

O
Obesity, 172–173
Octafluoropropane microbubbles, 780–781, 783
Octa-n-butoxy-substituted naphthalocyanine, 469
Octreotate, 409–410
Octreotide, 633, 659
Ocular inserts, 904
O-dianissidine dihydrochloride, 252–253
Olanzapine, 689–690687–688
Oligodendrocyte progenitor cells, 533, 816–817, 859
Oligodextran, 831
Oligonucleotides
antisense, 97, 242, 420–421, 426, 428–430, 433, 436, 488, 510, 828, 838–839, 850
CTFO (circular triple helix-forming oligonucleotides), 435
liposome-encapsulated Cy3-labeled, 429–430
magnetic bead-linked oligonucleotide probes, 438, 861
phosphoric diester-modified, 435
TFO (triple helix-forming oligonucleotides), 420–421, 426, 488, 504–505, 510
Oligouria, 355
Omeprazole, 30, 170–171, 298, 305–306, 419–420
Onchocerca volvulus, 452
Oncosis, 455–456
Oncosis, isoproterenol-induced, 573, 576
Oocyte retrieval prediction, 166–167
Ophthalmic artery dependent vision, 95–96, 122, 123
Ophthalmic delivery, 866–867, 904
Opiate peptides, 604, 626, 677
Opioid abstinence, 73–74
Opioid receptors, 96, 692–693, 873, 888–889
Opioid tolerance, 97
Optical coherence tomography, 867
OptiMARK, 543, 558–559
Optison, 765, 769, 771, 782, 784, 791, 797–798, 845
Oral cancer, 180, 413–414, 499–500
Organic anion transporter, 99, 364
Organophosphate, 97, 260, 280–281
Osteoarthritis, 554
Osteomyelitis, 444, 447–450, 608, 616–619, 623–625, 712–713
Osteopenia and osteoporosis, 341–342, 364–365
Osteosarcoma, 342, 423, 496, 625, 648–649, 881
Ouabain, 461
Ovalbumin, 240, 241, 265, 394–395
OVA-liposome, 831–832
Ovarian carcinoma, 430–431, 633–636, 654, 803, 829, 835–836, 865, 880, 894–897
Oxidases, mixed function, 314
Oxidation-activated fluorogenic probes, 67, 468–469, 484
Oxidation potential in tissues, 468–469
Oxidative stress, 250–251
Oximetry, 469
Oxotechnetium, 645
Oxotremorine, 66
Oxygen
bronchial constriction response, 375
hyperpolarized, 564
ventilation/perfusion mismatch, 380
Oxygen tension indicator, 469, 552
Oxytocin, 167
Oxytocin receptor complex expression, 650–651, 901
Oxytremorine, 280–281

P
P604, 746
P743, 744, 757
P7228, 545–546
Paclitaxel conjugate, 900
Paget's disease, 647–648
Pain and lameness disorders, 342
Pain sensation/threshold, 326–327
PAMAM-G4D-(1B4M-Gd)(64), 547
p-aminosalicyclic acid, 214
Pancreas
endocrine function, 129–130, 607–608
exocrine function, 212–216, 402, 487–488
magnetic resonance imaging (MRI), 560–562
neoplasia, 130, 212–219, 414–415, 497–498, 627–629, 634, 636, 653, 661–662, 895–897, 900–903
nuclear planar scintigraphy, 634, 636, 666
pancreatitis, 212, 214, 215, 294, 561–562, 666
ultrasonography, 804–805
Zollinger-Ellison syndrome, 212–219
Pancreolauryl test, 216, 487–488
Pandolol receptor, 874–875
Panic disorder, 74
Pannus, 555, 799
Papain-induced cartilage injury, 553
Papaverine, 352, 750, 763
PAPS, 317
Paraaminohippuric acid (PAH)
effective renal plasma flow (ERPF), 356, 362
excretion, 329–330, 366–367, 512
organic anion transporter, 99, 364
tubular absorption, 512
Paracetamol, 184–185, 202, 279
4-para-phenylvinyl estradiol analogues, 643, 878–879
Parasitology, 452–453
Parasympathic nervous system, 122, 600–601, 873–874
Parathyroid adenoma, 653, 655–656, 724–725
Parathyroid hormone, 176–177
Parkinson's disease, 70–71, 93, 97–99, 322, 486, 566, 598–599, 693–698, 815, 852, 875–876
Pars intermedia pituitary adenoma (PIPA), 145, 417
Patent blue V dye, 493
Patent ductus arteriosus, 42
PEBBLEs, 511, 860, 868
Pellegra, 74–75
Pentagastrin
achlasia, 181
carcinoid syndrome, 410
gastrinoma, 129, 217–218, 413
hepatic neoplasia, 280
thyroid carcinoma, 170–172, 416–417, 419–420, 664–665
VIPoma syndrome, 130, 415
Pentazocine, 659, 692–693
Pentobarbital, 85, 111
Pentylenetetrazol, 78–79, 82–83, 100, 105

PEO-PPO-PEO, 429
Peptide delivery, by liposomes, 840–841
Peptide nucleic acid (PNA), 435–436, 503–504, 574–575
Percuntaneous transluminal coronary angioplasty (PTCA) therapy, 581
Percutaneous computed tomography, 761
Percutaneous trans-hepatic cholangiography (PTC), 744–745
Perfluorcarbons, 565, 786, 857–858
Perfluorobutane, 789–790, 793–794, 846
Perfluorochemicals (PFCs), 523, 767–768
Perfluorodecalin (PFD), 780
Perfluorodichlorooctane (PFDCO), 780
Perfluorohexane (PFH), 780
Perfluorooctane (PFO), 780, 787
Perfluorooctyl bromide (PFOB), 780, 787
Perflutren microbubbles, 780, 784
Perfusion-metabolism mismatch, 465, 682–683
Peripheral artery disease
  arteriography, 773–774
  of lower limb, 19–20, 259
  platelets and, 259
  thrombosis, 594–595, 685, 870–871, 887
Peripheral nervous system, 62–63
Peripheral vascular disease, function and physiology, 36–47
Peritumor rim sign, 546
Permeation properties, of myocardial cells, 19
PESDA, 776
Pesticide, 102
P-glycoprotein (Pgp), 649, 881
Phage facilitated delivery, 861
Phagocyctic killing index, 251, 255
Phagocytosis, 251, 254–255, 494, 833, 863–864
Phantom studies, 518
Pharmaceutical delivery, 509–511
Pharmacogenetics
  acute lymphoblastic leukemia (ALL), 287–288
  antidepressants, 285
  attributes and perspectives, 283–285
  chemotherapeutic agents, 286
  concepts and disease examples, 281–288
  cytochrome P450, 286–287
  epilepsy, 286
  ethics and practices, 285
  extrahepatic, 322–335
  general hepatic biochemical metabolization, 289–290
  infectious disease, 286
  phase II metabolizing enzyme systems, 314–322
  phase I metabolizing enzyme systems, 290–314
  of prognosis and therapeutic response, 332–335
Pharmacogenomics, 289–290
Pharyngeal perforation, 748
Phased-array intracardiac echocardiography, 782
3-phase dynamic computed tomography, 776–777, 794–795, 800
Phase II metabolizing enzyme systems
  diagnostics, 278–279
  pharmacogenetics, 314–322
Phase I metabolizing enzyme systems
  diagnostics, 273–278
  pharmacogenetics, 290–314
Phase inversion harmonic imaging (PIHI), 797–798
Phenazocine, 873
Phenobarbital, 101–102, 290–294
Phenobarbitone, 102
Phenol red, 369, 461, 479–480, 513–514
Phenolsulfonphthalein (PSP)
  acute renal failure, 363–364
  gastrointestinal permeability, 205, 206
  glomerulonephritis, 511–512
  mucosal integrity and permeability, 487
  renal function, 361–362, 364, 366–368
  tubular absorption, 512, 513
  tubular excretion, 512–513
  tubular failure, 513
Phentolamine, 31, 62, 148
Phenylalanine, 330, 348, 355–356
Phenylbutazone, 173–174
Phenylephrine
  glaucoma, 119–120
  Horner's syndrome, 121
  long Q-T interval syndromes, 324
  myocardial glucose metabolism study, 18
  reversal with dapiprazole, 122–123
  sympathetic ophthalmic innervation, 126
  T wave lability, 13–14
Phenylethanolamide, 136
Phenylpropionic acid, 458
Phenytoin, 102, 308–309
Pheochromocytoma, 146–150, 417–418, 626–627, 632, 724, 901
Phlegmon, 791–792
Phleum pratensis, 377
Pholedrine, 120–121
Phorbol myristate acetate, 253–254
Phosphatidyl ethanolamine, 824
Phosphatidylinositol, 691
Phosphatidyl serine, 824
Phosphoric diester-modified oligonucleotides, 435
Phosphorothioate, fluorescein-labeled, 420–421, 504–505
Phosphorous excretion, 368–369
Phthalocyanine, 507
Physostigmine, 73, 92, 122
Phytohemagglutinin, 242, 247–248
Picrotoxin, 105
p-[^{123}I]-iodo-L-phenylalanine, 872
Pilocarpine
  glaucoma, 118, 119–120
  magnetic resonance imaging (MRI) and, 536–537
  parasympathic innervation, ophthalmic, 122
  pupillary response, 123
  RS 86 compared, 66
  xerostomia, 180–181
Pinacidil, 27, 477, 523–524
Piperidinyl nitroxide stable free radical derivative, 562–563
[^{18}F]-Piperidyl benzilate, 686
Piracetam, 81–82, 88–89
Piribedil, 75, 110
Piroxicam, 297
Pitressin tannate, 365
Pituitary-dependent hyperadrenocorticism (PDH), 136, 137, 143
Pituitary dwarfism, 150–151, 155, 167–168
Pituitary neoplasia, 149, 656, 661–662
Pituitary pars intermedia dysfunction, 142
Pituitary tumors, 133–134
PKH dyes, 226–227, 492
PLA nanospheres, 867
Plasmid-complexed liposomes, 801, 818, 820, 824–826, 840, 849
Plasmid DNA, 429, 432–435, 827–828
Plasmid-microspheres, 801, 820, 840, 849
Plasmids, 817
Platelet endothelial cell adhesion molecule-1 (PECAM-1), 884–885
Platelet GP IIb/IIIa receptor antagonist, 594–595, 685, 870–871, 887
Platelet GP IIb/IIIa receptor expression, 444, 579, 831
Platelets
  activation, 256–257
  adhesion, 257–258
  aggregation, 258–259
  antiplatelet properties of contrast agents, 735
  consumption, survival, 223–224
  [^{51}Cr]-labeled, 224
  delivery modalities, 814–815

endothelium interactions, 225–226, 476, 491–492
ex vivo, 256–259
fluorescent labeled, 16, 124, 125, 226, 476, 490–491
HMPAO-labeled, 594
[^{111}In]-labeled, 223–224, 593–594, 814–815
[^{99m}Tc]-labeled, 814–815
thrombasthenic, 257–258
Platelet-specific monoclonal antibody (P256), 40
PLGA, 854, 856
P7228 liposomes, 816
PMA (para-methoxyyamphetamine), 103
Pneumolysin, 249–250
Pokeweed mitogen, 242, 247–248
Polioencephalomalacia, 99–100
Poloxamine-908, 833, 858–859
Polyacrylic acid, 866–867
Polybutylcyanoacrylate nanoparticles, 859859
Polycrystalline iron oxide nanocompounds (PION), 551–552
Polycyclic aromatic hydrocarbons, 293
Polycystic kidney disease, 450, 729
Polycystic ovarian syndrome (PCOS), 166
Poly(D,L-lactic-Co-glycolic acid) multiphase microspheres, 495–496
Polyethylene glycol 400, 199
Polyethylene glycol (PEG), 26, 428–429
coated magnetite nanospheres, 798, 848, 863
ITG-PEG, 762–763
lipid-shelled microbubbles, 777–778
liposomes, 447–448, 617–618, 623, 800, 823, 834–837, 839–840, 842, 849
transferrin-PEG-polyethylenimine, 434–435
Polyethylenimine, 429, 434–435
Polyethylenimine polymers, 829, 851–852, 861–862
Polygalacturonic acid, 565–566
Poly-L-lysine, 844
Polylysine -[Gd]-DTPA, 539–540
Polymerized nanoparticle, 37
Polymer millrods, 856–857
Polyneuropathy, familial amyloid, 571, 597
Polyoxyethylene, 251, 864
Polyoxyethylene glycol 4000 stearate, 868
Polyoxypropylene, 251, 864
Polypeptide delivery, 511
Polysorbate 80, 859
Polystyrene fluorescent spheres, 514
Polyuria, 363
Poor metabolizers, 298, 305–306
Porphyrin derivatives, 429, 829, 851–852, 861–862
Portal vein, 744–745, 771–772
Portography, 759–760
Portosystemic shunt/vascular anomalies, 43, 277–278, 281
Positron emission tomography (PET), 677–729
artifacts, 677
cardiovascular, 677–685
central nervous system, 685–705
computed tomography and, 756, 758
gene expression, 705–706
glucose metabolism, myocardial, 18
hepatic, 706–708
immune system, 708
inflammation and sepsis, 708–709
metabolism, 709–712
musculoskeletal, 712–713
neoplasia, 713–729
special senses, 705
Potassium, 343–344, 462–463
Potassium ATP channel opener, 11
Povidone iodine dye, 179–180
PP101/PP149, 469
Pranlukast, 235, 384
Praunsnitz-Kustner (PK) test, 233
Pravastatin, 99
Prednisolone, 452
Prednisone, 118–119, 229
Premature thelarche, 168
Prinzmental's variant angina, 23–26
Prion plaques, 697
Probenecid, 368
Procainamide, 5–7, 7, 9–10, 15, 376
Progesterone, 164, 292, 329
Proguanil, 305–306
Prolactin, 72, 83, 89–90, 94, 105–106, 131, 164–168, 229, 337–338, 351–353
Promethaine, 78
Propanolol, 14–15, 36, 73, 161–162, 680
Propantheline, 186
Proparacaine, 122–123
Propidium iodine, 766, 854
Propylene glycol, 792
Prostaglandin E_2, 382
Prostate
carcinoma, 431, 556–557, 627–628, 634, 636, 657–658, 777, 804–806, 835–836, 850, 895–896, 903
ultrasonography, 777, 804, 805–806
Prostate-specific membrane antigen (PSMA), 836, 903
Protamine, 37, 777, 805
Protein C, 253
Protein kinase inhibitors, 469
Protein-losing enteropathy, 204–205
Proteoglycan depletion, 553
Provocative filling cystometry, 371
Provocative water cystometry, 370–371
Prussian blue, 566, 816–817
P-selectin, 256–257
Pseudoaneurysm, 792
Pseudohypoparathyroidism, 176–177
PS-TFO, 242
Psychosis, 327
Pteroylpolyglutamates, 201
Puberty, 168
PUFA, 383
Pullulan, 817, 861
Pulmonary artery pressure, 739–740
Pulmonary carcinoma, 658, 865, 878, 896
Pulmonary embolism, 529, 563, 594–595, 685, 756–757, 764, 870–871, 887
Pulmonary veins, 771
Pulse inversion harmonic imaging (PIHI), 792–793
Pupillary response, 123
Purkinje fiber action potential duration, 11
Pyelography, 731
Pyranine, 884
5-(3-pyridinyl)-4-azahomoadamantane 2a, 64
Pyridostigmine, 115, 160–161, 231
[^{18}F]-Pyrrolidyl benzilate, 686
Pyronin, 222
Pyruvate, 458

Q
QT-RR slope, 582, 602–603
Quantum dots (qdots), 556, 866
Quinidine, 313
Quinine, 291–292, 309, 391
Quipazine, 103

R
Raclopride, 695
Radiation-induced heaptic injury, 551–552
Radio allergosorbent test, 389–390

Radiographic image-enhancing agents, 731–764
adverse reactions, 731–741, 766
cardiovascular, 742–747
central nervous system, 747
gastrointestinal, 747–748
hepatobiliary, 748–749
monocytes and macrophages, 749
properties of agents, 741–742
renal, 749–750
reproduction, 750
Radioiodine, 605
Raffinose, 196
Ramipril, 322–323
Ranitidine, 313
RANTES, 228–229, 266, 393
[^{87}Rb], 27, 477, 523–524
Reactive oxygen species production, 19, 469, 470, 477, 495
RECK mRNA, 437
Rectal carcinoma, 545
Recombinant human beta$_2$ microglobulin, 871
Red acid dye, 179–180
Red blood cells
ex vivo, 260–261
fluorecent-labeled, 41, 479, 520
radiolabeled, 473–474, 595
Red blood cell velocity, 785–786
Reflectance confocal microscopy, 867
Reflex airway, 376
Reflex sympathetic dystrophy (RSD), 62
Rehnium-2, 659
Remethylation, 458
Renagrafin, 116–117
Renal artery, 372, 591–592, 667, 737, 745, 771–772
Renal system, 355–373
apoptosis, 457
computed tomography (CT) image-enhancing agents, 763
dysfunction, 305, 458, 519–520
failure, 355, 487–488, 749–750
fluorescent and pigment dye reagents, 511–514
magnetic resonance imaging (MRI), 562–563
neoplasia, 423, 562, 658–659
nuclear planar scintigraphy, 666–669
pharmacogenetics, 329–330
radiographic image-enhancing agents, 749–750
ultrasonography, 806–807
Renal tubular acidosis, 341–342, 364–365
Renal tubular function, 366–369, 668–669, 738, 904
Reperfusion injury, 20, 478, 524, 552, 786
Reproductive system, 351–354
endocrine system and, 164–170
fluorescent and pigment dye reagents, 514–515
neoplasia, 423–424
pharmacolgenetics, 328–329
radiographic image-enhancing agents, 750
ultrasonography, 807–808
Residual postembolization disease, 743–744
Resperidone, 327–328
Respiratory burst potential, 251–252, 253
Respiratory center depression, 116
Respiratory distress syndrome of newborns (RDS), 669
Respiratory system, 375–403
breath tests, 398–403
computed tomography (CT) image-enhancing agents, 763–764
disease conditions, 380–398
ex vivo, 264–266
fluorescent and pigment dye reagents, 515
lung cancer, 263–264, 437, 627–629, 633–634, 636, 649, 658, 660–661, 663–664, 726–727, 763, 828, 865, 878, 881, 892–893, 896–897, 903
magnetic resonance imaging (MRI), 563–565
nuclear planar scintigraphy, 669–673
pharmacogenetics, 330
physiology, 375–380
sepsis and inflammation, 450
Reticuloendothelial system, 251, 550–552, 864
Reticulosarcoma, 901–902
Retinal angiography, 123–124, 507
Retinal microglial cell development, 124, 508
Retinal microvasculature, 124, 476, 491–492
Retinal neuron visualization, 124–125, 508
Retinal vasculature, 125–126, 479, 508–509
Retinopathy, 46–47, 125, 479
Retroviral vectors, 811, 819–820
Retrovirus, 431–432, 433
RF-amide, 599
RGD-ultrasmall superparamagnetic particles of iron oxide (USPIOs), 531–532, 886, 887
RH414, 89, 485
Rhabdomyolyis, 263, 332, 348–349
Rhamnose, 191, 196, 198–199, 204–208
rhbeta$_2$mH5, 871–872, 883–884
Rhenium, 497
[Rhenium-N_2S_2]-E-aminotaxmoxifen, 643–644, 878
[Rhenium-N_2S_2]-Z-aminotaxmoxifen, 643–644, 878
Rheumatoid arthritis
as autoimmune disease, 232–233, 248
corticosteroids, 342–343
delivery modalities, 846
genetics, 316, 334–335, 427–428
inflammation and sepsis, 445–446
magnetic resonance imaging (MRI), 554–555
scintigraphy, 625
TPMT and, 321
ultrasound, 798–800
Rhinorrhea, cerebrospinal fluid, 77, 485
Rhodaminated LCDIO, 95, 409, 485, 543, 559, 859–860, 866
Rhodamine 123, 222
Rhodamine-labeled cationic liposomes, 837
Rifampicin, 268, 305, 309–310
Rifampil, 307–308
RNA, methylation of, 427
Robalzotan, 701–702
Rose bengal, 123–124, 507
Rotenone, 102
RS 86, 66, 686–687
(1R,2S)-4-[^{18}F]fluorometaraminol, 680
R-warfarin, 295
Ryanodine receptor, 331, 346–347

S
Salbutamol, 103, 376–377, 381, 386, 388
Salicylamide, 522
Saline infusion, 102
Salmeterol, 392
Salsolinol, 66
Salt sensitivity and regulation, 588, 603
SAMe (s-adenosyl-L-methionine), 70, 72, 79
SA node function/disease, 12–13
Sarcoidosis, 527, 540
Sarcoma, 634
S-carboxymethyl-L-cysteine, 317–318
Schilling test, 216
Schizophrenia, 74–75, 300–301, 327–328, 540, 689–690
Scleroderma, 168, 337–338
Sclerosis, 168
SCN5A gene mutation, 7
Scopolamine, 73, 93–94, 686
Secretin
biliary dyskinesis, 267

duodenal ulceration, 208–209
gastric antrum *versus* Zollinger-Ellison, 184
gastric ulceration, 188
gastrinoma, 216–219, 412–413, 488
pancreas exocrine function, 213–214
pancreatic duct function, 561
pancreatitis, chronic, 212, 215
VIPoma syndrome, 130
[^{75}Se]-D-selenomethionine, 634
[^{75}Se]-homocholic acid taurine (^{75}SeHCAT), 199–200
Seizures, 100–102. *See also* Epilepsy
alcoholic detoxification, 65, 82, 100
electroencephalography, 82–83
hypoglycemic-induced, 100–101
insecticides and nerve agents, 101
non-epileptic attacks, 102
SPECT, 697–699
Selective serotonin uptake inhibitors (SSRIs), 299–300
SELEX, 451
[^{75}Se]-methionine, 634, 638–639
Semiconductor nanocrystals, 37, 474
Semiconductor quantum dots (qdots), 556, 866
Sepsis
computed tomography (CT) image-enhancing agents, 760–761
fluorescent and pigment dye reagents, 515–516
positron emission tomography (PET), 708–709
scintigraphy, 441–443, 608–609, 612–614
Serine, 398–399, 458
Serotonin (5-HT), 76, 91, 103–105, 371–372, 376, 833
Serotonin (5-HT) receptor, 327–328, 700–702, 874–875
Serotonin transporter (SERT), 104–105, 702–703
[^{75}Se]-selenomethyl-norcholesterol, 604, 626, 677
Setastine, 74, 79–80
Sevoflurane, 303
SF_6 microbubbles, 768–769
SGAW, 382
Sheathlin, 179
SHI (2nd-harmonic imaging), 794
Shoulder, rheumatoid arthritis and, 799–800
SHU 563 A microbubbles, 770, 788–789, 791
SHU 508 microbubbles, 41–42, 480, 770–772, 788–789, 796–797, 803
Sick sinus syndrome, 12–13
Sigma receptors, 659, 881–882
Signal transducers and actiavtors of transcription (STAT), 304–305
(S)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy) pyridine, 64, 600, 874
Silicone, 755
Sincalide, 267
Sinerem, 746, 863
Single photon emission computed tomography (SPECT), 677–724
cardiovascular, 677–685
central nervous system, 685–705
computed tomography and, 756, 758
gene expression, 705–706
hepatic, 706–708
magnetic resonance imaging (MRI) compared, 539
metabolism, 709–712
musculoskeletal, 712–713
neoplasia, 713–724
Sinuses, 770
Sinus node reentrant tachycardia, 15
Skeletal muscle, 343–349, 589
Skin cancer, 498–499
Skin-prick test, 236, 388–390
Sleep disorders, 105–106
Sleep test, 347
Small unilamellar vesicles (SUV)-PEG liposomes, 800, 834, 849
SMANCS/lipiodol (S/L), 795–796
SmartMist, 904–905
S-mephenytoin, 305–306
S-nitrosylation, 330
S-[3-(N,N-dimethylamino)propyl]isothiourea (dimaprit), 183–184
SnoRNA-2 gene, 427
Sodium amobarbitol, 42–43, 61, 71
Sodium [^{13}C]-acetate, 189
Sodium deoxycorticosterone, 132
Sodium diclofenac, 843
Sodium fluorescein, 18. *See also* Fluorescein
Sodium hydroxide, 190
Sodium lactate, 74
Sodium nitroprusside, 32, 750, 763
Sodium-potassium ATPase, 461, 585–586, 870
Sodium salicylate, 276, 402
Sodium sulfanilic, 362
Sodium thiosulfate, 358
Somatosensory stimulation mapping, 540–541
Somatostatin analogues, 633
Somatostatin receptor, 263–264, 409–410, 421, 423, 485–486, 505–506, 599–600, 631, 633, 654–655, 660–662, 902–903
Sonazoid, 765–767, 793–794, 796, 845
SonoVue, 765, 775–776, 845
Sorbitol, 269
Soya lecithin, 824
Special senses, 117–127
fluorescent and pigment dye reagents, 507–509
magnetic resonance imaging (MRI), 544
positron emission tomography (PET), 705
SPECT (single photon emission computed tomography)
adenosine stress myocardial infusion, 55
cardiomyopathy, 245–246
dural arteriovenous fistulas, 114
hepatic excretory function, 271
myocardial ischemia, 57
myocardial perfusion assessment, 57–58
transient ischemic attack (TIA), 48–49
Spermidine, 900
Sphicter of Oddi dysfunction, 219, 267, 268, 792
Sphingomyelin, 495, 821–822
Spinal cord, 116–117
Spin-echo MR, 565
Spironolactone, 239–240
Spirulina breath test, 187
Splanchnic hemodynamics, 777
Spleen, 767–768
Spondylitis, 712–713
Squamous cell carcinoma
esophagus, 411–412, 557
head and neck, 333–334
oral, 180, 414, 499–500
skin, 421–422, 498–499
SR 4554, 720–721
Starch microspheres, 800, 834, 849
Statins, 324–325, 626
Stem cells
delivery modalities, 815–817
gene delivery and, 434
hematopoietic stem progenitor cell tracking, 226–227, 492
magnetic resonance imaging (MRI), 566–567
mesenchymal, 20, 478
migration, 93, 226–227, 478, 486, 566–567, 815–816, 876
primitive hemopoietic (PHSC), 222
Steroid 7-α-hydroxylase, 293
Steroid 15-α-hydroxylase, 293, 304–305
Steroid 16-α-hydroxylase, 304–305
Steroid hydroxylation, 293
Steroid metabolism, 307, 311
Stilbene derivatives, 68, 484, 688
Stimulated acoustic emission (SAE), 770, 788–789
Streptavidin-conjugated iron oxide nanoparticles, 864–865, 891–892

Streptokinase, 27–28
Stress echocardiography, 54, 779, 781, 784–786
Stress test, 47–48, 227–228
Stroke, 541, 594–595, 685, 704, 870–871, 887
Strontium, 202
S-T segment depression, 22
Styrene maleic acid neocarzinostatin, 795–796
Styrylbenzene, 687–688
Styrylxanthine derivatives, 758
Subcolloidal nanoparticulate, 866–867
Substance P, 689
Succinimidyl-6-biotinamido-hexanoate, 443
Succinylcholine, 263, 323, 345
Sucralose, 205
Sucrose, 191–192, 195, 204–208
Sugar permeability, 282
Sulfaphenazole, 308
Sulfate conjugation, 316–317
Sulfidoleukotriene, 390
Sulfobromophthalein, 271–272, 489
Sulfonamide, 298–299, 878
Sulfosuccinimidyl-6-biotinamido-hexanoate, 443
Sulfotransferase (SULT), 316–319
Sulfoxidation, 317–318
Sulfrapenazole, 297
Sulfur hexafluoride, 565, 781, 795
Sulphadimethoxine, 291–292
Sulphamethoxazole, 853
Sulphaphenazole, 291–292
Sulpiride, 72, 167, 534
Sumatriptan, 89–90
Superoxide production, 28, 253–256, 470
Superparamagnetic iron oxide (SPIO), 96, 527–528, 540, 545, 549–551, 863
Superparamagnetic solid lipid nanoparticles (SLN), 533, 858
Suprastin, 74, 79–80
Surfactant, 398, 515, 669, 864
Sympathetic nervous system, 62, 126, 582, 586–589, 600–603
Syncope, 32–35, 106–109
Synechia, 123
Synovial hypertrophy, 554
Synovitis, 554–555, 799–800
Systemic lupus erythematosis (SLE), 233, 247–249
Systemic sclerosis, 337–338

T
Tachycardia, 6–8, 11, 14–15
Tacrine, 314
Tardive dyskinesia, 286
Targeted delivery, 868–869
Tartaric acid, 77–78
Tau protein, 68, 482
Tauro 23 [^{75}Se]-selena-25-homocholic acid, 209, 445–446, 607, 615–616, 625
T3,4BCPC, 510–511
TC-2559, 874
[^{99m}Tc]-acid fibroblast growth factor 1, 592, 668
[^{99m}Tc]-annexin V, 456–457, 572–573, 627
[^{99m}Tc]-anti-abeta Fab, 596
[^{99m}Tc]-anti-CD15 IgM, 447, 608–609, 616
[^{99m}Tc]-anti-CEA, 642–643, 651, 890–891
[^{99m}Tc]-antifibrin Fab, 584
[^{99m}Tc]-antigranulocyte antibody, 449, 618–619, 623
[^{99m}Tc]-antilymphoma IgG fragment, 641–642, 899
[^{99m}Tc]-arcitumo Mab, 718
[^{99m}Tc]-ATSM, 464, 583
[^{99m}Tc]-biotin-PEG-distearoylphosphatidylethanolamine avidin, 614
[^{99m}Tc]-biscisate, 699
[^{99m}Tc]Bis(N-ethoxy,N-ethyldithiocarbamato)nitrido, 585, 591
[^{99m}Tc]-bleomycin, 651
[^{99m}Tc]-BMBuA, 844
[^{99m}Tc]-BMEDA, 844
[^{99m}Tc]-CCMSH, 646–647
[^{99m}Tc]-ciprofloxacin, 441, 620
[^{99m}Tc]-CO-MIBI, 649, 881
[^{99m}Tc]-dextran, 607
[^{99m}Tc]-dextran 70, 592–593
[^{99m}Tc]-dextran 500, 493
[^{99m}Tc]-diethylenetriamine pentaacetic acid (DTPA), 358–359, 591–592, 668–672
[^{99m}Tc]-diethylenetriamine pentaacetic acid (DTPA)-galactosyl human serum albumin, 609, 706–707
[^{99m}Tc]-diethylenetriamine pentaacetic acid (DTPA)-mannosyl-dextran, 640–641
[^{99m}Tc]-dimercaptopropionic acid, 666667
[^{99m}Tc]-dimercaptosuccinate (DMS), 666
[^{99m}Tc]-DMP444, 444, 579
[^{99m}Tc]-DMP728, 594–595, 685, 870–871, 887
[^{99m}Tc]-endothelin derivative, 592, 886
[^{99m}Tc]-epidermal growth factor, 629–630
[^{99m}Tc]-ethyl cysteinate dimer, 697–698, 703
[^{99m}Tc]-ethylenediaminediacetic acid-(HYNIC-TOC), 660
[^{99m}Tc]-exametazine-labeled leukocytes, 620
[^{99m}Tc]-field bean protease inhibitor (FBPI), 652, 873
[^{99m}Tc]-folic acid, 635, 879–880
[^{99m}Tc]-furifosmin, 663727
[^{99m}Tc]-G250, 658
[^{99m}Tc]-galactosyl-neoglycoalbumin, 609–610
[^{99m}Tc]-glucaric acid, 455–456, 573, 576, 582–584
[^{99m}Tc]-glucoheptonate (GHA), 666
[^{99m}Tc]-granulocytes, 446, 614–615
[^{99m}Tc]-hexamethylpropylene amine oxime (HMPAO), 594, 597–598, 692, 698–699
[^{99m}Tc]-hexamethylpropylene amine oxime (HMPAO)-[SSL-VIP] liposomes, 644–645, 835
[^{99m}Tc]-hippurate analogues, 668–669
[^{99m}Tc]-HMPAO-labeled-leukocytes, 608, 666
[^{99m}Tc]-HMPAO-labeled-platelets, 594
[^{99m}Tc]-HMPAO-labeled-WBCs, 441, 446–447, 620
[^{99m}Tc]-human serum albumin (HSA), 422, 444, 493, 589–590, 616–617, 640
[^{99m}Tc]-hydroxyethylene diphosponate, 449–450, 617
[^{99m}Tc]-hydroxy ethyl starch, 606–607
[^{99m}Tc]-HYNIC, 246, 263–264, 400, 446–448, 456, 457, 572, 590–591, 612, 614–615, 617–618, 623, 814, 885–886
[^{99m}Tc]-interleukin-8, 439, 446, 448–449, 611–615, 618–619, 624–625, 814
[^{99m}Tc]-liposome, 615, 825–826, 833
[^{99m}Tc]-macroaggregated albumin (MAA), 593, 671–673, 756
[^{99m}Tc]-MAG3, 592, 628–629, 666, 892–893
[^{99m}Tc]-malic acid, 666–667
[^{99m}Tc]-MAMA-bombesin, 628–629
[^{99m}Tc]-MAMA-chrysamine-G, 569–570, 595–596
[^{99m}Tc]-MDP, 447–448, 617–618, 622–624
[^{99m}Tc] meal-scan, 184, 188
[^{99m}Tc]-medronate, 645
[^{99m}Tc]-mercaptoacetyltriglycine-3, 372–373, 591, 667–668
[^{99m}Tc]-MIBI (methoxy-isobutyl-isonitrile), 577, 583–584, 630, 648–649, 662–663, 665, 716, 727, 881
[^{99m}Tc]-nitroimidazole, 463–464, 580–581
[^{99m}Tc]N-NOET, 585, 591
[^{99m}Tc-N_2S_2] -aminotamoxifen, 497
[^{99m}Tc-N_2S_2]-E-aminotaxmoxifen, 643–644, 878
[^{99m}Tc-N_2S_2]-Z-aminotaxmoxifen, 643–644, 878
[$^{99m}TcO_4$], 604–606, 652, 653, 724, 873
[^{99m}Tc]-oxotechnetium(V) complexes of AADT chelates, 645
[^{99m}Tc]-PEG liposomes, 447–448, 617–618, 623
[^{99m}Tc]-peroxynitrite-modified beta-VLDL, 622
[^{99m}Tc]-pertechnegas, 672–673
[^{99m}Tc]-phytate, 549, 610, 708, 863

[^{99m}Tc]-platelets, 815
[^{99m}Tc]-pyridoxylidene glutamate, 267, 610
[^{99m}Tc]-red blood cells, 595
[^{99m}Tc]-RP128, 444–445, 621–622, 812–813, 889
[^{99m}Tc]-RP517, 441, 580, 619, 813, 890
[^{99m}Tc]-Sc-UBI, 620–621
[^{99m}Tc]-sestaMIBI, 28, 30, 57, 188, 581, 583, 648, 655–656, 663, 678–679, 681–682, 725, 727, 776
[^{99m}Tc]-sigma$_2$ receptor ligand, 659, 882
[^{99m}Tc]-sulesomab, 444, 616–617
[^{99m}Tc] sulphur colloid, 186, 422, 493, 640
[^{99m}Tc]-surfactant, 669
[^{99m}Tc]-Tat-peptide-4, 639, 898–899
[^{99m}Tc]-T3,4BCPC, 644
[^{99m}Tc]-tetrofosmin, 58, 584–585, 648, 663, 678, 684, 727, 880
[^{99m}Tc]-tobramycin-serum albumin, 670
[^{99m}Tc]-tricine-(HYNIC-TOC), 660–661
[^{99m}Tc]-tricine-nicotinic acid-(HYNIC-TOC), 660
[^{99m}Tc]-TRODAT, 697, 698
[^{99m}Tc] tropane ligands, 97, 598, 599
[^{99m}Tc]-UBI, 442–443, 620–621
[^{99m}Tc]-(V)dimercaptosuccinic acid (DMSA), 652, 873
[^{99m}Tc]-very low-density lipoproteins (VLDLs), 622
[^{99m}Tc] tropane ligands, 696–697670
TEAPC-Chol, 827
Technetium pertechnetate, 604–605
Tegafur, 246–247, 292, 333
Teldane, 74, 79–80
Tei index, 779
Temozolomide, 714–715, 725–726
Tempo carboxylic acid (TCA), 563
Tensilon, 114–115, 230–231, 347–348
Tensostat, 187
Terbutaline, 856
Terfenadine, 74, 79–80
TES, 562–563
Testicular function, 168–170, 353–354
Testosterone, 170, 292, 293, 328–329
Tetanus, 62–63
T ethanate, 310
Tetracycline, 341, 496
Tetrahydroaminoacridine, 69
Tetrasulfonate, 123–124
Texas red-labeled dextran, 337, 422, 498
TFO (triple helix-forming oligonucleotides), 420–421, 426, 488, 504–505, 510
Thalamic tectorrotundal pathway, 109–110, 486
Thalamus, 791
Thalassemia, 227
[^{201}Thallium], 47–48, 57–58, 575, 578, 585, 587–589, 593, 600–601, 625–626, 629–630, 638, 652–653, 655–656, 663, 681–682, 684, 715–716, 719, 724, 873
[^{201}Thallium]-pentetreotide, 629, 713
Theophylline, 135–136, 201, 294
Thiamine, 99–100
Thiobarbituric acid, 224–225, 250–251
Thioflavin dye, 570, 597
Thioflavin S, 68–69, 482–483, 483
Thioflavin T, 68, 484, 688, 689
Thiopental, 89
Thioperamide, 69
Thiopurine methyltransferase (TPMT), 319–321
Thoracic imaging, 563–564
Thrombocytopenia, 223–225
Thrombocytopenia, autoimmune, 815
Thrombosis
  deep venous, 584, 685, 870–871, 887
  delivery modalities, 847, 870–871, 886–887
  hepatic artery, 792
  magnetic resonance imaging (MRI), 531–532
  nuclear scintigraphy, 40, 584, 593–595, 814
  single photon emission computed tomography (SPECT), 685
  ultrasound, 770, 772, 780, 787, 792
Thromboxane, 258
Thrombus detection, 478
Thymidine, 442, 467
Thymidine kinase, 425–426, 467–468, 573–574, 657, 705–706, 712, 720
Thymidylate synthase, 333
Thymoma, 723
Thyroid gland
  cancer, 170–172, 173, 416–417, 419–420, 633–634, 651, 662–665, 727, 890–891, 896–897, 903
  euthyroidism, 172
  hyperthyroidism, 174
  hypothalamus, pituitary, and thyroid axis, 170–176
  hypothyroidism, 172–175
  obesity, 172–173
  scintigraphy, 604–606
  thyroid function, 173–175, 605–606
Thyroid-stimulating hormone (TSH), 111–112, 131
  chorea-acanthocytosis, 77, 131
  fatty acids influence on plasma levels, 163–164, 176
  infertility, 352–353
  recombinant, 173–174
  scleroderma, 337–338
  sclerosis, 168
  thyroid cancer, 171, 419
  thyroid function, 173–175
Thyrotoxicosis, 606, 653
Thyrotropin, recombinant, 173, 419
Thyrotropin-releasing hormone (TRH), 72, 73, 94, 337
  acromegaly, 150
  anterior pituitary function, 130–131, 133
  chorea-acanthocytosis, 77, 131
  cranial trauma, 110–111, 131–132
  Cushing's disease, 137–138, 142, 145, 146, 417
  endometriosis, 164, 351–352
  gastric ulceration, 187–188
  growth hormone and, 150, 162–163, 163–164, 176
  Hallerman-Streiff Syndrome (HSS), 132
  hyperprolactemia, 167
  hyperprolactinemia, 133
  infertility, 166, 352–353
  multiple sclerosis, 229–230
  myasthenia gravis (MG), 347
  obesity, hypothyroid/euthyroid, 172–173
  Parkinson's disease, 98
  pituitary tumors, 133–134, 417
  prolactin suppression, 167–168
  stimulation test, 175, 227
  thalassemia, 227
Thyroxine (T4), 131, 173–174
Tiaranide, 338
Tic disorders, 75–76, 110
TmDOTP^{-5}, 38
TNF-cDNA, 829–830
Tobacco dependency, 64, 600, 874
Toborinone, 288, 360
Tolbutamide, 291–292, 297–298, 308, 461
Tolcapone, 322
Topoisomerase, 425, 652–653, 727–728, 882–883
Torsade de Pointes, 15–16
Tourette's syndrome, 75–76, 110
Tramadol, 370
Trans-cardiac conductance (TCC), 36
Trans-cranial color-coded sonography (TCCS), 771, 789
Trans-cranial gray-scale harmonic imaging, 789–790
Trans-cranial sonography, 791

Trans-cutaneous delivery, 825
Trans-esophageal pacing, 577, 583–584
Transfection agents, 566–567, 816–817
Transferrin, 219–220, 413
  magnetic nanoparticles, 533, 545–546, 816, 817
  PEG liposomes, 839–840
  receptors, 435–436, 574–575, 598, 817, 829, 839–840, 859
Transferrin-PEG-PEI, 434–435, 829–830
Transforming growth factor-α (TGF-α), 183–184
Transient ischemic attack (TIA), 48–49, 704
Trans-jugular intrahepatic portosystemic shunt placement (TIPS), 744
Trans-membrane carrier peptide, 556–557, 657–658
Trans-methylation, 315, 398–399, 458
Trans-mucosal delivery, 860
Trans-placental contrast media transport, 741
Trans-sulferation capacity, hepatocyte, 279
Trans-sulfuration, 315, 398–399, 458
Transthyretin, 483
Tranylcypromine, 313
Trauma
  bladder rupture, 372
  CNS, 110–112, 603, 703, 876–877
  corneal, 117
  cranial, 131–132, 485
  intestinal, 207–208
Trazodone, 78–79, 105
TREN-Me-3,2-HOPO, 522
Triakontatetraneuropeptide, 254–255
Tricuspid regurgitation, 788
Tricyclic antidepressants, 299–300
Triglyceride, iodinated, 759–760, 762–763
Triiodothyronine (T-3), 131, 141, 173–174, 176
Trilostane, 142
17 (1,3,5-tri-n-hexyl-2,4,6-triiodobenzene), 748
Triphenyltetrazolium, 27, 28, 470, 477, 523–524
Troleandomycin, 305–310
Tropicamide, 117–118, 122–123
TRX-20, 836
Trypan blue dye, 481, 860
Trypanosoma gambiense, 452
Trypsin-induced injury, 553
Tryptase expression, 391
Tuberculin antigen, 244
Tuberculosis, 243, 244
Tubular absorption, 512
Tubular acidosis, 341–342, 364–365
Tubular excretion, 512–513
Tuftsin, 444–445, 621–622, 812–813, 889
Tumor necrosis factor-alpha (TNF-α)
  allergic reactions, 228–229
  anti-TNF-α, 316, 334–335, 343, 427–428
  cDNA, 434–435
  cyclooxygenase synthesis, 265–266
  gene delivery, 860–861
  hepatitis, 244–245, 279–280
  immune hypersensitivity, 393
  inflammatory responses, 397–398
  microspheres, 495–496, 855
  multiple sclerosis, 230
  neutrophil metabolism and, 728–729
  RANTES, 266
  rheumatoid arthritis, 232–233, 334–335, 427
  systemic lupus erythematosis (SLE), 233, 247–249
TUNEL tissue analysis, 456–457, 572–573, 627
Tunicamicyn, 894–895
T wave alternans, 13
T wave lability, 13–14
2D-PAGE, 289
Tyrosinase promoter, 828–829
Tyrosine analogues, 604, 626, 677
Tyrosine hydrolase, 136
Tyrosine kinase, 864–865136

U
Ubiquicidin, 442–443
[U-^{14}C]-palmitate, 691, 710–711
UDP-glucuronosyl transferase, 322
Ulcers, 338
Ultrasmall superparamagnetic particles of iron oxide (USPIOs), 526, 530–532, 542, 550–551, 555, 558, 562, 863–864
Ultrasonography, 765–808
  aortic stenosis, 51
  atherosclerotic plaque deformation, 51–52
  biliary colic, 267, 268
  bladder rupture, 857
  cardiovascular, 768–788
  central nervous system, 788–791
  contrast-enhancing agents, 40–41, 765–768
  gastrointestinal, 791–792
  general concepts, 765–768
  hepatic, 792–798
  immune system, 798
  musculoskeletal system, 798–800
  neoplasia, 800–804
  nitroglycerin-mediated vasodilation, 59–60
  pancreas, 804–805
  pharmaceutical delivery, 853–854
  prostate, 805–806
  renal system, 806–807
  reproduction, 807–808
  vascular blood flow, 40–42
UMB68/UMB75, 106
Urea, 750, 763
Urea cycle, 275, 277–278
Urinary bladder, 514
  cancer, 370, 423, 500, 651, 890–891
  contractions, 11–12, 370
  function, 76, 103–104, 370–372
  rupture, 807, 857
Urinary system, 355–373
  neoplasia, 423
  ultrasound, 806–807
Urine 17-OH, 132, 146
Urography, 750, 763
Urolithiasis, 372
Urticaria, 338

V
Valine metabolism, 379
Valproate, 80, 81, 84, 88–89
Varicoceles, 169–170
Vascular amyloid deposits, 570
Vascular blood flow, 40–42, 479–480, 769–772, 785–787, 800–801
Vascular blood pool, 589–590
Vascular density, 777
Vascular endothelial growth factor (VEGF), 762, 884, 886
Vascular extravasation, 46, 480–481
Vascular grafts, 44, 49–50, 106–107
Vascular hypertension, 591–592, 667
Vascular imaging, MRI, 528–532
Vascular malformations, 42–44, 43, 277–278, 281, 743–744
Vascular patency, 769
Vascular perfusion, 44–45, 112–114, 338–339, 461, 474, 479–481, 529, 563, 592–593, 703–705
Vascular permeability, 45–47, 125, 444, 479–481, 616–617, 746
Vascular spasm, 578–579
Vascular thrombosis, 584, 593–595
Vasoactive intestinal peptide, 130, 415, 604, 626, 644–645, 677, 835

Vasodilatory response, 47, 59–60
Vasopressin, 167, 363, 366
Vector-mediated gene (oligonucleotide) delivery systems, 817–820
Vecuronium, 115, 116, 231, 232
Vena cava
  cavography, 746–747, 757
  thrombosis of, 595
  ultrasound, 771–772
Ventilation/perfusion, 380, 671–673
Ventricular arrythmia, 15
Ventricular (myocardial) function, 59, 782–784
Ventricular physiology, 781–782
Ventricular wall motion, 59, 783–784
Ventricular wall motion score index (WMSI), 55, 782
Verapamil, 880
Vertebral artery, 770
Vesicoureteral reflux grading (VUR), 806–807, 857
Vestibular disease, 126–127
Vinorelbine, 648
VIPoma syndrome, 130, 415
Viral infections, 451–452
Virosomes, 835–836
Visual pathway staining, 121, 509
Vitality staining, 481–482
Vitamin B_{12}, 200–201
Vitamin D, 201–202, 203, 277
Von Willebrand's disease (vWD), 257–258
Von Willebrand's factor, 259
Von Willebrand's factor antigen (vW Fag), 227–228

W
W7, 15–16
Wada test, 43, 61, 71, 84–88, 597–598, 698
Walpole's buffer solution, 372
Warfarin, 295, 299
Water diffusion coefficients, 38–39, 476–477
Water provocative test, 118
WAY-133537, 356
Whiskey, oral, 170, 420
White blood cells, HMPAO-labeled, 441, 620
Wideband harmonic imaging (WHI), 796, 801
WIN 22181, 529–530
Wolff-Parkinson-White Syndrome, 5, 11, 14–15
WST-1, 251–252, 256
Wuchereria bancrofti, 452–453

X
X-34, 68–69, 483
Xanthene, 507
Xenobiotic metabolism, 461, 479–480
Xenon (^{129}Xe), 564–565
Xenon (^{133}Xe), 672, 673
Xenotransplants/xenografts, 632, 638, 718, 894
Xerostomia, 180–181
XR9576, 648
X-ray micro-computed tomography, 764
Xylazine, 153
Xylocaine, 112
Xylose, 191–192, 196, 203, 204–208, 212, 213

Y
[^{90}Y]-anti-CEA Mab, 569
[^{90}Y]-DOTAO-D-Phe_1-Tyr_3-octreotide (DOTATOC), 631
Yohimbine, 74

Z
ZD6169, 356
Zinc sulfate, 242–243, 866
Zollinger-Ellison syndrome, 129, 184, 208–209, 212–219, 412–413, 488
Zyn-205 linker, 811