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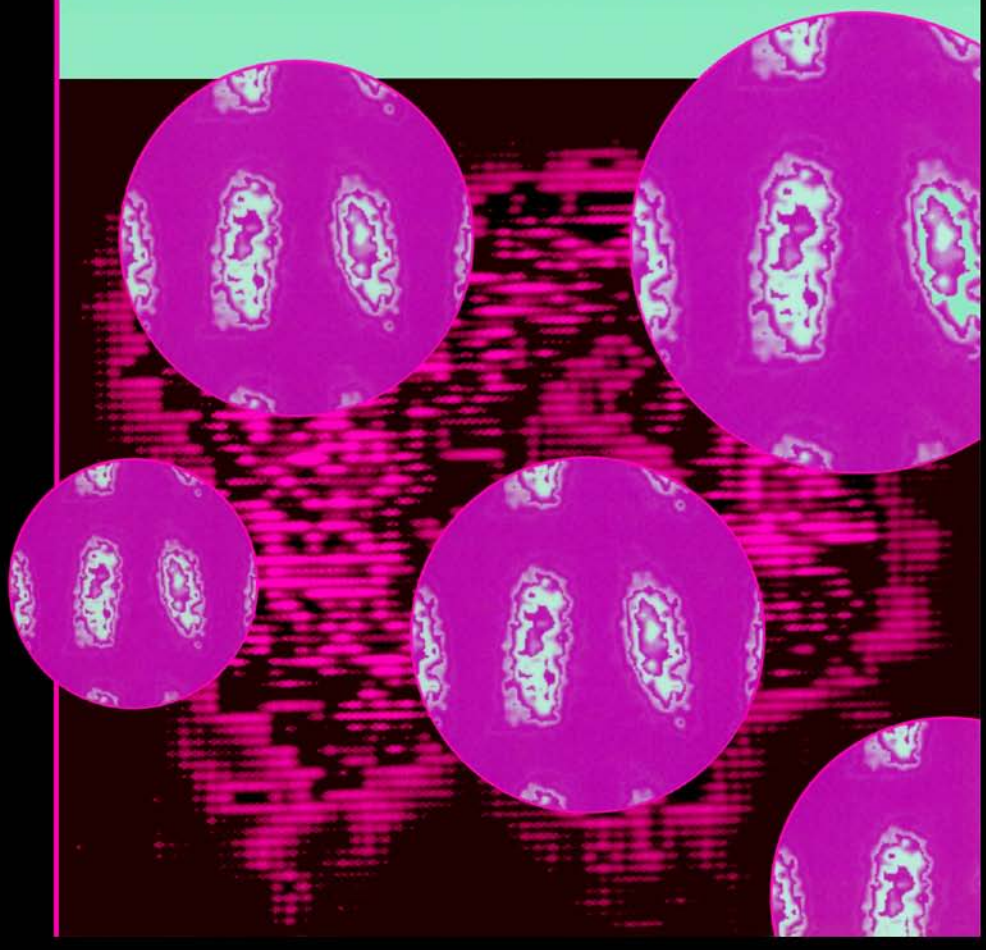
Nuclear Medicine in Pharmaceutical Research

Edited by
Alan Perkins and Malcolm Frier



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Edited by

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Preface

Radioactive tracers are playing an increasingly important role in the study of drug formulation and drug delivery systems, ranging from the discovery of new drugs to the *in vivo* evaluation of drug delivery in humans. This book defines the role and scope of nuclear medicine imaging techniques (gamma scintigraphy) in pharmaceutical research, providing a wealth of information from clinical trial data. As the editors state: *'no other technique can localize so precisely the site of disintegration of a tablet in the GI tract, the penetration of an aerosol in the lung or the residence time of a drug on the cornea'*.

There are 9 chapters covering all aspects of scintigraphic imaging applied to pharmaceutical research, ranging from the regulatory requirements, ethical submission and approval for study in normal human subjects and patient groups, through to detailed descriptions of specific applications. The introductory chapters provide clear descriptions of the basic instrumentation, techniques, the principles of GMP applied to radiolabelling procedures and the importance of the validation of products and procedures.

The following chapters provide detailed information from scintigraphic studies of pulmonary, nasal, oral, ocular and intravenous drug formulations. A Note in Proof refers to the application of PET imaging techniques in this field.

This book will be a valuable aid to all with interests in drug delivery, whether working in pharmaceutical research, academia or clinical sciences.

A.C.Perkins and M.Frier (Eds)
University Hospital, Nottingham, UK

Foreword

Huge sums of money are invested annually by the pharmaceutical industry into improved treatments for diseases including cancer, arthritis and AIDS. In the United Kingdom alone, almost one fifth of all industrial research and development is performed by the pharmaceutical industry, which in turn puts back more than one fifth of its gross output into research and development. By its very nature, pharmaceutical research is complex, lengthy and costly. Only one or two compounds in 10,000 will eventually reach the market, and even then there is no guarantee of commercial success.

In this kind of environment, demand for resources financial, material and intellectual is extremely high and research and development methods which can improve efficiency and cost-effectiveness are to be welcomed.

The application of nuclear medicine technology to the study of drug formulation and the development of new drug delivery systems is one such method. Its strength is that it can provide graphic evidence of the behaviour of drug formulations in the actual patients for whom that drug has been developed. Not only that but the data obtained are quantifiable, and allow direct comparisons to be made between different formulations in the same subjects. The outcomes of these studies can be demonstrated in optimization of dosing regimes, improved precision in targeting and control of drug delivery, all of which contribute to improvements in compliance and cost-effectiveness.

It is more than 20 years ago now that workers in the United States and in Sweden first demonstrated the potential of gamma scintigraphy as a non-invasive technique for investigating the fate of tablets and capsules in the gastrointestinal tract of man. In the intervening years the technique has been applied to a range of formulations and delivery systems and has led to a substantial increase in knowledge about their *in vivo* behaviour. Probably the most significant information to emerge relates to the variability of human physiology, as can occur between individuals, or within the same individual in health and disease. Certainly enough information on gastrointestinal physiology has emerged to have a significant impact on the design of dosing regimes for orally-administered formulations. As a tool

it has its limitations, primarily related to the range of suitable radionuclides available for imaging. A number of novel approaches have been introduced to overcome some of these limitations, including neutron activation, and the use of novel radiopharmaceuticals as drug models. Undoubtedly, improvements in accessibility to PET technology will have an increasing impact on the ways in which the techniques can be applied, and offer the potential of extending studies beyond formulation biodistribution into drug absorption and metabolism.

Proof of the robustness of the technique can be seen in the fact that viable and successful commercial service organisations have spawned in its wake, and the pharmaceutical industry as a whole makes good use of this resource. The range of material documented in this volume is an indication of how wide the applications are and evidence of the importance of scintigraphic studies in the development of new and effective drug delivery systems.

Dr Trevor Jones
Director General
Association of the British Pharmaceutical Industry

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Ethical and Regulatory Approval for Human Studies

A.WARBICK-CERONE

Science does not always progress in a continuous flow. It is true that we are constantly gaining knowledge, but it is quite common that new ideas, once they are enunciated and partially developed, lie dormant for a period of time until they are resurrected by someone who understands their usefulness, and new strides forward are made. Lelio Colombetti, PhD

1.1 Introduction

Ethical and regulatory controls in research involving human subjects are intended to encourage research without compromising high standards of health and safety.

In recent years, regulatory agencies (Health and Welfare Canada, 1990) have begun to apply the practices of risk assessment (Bailar *et al*., 1993) and cost-benefit analysis. Some countries are more highly advanced in this process than others, using risk assessment and cost-benefit principles extensively in designing regulations relating to health, safety and the environment.

Over the last decade, the development of innovations in risk assessment has promised not only improvement in the scientific treatment of risk but more analytical approaches to risk management, resulting in more consistent health protection policies.

The approval of human studies in research and clinical trials encompasses both ethical and regulatory factors. Control of risk may be achieved in a variety of ways. In the past, regulation was widely used as a risk management tool. More recently, non-regulatory options for controlling risk have received considerable attention, including economic, advisory or technical solutions to risk management problems. Of the different options available regulation still remains the most widely used for clinical trials, while research is in general managed by non-regulatory means.

The morals and principles applied to the conduct of medical or pharmaceutical research will only be as ethical as the current moral and ethical limitations placed on us by our societies and by the education and knowledge achieved through the preceding years of medical and pharmaceutical research.

Research using radiopharmaceuticals is subject to the same ethical scrutiny as other drugs. The highly sensitive issue of radiation exposure of the study subject adds additional criteria to the research parameters which must also be evaluated. In this respect, the use of well characterized radiopharmaceuticals in human studies is subject to less critical examination than new chemical or biological entities radiolabelled with novel radionuclides. The purpose of this chapter is to review the current morals and regulatory trends which govern proposed human research studies using radiopharmaceuticals.

It is important to define and clarify the distinction between research and clinical trials as this will in some cases determine the role of regulatory agencies in the study being undertaken. In general, basic radiopharmaceutical research will be used to obtain information relating to the metabolism (including kinetics, distribution and localization) of the radioactive drug or to investigate human pathology, pathophysiology or biochemistry, but not for immediate therapeutic, diagnostic or similar purposes which are components of a clinical trial.

Drugs used in clinical trials are subject to the regulatory requirements of the regulatory agency in the country in which the trial will be performed. In addition, the requirements for Phase I, Phase II and Phase III clinical trials may vary from one regulatory agency to another. The requirements (Raven, 1993) for these types of studies will not be the subject of this chapter.

1.2 Essential Elements in Organizing a Research Study in Humans

1 Preparation of the research protocol

2 Review of the protocol by an ethics committee

3 Responsibilities of the investigator

4 Data handling and archiving

5 Statistics and quality assurance

1.2.1 The Research Protocol

The research protocol should be clear and sufficiently detailed to allow for review by the ethics committee and to enable organized progression of the study. The following elements should be included:

1 Introductory statement and background

2 Study objective/aim

3 Subject selection criteria

4 Description of the study design

5 Materials/supplies

6 Dosage and administration

7 Product preparation and quality control

8 Laboratory procedures

9 Clinical procedures

10 Statistical analysis—how data will be analysed

11 Drug adverse reaction, drop out, replacement of study subjects, test article accountability

12 Informed consent documents

13 Ethics Review Board/Radioisotope Committee approval

14 Data recording and retention of information

15 Discussion of the use of the results

16 How deviations from protocol will be dealt with

It is important to determine how long records should be retained after the study is completed. Two years is often used as a guide, however industrially sponsored research may be retained for longer than 10 years.

Because the drug substances being used in the research study are radioactive the following essential criteria should be addressed in the research protocol.

Pharmacological dose

The amount of active ingredient or combination of active ingredients should not cause any clinically detectable pharmacological effect in the human subject. New chemical entities must be assessed to determine if a pharmacological response is possible in the doses intended for humans.

Radiation dose

The radiation dose of the administered radiopharmaceutical must be considered. Good estimates of the absorbed radiation dose can be made when information on the radionuclidic properties and decay schemes, the amount of radioactivity injected, and the biological fate of the radiopharmaceutical are known. Current knowledge of radionuclidic properties is adequate. Biological data may be limited when given the unique nature of a radiopharmaceutical or drug delivery system which may be under development.

The amount of radioactive material to be administered should be such that the subject receives the smallest possible radiation dose. The estimated amount of radioactivity to be administered to the subject may be based on preliminary animal data, from published literature or from other valid human studies, and the researcher's

knowledge of acceptable doses for other approved radiopharmaceuticals. One must always be aware that the biodistribution may be species specific and hence the biodistribution observed in the animal model may not be directly applicable to man. The dose estimates will reflect the dose for healthy humans so where the research study is to involve humans with a disease state, this must be taken into consideration.

The radiation dosimetry should be presented as three separate components:

(a) **Raw data used to derive the calculations.** Target organs and the organs at risk should be clearly identified on the basis of tissue distribution studies in animals. The parameters of absorption (if applicable), distribution, metabolism and excretion can be measured. Raw data should be presented in tabular form showing various time points and the percent administered dose/whole body (or per gram provided the mass of the organ is stated) and decay corrected to the time of injection. This table should be prepared for the radiopharmaceutical being proposed and for the principal radionuclidic and radiochemical impurities if their tissue distributions are substantially different from that of the radiopharmaceutical. The decay scheme for the principal radionuclide and the relevant radionuclidic impurities should also be included.

(b) **Corroborating data such as assumptions, models, etc. used to calculate the final dose estimates.** This component presents all sources of data, a description of models used in the dosimetry calculations and a statement of any assumptions, etc. used in any of the dose calculations. A table should summarize all the relevant physical and biological parameters used for calculation of each organ dose including: the fractional uptake of administered radioactivity in each organ, the biological half-life of radioactivity in each organ and the cumulative radioactivity for the principal radionuclide, and all relevant radiochemical and radionuclidic impurities.

(c) **A summary of final dose estimates in tabular form.** A table should be used to summarize all of the organ dose estimates for all relevant phantoms (test objects and simulations) for the radiopharmaceutical and all radionuclidic and radiochemical impurities that might be present in the final dose form. An estimate of the effective dose equivalent (EDE) per unit of administered radioactivity (mSv/MBq or mrem/mCi) and the EDE (mSv or mrem) for the total amount of administered radioactivity recommended for each phantom should be provided. The final dose estimates should include contributions from the following sources:

(i) The radionuclide used in the formulation of the drug.

(ii) Any radionuclidic impurity, if present in an amount equal to or greater than 1% of the radioactive concentration of the principal radionuclide, or, if the radionuclidic impurity at any concentration could contribute substantially to the patient dose by virtue of its physical decay characteristics or its pharmacokinetic or pharmacodynamic behaviour. It is incumbent upon the researcher to show that such a contribution to the dose does not occur.

(iii) Any radiochemical impurity, present in an amount equal to or greater than 5% of the administered radioactivity if the tissue distributions of the radiochemical impurity and the radiopharmaceutical are substantially different.

The United States Food and Drug Administration (FDA) sets limits on the radiation dose to an adult research subject (United States Food and Drug

Administration, 1996). They indicate that under no circumstances should the radiation dose to an adult research subject from a single study or cumulatively from a number of studies conducted within 1 year be generally recognized as safe if such dose exceeds the following:

	Rems	mSv
Whole body, active blood-forming organs, lens of the eye and gonads:		
Single dose	3	30
Annual and total dose commitment	5	50
Other organs:		
Single dose	5	50
Annual and total dose commitment	15	150

Furthermore: 'For a research subject under 18 years of age at his last birthday, the radiation dose shall not exceed 10 percent of that set forth in the above table.'

The majority of work using radiopharmaceuticals for the study of drug formulation and drug delivery systems in normal subjects results in very low radiation doses of the order of 0.1 Rem (1 mSv).

Human research subjects

Research subjects should be at least 18 years of age and legally competent. There may be special situations where the study presents a unique opportunity to gain information not currently available and requires the use of subjects less than 18 years of age. In this case the lack of a significant risk would have to be proven to the committee performing the assessment of the study protocol. In rare cases involving young children, a qualified paediatric consultant may be asked to support the review of the protocol.

For female subjects of child-bearing age confirmation that the subject is not pregnant is necessary before participation in the study is allowed.

Quality of the radioactive drug

The radioactive drug used in the research study should be prepared and tested to meet a standard no less than that required for any drug which would be used in a clinical trial. It is the responsibility of the researcher to ensure that his or her research team possesses the expertise to adequately evaluate the pharmaceutical elegance and safety of the drug substance. Where the researcher manufactures the drug substance, Good Manufacturing Practices should be applied.

There are no ideal radiopharmaceuticals, only the best compromise between the physical and chemical characteristics of the radionuclide and the chemical entity which will combine to form the radiopharmaceutical. When the radionuclide

and the chemical or drug entity are matched, other factors must be considered (NCRP, 1982), namely:

- the optimal radiochemical characteristics
- the toxicity of the radiopharmaceutical content and toxicity of non-radioactive constituents
- the biodistribution
- sterility and apyrogenicity
- immunogenicity, if applicable

The researcher's choice of the appropriate radionuclide (NCRP, 1982) for the study being planned will be affected by the following:

- the chemical nature of the radionuclide
- the nature of the radionuclidic emissions
- the half-life of the radionuclide
- the radionuclidic purity of the radionuclide
- the availability and cost of the radionuclide

The researcher is responsible for ensuring that the final drug product is safe for human use, that appropriate controls are applied during the manufacturing process and that appropriate quality control tests yielding acceptable results are performed prior to use of the product in the human study. This applies whether the researcher prepares the product him or herself or whether the work is contracted out. The researcher is ultimately responsible for the quality of the product.

Pharmacopoeias may be consulted for suggested quality control test methodology. Tests such as chemical, pharmaceutical, radiochemical and radionuclidic assessments of identity, strength, quality and purity may be required based on the type of product being prepared. It may also be necessary for the researcher to demonstrate that the preparation of the product results in a product of uniform and reproducible quality which will lend significance to the research study. Calibration of equipment used to measure radioactivity may require consideration and may be an integral component of the study design.

Radioactive drugs which are parenterals are subject to requirements for sterility and pyrogen testing. Additional tests may be required if the product is a biologic.

Researchers may need to consult other experts to ensure that the product intended for human use has been adequately characterized and tested prior to administration to the human subject.

- Completion of certain tests, for example sterility or pyrogen tests, may not be possible prior to release of products containing short-lived radionuclides. These tests may be in progress and results may not be available at the time of product release. For these types of products strict in-process controls become extremely important.

- Potential and actual impurities should be considered for their possible effect on the study subject and for potential influences on radiochemical purity and biodistribution.
- Specifications and acceptable tolerances should be established for the product and for ingredients to ensure that each batch of product is identical.
- The radionuclidic purity and the nature and level of radiochemical impurities may change over the useful life of the drug product and should be known.

Adverse reactions and altered biodistributions

The researcher should carefully assess the situations in which an adverse reaction or an altered biodistribution has occurred in the study subject. It may be necessary to advise the ethics committee of the incident and to reassess the study protocol.

Genotoxicity testing

The researcher must establish a basic understanding of the pharmacology of the drug substance and when warranted genotoxicity testing may be required. Genotoxicity tests should be performed using the final radiolabelled drug product rather than the drug substance which has not been radiolabelled. It is important to predict whether the radiopharmaceutical will be incorporated into the cell nucleus and if so, it will be necessary for the researcher to comment on the associated risk. This information should then be communicated to the study subject.

1.2.2 Decision Making and Ethical Considerations

Governments promulgate regulations for the purposes of managing risk. In some instances, risk assessment is explicitly required and forms a fundamental basis for decision making. In other instances, the role played by risk assessment reflects many factors, including the influence of special interest groups, political pressures and public sensitivities. Above all, regulatory agencies are charged with risk management activities, i.e. to regulate risk (Belzer, 1992).

The use of human beings in radiopharmaceutical research must be deemed essential for scientific reasons and should be based on sound data from preliminary research on animal or other models, wherever possible.

In many countries it is an ethics committee who will have the responsibility of reviewing all proposals pertaining to basic research and clinical trials which will take place in the affiliated institution. Ethics committees are independent bodies which will include medical and non-medical members who are required to give consideration to clinical trials and human research in the context of safety, integrity and human rights. The names given to these ethics committees vary throughout the world. The terms Ethics Committee, Research Ethics Committee and Institutional Review Board are just a few that are used.

Not all countries apply the same criteria to ethics committees, to the extent that there is sometimes no recommendation made for the composition of the committee. Whatever the situation, there must be a minimum standard for the ethics committees, so as to ensure that the interests of human subjects are protected. However, in general, there seems to be a consensus that the committee should be constituted as follows:

1 There are no fixed numbers of committee members and academic backgrounds will vary.
2 There should be sufficient members who are qualified by experience and expertise.
3 There should be a mix of male and female members.
4 There should be at least one or more non-scientific persons.
5 There should be at least one member not affiliated to the institution (for those committees which are struck within an institition).
6 There should be no conflict of interest.

It is recommended that the committee not be static, to ensure that proper expertise is available when a particular research proposal is to be evaluated. It may also be important to ensure that at least one member of the committee has ethics expertise gained through formal training in ethics, or has practical or academic expertise in research ethics. A national body which certifies, accredits, approves or sanctions research ethics boards, exists in some countries and has merit.

The European Community (EC) does not have a firm requirement on the composition of the ethics committee, however, individual countries have established their own guidance documents on medical research. The Canadian *Guidelines on Research Involving Human Subjects* (Medical Research Council of Canada, 1987) describes the composition of such a committee.

In some instances the committee may be independent/regional or conversely it may be affiliated to a hospital or university. Regional committees exist in the Nordic countries as well as in the UK where Local Research Ethics Committees (Department of Health, 1992) have been established. In this situation the committee members remain at arm's length from the researchers and their institution and so there is less chance of a conflict of interest. On the other hand, ethics committees at local institutions and communities will allow community ethics to be brought to bear when reconciling cultural differences of populations (Klassen, 1996).

Committees may be either non-profit or profit organizations. It is also noteworthy that some countries recommend insurance to cover the civil responsibilities of the investigator/sponsor or to provide compensation for possible injury to the study subject.

In general, it is agreed that the role of the ethics committee is to safeguard the rights and welfare of the human subject by ensuring that:

- the question addressed by the research activity is a worthwhile one

- study subjects are not unnecessarily or excessively exposed to risk
- the benefits of the research outweigh the risk
- the investigator effectively excludes special groups of patients in whom the risk of participation would be particularly greater
- appropriate informed consent is sought and documented
- appropriate provision has been made for privacy and confidentiality
- vulnerable subjects are protected

The Declaration of Helsinki (1964), the United States Food and Drug Administration regulations and the EC Guideline requirements for subject protection are essentially comparable.

In giving consideration to the special concerns pertaining to radiopharmaceuticals, the United States Food and Drug Administration has, in their Code of Federal Regulations, defined a Radioactive Drug Research Committee. The membership of this committee is structured so as to encompass authoritative individuals who are most qualified to evaluate a research project involving a radioactive drug. The membership includes a physician recognized as a specialist in nuclear medicine, a person qualified by training and experience to formulate radioactive drugs, a person with special competence in radiation safety and radiation dosimetry, and two individuals qualified in various disciplines pertinent to the field of nuclear medicine. The membership is intended to be sufficiently diverse to permit expert review of the technical and scientific aspects of the proposal submitted to the committee. The addition of consultants in other pertinent medical disciplines is encouraged.

The Radioactive Research Committee is responsible for giving consideration to the following criteria:

- pharmacological dose
- dose of radioactivity
- radiation exposure
- assessment of the requirements for the qualified investigator using radioactive material
- appropriate licensure to use radioactive materials
- selection and consent of research subjects
- quality of radioactive drugs
- research protocol design
- reporting of adverse reactions
- approval of the appropriate ethics committee

The Committee will submit ‘Reports on Research Use of Radioactive Drugs’ to the FDA annually, providing a broad scope of descriptive information on the conduct of the study. Periodically, the FDA will monitor the activities of the

Committee by reviewing annual reports, the minutes of the Committee meetings and full protocols for selected studies, and through on-site inspections.

The detailing in the federal regulations of the criteria which must be reviewed and assessed by the Radioactive Drug Research Committee encompasses those criteria which will ensure that the drug is safe for use and that due consideration has been given to the radioactive nature of the drug. These criteria have already been outlined in this chapter.

Other countries prefer to use a non-regulatory approach, applied through the use of a guidelines document. The Atomic Energy Control Board in Canada has developed a parallel philosophy to the FDA. The *Guidelines for Research on Human Subjects using Radionuclides* (Atomic Energy Control Board, 1993) provide guidance and recommendations to organizations and investigators on the requirements for undertaking research in healthy human subjects using radioactive materials. The guidelines outline the composition of the Scientific and Ethical Review Committee, which is charged with reviewing all research proposals for an institution as well as for those studies involving radiation. For the purposes of research in humans using radionuclides, a specialist or specialist group with expertise in the use of ionizing radiation is considered essential. This recommendation parallels the activities of the United States Radioactive Drug Research Committee. Recommendations for the operational activities of the Scientific and Ethical Review Committee are detailed in the guidelines including the close monitoring of the research project by this committee.

Who will ensure that ethics committees are appropriately structured and function as recommended in guidance documents developed by health agencies or professional interest groups? Who will ensure that in the case of a radiopharmaceutical, at least one member of the committee or one invited member has academic and practical training in the development and testing of this class of drug? In situations where the activities of the ethics committee are not audited by a regulatory agency or professional interest group, the professional integrity and responsibility of the members of the ethics committee are paramount in ensuring compliance with acceptable standards.

1.2.3 The Investigator's Responsibility

The principal investigator or researcher is tasked with the preparation of the study protocol and accepts responsibility for all aspects of the study. For this reason, this individual must possess appropriate qualifications gained through academic and practical training in the scientific field directly related to the study proposal. The *ICH Harmonised Tripartite Guidelines for Good Clinical Practice* (1996) outlines the detailed responsibilities of the investigator, which will also be applicable to a researcher.

The Case Report Form

The Case Report Form represents the only tangible piece of historical documentation or collection of pieces of information pertaining to the research

study (Raven, 1993). These forms will contain all the information about the research study and the results. The forms will match the research protocol and be completed by the responsible researcher or associate. The results will be appropriate for passing on to a statistician, if applicable, for analysis.

In general, the Case Report Form will include several elements, depending on the nature of the research study. These may include:

- information and consent forms
- patient selection check-list
- patient medical history and medication record, if appropriate
- laboratory assessments (biodistribution, absorption, metabolism, excretion, etc.)
- adverse reaction form
- end of study/withdrawal form

Data archival and recording procedures

Two basic type of records will be retained by the investigator:

1 Subject records
2 Study protocol

The investigator will be responsible for preparing and maintaining adequate accurate subject histories, demographic data and pertinent observations for the research study.

Depending on the complexity of the research study and the need to acquire detailed information for future processing, the following types of generic data collection may be prepared (Spilker, 1987). The reference check-list below is intended to apply to clinical trials but one can easily see that many of the criteria are applicable to radioactive drugs in the research setting.

1 Patient demographics (initials, name or unique identifier, address, date of birth, sex)
2 General medical history, including concomitant medication history
3 Specific medical history, focusing on the specific medical problem being evaluated
4 Physical examination including vital signs, height and weight; forms for both complete and abbreviated examinations may be prepared
5 Blood sampling: profile of time after drug administration
6 Urinary, faecal or other sample: profile of time after drug administration
7 Clinical chemistry
8 Diagnostic radiology reports: X-ray, CT, MRI, ultrasound

9 Electrocardiogram
10 Other laboratory examinations (e.g. pathology samples and reports, biological samples and reports, physiological examinations)
11 Neurological examination (EEG) and/or other specialized tests
12 Drug preparation, quality control, dispensing and administration record
13 Efficacy measurements: scoring for scans, comparison with other diagnostic modalities, etc.
14 Quality of life measurements
15 Adverse reactions—summary form for study
16 Adverse reactions—specific form for exact times of onset and cessation
17 Study subject follow-up
18 Termination record
19 Patient withdrawal
20 Investigator's comment log

The drug dispensing record above is of significant importance as it will provide information on the pharmaceutical quality of the radiopharmaceutical used in the research study. Detailed information on the source of the raw materials or finished product, preparative information, quality control data and relevant stability data may be of utmost importance when performing a retrospective review of the research study results.

1.2.4 Statistics and Quality Assurance

Statistical methods to be employed to analyse data should be defined as a component of the research protocol. Rationalization for population and sampling size(s) must be provided. Information on the level of significance to be applied and factors which would contribute to termination of the trial are important. The application of statistical analysis for the data collected during the research study will be dependent on the quality of the data collected.

The researcher must ensure that Good Clinical Practice (GCP), Good Manufacturing Practices (GMP) and Good Laboratory Practice (GLP) are applied throughout the various phases of the study protocol. All observations and findings should be verified. Quality control must be applied consistently and at each stage of data handling. Unless the entire procedure, including the analysis of data, is adequately controlled there is the risk of failure and hence a waste of human and financial resources with undisputable associated ethics concerns. By being able to demonstrate that a quality assurance programme is in place, the researcher ensures that merit has been incorporated into the research project. In general, guidelines for GCP, GMP and GLP exist throughout the world. The Committee for Proprietary Medical Products (CPMP) of the EC has adopted the ICH

Guidelines for Good Clinical Practice. Other countries are also following this move, including Japan, the United States and Canada.

1.3 Summary

It is clear that, within limitations, the same study criteria will apply in the research setting as for a clinical trial. These criteria ensure that the welfare and interests of the study subject are being protected while at the same time ensuring that the study will be viable. There is no intent to create impediments to research and at all costs unjustified impediments should be avoided. The results of human research can often advance knowledge, relieve suffering and promote welfare. Impediments to research may be as unethical as those that violate the dignity and safety of human subjects. We rely on the ethics committees of the world to balance such needs.

Radiopharmaceuticals have a very special role to play in research. In a non-invasive manner, radiopharmaceuticals can be used to produce kinetic information on the drug and metabolite or can enhance our knowledge of human pathology, pathophysiology or biochemistry, while at the same time respecting the health and safety of the human subject.

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2

Instrumentation, Imaging, Data Analysis and Display

A.C.PERKINS

2.1 Introduction

The initial medical successes early in the 20th century using radium for the treatment of cancer were followed by the use of radioiodine in the diagnosis and treatment of thyrotoxicosis and thyroid cancer. Over the past 50 years a broad range of gamma and beta-emitting radionuclides has been incorporated into radiochemicals and radiopharmaceuticals for routine use as biological tracers and as diagnostic and therapeutic agents used clinically in hospital laboratories and clinics (AAPM, 1987; Anderson and Bergmann, 1994). These tracers are increasingly being used as a means of monitoring drug release, deposition dispersion and kinetics. This chapter outlines the basic instrumentation and technology associated with the application of scintigraphy to drug formulation research.

2.2 Radioactive Tracers

Radioactive decay results in a range of different products, e.g. alpha, beta, gamma and X-rays. Alpha and beta emissions are generally regarded as particulate forms of radiation (alpha being the nucleus of the helium atom and beta being an electron), whereas gamma rays are termed 'photons', these being similar in nature to X-rays. The electrostatic interaction between these forms of radiation and matter is known as ionization, hence the term 'ionizing radiations'. The process of ionization results in electrons being stripped away from the atoms and molecules of the medium with which the radiation interacts, leaving oppositely charged ion pairs. Alpha emitters are not normally used in medicine, their role being confined to the realms of experimental tumour therapy. Beta emitters are used for laboratory-based *in vitro* diagnostic procedures, however they are also used in relatively large amounts for *in vivo* targeted therapeutic procedures. In the pharmaceutical industry,

beta-emitting radionuclides, for example carbon (C-14) and sulphur (S-35) are important analytical tools used in drug discovery and development, mainly for metabolic and pharmacokinetic studies.

Due to their penetrating nature, gamma rays, which are of similar energy to diagnostic X-rays, are suitable for *in vivo* imaging procedures. In industry sealed solid gamma ray sources, such as Co-57, are used for several purposes ranging from process control and thickness gauging of powder beds to terminal sterilization.

All radioactive materials used in medicine are artificially produced either by neutron bombardment in a nuclear reactor or by irradiation of an appropriate target using charged particles from a particle accelerator. Radionuclide production in a nuclear reactor (activation) results in neutron-rich radionuclides which decay to give off electrons and gamma rays. One commonly used method for the production of radionuclides from a reactor is to separate out the fission fragments from spent uranium fuel rods. This is the major source of Mo-99 for the production of the generators which are used in hospital radiopharmacies for dispensing Tc-99m, the main radionuclide used in nuclear medicine imaging. Tc-99m is a pure gamma emitter with a physical half-life of 6 hours and is considered almost ideal for routine diagnostic imaging.

Charged particle accelerators (linear accelerators and cyclotrons) produce beams of protons, deuterons and helium ions and are used to produce neutron-deficient radionuclides such as In-111 and F-18 that decay by electron capture and emission of X-rays, gamma rays or positrons (positively charged electrons). A list of the most common gamma-emitting radionuclides used in nuclear medicine imaging is given in Table 2.1. The combination of the radionuclide and the ligand in a form suitable for human administration is known as a radiopharmaceutical.

Unlike the majority of medical imaging modalities, radionuclide imaging depicts function rather than anatomy. In medical diagnosis this technique offers a non-invasive method for the measurement of organ function, tissue perfusion and receptor binding using suitably labelled molecules or compounds (Anger, 1964; Bilinghurst and Fritzberg, 1981). Providing a suitable radiolabel exists, nuclear medicine techniques can demonstrate the *in vivo* biodistribution and kinetics of a ligand which may be a drug, a carrier or a biodelivery vehicle (BSI, 1992; Davis *et al*., 1996). In general radiopharmaceuticals are licensed pharmaceutical products which can be prepared from kit formulations (Ell and Holman, 1982). In many research applications these kits may be safely used for applications other than those for which the licence applies. For example, an intravenous formulation may be incorporated into a research product intended for oral administration (e.g. Tc-99m DTPA and Tc-99m-tin colloid which are used as non-absorbable markers in gastrointestinal studies). Regulatory restrictions governing the use of both medicinal products and radioactive substances apply to activities within radiopharmacies and nuclear medicine units (Goris and Brander, 1983; Grigg, 1976). Consequently the dedicated facilities, equipment and personnel are largely restricted to hospitals and academic institutions and are not usually found in industrial establishments.

Table 2.1 Radionuclides used in nuclear medicine

Radionuclide	Type of decay	Principal photon emission (keV)	Physical half-life
Single photon emitters (gamma camera and SPET imaging)			
Technetium-99m[a]	Electron capture	140	6 h
Krypton-81m	Isomeric transition	191	13 s
Indium-111	Electron capture	173, 247	2.8 days
Iodine-123	Electron capture	160	13 h
Iodine-131	Beta	360	8 d
Thallium-201	Electron capture	78	73.1 h
Samarium-153	$Beta^-$	103	47 h
Erbium-171	$Beta^-$	296, 308	7.5 h
Positron emitters (PET imaging)			
Oxygen-15	Positron emission	511	2 min
Carbon-11	Positron emission	511	20 min
Nitrogen-13	Positron emission	511	10 min
Fluorine-18	Positron emission	511	110 min

Generator-produced positron emitters

Parent	(physical half-life)	Daughter	(physical half-life)
Strontium-82	(25 days)	Rubidium-82	(76 s)
Zinc-62	(0.2 h)	Copper-62	(9.7 min)
Germanium-68	(288 days)	Gallium-68	(68 min)

[a] Tc-99m is a generator product from molybdenum-99 (physical half-life 66 h)

2.3 Radiation Detectors

The most commonly used radiation detectors are based on either a gas-filled chamber, an organic or inorganic scintillator, or a solid state semiconductor. Gasfilled detectors include ionization chambers, proportional counters and Geiger-Muller tubes. Detection basically involves the collection of charged ions which are attracted to positively and negatively charged electrodes. The current produced can be amplified and registered by an appropriate instrument, usually an electrometer. The radionuclide calibrator used in the radiopharmacy is an example of such an instrument.

Scintillation detectors are based on organic and inorganic scintillators, which have the capacity to emit a flash of visible light as a result of electrons released due to absorption of a gamma or X-ray photon (photoelectric effect). The main types of scintillator used are sodium iodide, $NaI(_{Tl})$, and caesium iodide, $CsI(_{Tl})$, doped with thallium or crystals of bismuth germanate (BiGeO). These materials

have a high atomic number and are efficient at absorbing gamma and X-ray photons, although at higher photon energies thicker crystals are required. The scintillation crystal is hermetically sealed in a light-tight aluminium can with a glass rear face for optical coupling to a high gain photocell known as a photomultiplier tube. Synthetic crystals are produced in a variety of shapes and sizes for the detection of gamma radiation. For example, crystals with holes or wells drilled in them are used for highly efficient counting of small volumes in sample tubes (well counters). The crystal-photomultiplier tube combination is found in a range of instruments including the simple hand-held contamination monitor, automatic sample counter and the imaging camera. Sample counters include manual and automatic well counters for the assay of gamma-emitting radionuclides. These are relatively inexpensive instruments with high sensitivity which are widely used in hospitals and industry for the *in vitro* assay of radioactive substances.

Solid state semiconductor detectors such as CdTl and Ge(Li) are used for a number of more specialized applications. However, they lack the sensitivity of scintillation detectors and are therefore not as widely used for imaging.

2.4 The Radionuclide Calibrator

Because the majority of nuclear medicine investigations involve the administration of a radiolabelled compound or conjugate to humans it is crucial that the amount of radioactivity is accurately known. One of the fundamental principles of any procedure involving the use of radiation is that the amounts should be kept as low as reasonably achievable (ALARA). It is therefore important that the prescribed amount of a radionuclide administered for diagnosis, treatment or research should not be exceeded. This places stringent requirements on the instrumentation used for the measurement of the amount of a radionuclide to be administered to either a patient undergoing a diagnostic procedure or to a human volunteer participating in a research trial.

The radionuclide calibrator is the standard instrument which is found in all radiopharmacy units for measurement of the amount of activity of a gammaemitting radionuclide (Figure 2.1). This instrument is a high-performance ionization chamber with a calibrated digital read-out which will read directly in the units of radioactivity, bequerels (Bq) or megabecquerels (MBq) in Europe[1] and curies (Ci) or millicuries (mCi) in the United States[2]. The ionization chamber is designed in the form of a cylinder with a hollow centre and an opening at one end. The sample to be measured is placed in a plastic or nylon holder which is then dropped down inside the chamber. After a brief delay to allow integration of the count rate obtained from the sample, the activity can be read directly on a digital display. A range of factors can be pre-set to include a setting specific for each radionuclide. Providing the instrument is calibrated for the appropriate radionuclide and the volume of the sample is known, the specific activity can be

[1] Where 1 Bq = the disintegration of one atom per second.
[2] Where 1mCi = 37 MBq

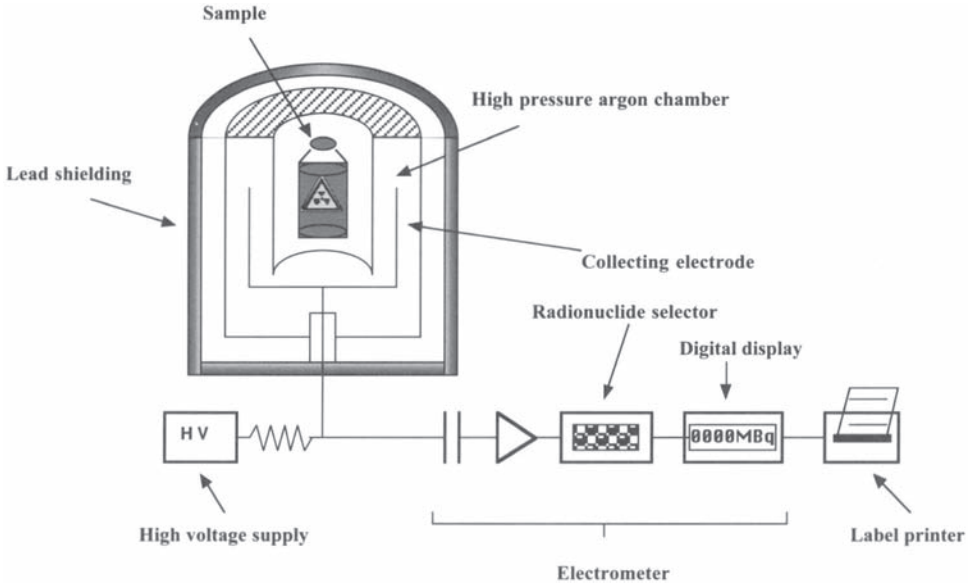

Figure 2.1 Schematic diagram of a radionuclide dose calibrator

calculated to give the activity per millilitre of the sample (MBq/ml). Alternatively, if the weight of the sample is known, the specific activity may be expressed as MBq/mg. Calibrators used for the dispensing of radiopharmaceuticals are usually connected to a printer to record the nature and volume of the radiopharmaceutical, the activity, and other details such as batch number and expiry time.

It is essential that the accuracy of the radionuclide calibrator is known. This should be checked on a regular basis as part of a routine quality assurance programme according to good manufacturing and clinical practice (GMP/GCP). The readings obtained from the calibrator should be checked on a daily basis for all radionuclide settings using a long-lived reference source such as Co-57 or Ra-226 to ensure that the measurements are within the required tolerances (Hevesey, 1923).

2.5 The Gamma Camera

The gamma camera or scintillation camera is the main instrument used for nuclear medicine imaging. First developed in 1956 by Hal Anger in the United States (IPSM, 1985) this device was originally based on a single circular scintillation crystal coupled to an array of seven photomultiplier (PM) tubes (The Anger Camera). Modern gamma cameras operate on a similar principle although the detector crystal may be rectangular with over 90 PM tubes. Systems are manufactured by a number of major electrotechnical companies with interests in

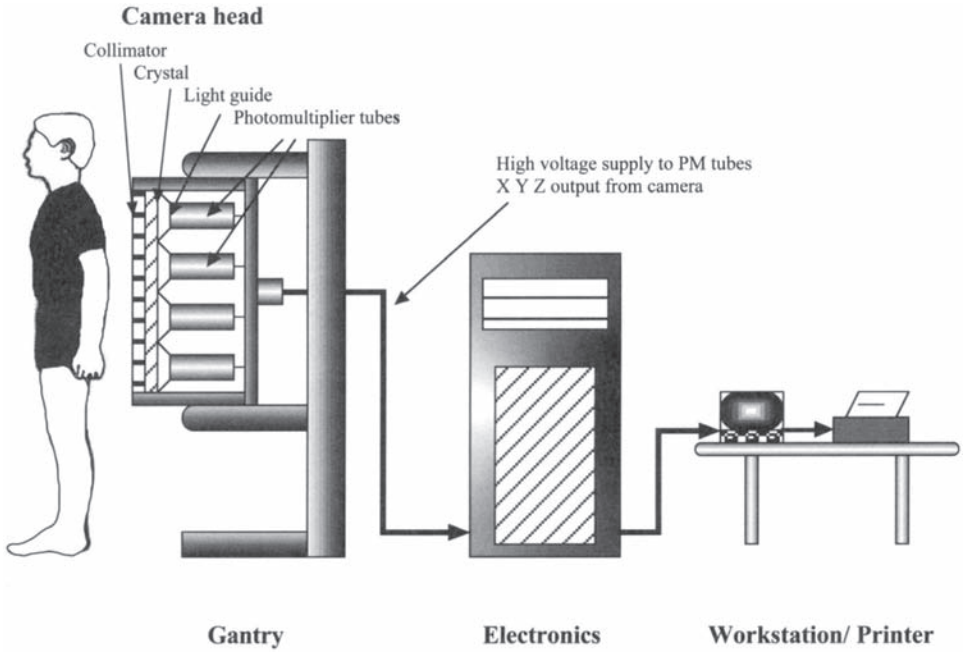

Figure 2.2 Schematic diagram of a gamma camera and computer system

medical imaging, for example International General Electric, Siemens and Toshiba. A schematic diagram of a camera and computer system is shown in Figure 2.2.

The gamma camera comprises one or more large detector heads shielded in lead to reduce the ingress of background radiation. The detector head is mounted onto a mechanical gantry which may be a cantilevered, counterbalanced system for manual positioning of the detector above or below the patient, or the head may be motor driven using a push button control (see Figure 2.3). On the face of the head is a removable lead plate called the collimator. This has a series of fine holes to channel the gamma photons onto the detector crystal. Any gamma ray not passing parallel with the holes is absorbed by the surrounding lead septa. Different types of collimator with varying thicknesses of lead septa between the holes are used for imaging radionuclides, producing different energies of gamma rays. These play an important role in the formation of the image and especially in reducing the amount of scattered radiation. The collimators are commonly referred to as being low energy (140 keV maximum), medium energy (150–300 keV) and high energy (300–400 keV). For example the different collimators would be used accordingly: Tc-99m = low energy, In-111 = medium energy and I-131 = high energy. For each energy range, parallel hole, diverging hole, converging hole and pin hole collimators are available depending upon the degree of magnification required. However, because images are generally acquired by computer any magnification is usually achieved electronically.

The single most expensive component in the scintillation camera is the $NaI(_{Tl})$ crystal, which is synthetically grown to the manufacturer's specification.

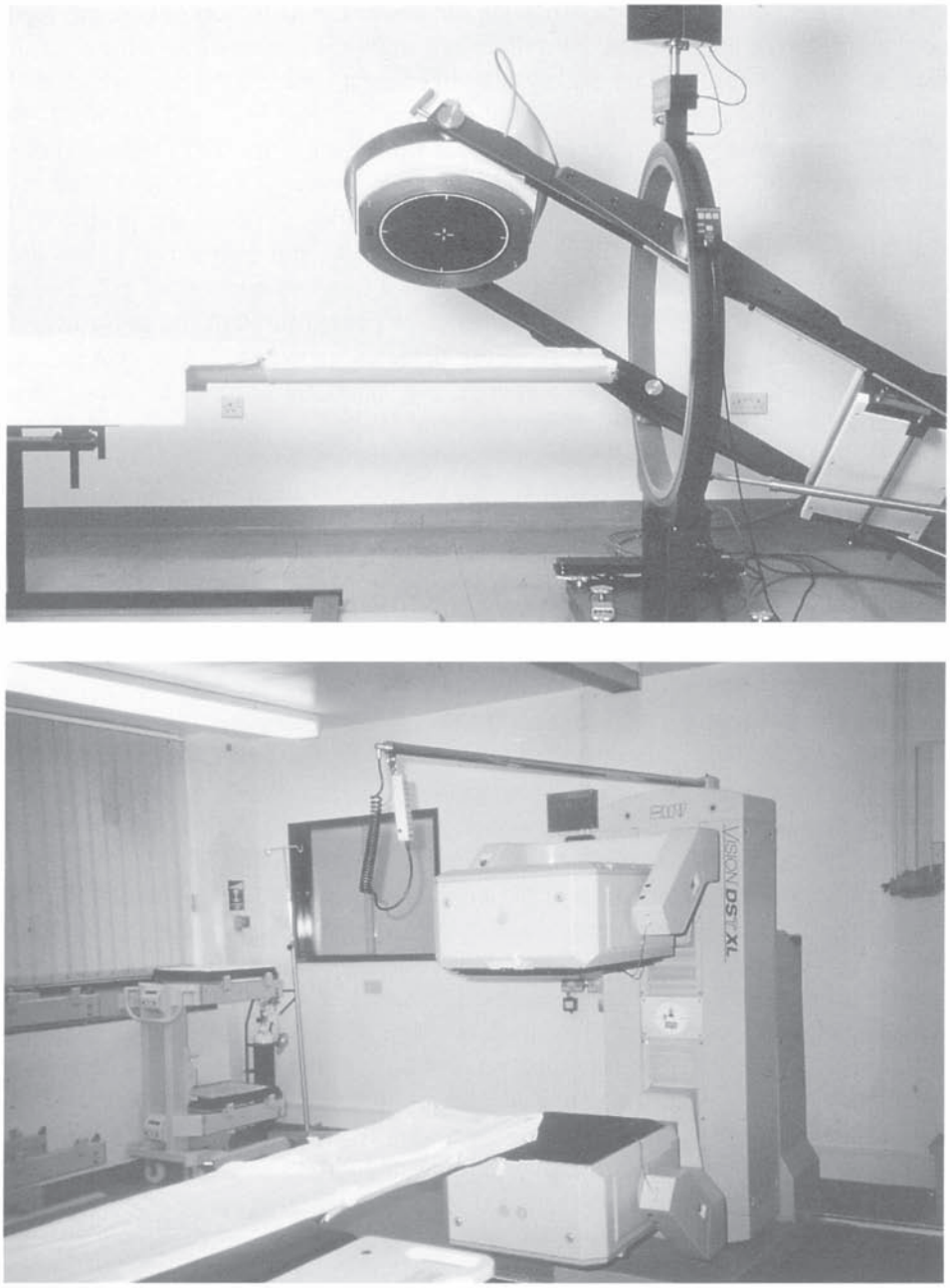

Figure 2.3 (A) A single head gamma camera system. (B) A dual head gamma camera system

The crystal is extremely fragile and mechanical shock can result in cracking of the crystal, rendering the camera inoperable. The hygroscopic nature of the crystal requires it to be hermetically sealed to prevent the ingress of moisture. Any sudden change in temperature can also result in spontaneous fracture of the crystal.

During operation gamma photons are channelled onto the crystal by the lead collimator, hence an image of the radioactive source is projected onto the crystal. Gamma photons absorbed by the crystal will cause a visible light flash which will be detected by the array of photomultiplier tubes (see Figure 2.2). Typically 10% of the gamma energy is converted into light producing some 3000 light photons of 400 nm wavelength for each 100keV gamma energy absorbed. Following the detection of each light photon a digital network calculates the position of the detected event in space. For each gamma photon, three electrical pulses are produced, two spatial coordinates X and Y and a summed energy pulse Z. The Z pulse contains the energy information which is passed through the pulse height analyser to discriminate the energy of the detected gamma ray. The camera can be tuned into the required gamma energy in a manner analogous to tuning in a radio to a broadcast frequency. If accepted within the selected energy window the detected pulses are registered and positioned on the image at the appropriate X,Y coordinates. The image is built up over a period of time until sufficient counts are collected.

The high voltage electrical supply and associated electronics are usually situated in a cabinet in close proximity to the head. Data collection is set and controlled by an acquisition module with TV monitor and keyboard.

2.6 Image Acquisition

Scintigraphic images may be collected for a set number of counts or a set period of time. The resultant view is known as a planar view. The data are displayed as a digital image, usually in a matrix of 64×64, 128×128 or 256×256 pixels in the same way as any other form of digital image. Gamma camera images are not high-resolution anatomical images and therefore high pixel densities are not required. It is usual for data to be handled by a computer network to allow viewing, data archiving and processing of images. The image is scaled relative to the pixel with the highest count rate. This pixel is given a 100% value and each of the remaining cells are assigned a value proportional to the counts recorded in that cell. The final image is displayed using an appropriate colour scale normally on a thermal scale (varying density of red) or a scale with red and yellow indicating high activity, through to blue indicating low activity. The image viewing and processing workstation may be situated at a location remote from the camera room. Image printers provide colour and black and white hard copy output to paper or film.

The following modes of data acquisition are used:

1 Static acquisition

2 Dynamic acquisition

3 Dual energy acquisition

4 Cardiac gated acquisition

5 Tomographic acquisition

2.6.1 Static Acquisition

Static images are recorded as a single set matrix over a set period of time or counts. The time of data collection will depend upon the amount of radioactivity administered. In clinical practice the camera is placed a few centimetres from the patient and the gamma rays counted over a set period of time, normally of the order of 30 s to 5 min to give sufficient counts within the image. A static study represents the distribution of the radiopharmaceutical at a set period of time after administration and is used when pharmacokinetics and transit are relatively slow.

2.6.2 Dynamic Acquisition

In a dynamic study a series of frames of a given matrix size are acquired over the total length of the study. The acquisition parameters stated are the matrix size, frame time (seconds) and the total number of frames. This type of study is used for assessing function with time, for example as in a renogram for the assessment of kidney function, or for monitoring the swallowing of a radiolabelled drink, meal or oral dose form. Data are usually expressed in the form of activity time curves generated from a set of regions defined on the dynamic images.

2.6.3 Dual Energy Acquisition

In dual radionuclide or dual isotope studies the counts from two energy windows are simultaneously acquired into separate matrices of the same size. In this way it is possible to record the images of two radiopharmaceuticals simultaneously administered to a patient. The images may later be digitally compared or even subtracted pixel by pixel by computer to highlight differences.

2.6.4 Cardiac Gated Acquisition

It is possible to control data acquisition by triggering from a physiological signal such as an ECG waveform or respiratory gate. In nuclear medicine the most common study of this type is the cardiac gated study. Synchronization of data recording is obtained from ECG electrodes. Data are used to calculate the left ventricular ejection fraction which is of value in monitoring the effects of drugs used in cardiology.

2.6.5 Tomographic Acquisition

A tomogram is a slice. Emission tomography may be performed using a dedicated scanner, or using a rotating gamma camera system, to display the data as slices taken at chosen planes through the subject or patient. Using the conventional gamma

emitting radionuclides such as Tc-99m tomography is usually performed by rotating the gamma camera system around the patient (IPSM, 1988, 1992a). The term applied to this procedure is single photon emission computed tomography (SPECT or SPET). This term signifies the detection of only one gamma photon to produce an image data point and is distinctly different to positron emission tomography (PET) in which two photon events arising from a positron-emitting radionuclide are necessary for an accepted event. Most SPET imaging is performed with a single or dual head gamma camera mounted on a gantry to facilitate circular rotation of the detector 360° around the patient. Data are acquired as a series of dynamic planar matrix views, typically 64 views in a 128×128 matrix. The main advantages of SPET include an increase in the image contrast, the visualization of data slice by slice and the possibility of additional filtering of image data. Careful system set-up and calibration is required for SPET imaging. The assessment of the image acquisition and reconstruction parameters using phantoms (test objects) is also necessary to maintain high performance of the equipment (IPSM, 1992b). The main variables affecting the collection of image data using rotating gamma cameras are:

- Size of the image matrix
- Number of angular increments for data collection
- 180° or 360° rotation
- Choice of collimator
- Increment acquisition time
- Detector uniformity correction
- Centre of rotation correction

2.7 Image Reconstruction and Display

Tomographic images are reconstructed by computer, using the same mathematical process (filtered back projection) as used in X-ray computed tomography. The data in the planar views are projected onto the image matrix in the tomographic image plane, this process being repeated for all angles around the patient. To remove unwanted contributions from the reconstructed image, each data point has an associated negative part projected onto the image. Once reconstructed the tomographic images may be viewed on the display as contiguous slices through the subject. The main image processing variables are:

- Choice of image pre-filter
- Choice of reconstruction filter
- Attenuation correction
- Scatter correction
- Slice orientation

Images may be displayed as orthogonal, axial, coronal and sagittal slices, or as oblique cuts through any chosen angle. The three-dimensional pixel is known as a voxel. Once in this form the data may be processed and viewed as a three-dimensional image using volume rendering computer software (Jarritt and Acton, 1996). This process is computationally intensive but is now widely used as a result of the availability of relatively inexpensive and powerful computer workstations. Depth cues are added by illumination to give depth in the image. Rotation of the information adds to the effect.

SPET provides the most accurate means for the quantification of volume and the absolute quantification of organ uptake of single photon tracers. Volumes may be measured providing the pixel size and slice thickness are known.

2.8 PET Imaging

Positron emission tomography uses radionuclides which decay by the emission of positrons. Positrons are positively charged electrons which are short-lived and travel only a short distance of 1 or 2 mm before being stopped. They readily combine with an electron to produce two gamma photons, each having energy of 511 keV, which are emitted in directions 180° opposed to each other. Diagonally opposed detectors and a coincidence network is used to accept the two photons produced from one positron event (Figure 2.4). Imaging is usually performed on a dedicated PET scanner comprising a circular array of detectors and looking

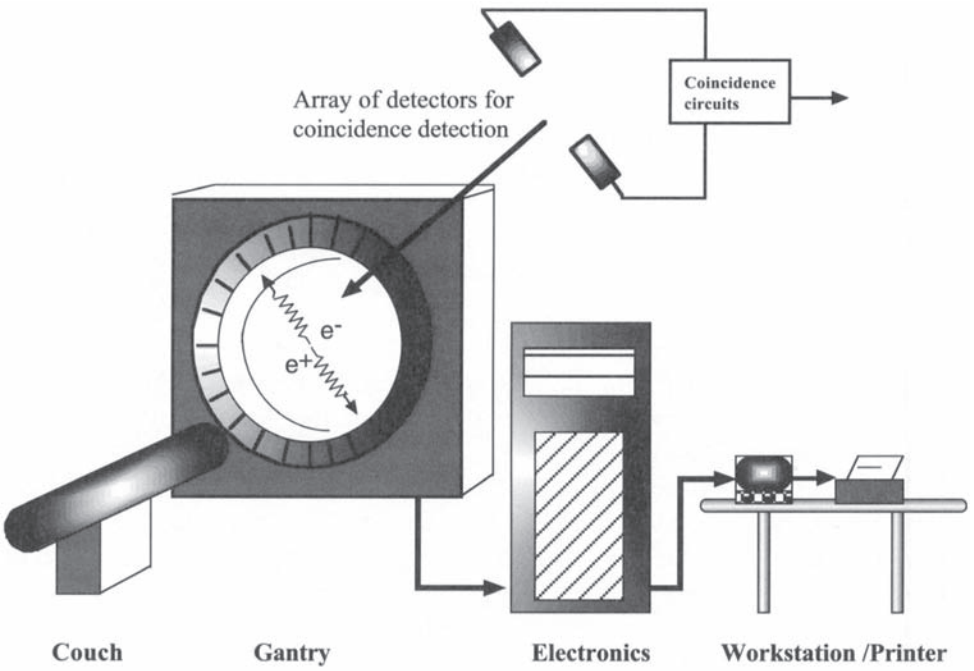

Figure 2.4 Schematic diagram of a PET camera system

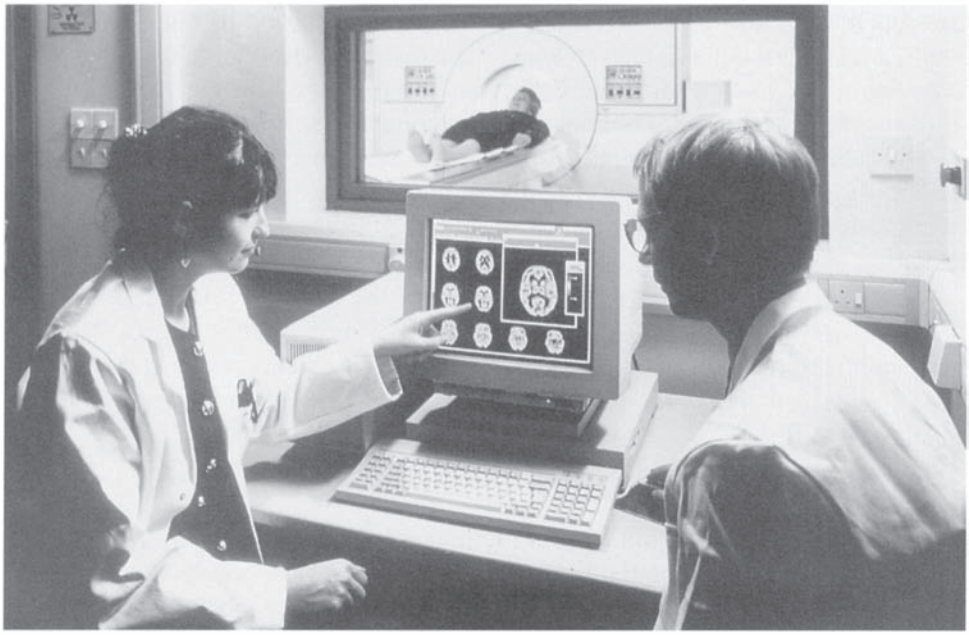

Figure 2.5 A PET camera and image workstation. *(Photograph courtesy of Dr Tom Nunan, St Thomas's Hospital, London.)*

much like a CT scanner (Figure 2.5). PET scanners have higher spatial resolution than SPET. Transmission sources are used to correct for the attenuation of gamma photons in the patient, thus increasing the accuracy of data quantification. Positron-emitting radionuclides generally have short physical half-lives. A cyclotron is therefore required for the production of radionuclides in close proximity to the PET scanner. Rapid automated labelling techniques are required for radiopharmaceutical production, thus adding to the expense of the technique (Jones, 1996).

A standard gamma camera may be used for detecting single 511 keV gamma rays from positron emitters provided high energy collimation is used (NEMA, 1986), however, because of the thin crystals in standard cameras the sensitivity of detection is low and image quality is poor. The development of dual headed gamma camera systems with the ability for coincidence detection of opposed 511 keV photons will increase the availability of this technology to a larger number of centres (Parkin *et al*., 1992).

One of the main advantages of PET is in the range of biologically active elements which can be studied. Organic chemicals with elements having positronemitting radionuclides include carbon-11, nitrogen-13, oxygen-15 and fluorine-18. A list of the main tracers used is given in Table 2.1. PET investigations allow the measurement of physiological processes such as blood flow and tissue perfusion, energy and fatty acid metabolism, protein synthesis and receptor binding (Perkins, 1996; Perkins and Frier, 1996). In this way PET imaging has provided some of the fundamental scientific information relating

to the understanding of human physiology. In routine clinical practice F-18 is emerging as one of the most useful tracers in oncology for tumour detection (Rigo *et al*., 1996).

2.9 Image Processing and Data Analysis

Once the images have been stored by computer mathematical operations may be performed to improve the visualization of the data (Sampson, 1994). Simple transformations include windowing the display threshold (lower) and saturation (upper) levels and image smoothing using filters to extract features from the images. Vertical and horizontal movement (translation) of the image may be carried out by moving pixel values one column or one row at a time.

One of the most powerful features of scintigraphic imaging is the ability to quantify the image data. Each pixel in the image represents the number of detected gamma rays from that area of the subject. It is therefore possible to quantify the uptake over specific portions of the image. This is achieved by defining a region of interest (ROI) in the computer image (see Figure 2.6). In this way the uptake may be expressed as a percentage of the administered dose or directly in MBq.

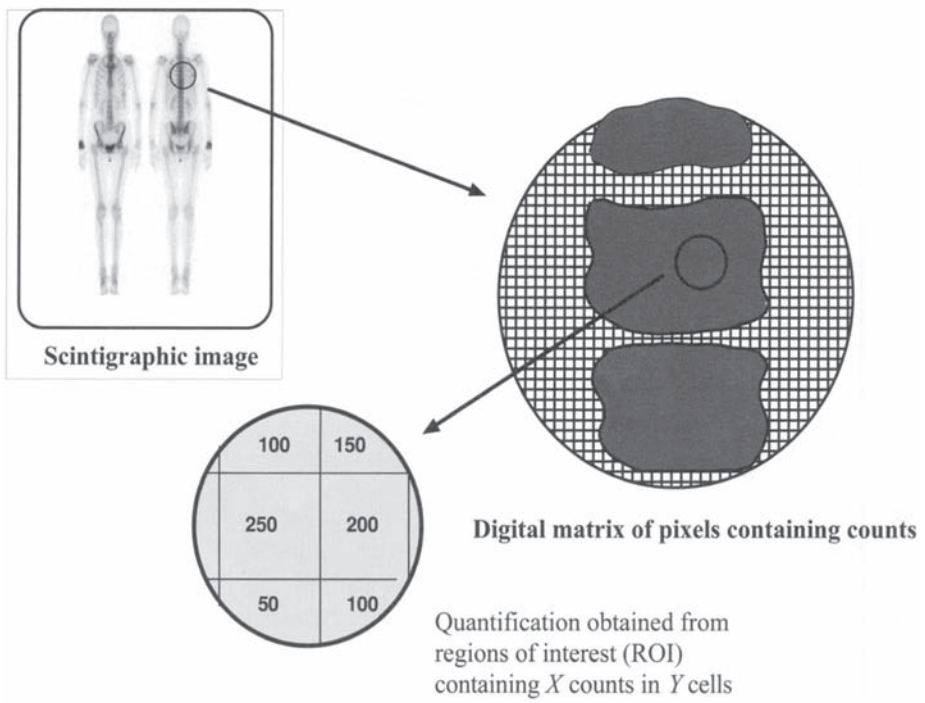

Figure 2.6 Quantification of radioactivity in the image is performed by defining ROIs which include a set number of pixels. The count rates in all the pixels are then summed to give the numerical value

There are a number of aspects to be considered when quantifying data from scintigraphic images. The main points are:

1 *The time of image acquisition.* The counts obtained from the images are dependent on the time of data collection. It is the count rate which is being measured from the images.

2 *Background subtraction.* It is necessary to subtract a constant value from every pixel in the ROI to account for background levels of radioactivity.

3 *Decay correction.* It is necessary to account for radioactive decay with time, especially if a study is performed over a significant time period when compared to the physical half-life of the radionuclide.

4 *Gamma ray attenuation.* Gamma rays are attenuated at depth in the patient. If the attenuation coefficient is measured using a transmission source an appropriate correction can be made. The methods used for quantification of residence and uptake will depend upon the nature of the study and the site of deposition. Calculation of the geometric mean of the count rates in paired anterior and posterior planar views is often used to provide a more accurate measurement of quantification. This may be given by:

$$C_c = \sqrt{[C_a \times C_p]}$$

where C_c = corrected count rate, C_a = anterior count rate and, C_p = posterior count rate.

5 *Activity time curves.* By defining ROIs on a dynamic image series recorded over a period of time, the count rates may be used to generate activity time curves. Providing there has been no movement of the subject, the count rate obtained from the ROI in each frame of the series can be used as a data point on the activity time curve. This technique can be used to measure rates of uptake, dissolution, spread and relative function. Once the curves have been generated a number of parameters may be measured, for example gradients and areas under the curve.

2.10 Quality Assurance

Good clinical practice demands that the equipment is maintained in optimum working order. This is particularly important in longitudinal studies, because variation in equipment performance over time can adversely affect the results obtained from an imaging study. Quality can only be assured if adequate measurements are carried out on a regular basis. Equipment calibration procedures should be performed at daily, weekly and monthly intervals. A quality assurance programme must incorporate written procedures for equipment calibration and records of the physical measurements made should be kept for audit purposes. This applies to all aspects of the process from the gamma camera hardware to

validation of the software used to generate the numerical data. Gamma camera calibration procedures using phantoms and test objects are well described in the literature (Wallis and Miller, 1991; Van Lingen *et al*., 1992; Stokin and Pike, 1993; Sharp *et al*., 1998). Parameters such as detector uniformity, sensitivity, count rate performance, spatial resolution and spatial distortion should all be measured. In addition it is essential that regular checks should be made to ensure the safe operation of equipment.

The key feature of all aspects of gamma scintigraphy as applied to pharmaceutical research is to validate all procedures and techniques carried out. This applies to all stages in the execution of a study, from radiolabelling and formulation manufacture through to data acquisition and image analysis.

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3

Good Manufacturing Practice in the Preparation of Pharmaceutical Dosage Forms for Scintigraphic Evaluation

M.FRIER

3.1 Introduction

Increasing use is being made of scintigraphic techniques in the development and evaluation of drug delivery systems. Use of the gamma camera to image the *in vivo* distribution of pharmaceutical formulations radiolabelled with a suitable gamma-emitting radionuclide enables the quantification of biodistribution, and determination of sites of release and kinetics of drug delivery from novel carrier systems and devices. Combining radionuclide tracer techniques with other methods of physiological measurement allows correlation to be established between the observed pharmacological effects and the precise site of delivery. Information gained from such studies is frequently incorporated into dossiers for submission to regulatory authorities for the purposes of product registration.

Nuclear medicine imaging techniques are recognized for their ability to provide information on physiological processes, rather than on anatomical structures. Most medical imaging modalities such as conventional radiology, CT, ultrasound and magnetic resonance provide high resolution anatomical definition. Gamma camera images, on the other hand, provide a functional map of physiological processes. The radiopharmaceutical tracer, containing a suitable gamma-emitting radionuclide, is administered to the patient and its fate monitored *in vivo* using a gamma camera. Recently this technology has been applied to the study of the *in vivo* behaviour of drugs and drug delivery systems, and commercial interest in the use of these techniques has been growing steadily. Scintigraphic imaging techniques, of the type used in diagnostic nuclear medicine, offer great potential in the study of the behaviour of drug formulations and drug delivery systems in human subjects. Application of the technique, however, presupposes that the drug, or one of the components of the formulation, can be radiolabelled with a suitable gamma emitter without altering the product characteristics. The radiolabelling stage is followed by imaging with a gamma camera and the information obtained

can be used to monitor *in vivo* transit, dispersion and deposition. This technology has been applied in the assessment of a wide range of pharmaceutical formulations and new drug delivery systems, including tablets, capsules, metered dose inhalers, nasal sprays and ophthalmic formulations. Such studies provide data on the nature and characteristics of a product, which may in turn be included in product registration dossiers for submission to regulatory authorities, providing both fundamental scientific data and supporting information. There are now numerous examples of published studies that have been utilized in this way.

Medicinal products intended for investigational purposes are presently not the subject of marketing or manufacturing legislation within the European Community. There is agreement, however, that Member States may require compliance with the principles of good manufacturing practice (GMP) during the manufacture of products intended for use in clinical trials. This agreement is based on the suggestion that it is illogical for experimental products to be exempt from the controls which would apply to the formulations of which they are the prototypes. It is recognized that manufacture of investigational products may not take place under a set routine, and that there may be incomplete characterization of the product.

It is perhaps useful at this point to introduce some definitions. European Community guidance on the application of GMP principles to the manufacture of materials for use in clinical trials (HMSO, 1997) defines the term 'clinical trial' as 'any investigation in human subjects intended to discover or verify the clinical, pharmacological and/or other pharmacodynamic effects of an investigational product(s) and/or to identify any adverse reactions to an investigational product(s), and/or to study absorption, distribution, metabolism, and excretion of an investigational product(s) with the object of ascertaining its safety and/or efficacy'. An 'investigational medicinal product' is described as a 'pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorisation when used or assembled (formulated or packaged in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use'.

It is clear that scintigraphic studies of pharmaceutical dosage forms containing radioactive components fall within the scope of these definitions, and that application of the principles of GMP be considered implicit in the preparative stages. However, the need for GMP may raise certain logistical problems because of the requirement to introduce radioactive, often short-lived, tracers into pharmaceutical dosage forms. Commercial pharmaceutical manufacturers are generally able to produce small batches of non-radioactive investigational products in dedicated, or pilot-scale plant, under conditions identical with, or very similar to those used in full-scale manufacture. However, it is rare to find commercial manufacturers with on-site production facilities for the manipulation of radioactive materials. In these circumstances, dosage forms must be modified after manufacture in premises not under the direct control of the manufacturer. There may be circumstances in which the whole of the manufacturing process has to be contracted out to sites appropriately authorized to handle radioactive substances.

Techniques for the incorporation of radionuclides vary in complexity, ranging from simple physical manipulation of preformed tablets to complete fabrication of complex, controlled-release dosage forms. It is proposed to identify the important elements of GMP, and to describe the preparation of a number of differing dosage forms of varying complexity, to describe how the principles of GMP may be applied. Post-production radiolabelling using neutron activation offers distinct advantages in the achievement of GMP standards by permitting manipulation of non-active components and products in dedicated or pilot-scale facilities, with subsequent irradiation of the complete product.

3.2 Elements of Good Manufacturing Practice

Good manufacturing practice is that part of quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use. It is concerned with both production and quality control, and requires the implementation of a highly effective quality management system, which assumes greater significance in the manufacture of investigational medicinal products, because of the non-routine nature or increased complexity of the operations involved. The quality management system should extend to cover the following main areas, which will be discussed in turn.

3.2.1 Premises

Any premises where investigational product manufacture takes place should be secure, and provide a constant, controlled environment. It is recognized that, unlike routine manufacture, different products may be handled in the same premises and at the same time, which reinforces the need to have appropriate procedures in place to avoid product mix-up and cross-contamination. Premises should be cleaned using appropriate, written and recorded procedures, and an operational log of all procedures performed within the premises should be maintained.

3.2.2 Personnel

Any staff involved in preparation procedures should be appropriately qualified and trained, and the training should include some formal, documented instruction in GMP. The nature of investigational manufacture is such that the numbers of staff involved will be small, although ideally, there should be separate people involved in production and quality control. This may not always be possible, but at the very least, release procedures should be agreed with quality control prior to the start of the study, and at every stage of the manufacturing operation, two members of staff should be involved to provide independent checks.

3.2.3 Protocols

It is essential that starting materials and finished products are clearly specified in protocols, which also include criteria for release of the finished product. Processing instructions need to be in writing, and provision made for recording of batch manufacturing details. However, it is recognized that the product specifications and processing instructions may vary during the developmental stages, and this is quite acceptable providing the rationale is explained, and any changes are authorized and recorded.

3.2.4 Processing

All processing should be performed in accordance with written instructions. Variations in procedure are permissible, but prior authorization should be sought, and the nature of the variation, together with the reasons, recorded. Validation of procedures is crucial, because, by their nature, they are investigational and non-routine. Validation can be direct, or may in some cases have to be performed parametrically as, for example, when assessing the effect of a radiolabelling procedure on a pharmaceutical product. It is often convenient to test the process by performing the required manipulations in the absence of the radioactive component.

3.2.5 Plant and Equipment

As for processing, the keyword here is validation. Any equipment or plant used must perform reliably within known parameters, and it is usual to carry out validations at three stages, namely on installation, during routine operation, and during performance of the actual process in question. Validation at installation is simply to confirm that the equipment conforms to its specifications. Validation during operation establishes the working parameters, and it is expected that measurements taken during performance of the actual procedure will fall within those parameters. Appropriate cleaning of plant and equipment is essential not only to avoid cross-contamination but to ensure that materials used in cleaning do not have a detrimental effect on the products themselves. It is therefore essential that cleaning procedures be validated, documented and recorded. Equipment including, for example, balances and ionization chambers, is calibrated using test weights or radioactive sources with certification traceable to national standards. Usage of equipment is continually recorded in the form of an operational log.

3.2.6 Products

It is usual to estimate the yield of any production process. In the context of preparing a radioactively labelled product, this will usually take the form of an

estimate of radioactivity, and will need to take into account time differences between preparation and dosing, to compensate for radioactive decay. Actual and theoretical yields should be reconciled, and any discrepancies investigated. As already described, product release is only permitted in accordance with a standard operating procedure which specifies the release criteria. If storage is required between preparation and use, the storage area should be secure and, ideally, dedicated. Surplus or unused materials may only be disposed of or destroyed in accordance with written standard operating procedures.

3.3 Representative GMP Studies

In most respects, these elements of GMP are identical with those which apply in the routine manufacture of medicinal products. Available guidance does recognize the investigational nature of clinical trials manufacture, and identifies the need to be flexible in certain areas. The small numbers of staff involved, and the developmental nature of the procedures, are two examples of the way in which this flexible approach is applied. In order to illustrate these points further, it is proposed to describe the preparation procedures involved in three studies involving manufacture of radioactively labelled investigational medicinal products under GMP conditions. The products differ considerably in complexity, and in the extent to which they can be prefabricated in a commercial GMP manufacturing facility.

3.3.1 Film-coated Tablets for Oesophageal Transit

Measurement of oesophageal transit in normal subjects can provide information on the swallowing patterns of different formulations such as tablets and capsules where the shape, density and nature of the surface coating may affect the rate of transit. These patterns may be exaggerated in elderly subjects, or in patient groups with pathophysiological conditions affecting the motility of the oesophagus. Such studies are especially valuable in the evaluation of formulations for the delivery of irritant drugs which have the potential, should they become lodged in the oesophagus, to cause oesophageal inflammation or ulceration caused by localized drug release. The dosage form chosen for the study was an oval, filmcoated compressed tablet. A hole 1.5 mm in diameter was drilled into one end of the tablet to a depth approximately half the length of the tablet's long axis, and radiolabelling accomplished by filling the hole with an ion-exchange resin containing Tc-99m pertechnetate. The resin was sealed in place with a small waterresistant plug. Technical details of this study, together with results and outcomes, are described in detail in Chapter 5.

The first and most important stage of the study involves validation, to demonstrate the effect on tablet performance of the drilling, filling and sealing manipulations. This requires measurement of standard tablet performance parameters, including disintegration time and hardness, pre and post drilling

and filling. Averages of pre and post drilling weights, pre and post filled weights and pre and post sealed weights can also be incorporated into the standard operating procedures describing parametric release criteria. Another major consideration in adopting GMP procedures concerns the quality of materials used. The tablets used in this particular study were placebos, manufactured specifically for clinical trial use in a dedicated commercial production plant. Some materials may be used in ways for which they are not intended, and may not be approved for medicinal use at all, although they may still be safe for use in controlled clinical trials. This places great emphasis on the need to establish the quality of materials used, ideally by using products of medicinal quality, but in situations where this is not possible, by obtaining appropriate certificates of analysis, and by application of some form of identification check. The sealant used was in fact a surgical bone cement, intended for insertion into bony cavities, and, as such, was considered perfectly acceptable for oral administration.

The equipment used in the study was very simple, comprising a small electrically-powered drill. However, even the use of comparatively simple equipment raises a number of important GMP principles. Cleaning, particularly of the drill bit, is crucial, firstly to prevent contamination of the tablets, secondly to avoid cross-contamination, and thirdly to ensure reproducibility of hole size and prevent tablet cracking by avoiding build-up of film coat residues on the bit. Choice of cleaning agent is also important, both in being effective, and in leaving no residues. Any required lubrication of the drill motor should be performed with approved lubricants.

Some parametric release criteria have already been mentioned. Other direct criteria must be established, including visual inspection at post drilling, post filling and post sealing stages, and in the calibrated radioactivity levels of individual tablets. These criteria should be agreed by the principal investigator and by the persons responsible for manufacture and quality assurance.

3.3.2 Metered Dose Inhalers for Pulmonary Drug Delivery

Gamma scintigraphy is a well-established technique for assessing the performance of nebulizers, metered dose inhalers and powder inhalers, and in determining pulmonary drug deposition. By defining appropriate regions of interest on computer images an accurate quantitative measure of the proportion of drug reaching the lung may be made. The measurement of regional deposition may be assessed quantitatively in combination with a Kr-81m gas ventilation image to measure the ventilation space of the lung. Total deposition is assessed by additionally imaging the administration unit pre and post dosing. It is possible to assess the effect of different variables, such as metered volume or propellant vapour pressure, and also the effectiveness of devices such as spacers in improving the proportion of drug reaching the lungs or in reducing the proportion reaching the stomach. The technique is especially useful for the regional assessment of drug deposition in patients where the airway may be abnormal,

and may help in choosing the most appropriate delivery systems for use in different types of lung pathology. It may also help in highlighting shortcomings in technique in the use of metered dose inhalers, and lead to a broader application of this technology to drug delivery.

There are many published examples of studies involving the use of radiolabelled metered dose inhalers (for example, Hardy *et al*., 1996) and reference should also be made to Chapter 4, which describes in detail the technical aspects and outcomes. Preparation procedures are, however, generally more complex than for the tablet study just described, and numerous GMP issues are raised. In the example chosen, metered dose inhalers containing 100 μg per actuation of a solid drug dispersed in a CFC propellant, were radiolabelled with Tc-99m pertechnetate to assess the effectiveness of a spacing device. Introduction of the radiolabel requires opening of the canister, and addition of pertechnetate, followed by resealing of the canister. GMP considerations primarily revolve around validation and the establishment of release criteria. The main steps in the radiolabelling sequence can be summarized as follows:

1 Extract Tc-99m pertechnetate into butanone.

2 Place butanone solution into empty MDI canister, and evaporate to dryness under a stream of air by the application of gentle heat.

3 Freeze the contents of a standard, filled MDI canister using liquid nitrogen.

4 Open the frozen canister using a suitable cutter, and pour the liquid contents into the dry canister containing Tc-99m pertechnetate.

5 Crimpseal the radioactive canister using a fresh actuator valve.

6 Check the sealed canister for leaks by immersion in water.

Materials and equipment which come into contact with the final product are butanone, air used in drying, new canisters and actuators, and the cutter used in opening. GMP principles apply, and where appropriate, certificates of analysis should be obtained, identity checks performed, and suitable cleaning procedures adopted.

Two other areas of concern with respect to GMP are validation, and definition of release criteria for the final product. Validation is concerned principally with establishing the fact that the radioactive canister has the same performance characteristics as that of the original. Drug delivery per actuation, and particle size distribution of the delivered drug must be determined.

However, because penetration and localization of the drug are to be determined by gamma camera imaging of the radioactive content, it is also important to establish concordance between the particle size distribution of the drug and radioactivity. Results of a size distribution study comparing drug and activity distributions determined using an Andersen impactor are shown in Figure 3.1. Drug delivery per actuation and particle size distribution constitute two parametric release criteria. Direct release criteria include radioactivity content of the final canister and activity delivered per actuation, determined by direct measurement.

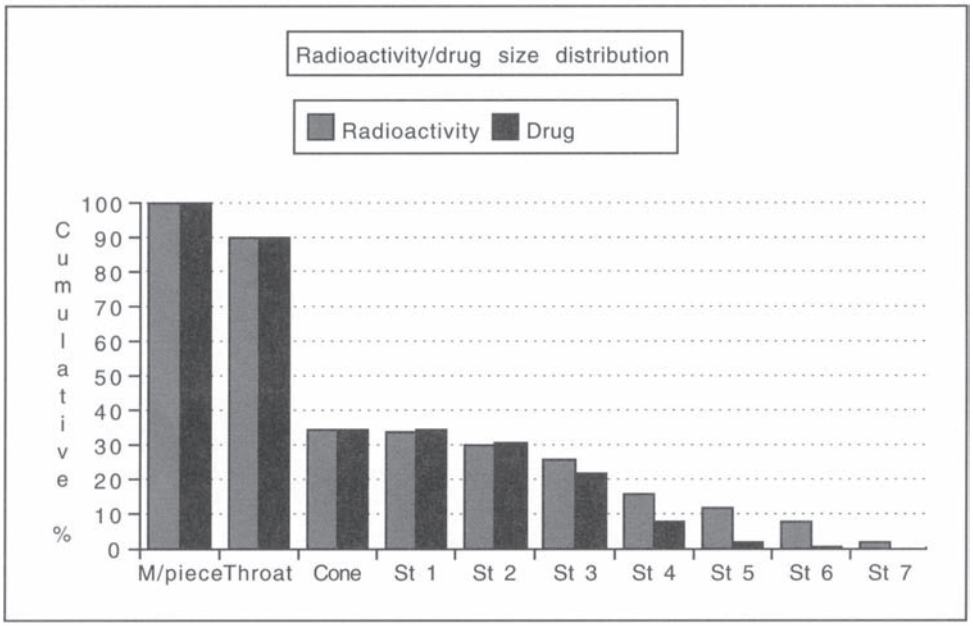

Figure 3.1 Distribution of drug and radioactivity following analysis of the output of a metered dose inhaler on an Andersen impactor

3.3.3 Gastroresistant Pulsed Release Delivery Systems

Many orally administered drug formulations are designed to deliver drugs in a controlled way, either by releasing the drug gradually over time to sustain plasma levels following a single administration, by releasing the drug in response to the particular pH environment, or by containing some form of programming device to provide delivery in a pulsed or timed manner. Gastro-resistant formulations protect potentially sensitive drugs from the acid environment of the stomach, and also help to provide more controllable drug absorption, because of the extreme variability of gastric emptying compared to the much less variable kinetics of intestinal transit.

Wilson *et al*. (1997) have described a scintigraphic evaluation in human subjects of a gastro-resistant, pulsed release delivery system, designed to target drug delivery to the colon. The technical details of this study are also described in Chapter 7. Two different radionuclides are required. Introduction of the radiolabels requires complete fabrication of the dosage form. GMP considerations primarily revolve around quality of source materials, validation and the establishment of release criteria. The main steps in the radiolabelling sequence can be summarized as follows:

1 Add Tc-99m DTPA to a mixture of sucrose and sodium docusate, and dry.

2 Fill low-density polyethylene capsule bodies with the radioactive sucrose mixture.

3 Plug the capsule body necks with hydrogel polymer plugs.

4 Place In-111 resin in enteric-coated gelatin capsule caps.

5 Place a cap on to each hydrogel-plugged body.

6 Seal the join between body and shell using a gastro-resistant polymer solution.

All materials described form part of the final dosage form. GMP principles apply, and where appropriate, certificates of analysis should be obtained and identity checks performed. Only simple equipment, such as spatulae, is required, but appropriate cleaning procedures should be adopted to prevent cross-contamination. Validation primarily involves establishing correlation between observed dispersion of capsule contents under controlled conditions, and the measured release of radioactivity. Release criteria include fill weight and radioactive content.

3.3.4 Neutron Activation

An elegant approach to radiolabelling, and incidentally one which addresses many GMP issues, is to incorporate the non-radioactive oxide of either samarium-152 or erbium-170 into the formulation during manufacture. Following neutron activation in a nuclear reactor the radioactive products Sm-153 or Er-171 may be used for imaging (Parr and Jay, 1987; Awang *et al*., 1993). Both samarium and erbium oxide are non-absorbable within the gastrointestinal tract and are suitable for studying slow-release formulations. These techniques allow manufacture of products in GMP facilities because no handling of radioactive material is involved until the final irradiation. They also address the situation where production processes are lengthy or multistage, for example in the production of multi-layered compressed tablets, or sugar-coated formulations, because they avoid the problem of lengthy handling of radioactive products, or of excessive radioactive decay occurring during the production process. It is still necessary, however, to consider quality of source materials, particularly of the erbium and samarium oxides themselves, and validation is crucial, as significant changes can be induced by the irradiation procedure itself, which can bring about substantial heating of the target material.

There is no simple description of Good Manufacturing Practice. The most important aspect is for it not to be seen as something ‘extra’ added to a process but for it to be implicit at every stage involved in the preparation of pharmaceutical products for use in clinical trials. Seen from the regulatory affairs viewpoint, adoption of the principles of Good Manufacturing Practice, Good Clinical Practice and Good Laboratory Practice is now essential to the acceptability of any supporting data included in product dossiers. Adoption of the principles from the very outset helps prevent later compromise in the study design, and gives a high level of confidence in the quality of any results obtained.

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4

Drug Delivery to the Respiratory Tract

J.G.HARDY

4.1 Introduction

Radionuclide imaging has a well-established role in the development and evaluation of products for drug delivery to the respiratory tract. The principal application is in monitoring lung deposition of inhaled aerosols. Radiolabelling of the aerosol allows quantification of the distribution of the formulation. This is particularly relevant, because most inhaled formulations are administered for their topical actions. Nuclear medicine techniques are also applied to the monitoring of nasal formulations, mainly for determining deposition sites and measuring the rates of clearance of formulations from the nasal cavity.

Administration of drugs directly into the lungs provides a high therapeutic index, by allowing relatively low doses to be deposited to achieve high local concentrations at the sites of action. The main factor influencing lung drug deposition is particle size (Stuart, 1973). Particles having mass median aerodynamic diameters greater than 5 μm tend to deposit by impaction in the oropharynx and the large airways. Those between 0.5 and 5 μm diameter deposit in the lungs primarily by sedimentation, while deposition of the finer particles is largely dependent on diffusion. Radionuclide studies have played a major role in investigations of the effects of particle size and dosing procedures on lung deposition.

Therapeutic aerosols are administered using a wide range of devices such as nebulizers, pressurized metered dose inhalers and powder inhalers. Monitoring the deposition of radiolabelled aerosols provides a direct measure of the efficiency of such devices. Additionally, aerosol delivery systems have been developed specifically for the administration of diagnostic aerosols for lung ventilation studies (Mackey *et al*., 1994).

As with the lungs, drugs are administered to the nasal cavity primarily for their local action, but there is increasing interest in this route for systemic delivery. Key factors readily amenable to investigation by gamma scintigraphy are the site and extent of drug deposition and the residence time in the nasal cavity.

4.2 Radiolabelling and Imaging

The radiolabelling of respiratory formulations is dependent on the nature of the product. Aqueous solutions for nebulization are often radiolabelled by the addition of Tc-99m-labelled diethylenetriaminepentaacetic acid (Tc-99m-DTPA). This tracer is absorbed from healthy lungs with a half-time of approximately 1 h (Jones *et al*., 1980) and rapidly cleared from the blood by the kidneys. The swallowed portion of the dose is excreted without absorption from the gastrointestinal tract, allowing differentiation between the fractions deposited in the lungs and the oropharynx. In situations where administration may be relatively prolonged, use of a less rapidly absorbed tracer such as Tc-99m-labelled albumin may be advantageous. Additionally, if the rate of clearance is to be monitored, for example of a solution deposited in the nasal cavity, the tracer distribution should represent that of the constituent of interest.

Most pressurized metered dose inhalers contain a suspension of the drug particles in the propellant. It is important that the distribution of the radiolabel should reflect that of the drug particles and that it is unaffected by the radiolabelling procedure. The most commonly adopted procedure involves extracting Tc-99m-pertechnetate into an organic solvent and evaporating the solution to dryness in an empty aerosol canister. Using a cold transfer process, the contents of a canister of the formulation to be labelled are added to the technetium-containing canister and a metering valve attached (Hardy, 1997). Confirmation of satisfactory radiolabelling is achieved using a multistage impinger to compare the drug and radiolabel distributions from the labelled preparation with the drug distribution from a non-labelled canister of the same product.

The radiolabelling of a propellant-soluble pressurized metered dose inhaler involves the addition of a propellant-soluble radiopharmaceutical, for example Tc-99m-hexamethylpropyleneamine oxime, or direct labelling of the drug molecules. As with the suspension formulations, the drug and tracer distributions should be assessed *in vitro* in order to validate the labelling procedure.

Powder inhalers usually contain either pure drug or a blend of the drug with a carrier, often lactose. The diameters of the carrier particles are generally much larger than those of the drug particles (Figure 4.1). In order to ensure that the radiolabel is associated only with the drug particles, it is necessary to label the drug prior to blending with the carrier. The radiolabel, normally a compound of technetium-99m, is dissolved in a volatile solvent in which the drug particles are insoluble. The radiolabelled solution is added to the drug powder and the solvent evaporated. If necessary, the drug particles are disaggregated and blended with carrier before being loaded into the inhaler. The labelling procedure should be validated using impinger deposition studies.

For lung deposition studies, the distribution of the radiolabelled formulation should be imaged immediately following administration. Once in the lungs, weakly bound tracer is likely to redistribute or clear at a different rate from that of the drug. For planar gamma camera imaging, normally an anterior and a posterior image are recorded to include the lungs and stomach. A lateral image is also

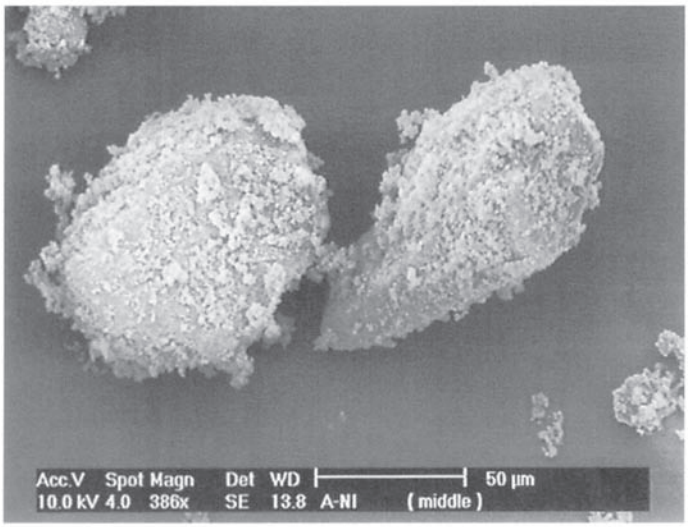

Figure 4.1 Blend of micronized salbutamol sulphate powder with lactose carrier particles

taken of the head and neck. Quantification requires correction for tissue attenuation of the counts detected (Forge *et al*., 1993). Use of a multi-headed gamma camera may permit tomographic imaging before significant redistribution of the tracer has occurred. Tomographic imaging offers the potential of closely relating drug distribution to lung morphology (Fleming *et al*., 1996).

The distribution and clearance of formulations deposited in the nasal cavity can be monitored from lateral images of the head recorded at intervals, typically for up to 4 h following administration. Images of the thorax taken soon after dosing may be helpful in confirming the lack of inhalation of nasal sprays into the lungs.

4.3 Aerosol Generation

Therapeutic aerosols are generated by three types of device: nebulizers, pressurized metered dose inhalers and dry powder inhalers.

4.3.1 Nebulizers

Nebulizers are used for converting aqueous solutions and suspensions into respirable droplets. They are of two basic types: jet nebulizers which rely on a stream of air to generate the aerosol, and ultrasonic nebulizers in which droplets are produced by the high frequency vibration of a piezoelectric crystal.

Nebulizers are useful for the delivery of relatively high dose treatments to patients with severe chronic obstructive pulmonary disease or asthma. They are

also commonly used for the administration of antibiotics. Depending on the device and the formulation, the fill volumes range from about 2 to 6 ml, with nebulization taking 10–30 min. The aerosols delivered from nebulizers have mass median aerodynamic diameters (MMAD) of typically 1–5 μm; the actual values reported for a particular nebulizer-formulation combination being dependent on the measurement technique adopted. Differences in the design and operation of the nebulizers results in considerable variability in drug delivery, both in terms of particle size distributions and the rates of aerosol generation (Smith *et al*., 1995).

Radionuclide imaging studies have been applied to the characterization of nebulizer performance. The deposition of tracer in the lungs tends to be low, generally less than 20% of the amount loaded into the device (Hardy *et al*., 1993a). A study comparing eight different nebulizers to administer pentamidine aerosols to patients with AIDS showed that the average doses deposited in the lungs ranged from 1 to 5% (Thomas *et al*., 1991). Most of the drug remains concentrated within the device or deposited on the delivery tubes and baffles intended to filter out the larger droplets. Additionally, aerosol may be wasted by generation during the non-inspiratory phase of respiration. In general, nebulization occurs more rapidly using ultrasonic nebulizers than with jet nebulizers. The droplets generated, however, tend to be larger from the ultrasonic nebulizers, resulting in relatively more central lung deposition.

The physicochemical nature of the formulation also affects lung deposition. Chan and colleagues (1994) used radiolabelled solutions to show that following inhalation of similar sized aerosols, hypotonic droplets deposited more peripherally than hypertonic droplets. The effect was more apparent at low droplet concentrations in the airways and was attributed to the tendency for hypotonic droplets to shrink whilst the hypertonic droplets grew by taking up moisture. Other formulation considerations include the surface tension of the liquid. The addition of up to 1% surfactant, for example to facilitate nebulization of a suspension, decreases surface tension and tends to increase the MMAD of the aerosol (McCallion *et al*., 1996).

Radionuclide imaging studies are useful in the assessment of administration techniques. It has been shown, for example, that about twice the amount of aerosol deposits in the lung when inhaled through the mouth compared with nasal breathing (Everard *et al*., 1993, Chua *et al*., 1994). The distributions within the lungs were, however, the same for both oral and nasal inhalation (see Figure 4.2, colour section). Such information is particularly relevant in the treatment of infants, because they inhale primarily via the nose even when their mouths are open (Chua *et al*., 1994).

Lung deposition data, provided by gamma scintigraphy, can be used to help in the selection of the most appropriate system for delivery of a particular treatment. For example, the choice of nebulizer for the administration of pentamidine as a prophylaxis against *Pneumonocystis carinii* pneumonia in patients with AIDS has received considerable attention (Thomas *et al*., 1991, Smith *et al*., 1992). Nebulizers resulting in high peripheral lung deposition with relatively low oropharyngeal deposition provide the most effective therapy whilst minimizing the unpleasant side-effects of the treatment. Another situation in which the choice

of nebulizer may be important is the administration of gentamicin in cystic fibrosis. It has been demonstrated by Smaldone (1994) that the highest sputum levels are associated with more central deposition of the drug.

Drug delivery from nebulizers is, in general, poorly controlled. With the introduction of a wider range of inhaled drugs, it is becoming increasingly important to ensure more precise dosing. The ways in which nebulizers are used, for example in terms of solution volume and air flow rate for each pharmaceutical formulation, need careful consideration. Gamma scintigraphic studies can help in ensuring that the formulation is administered from the most suitable device to provide deposition appropriate to the condition being treated.

4.3.2 Pressurized Metered Dose Inhalers

Pressurized metered dose inhalers (MDIs) have been available since the mid-1950s and by 1996 annual production was in excess of 400 million. They are readily portable and provide a convenient means of dosing patients having a wide range of respiratory diseases. Approximately 80% of inhaled asthma therapy is delivered by MDIs.

Most metered dose inhalers comprise a metal canister fitted with a metering valve and containing drug suspended or dissolved in chlorofluorocarbon (CFC) or hydrofluoroalkane (HFA) propellants. The canister is fitted into a plastic actuator from which the dose is delivered. The majority of currently available MDIs contain suspensions of drug particles 1–2 μm in diameter. Each canister contains typically 100–200 doses of between 20 μg and 5 mg drug per actuation. Drug delivery occurs over about 0.1 s; the spray velocity averaging about 10 m/s over the first 5 cm. This results in many patients experiencing difficulties in using MDIs correctly. The main problem, which may affect half the users, is an inability to coordinate actuation of the inhaler with inspiration of the dose. Additionally, many patients cease inhaling in response to the propellant impacting on the throat. In order to improve drug delivery, MDIs are frequently used together with a spacer device attached to the actuator mouthpiece. Breath-actuated inhalers have also been developed in order to overcome the coordination problem.

Initial studies (Newman *et al.*, 1980, 1982; Lawford and McKenzie, 1983), monitoring radiolabelled particulate deposition and changes in lung function in response to bronchodilators administered from MDIs, indicated that for optimum drug delivery MDIs should be actuated early during a slow inspiration followed by breath-holding. In a recent study, Farr and colleagues (1995) used a microprocessor-controlled MDI to deliver Tc-99m-labelled salbutamol particles at three inspirational air flow rates and during early and late stages of inhalation. Each administration was followed by breath holding for 5 s. The maximum average lung deposition of 19% was achieved when the inhaler was actuated at an air flow rate of about 90 l/min, early during inspiration. The corresponding values of 14 and 8% were obtained with air flow rates of 30 and 250 l/min, respectively. Lung deposition was reduced by delivery of the dose at a late stage during inhalation.

Table 4.1 Lung deposition in healthy subjects from metered dose inhalers

Drug suspension	Dose/actuation (μg)	Lung deposition (%)	Reference
Sodium cromoglycate	5000	9	Newman *et al.* (1991b)
Nacystelyn	2000	12	Hardy *et al.* (1993b)
Budesonide	200	18	Thorsson *et al.* (1994)
Salbutamol	100	19	Farr *et al.* (1995)
		22	Melchor *et al.* (1993)
		24	Biddiscombe *et al.* (1993)

The proportion of the lung dose depositing peripherally was not significantly affected by the dosing procedure. Oropharyngeal deposition was about 60% with low air flow at actuation and increased to around 70% with the highest air flow rates. For all the administration conditions only about 1% of the dose was exhaled. These findings confirm that the inhaler should be actuated early during inspiration at a relatively slow air flow rate.

Lung deposition from MDIs is influenced by the formulation. Radionuclide imaging studies have shown that for drug suspensions, the proportion of the dose depositing in the lungs tends to increase as the drug content per actuation decreases (Table 4.1). This is probably due to the generation of a larger proportion of droplets containing more than one drug particle at higher drug concentrations. This will result in an increase in the average aerosol particle size and hence a reduction in lung deposition. Even with low drug concentrations, lung deposition is generally less than 20% of the dose from the canister, with approximately 65% impacting in the oropharynx and the remainder depositing in the actuator.

With suspension MDI drug formulations the minimum aerosol size is that of the drug particle. For a solution formulation, dispersion and evaporation of the propellant governs the droplet size distribution. Studies with a CFC propellant soluble radiolabelled tracer, Tc-99m-hexamethylpropyleneamine oxime, resulted in lung depositions of about 40% (Ashworth *et al.*, 1991). Lung deposition was independent of the actuator orifice diameter, indicating that evaporation of the droplets was of overriding importance. This has been supported by a study by Harnor and colleagues (1993) in which lung depositions of 51 and 65% were achieved by dosing with a propellant having relatively low and high vapour pressures, respectively. The propellant having the higher vapour pressure would be expected to evaporate more rapidly, resulting in smaller inhaled droplets. Thus there is potential for significant improvements in lung deposition by modifications to the formulations.

Due to the ozone-depleting effects of CFCs, their use is being phased out and in MDIs they are being replaced by HFA propellants. Differences in the physicochemical properties of the two types of propellants have necessitated

modifications to device components and drug formulations. The main effect on drug delivery results from some drugs, for example beclomethasone dipropionate (BDP), being suspensions in CFC propellants but solutions in the HFAs. Leach (1996) measured lung depositions of 51% for a BDP-HFA formulation compared with 4% for a BDP-CFC product, with a corresponding reduction in oropharyngeal deposition to 29% from 85%. Although these lung deposition values may be overestimated due to the count rates not having been corrected for tissue attenuation, they confirm the improvement that can be achieved with solution MDIs.

The study by Leach (1996) included a comparison of the lung deposition of aerosol from a BDP-HFA solution MDI in asthmatic patients and healthy subjects. Similar values were found for both groups. This finding is in agreement with the data reported by Melchor *et al.* (1993) for a salbutamol suspension MDI. The mean total lung deposition in the patients (18%) was not significantly different from that in healthy subjects. A greater proportion of the lung dose deposited peripherally in the healthy subjects than in the patients (see Figure 4.3, colour section), 44% compared with 30%. Even with trained, healthy subjects being dosed under close supervision, the proportions of the dose depositing in the lungs may vary by a factor of two or more. For example, in six subjects inhaling salbutamol the mean lung deposition was 24% with a range of 15–37% (Biddiscombe *et al.*, 1993) and in six dosed with nacystelyn the mean value was 12% with a range of 9–16% (Hardy *et al.*, 1993b). With patients adopting poor inhalation techniques, the variability is much greater. For eight asthmatic patients inhaling salbutamol from an MDI, lung deposition ranged from less than 1% to 28% (Newman *et al.*, 1991a).

The inability of patients to coordinate well the actuation of the MDI with inhalation has been addressed by the introduction of breath-actuated inhalers. These devices are designed to depress the canister and deliver a dose automatically at a predetermined inspiratory air flow rate, of typically 20–30 l/min (Fergusson *et al.*, 1991; Hardy *et al.*, 1996). In a study using radiolabelled salbutamol, Newman and coworkers (1991b) showed that patients unable to coordinate correctly had, on average, lung depositions of 7% using standard MDIs, which was less than half that achieved in patients with a good technique. In the same group of poor coordinators lung deposition from a breath-actuated inhaler averaged 21%, comparable with that attained by good coordinators. Thus by ensuring drug delivery at a relatively slow inspirational air flow, the drug should be delivered early during inspiration, thereby aiding lung deposition.

An alternative approach to improving lung deposition in poor coordinators is to actuate the MDI into an aerosol holding chamber, a large volume spacer, from which the patient subsequently inhales. This eliminates the need for precise synchronization between actuation and inhalation. Such spacers typically have volumes of about 700 ml. The larger aerosol particles are removed by impaction on to the spacer walls, whilst the contained aerosol droplets evaporate, resulting in finer particles for inhalation. Much of the dose may, however, be lost rapidly to the spacer walls due to the electrostatic attraction between the aerosol and the spacer (Barry and O'Callaghan, 1995). Depending on the formulation, the spacer

material and design, and the interval between actuation of the MDI and inhalation, the patient may receive a similar, greater or lower dose to the lungs than from a correctly used MDI. Most poor coordinators would, on average, be expected to achieve an improved dose to the lungs following administration via a large volume spacer.

Melchor and colleagues (1993) investigated the effect of a large volume spacer on the deposition of Tc-99m-labelled salbutamol from an MDI. The drug was administered to both healthy volunteers and asthmatic patients, trained in the correct use of the inhaler. Use of the spacer did not affect total lung deposition in either group. A greater proportion of the lung dose, however, deposited deeper in the lungs when administered via the spacer. The oropharyngeal dose in the patients was reduced from 50% to 6% by use of the spacer: deposition in the spacer being 45%. In contrast, Newman and coworkers (1996) found increased lung deposition, from 15% to 28%, of Tc-99m-labelled flunisolide in healthy subjects dosed from an MDI via a 250 ml spacer. This was accompanied by a reduction in oropharyngeal deposition from 67% to 27%. Such studies serve to illustrate the variable effects of spacers, even when used under optimum conditions.

Reduction in oropharyngeal deposition can be achieved with a small spacer, having a volume of about 50 ml. Such spacers function as extensions to the actuator mouthpieces, rather than as holding chambers. They are effective for a wide range of MDI formulations: with nacystelyn 2 mg/actuation oropharyngeal deposition was reduced by 85% (Hardy *et al*., 1993b); with beclomethasone dipropionate 100 μg/actuation by 80% (Hardy *et al*., 1996); and with a propellant soluble drug by 77% (Ashworth *et al*., 1991). With the propellant-soluble drug, lung deposition increased from 38% to 57%. Thus for patients who are good coordinators, or breath-operated inhaler users, a small volume spacer provides a convenient alternative to a larger spacer as a means of reducing oropharyngeal deposition.

4.3.3 Dry Powder Inhalers

Dry powder inhalers are of two types, those containing drug predispensed in individual doses in capsules or blisters and those having a reservoir of powder from which doses are metered in the devices. Pure drug powder of a particle size suitable for inhalation into the lungs tends to be autoadhesive, resulting in poor flow properties. The powders used in inhalers, therefore, are either loose aggregates of pure drug or blends of the drug with a carrier material, often lactose, of larger particle size as shown in Figure 4.1. Because it is usually the drug distribution that is of interest, radiolabelling of blends requires that the drug particles are labelled before mixing with the carrier. To obtain meaningful drug deposition data for commercial powder inhalers requires that the radiolabelled powders match those of the original formulations.

Most powder inhalers rely on the patients' inspiration to withdraw the drug from the inhaler and to disperse the particles. Sufficient air flow must, therefore, be generated through the device. Drug delivery from powder inhalers is further

complicated by the different resistances of devices to air flow (Clark and Hollingworth, 1993). These factors have to be taken into account for both the *in vitro* and *in vivo* evaluations of powder inhalers. Whilst, for example, an air flow rate of 60 l/min can be regarded as low through a low resistance inhaler such as the Rotahaler®, this air flow rate could not be achieved by healthy subjects using the high resistance Pulvinal® inhaler (Pitcairn *et al*., 1994). Gamma camera studies have been used extensively in the assessment of factors, such as inhalation flow rate, affecting lung drug deposition from powder inhalers.

A study of sodium cromoglycate delivery from capsules in the Spinhaler® has been undertaken by Newman and colleagues (1994b). Inhalation at 120 l/min with the head tilted back resulted in lung deposition of 13%, while the corresponding value for inhalation at 60 l/min was only 6%. Approximately one-third of the dose remained associated with the device. In the same study, the effect of breath holding and head position were investigated. Lung deposition was not significantly improved by breath holding for 10 s. With the head held in the normal position, lung deposition increased to 17%. A relatively low lung deposition of 8% was reported for sodium cromoglycate from a capsule delivered using the Rotahaler® (Vidgren *et al*., 1990).

Total lung depositions of 12% and 11% of salbutamol following blend delivery from blisters were not significantly different in healthy subjects and asthmatic patients, respectively (Melchor *et al*., 1993). These values were significantly less than the corresponding values of 22% and 18% from a pressurized metered dose inhaler. As with the MDI, the drug was deposited more centrally in the lungs of the asthmatic patients. Salbutamol lung deposition from a blend in the multidose Pulvinal® powder inhaler was 14% when inhaled with maximum effort, at an air flow rate of 46 l/min (Pitcairn *et al*., 1994).

Much higher lung drug deposition values have been reported for the Turbohaler®, a multidose inhaler delivering pure drug. Most asthmatic patients can achieve a peak inspiratory air flow rate in excess of 60 l/min through this device (Persson *et al*., 1997). Using gamma scintigraphy the lung depositions of budesonide and terbutaline sulphate inhaled at approximately 60 l/min were 28% and 27%, respectively, of the metered dose (Borgström *et al*., 1994). When inhaled at 35 l/min, the lung deposition of budesonide was reduced to 15%. This reduction in lung deposition was accompanied by an increase in oropharyngeal deposition from 58% to 67%. Similar lung deposition data have been obtained from studies monitoring drug absorption and excretion. Such studies require the administration of charcoal to block absorption of the drug deposited in the mouth and throat. A slightly lower lung deposition value of 21% was obtained by analysis of terbutaline excretion in the urine following inhalation from the Turbohaler® (Borgström *et al*., 1992). A comparison of budesonide lung deposition, by measuring blood concentrations of the drug inhaled from the Turbohaler® and a pressurized MDI, showed the Turbohaler® to be twice as efficient as the MDI (Thorsson *et al*., 1994).

Gamma scintigraphy has been used extensively in the development and assessment of powder inhalers. The two factors having the greatest influence on lung deposition are inhaler design and inhalation flow rate. Improvements

in the design of powder inhalers is leading to more efficient and reproducible drug delivery. At the same time the inhalation route appears more attractive for the administration of controlled release formulations and of systemically acting drugs having poor bioavailability following adminstration via the gastrointestinal tract.

4.3.4 Particle Clearance from the Lungs

The fate of inhaled particles is particularly important for formulations intended to provide drug release over prolonged periods.

Insoluble particles depositing in the bronchi and bronchioles are cleared from the lungs by mucociliary clearance. Depending on the depth of penetration into the lungs, retention times in the ciliated airways are likely to range from a few minutes to several hours, with negligible amounts being retained at 24 h (Lay *et al*., 1995). In contrast, insoluble particles depositing in the alveoli are retained for prolonged periods. Studies in healthy volunteers have shown that about 50% of 5 μm diameter particles, gently inhaled followed by breath holding, are retained in the lungs at 24 h (Agnew *et al*., 1985). After inhaling particles 5 nm in diameter there is very little clearance over the same period, indicating that almost all of the dose is deposited in the alveoli (Burch *et al*., 1986).

Particles deposited in the alveoli are cleared by phagocytosis by alveolar macrophages and by translocation of the particles across the alveolar epithelium (Bowden, 1987). The alveolar macrophages migrate to the terminal bronchioles, with a clearance half-time of several months, from where they are removed by mucociliary action. The particles in the connective tissues are likely to be phagocytosed by interstitial macrophages and remain relatively immobile.

To provide sustained delivery of drug within the lungs in excess of a few hours requires the particles to be deposited in the alveoli, to avoid clearance by mucociliary action. Within the alveoli the particles are likely to undergo phagocytosis, which may prevent effective delivery of the drug to its site of action. Studies using radiolabelled tracers will be useful for determining the fate of such formulations.

4.4 Nasal Drug Delivery

The nose acts as a filter for removing particles from the inhaled air. Most of the particles deposit anteriorly in the nasal cavity, in the region free of cilia. Clearance from this region is relatively slow and occurs by traction of the mucus layer into the ciliated posterior two-thirds of the nasal cavity. In healthy subjects, ciliary action clears mucus into the nasopharynx at approximately 5 mm/min, resulting in a particulate residence time in the ciliated region of about 15 min (Proctor *et al*., 1973). Mucociliary function can be greatly affected by pathology, for example the common cold can both increase and decrease nasal clearance (Proctor *et al*., 1973; Bond *et al*., 1984). Other conditions, such as cystic fibrosis,

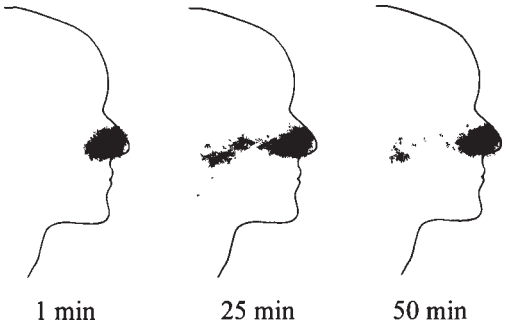

Figure 4.4 Deposition and clearance of a nasal spray solution

chronic sinusitis, polyposis, Sjögren's syndrome and Karagener's syndrome have been shown to decrease particulate clearance from the nasal cavity (Sakakura *et al*., 1983; Lee *et al*., 1984). Gamma scintigraphy has been extensively applied to the study of nasal mucociliary function.

Drugs are administered into the nose for both topical action in the nasal cavity and systemic action. The nasal route of drug delivery is attractive, because administration is relatively easy, enzymatic drug degradation is low and absorption is rapid. The permeability of the nasal mucosa to drugs, however, decreases with increasing molecular size. Additionally, the mucus layer provides a barrier between the drug and the underlying epithelium. Drug delivery can be enhanced by the application of formulations designed to prolong retention in the nasal cavity and the incorporation of penetration enhancers (Dondeti *et al*., 1996).

It has been shown by gamma scintigraphy that nasal sprays deposit mainly in the non-ciliated anterior third of the nasal cavity. Clearance from the deposition site is predominantly via the inferior meatus and into the pharynx, with little spreading over the turbinates (Figure 4.4). The deposition pattern is little affected by the type of spray, being similar for mechanical pumps (Aoki and Crawley, 1976; Hardy *et al*., 1985), a pressurized metered dose inhaler (Newman *et al*., 1987) and a powder insufflator (Illum *et al*., 1987). Changes in spray cone angle of the pumps (Bond *et al*., 1984), tilting of the device during dosing (Newman *et al*., 1987) and gentle or vigorous inhalation (Newman *et al*., 1994b) had no significant effect on deposition. Clearance of non-viscous solutions of Tc-99m-labelled human serum albumin followed a biphasic pattern with an overall half-time of typically 20–30 min (Aoki and Crawley, 1976; Hardy *et al*., 1985). None of the spray was detected in the lungs.

Nasal drops, applied with the subjects supine followed by tilting of the head, resulted in extensive coverage of the walls of the nasal cavity (Hardy *et al*., 1985). Greater coverage was achieved by increasing the solution volume from 30 µl to 90 µl. This deposition in the ciliated regions enhanced the overall clearance rates compared with the solution applied by spray.

Residence of a spray formulation in the nasal cavity can be prolonged by increasing the viscosity of the solution using hydroxypropyl methylcellulose

(Pennington *et al*., 1988). Although the deposition patterns were the same for all the solutions investigated, the clearance rates decreased with increasing viscosity. For the solution with the highest kinematic viscosity studied, 430 mm^2/s at 20°C, the clearance half-time was 2.2 h. Illum and colleagues (1987) investigated albumin, starch and DEAE-dextran microspheres as potential nasal drug delivery systems. The powders form bioadhesive gel-like structures when in contact with the mucus. The powders were labelled with technetium-99m and shown to deposit anteriorly in the nasal cavity, with little reaching the turbinates. Slowest clearance, with a half-time of about 4 h, was obtained with the DEAE-dextran.

The systemic activity of nasally administered drugs is dependent on the nature of the formulation and the mode of administration. Gamma scintigraphy provides a useful tool for monitoring the distributions of formulations in the nasal cavity and for investigating the effects of modifications designed to enhance retention.

4.5 Conclusion

There is increasing interest in the potential of the respiratory tract as a route of administration for drugs being developed as a result of advances in biotechnology (Clark *et al*., 1996; Pouton *et al*., 1996). Alongside the pharmaceutical developments, devices are being improved to deliver doses more accurately and more efficiently to their sites of action. Gamma scintigraphy has a well-established role in the assessment of inhalation products. There are likely to be increasing demands for such studies in this area of rapid scientific advancement.

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5

Scintigraphic Study of Oesophageal Transit and Retention

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5.1 Introduction

Nuclear scintigraphic study of oesophageal transit, introduced by Kazem (1972), is a diagnostic test that examines the function of the oesophagus. It is designed to complement the anatomical data obtained though endoscopy and radiological techniques and may complement the physiological data obtained through manometry. Mechanical obstructions to the oesophagus can be identified through radiological studies and endoscopic techniques, but abnormalities in the motor function are better demonstrated by nuclear scintigraphy.

While gastro-oesophageal nuclear scintigraphy has been available for over two decades, a standardized clinical procedural approach to this test has yet to be defined. Generally, in oesophageal nuclear scintigraphy the patient is asked to swallow a radioactive bolus while positioned upright or supine with a gamma camera positioned over the anterior or posterior chest with its field of view extending from the cervical to the upper abdominal region. Typically, the camera is equipped with a low-energy all-purpose collimator, and is interfaced with a computer. During continuous image acquisition the patient is asked to swallow the radioactive bolus and to subsequently swallow at 15–30 s intervals with data acquisition for up to several minutes.

The most frequently used radioactive bolus is 5–10 MBq technetium-99m (^{99m}Tc) sulphur or tin colloid in 10–20 ml water. The ^{99m}Tc sulphur colloid in water is chosen for the short half-life of the radionuclide, the low radiation burden to the patient, physiological compatibility, and its lack of absorption across the gastrointestinal tract. There is no accepted standard patient positioning and patients may be investigated supine or upright.

Marshall *et al*. (1994) studied patients with achalasia before and after treatment in the upright and supine positions. They found that in patients with symptomatic relief following treatment, only upright oesophageal emptying study correlated with symptomatic relief. The authors attribute the difference in oesophageal

emptying rates in the supine and upright positions to the advantage of gravity and suggested that the upright position better reflected posture when food or drink is swallowed. Imaging in the supine position eliminates the influence of gravity, and relies principally on the motor function of the oesophagus.

Quantitation of oesophageal transit was introduced by Tolin *et al*. (1979). Data were acquired following the swallowing of a single radioactive bolus of water. The count rate derived from computer drawn regions of interest around the oesophagus were used to determine oesophageal transit rates with the use of the formula:

$$C_t = E_{max}\text{-}E_t \times 100\%$$

where C_t is the percent oesophageal transit at time t, E_{max} is the maximal count rate in the oesophagus and E_t is the oesophageal count rate at time t.

Blackwell *et al*. (1983) relied upon computer analysis of imaged data. They divided the oesophagus into three areas of interest between the cricoid cartilage and the stomach, designated as proximal, middle and distal portions. Total oesophageal transit time was defined as the elapsed time between the appearance of radioactivity in the proximal oesophagus until the clearance of 90% of the peak activity from the distal oesophagus.

5.1.1 Oesophageal Transit Times

Normal oesophageal transit time in adults for a liquid is considered to be less than 15 s (Blackwell *et al.*, 1983). Various disease states can alter oesophageal transit times and oesophageal emptying times. Achalasia is characterized by a failure of the oesophagogastric sphincter to relax with swallowing. In these patients oesophageal emptying may be prolonged, with emptying for patients studied in the upright position at time-points of 2, 5 and 10 min of 3% (±3%) 10% (±11%) 14% (±14%) respectively (Marshall *et al*., 1994). This demonstrates a large amount of liquid retained in the oesophagus for prolonged periods. Patients with progressive systemic sclerosis experience hypomotility of the oesophagus that can be demonstrated by oesophageal scintigraphy (Wegener *et al*., 1994). These investigators reported median oesophageal transit times of 34.5 s in patients with progressive systemic sclerosis versus 8 s in their normal control group. Limburg *et al*. (1995) studied oesophageal transit in patients with Raynaud's phenomenon. These investigators found that 24 of 25 patients with primary Raynaud's phenomenon had normal oesophageal transit times (median 6.5 s). In patients with secondary Raynaud's phenomenon, oesophageal transit time was 34.5 s, and oesophageal retention of radioactivity after 60 s was 37% ± 16%.

Oesophageal transit studies in normal subjects following the single swallow of ^{99m}Tc sulphur colloid in 10–20 ml water may demonstrate considerable intrasubject variation. Klein and Wald (1987) found that normal subjects occasionally have aberrant swallows characterized by retrograde motion in the first swallow, and high residual fraction. Jorgensen *et al*. (1992) found that by

making double determinations with the single swallow method that intra-subject variations of mean transit time could be significantly reduced. Tatsch *et al*. (1991) suggested that variation could be reduced by the acquisition and processing of summed data from six multiple consecutive swallows. These investigators gave normal subjects a radiolabelled test bolus at six sequential 30 s intervals. The data from each swallow were condensed and summed into a single image. They found that normal subjects had a high frequency of abnormal single swallows, 16% for liquid and 25% for solid, as compared with their respective multi-swallow summed data. Conversely, patients with pathological emptying rates in the summed images had a high frequency of normal emptying rates, 36% for liquids and 27% for solids.

It is unclear whether age has an influence on oesophageal transit rates. Klein and Wald (1987) reported an age-related effect. They found retarded transit of a small amount of the aqueous bolus, possibly caused by oesophageal adhesion of ^{99m}Tc sulphur colloid which they attributed to diminished saliva production, and mucosal changes. However, Blackwell *et al*. (1983) and Jorgensen *et al*. (1992) found no age-related influence on transit times for ^{99m}Tc labelled liquids in mixed sex groups of normal individuals studied by a single swallow technique in the supine position.

5.1.2 The Oesophageal Transit of Pharmaceutical Dosage Forms

The oral delivery of pharmaceutical dosage forms is still the preferred mode of drug administration. Immediate release solid oral dosage forms are designed to pass through the oesophagus and to disintegrate and subsequently dissolve in the stomach, and be in a solution form for absorption in the small and/or large intestine. It is assumed that oral dosage forms would be ingested with water or an appropriate fluid which would ensure the rapid delivery of the dosage form to the stomach. Once in the stomach the gastric contents would disintegrate the dosage form and the drug would be available for dissolution and subsequent absorption.

As early as 1959 it was demonstrated that solid oral dosage forms are often delayed in their transit through the oesophagus (Praetorius and Faber, 1950). However, it was not until 1970 that the clinical significance of delayed oesophageal transit and oesophageal injury appeared from independent reports from Spain (Juncosa, 1979), the United States (Pemberton, 1970) and Great Britain (Pemberton, 1970). The first term used to describe the oesophageal injury was ‘pill erosion’, because physicians were the first health care professionals to report this type of injury. The term is still used today even though there are very few pharmaceutical pill formulations on the market. One of the first reported cases was the oesophageal obstruction and ulceration caused by an oral potassium chloride capsule (Pemberton, 1970). Since then, over 700 cases of oesophageal injury of varying degree have been reported in the literature. Potassium chloride dosage forms have caused at least 16 oesophageal strictures and four oesophageal haemorrages (Kikendall *et al*., 1983; Bott *et al*., 1987; Kikendall and Johnson, 1991; Bonavina *et al*., 1987; Carlborg *et al*., 1978; Henry *et al*., 1983; Lubbe *et*

al., 1979; McCall, 1975; Netter *et al*., 1988; Pemberton, 1970; Rosenthal *et al*., 1974; Sumithran *et al*., 1979; Whitney and Croxon, 1972). Four patients taking slow-release potassium chloride tablets haemorrhaged to death from oesophageal ulcers, three of which had penetrated the aorta (McCall, 1975), the left atrium (Sumithran *et al*., 1979) or a bronchial collateral artery (Henry *et al*., 1983). Specific drugs which have been reported to cause lesions in the gastrointestinal tract are emepronium bromide, doxycycline and other tetracyclines/antibiotics, iron preparations, quinidine, non-steroidal anti-inflammatory drugs, and at a lower frequency of injury alprenolol, ascorbic acid, captopril, sustained-release theophylline, and zidovudine (AZT). The site of oesophageal pill-induced lesions is most frequently the mid-oesophagus, but lesions also occur in the distal part of the oesophagus above the lower oesophageal sphincter (Perkins *et al*., 1994; Weihrauch, 1994). This is because the oesophagus shows in these sections physiological narrowing caused extrinsically by the aorta and main bifurcation of the bronchial system and intrinsically from the functional anatomy of the lower oesophageal sphincter.

The clinical implications of delayed oesophageal transit vary from delayed drug absorption to severe oesophageal ulceration which can result in hospitalization. The majority of oral dosage forms are absorbed from the small intestine and any delay proximal to this site will delay drug absorption. Studies have shown that delayed oesophageal transit prolongs the lag time for drug absorption and significantly lowers the peak blood concentrations (Channer and Roberts, 1984a,b, 1985). This phenomenon is of clinical significance for hypnotic and analgesic drugs which require rapid absorption in order to achieve a therapeutic effect.

5.2 Clinical Presentation and Diagnoses of Oesophageal Adhesion

Individuals who swallow solid oral dosage forms may experience oesophageal adhesion because delayed oesophageal transit is a common occurrence in persons with normal oesophageal motility and a normal gastrointestinal history. Patients of all ages have been injured by dosage form adhesion in the oesophagus (Henry *et al*., 1983). The majority of injured patients have no prior oesophageal symptoms and present with sudden onset of constant retrosternal pain, burning, dysphagia (difficulty swallowing), odynophagia (pain upon swallowing) and nausea. The pain is usually constant and may even increase in severity for the next few days before subsiding over the next few days or weeks. Some patients recall that the dosage form seemed to adhere in the oesophagus before the onset of symptoms and others report that symptoms began after swallowing the dosage form at bedtime or without water (Kikendall *et al*., 1983). Generally the patient awakens with acute symptoms approximately 2 h post-dosing or in the morning (Wienbeck *et al*., 1988; Kikendall, 1991). If the patient has a normal history and presents with acute retrosternal pain and odynophagia after ingestion of a caustic dosage form the diagnosis of ‘pill-induced’ oesophageal injury is obvious and may not require confirmation unless the patient fails to recover. If the symptoms are severe or unusually

persistent, endoscopy may be required for an accurate diagnosis. Endoscopy usually shows one or more discrete, shallow oesophageal ulcers (Kikendall, 1991). The surrounding mucosa usually appears normal, but circumferential lesions several centimetres in length have been reported (Kikendall, 1991). In rare cases, deeper injury results in mediastinitis or haemorrhage due to penetration of the left atrium or a major blood vessel (Rosenthal *et al*., 1974).

5.3 Factors Affecting Oesophageal Adhesion

5.3.1 Age

Patients of any age are susceptible to oesophageal injury. Although younger patients tend to have a normal gastrointestinal history, oesophageal injury has been reported in patients aged from 3 to 90 (Kikendall, 1991). Generally the types of medication taken by the various age groups are different, e.g. young female patients are treated with antibiotics for urinary tract infections and the elderly are treated with drugs to treat arrhythmias and hypertension. Elderly patients are particularly at risk because they have decreased mucus flow and low amplitude peristaltic propulsion.

5.3.2 Practice and Habits of Administration

The improper ingestion of a pharmaceutical dosage form contributes to oesophageal adhesion and potential injury. It is now generally accepted that oesophageal transit is markedly affected by the posture of the subject and the amount of fluid used to swallow the dosage form (Channer and Roberts, 1984b). If tablets are taken without water, the risk of adhesion is greatly increased and the dosage form may remain in the lower oesophagus until it disintegrates (Hey *et al*., 1982). Potentially more dangerous is ingestion of the dosage form at bedtime while lying down, for oesophageal transit rate, saliva production and swallowing frequency are all reduced in the supine position. Recent reports document oesophagitis with alendronate, an aminobisphosphonate, if the uncoated tablets are not taken with 6–8 oz. of water or if ingested in the supine position (De Groen *et al*., 1996).

5.3.3 Formulation Effects

Over the past decade there have been numerous publications describing the oesophageal transit of pharmaceutical dosage forms. The technique of gamma scintigraphy is currently the preferred method of monitoring oesophageal transit. It is capable of following the transit of any radiolabelled dosage form and also identifying the site of adhesion. The generally accepted normal oesophageal transit time in healthy individuals of pharmaceutical formulations is of the order

of 10–14 s and the overall incidence of oesophageal adhesion is approximately 20% (Wilson and Washington, 1989). There are numerous factors that affect the oesophageal adhesion of pharmaceutical dosage forms. These pharmaceutical attributes include: (a) shape of the dosage form, (b) size of the dosage form, (c) surface characteristics of the dosage form (i.e. surface area, adhesive properties), and (d) density. Confounding factors include the volume of fluid administered with the dosage form and the body position (erect or supine). It appears that administration of dosage forms with little or no water and swallowing in the supine position are the greatest factors which enhance the probability of adhesion. Large oval tablets taken with little or no water in the supine position have the greatest tendency to adhere to the oesophagus (Wamburg *et al*., 1983). It is difficult to rank pharmaceutical dosage forms in terms of oesophageal adhesion, but it is generally accepted that the formulations most prone to adherence are gelatin capsules, film-coated tablets, uncoated tablets and sugar coated tablets, which show the least potential to adhere (Marvola *et al*., 1982; Swisher *et al*., 1984).

If the dosage form adheres in the oesophagus, the active ingredient slowly dissolves and creates a high drug concentration on the mucosal surface of the oesophagus. Therefore, certain classes of drugs are problematic and have a higher potential to cause oesophageal injury. Particularly problematic are drugs which when dissolved have a pH below 2–3, drugs with cytotoxic activity (caustic) and/or the local development of a hyperosmolar solution which causes mucosal desiccation.

The data presented in the literature are often confusing and at times conflicting, as there is no standardized procedure for conducting and reporting the results of oesophageal transit studies. The data on the formulation used in transit studies is often incomplete and investigators vary the dosage form administration technique (volume of fluid and body position) to meet the intent of the study. In addition patient age, disease state and number of patients vary significantly. Therefore, if oesophageal adhesion is an important parameter to monitor, it is recommended that oesophageal transit studies be conducted using a specific formulation in a representative subject population.

5.4 Case Studies

Scintigraphy can be used during the design and development phases of various pharmaceutical dosage forms and in this chapter two examples will be presented on how scintigraphy was utilized to meet very different project objectives.

5.4.1 Design of a Solid Oral Formulation with Rapid Oesophageal Transit

In this example (Perkins *et al*., 1994), a solid oral formulation was being designed specifically to treat the elderly population (>55 years). Although never

confirmed, there was a concern about the inherent irritation potential of the drug substance and, because the target population was elderly, there was concern about oesophageal adhesion, especially if the product was taken incorrectly. The study was conducted to screen two formulations for oesophageal adhesion and the dosage form with the fastest transit would be selected for further development. It was decided to use placebo formulations, because the external surface characteristics of the placebos were identical to those of the actives. As mentioned, there is no universal study design to evaluate oesophageal transit and the following key points were addressed before conduction of the study: (1) the population must be defined in terms of age and disease state and number of subjects, (2) the dosing condition must be clearly defined in terms of fluid intake and body position, (3) the criteria for adhesion must be defined as some researchers use the criteria of >20 s for adhesion and others use >90 s or longer, (4) the dosing condition, volume of water and body position must be defined.

The answers to these questions must be defined based on the intent of the particular study. An advantage of oesophageal transit studies is that they can often be conducted without the active ingredient and a suitable placebo with the same physical properties, including surface characteristics, may be substituted. This is particularly important for drug candidates in development, as it may simplify the regulatory requirements needed to conduct the study. Ideally the transit study should be conducted early in a formulation development programme in order to have time to use the results of the transit study to design the optimum formulation for Phase III clinical trials. For drugs in Phase III or for commercial formulations, transit studies can be used to determine the appropriate administration technique for the dosage form in the target population.

The dosage forms were enteric-coated tablets and #3 hard gelatin capsules containing enteric-coated beads. The tablets were modified oval shaped (5.7×11.5 cm) weighing 240 mg with a density of 1.2 g/cm. The enteric coating consisted of a Eudragit L100–55 (Rohm Pharma, Darmstadt, West Germany) polymer system with an average film thickness of 50 microns. The #3 gelatin capsule contained roughly 400 enteric coated 20/25 mesh Nu-Pareil beads (Crompton and Knowles, Marwah, NJ) coated with the same Eudragit L100–55 polymer system with an average film thickness of 40 microns. The dosage forms were radiolabelled by the incorporation of approximately 3 MBq of ^{99m}Tc-sodium pertechnetate adsorbed onto 5 mg of Amberlite resin per dosage form using the method described by Wilson and Hardy (1985). The ion exchange resin was IRA 416 (Cl), particle size 0.3–1.2 mm (BDH Laboratories). At the time of incorporation the activity of ^{99m}Tc is calculated to yield a 3 MBq dose (range 2.5–3.5 MBq at the time of dosing).

The target population for use of the product is the elderly and all subjects that participated in the study ranged in age from 50 to 79 years. Twenty-three subjects participated in the crossover study. It was decided to administer the dosage form with 50 ml of room temperature tap water as this represents a typical volume of ingested fluid and subjects were seated erect in front of the gamma camera for imaging. The body position and fluid intake are crucial variables which must be

Table 5.1 Oesophageal transit times (seconds) for radiolabelled capsule and tablet formulations obtained from a crossover scintigraphic study in 23 elderly subjects (taken from Perkins *et al.*, 1994)

Subject	Capsule	Tablet
1	7.5	14.0
2	9.0	6.0
3	3.0	3.0
4	4.5	3.0
5	6.5	3.5
6	16.0	4.0
7	14.5	2.0
8	3.5	4.0
9	4.5	1.5
10	36.0	2.5
11	17.5	6.0
12	5.0	8.0
13	35.0	2.0
14	5.0	4.0
15	9.5	2.5
16	38.5	6.5
17	1.5	1.0
18	15.5	4.0
19	174.5	2.5
20	7.5	2.5
21	11.0	8.0
22	15.0	2.0
23	40.0	7.0
Mean	20.9	4.33
Sample SD	35.6	2.95
Range	1.5–174.5	1.0–14.0
Population SD	34.8	2.88

controlled in the design of the study. The oesophageal transit was monitored for 10 min after dosing.

The agreed criteria for normal oesophageal transit in this study was ‹20 s. The ‹20 s criteria is less than the criteria used in other published studies where 60 and 90 s have been used as the upper limit (Hey *et al.*, 1992; Jorgensen *et al.*, 1992). The results of the oesophageal transit are shown in Table 5.1.

The results clearly demonstrate the more rapid transit time of the entericcoated tablet compared to the hard gelatin capsules. Five subjects had delayed oesophageal transit (>20 s) with the capsule formulation and there were no delays with the enteric-coated tablet formulations. The gelatin capsule upon hydration in the mouth

becomes tacky which may impair transit, or its lighter density may delay transit, whereas the enteric coating on the tablet does not hydrate in the mouth and traverses the oesophagus with no signs of oesophageal adhesion. Although the transit times were not significantly different, the enteric-coated tablet has a more rapid transit time vs. the hard gelatin capsule formulation.

5.4.2 Design of an Oesophageal Coating Formulation

The function of the oesophagus is to deliver discrete boluses of swallowed material to the stomach. Swallowing begins as a voluntary oral event involving coordinated movements of the jaw and tongue, followed by reflex movement of the posterior tongue, pharyngeal and parapharyngeal muscles, upper oesophageal sphincter, oesophageal body and lower oesophageal sphincter. The coordinated patterns of motion of the oesophagus result in a very efficient propulsion of virtually all of the bolus, leaving very little residual material in the oesophagus. In certain clinical conditions, such as gastro-oesophageal reflux disease (GERD), it would be advantageous to coat the oesophageal mucosa and protect it from damage caused by refluxed gastric acid and enzymes. The oesophagus, however, is very efficient at swallowing virtually all of a bolus, leaving very little residual coating. To study the effectiveness of oesophageal coating (deposition and clearance) requires a scintigraphic method that can detect passage of the swallowed bolus as well as residual deposition on the oesophageal mucosa and clearance of the radioactive isotope over time.

One active formulation that has been studied scintigraphically for its oesophageal coating efficacy is sucralfate. Sucralfate (Carafate®) is a complex salt of sucrose sulphate and aluminium hydroxide that, when exposed to the acid of the stomach, forms a sticky paste that preferentially adheres to damaged mucosa and forms an acid-resistant layer. Coating intact, healthy mucosa is more of a challenge. Recent studies suggest that sucralfate, delivered as a stable fluid gel (Gastrogel®), may show superior adhesion to damaged and normal oesophageal mucosa without prior exposure to gastric acid. An example of how scintigraphy can be applied to the study of oesophageal coatings is a study conducted at University Hospital, Nottingham, to determine the relative anatomical coating and clearance rate of sucralfate, sucralfate gel and placebo in the oesophagus of 12 normal, healthy, male subjects.

The radioisotope used in this study was technetium 99m (^{99m}Tc), a gammaemitting isotope. The radiolabel was added to each of the unit doses on each study day, in an equal volume to deliver 2 MBq (74 μCi) ^{99m}Tc in each dose of test product. After the radiolabelled unit doses were prepared, they were scanned by the scintillation camera in a ‘neck phantom’ to obtain the total gamma counts contained in that dose. A ‘neck phantom’ is a device that approximates the attenuation of the gamma counts induced by the soft tissues of the neck, allowing more accurate correlation of *in vivo* and *ex vivo* counts. Subjects were then positioned in front of the scintillation camera and the dose was administered. A 10 min continuous scintigram was obtained with the camera in dynamic mode to

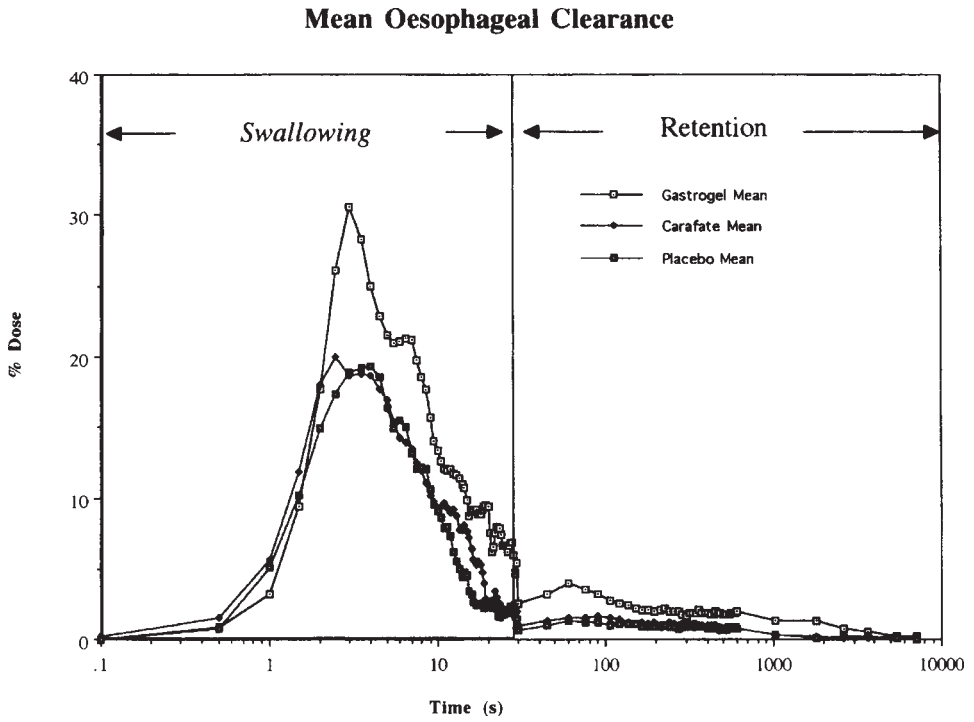

Figure 5.1 Retention of oesophageal coating formulations obtained from serial scintigraphic images recorded over a 3 h period (n=12 for each data point)

record the swallow and initial coating. Immediately after the 10 min continuous scintigram, the empty subject unit dose was scanned in the neck phantom to determine the gamma counts remaining in the syringe. The gamma count left in the syringe was subtracted from the total gamma count to determine the gamma count of the actual dose given. Static scintigraphic counts were then taken every 15 min for 1 h, and every 30 min for 5 h. An example of a scintigraphic plot of swallowing and oesophageal clearance is shown in Figure 5.1.

In this study, the primary effectiveness measures include retained fraction, duration and area under the curve (AUC) for retained fraction, for total oesophagus and upper/mid/lower regions of the oesophagus. Duration is a measure of time that ^{99m}Tc was detectable at 2 × background, and represents the duration of exposure of the treatment to the oesophageal mucosa. The duration of ^{99m}Tc detected at 2 × background in the total oesophagus was 59 min for sucralfate gel, 40 min for sucralfate, and 12 min for placebo. Area under the curve is a measure that represents the total exposure of the oesophagus to a treatment by quantitating the amount and duration of drug retention. Sucralfate gel had a significantly greater AUC compared to placebo for all regions of the oesophagus and for total oesophagus. Sucralfate gel also had a greater AUC compared to sucralfate for total oesophagus and upper and mid oesophagus. These results show that sucralfate gel has superior mucoadhesive capabilities compared to sucralfate and placebo when measured as AUC.

Retained fraction is a measure of the radioactivity retained in the oesophagus over time compared to the total administered dose. As calculated in this study, retained fraction is derived by dividing the AUC (calculated for each treatment group) by the theoretical AUC for the total dose administered (for each group) at time points between 30 and 600 s. The retained fraction in the total oesophagus for sucralfate gel, sucralfate and placebo was 2.15%, 0.99% and 0.88%, respectively. The theoretical AUC is based on the time interval between t_0 through 2 × background. These results show that sucralfate gel has a more than two-fold greater retained fraction compared to sucralfate and placebo. At 30 min after dosing, sucralfate gel exhibited a seven to 10-fold superiority in retained fraction, and still appeared to be slightly retained in the oesophagus 2 h after dosing.

This study demonstrated that gamma scintigraphy can be applied to determine the efficacy of oesophageal coating of different formulations of the same drug active. Sucralfate gel shows significantly greater cumulative oesophageal retention, as demonstrated by retained fraction, AUC and duration, compared to sucralfate and placebo, in healthy volunteers.

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6

Scintigraphic Study of Drug Delivery to the Stomach and Small Bowel

N.WASHINGTON and C.G.WILSON

6.1 Introduction

The mid-sections of the gut in humans have evolved active transport systems for optimal extraction of nutrients from a complex and varied diet. However, not all compounds which are presented to the gut wall are desirable for absorption. Ingested food may contain small amounts of components which are harmful, for example, plant alkaloids and complex phenolic compounds which arise during cooking. These compounds are not absorbed by active mechanisms but enter the bloodstream by dissolving in the membrane lipid (transcellular transport) or by opportunistic entry between the enterocyte gaps (paracellular transport). They would accumulate in tissues but for mechanisms which act to reduce concentrations by microsomal oxidation, conjugation and secretion. This is a major function of the liver. In the case of drugs, which obviously possess no nutritional benefit, the offending material is present in a great excess and Fickian diffusive processes usually win against secretory and metabolic activity which attempts to eliminate the substance. The overall effectiveness of this process depends on the concentration of the drug, blood flow and the efficiency of intestinal wall metabolism and hepatic extraction. The rate of presentation of drug to the systemic circulation is a function of the rate of gastric emptying, because the small intestine is, *par excellence,* the organ of absorption.

The stomach has a unique ability to store and to process food whilst separating out non-nutritious components for rapid disposal; hence the presence or absence of nutrients can dramatically affect the gastric emptying times of dosage forms. The interplay between gastric emptying and the absorption profile of a drug depends on numerous factors including:

- type of food ingested, e.g. calorific value and particle size and shape
- pH of food
- density of the food

- volume of the food
- physicochemical characteristics of the drug, e.g. solubility and ionization state at different pHs
- density of the dosage form
- size of the dosage form
- integrity of the dosage form in the gastric environment, e.g. enteric-coated formulations

In the optimization of drug formulations, these interactions of the formulation and physicochemical properties of the drug with physiological processes is extremely important. Drugs which are poorly soluble at physiological pHs usually have low bioavailability. For example, the drug acyclovir is acid soluble, but demonstrates a sharp reduction in solubility at intestinal pH. Theoretically, slowing the rate of gastric emptying should favour absorption by prolonging delivery of this drug to the relatively acidic conditions of the proximal small intestine. The duodenum is relatively short and transit through this region is rapid so increasing the exposure of the drug to the first few centimetres is critical to improve absorption. Towards the end of the duodenum, the Brunner's glands and pancreatic secretions will raise the pH of the intestinal contents from pH 1.5–2 to between 5 and 6, producing a sharp pH gradient (Oversen *et al*., 1986) and decreasing the solubility of the drug. Sipping acyclovir suspension over a 4 h period markedly increases absorption (Lewis *et al*., 1986). A logical method of prolonging the rate of presentation of the drug to the upper duodenum would be to administer the drug with food. However, food will dilute and buffer the gastric acid which may reduce drug solubility, and slower rates of presentation will also favour increased first pass metabolism by the gut wall and liver, leading to reduced bioavailability. It is hard to predict the overall effect and a combination of gamma scintigraphy, to follow the passage of the drug through the gastrointestinal tract, with pharmacokinetic analysis, becomes invaluable (Wilson and Washington, 1988). The combination of techniques was used to examine the marked effect of food on absorption. A heavy breakfast decreased acyclovir absorption compared to a light breakfast even though gastric emptying was prolonged from 47 (±15) min to 108 (±44) min by the greater calorific value of the meal (Wilson *et al*., 1987). These data indicate that the controlling parameter for absorption was reduction in solubility of the drug produced by food intake and that slowed gastric emptying was not an advantage.

Compliance is thought to be increased when therapy is presented as controlled or delayed release formulations. The release process must be sufficiently robust to survive the diversity of diets across the patient population. Reducing dosing frequency necessitates incorporation of larger doses of drug in each unit with the associated risk of premature high peaks or overlong delays in achieving effective systemic concentrations. Innovative attempts to produce line extensions are often dogged by poor *in vivo* performance even in the face of satisfactory *in vitro* data; gamma scintigraphy becomes a valuable tool to aid in the interpretation of the

unexpected pharmacokinetic behaviour. Sustained release ibuprofen formulations, for example, frequently produce twin peaks in the plasma concentration-time profile. Scintigraphy revealed that the dip from plateau levels was produced as the units went through the caecum where the pH is low and the dissolution is suppressed (Wilson *et al*., 1989).

6.2 Physiological Factors Affecting Transit

The stomach possesses the ability to deliver a constant calorific load to the small intestine whilst emptying the three phases of a meal, i.e. liquid, digestible solid and indigestible solid, at different rates. Typical values for 50% emptying of the stomach (T_{50}) for 10 oz. of a soft drink, scrambled egg and radio-opaque markers are 30±7min, 154±11 min and 3–4 h respectively (Feldman *et al*., 1984). The operation of control mechanisms of the upper digestive system can be demonstrated by comparing transits of meals with identical calorific values, but different fat content. The high fat meal empties from the stomach at 5.8 h (T_{50}) compared to 3.0 h for a low fat, high carbohydrate meal (Washington *et al*., 1994). Small intestinal transit is similar in both cases (3.1 h low fat meal, 2.7 h high fat meal) proving that the stomach provides the major regulatory mechanism. To further complicate the situation, it is not just the fat content of a meal which influences gastric emptying, but also the way in which the meal is cooked, for example, gastric emptying is significantly delayed if the fat content of the meal is fried (Benini *et al*., 1994).

The stomach also secretes hydrochloric acid which protects the body against the bacterial load ingested in the food, whilst maintaining the correct pH for pepsin to function. An adult consumes an average of 3–4 kg of food and drink daily (Rose, 1978) and the volume of gastric juices produced may be up to twice that of each meal (Malagelada *et al*., 1984). The flow of secretions is not constant and the greatest secretory activity occurs in the stomach within the first hour of eating. The empty stomach has a volume of approximately 50 ml which increases to about 1 litre when full. Initially, a meal will buffer the gastric contents, raising the pH, usually to 3 and above, depending upon the nature of the meal. The pH will then begin to fall as the food empties and sufficient gastric acid is produced to reduce the pH.

Carefully constructed studies have unexpectedly masked the sources of the physiological variation which occurs within the population. Many trials exclude female patients and data is only obtained from healthy young men. However, significant differences occur between motility patterns in men and women; for example, the emptying of solid food in normal young, pre-menopausal women is slower than in age-matched men, even during the first 10 days of the menstrual cycle (Knight *et al*., 1997). It is thought that this is primarily due to altered distal gastric motor function, because decreased antral contractility can be demonstrated by dynamic antral scintigraphy and antroduodenal manometry. Gamma scintigraphy is one of the few techniques which allows studies to be performed in young women of child-bearing age provided that they are not pregnant at the

time of study. The age of the subjects is also an important consideration; in the elderly, the rate of gastric emptying for solid and liquids may be significantly reduced, even though orocaecal and whole gut transit are not affected (Clarkston *et al*., 1997).

6.3 Choice of Radiolabels

A distinct advantage in the scintigraphic study of drug formulations is the fact that the meal and formulation can be labelled with probes based on different radionuclides, allowing simultaneous measurements of both phases. This is of particular value in gastric emptying and small intestinal transit studies. In practice, the choice is usually limited to two radionuclides, ^{111}In with ^{99m}Tc, whose gamma ray energy spectra differ significantly. It is important that the gamma ray energies should be appropriate for use with the gamma camera and that radiation dosimetry is kept as low as possible.

The selection of the radionuclide must be taken into consideration when designing a study. To assess gastric emptying Tc-99m is a suitable radionuclide whose short half-life (6 h) is not a restriction. On the other hand, indium-111, which has a longer half-life (2.8 days) would be a better choice for a dose form designed to deliver drug to the colon, as transit through this region can take 6–24 h. When choosing a suitable radiopharmaceutical, the decision has to be made as to whether the information required is position of the dose form within the gastrointestinal tract, or whether the release characteristics of the drug from the dose form need to be modelled. For example, for a study of the effectiveness of an enteric coat in resisting degradation in the stomach, the release of the radiolabel has to be indicative of release of the drug (Catteau *et al*., 1994). To predict the behaviour of multiparticulate dose forms, beaded resins such as Amberlite are often used. Fractions can be sieved to ensure that the mean particle size of model and formulation are similar. The reasonable assumption is made that both will transit through the gastrointestinal tract at the same rate.

If two phases are to be studied simultaneously, e.g. water and a controlled release tablet, it is advantageous to put the ^{99m}Tc into the most diffuse phase, i.e. the water, and to label the tablet with ^{111}In. Approximately 30% of the indium gamma rays appear in the technetium channel and hence the overlap is minimized and error is reduced if the indium is present as a discrete source. Although a correction can be made for the overlap of energy, it is an approximation because the actual value varies from person to person due to differential absorption by soft tissue and bone. If the ^{99m}Tc can be administered and imaged prior to the ^{111}In, the channel cross-talk for each person can be calculated from the increase in counts in the technetium channel. Problems may arise further down the gastrointestinal tract when the controlled release tablet breaks up and spreads. By this time, the technetium will have decayed through a half-life and the indium image will be predominant; the reduction in count rates due to radioactive decay can result in statistical errors and spatial information may be lost.

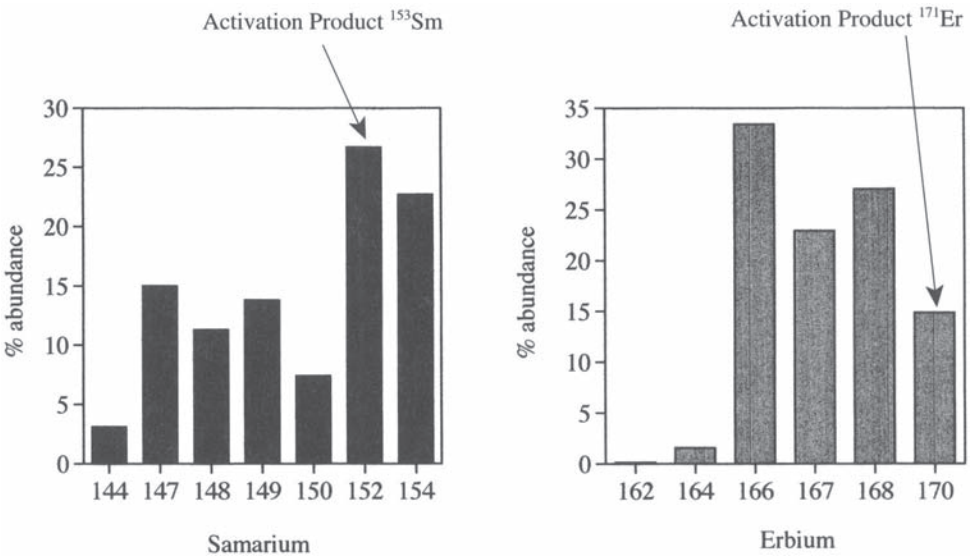

Figure 6.1 Natural isotopic abundance of samarium and erbium

6.3.1 Neutron Activation

Many sustained release formulations are too complex to be made on a small scale, or require manufacturing processes that must be performed in a pilot plant (for example coating). This can make production of radiolabelled formulations suitable for gamma scintigraphy difficult because the manufacturing equipment would become contaminated with radioactivity. Furthermore, extremely large amounts of activity may be needed. A possible solution to this is to incorporate into the dose forms an isotope which is not radioactive, but can be converted to a gamma emitter by neutron activation. Certain isotopes have extremely high cross-sections for neutron capture, and during irradiation in a nuclear reactor, absorb neutrons to form higher mass isotopes of the same element (i.e. having the same atomic number but higher atomic mass). The technique was first described by scientists from the University of Kentucky in the mid-1980s (Parr *et al*., 1986).

The most popular elements for this technique are samarium and erbium, normally used as oxides. Although all the naturally occurring isotopes are activated to some extent, the isotopes ^{170}Er and ^{152}Sm have the highest neutron capture cross-sections, although they naturally occur with 14.88% and 26.7% abundance respectively (Figure 6.1). The half-lives of the activation products (^{171}Er and ^{153}Sm) are 7.5 h and 46.7 h respectively. Initially the high cost of the enriched erbium or samarium needed for tablet preparation prevented the techniques from being widely used, however, it was subsequently discovered that cheaper non-enriched substrates could be employed (Watts *et al*., 1991). Insignificant amounts of the other samarium nuclides were produced and the technique was utilized to follow the oral delivery of labelled microspheres in man (Watts *et al*., 1994). Typically, to label drug formulations, a small amount (between 200 μg and 2 mg) of an

enriched $^{170}Er_2O_3$ or a samarium oxide is incorporated prior to the manufacture. Parr and co-workers describe the correlation of gastrointestinal transit and the plasma concentration-time profile of a ^{171}Er-labelled ibuprofen tablet which contained 0.18% of the oxide by weight (Parr *et al*., 1987).

Daughter radionuclides of lower atomic weight elements (carbon, hydrogen, oxygen and nitrogen) are not formed during the irradiation process, although the generation of short-lived isotopes of potassium and sodium may be a problem. Irradiation may also result in structural changes of the formulation, causing an alteration in physical properties, so it is important to carefully examine the effects of the neutron activation process (Watts *et al*., 1993a, b). Waaler and co-workers (1997) described the effects of thermal neutron irradiation on the release rates of crystals of acetylsalicylic acid (ASA) microencapsulated in ethylcellulose which were exposed to neutrons at a flux of 1×10^{13} n/cm^2. Disintegration was prolonged and after 15 min irradiation was inhibited almost completely. Irradiation appears to degrade the ethylcellulose and cause changes in the crystal structure of the ASA. In comparison, irradiation of ethylcellulose-coated paracetamol tablets for up to 4 min caused no changes (Waaler *et al*., 1997).

The radiation dosimetry from samarium is higher than ^{111}In or ^{99m}Tc, but it is still well within accepted limits. In a study of an enteric-coated, spherical pellet naproxen formulation containing 2 mg samarium oxide radiolabelled by neutron activation, which resulted in 1 MBq samarium-153 per capsule, the effective dose equivalent was of the order of 0.8 mSv (Hardy *et al*., 1991).

6.4 Pharmacoscintigraphy

Within clinical studies, a new discipline has evolved which has been termed by some workers 'pharmacoscintigraphy'. In most investigations employing a combined pharmacokinetic and scintigraphic technique, authors have attempted to relate blood levels to position of the formulation in the gastrointestinal tract. This has been useful in explaining intersubject variance, identifying windows of maximum absorption, and examining the effect of food on gastrointestinal transit.

Pharmacoscintigraphy is important in the repertoire of techniques available to study the interplay between physiology and pharmaceutics, but it should be remembered that it is an additional component of the formulation which is labelled rather than the drug itself. If the labelled component and the drug separate during transit, the area occupied by each may be completely different. On occasion, this can lead to unwarranted conclusions being drawn and it is essential that the relevant physiology is considered when interpreting results. In a recent study, gamma scintigraphy was used to follow the absorption of a hypolipidaemic drug (CGP 43371) which was administered with activated samarium-153 oxide in a separate capsule (Sun *et al*., 1996). The peak plasma concentrations of drug were reached 6 h after administration, which corresponded to the arrival of the capsules at the terminal ileum. The authors interpreted this data as evidence that the primary absorption site was in the ileum. However, this interpretation is not compatible with the observation that longer gastric residence improved the oral absorption

Figure 6.1 Natural isotopic abundance of samarium and erbium

Drug	Dosage form	Food effect	Reference
Acetylsalicylic acid	SR tablet	?	Wilson *et al.* (1984)
5-ASA	SR tablet	No	Hardy *et al.* (1993)
Acyclovir	Suspension	Yes	Wilson *et al.* (1987)
Amoxycillin	CR tablet	Yes	Gottfries *et al.* (1996)
Beclomethasone	CR tablet	?	Steed *et al.* (1994)
Buflomedil	SR tablet	Yes	Wilson *et al.* (1991b)
Cimetidine	Tablet/solution	?	Adkin *et al.* (1995a)
Erythromycin	Pellets	Yes	Digenis *et al.* (1990)
Ibuprofen	SR tablet	Yes	Parr *et al.* (1987)
Ibuprofen	SR tablet	Yes	Wilson *et al.* (1989)
Levodopa	Suspension	?	Roberts *et al.* (1996)
Morphine	CR tablet	?	Olsson *et al.* (1995)
Nifedipine	SR pellet	Yes	Wilding *et al.* (1992a)
Riboflavin	Capsules	?	Dansereau *et al.* (1997)
Rifampicin	Capsules	Yes	Washington *et al.* (1994)
Theophylline	SR pellet	No	Wilson *et al.* (1991a)
Tiaprofenic acid	SR tablet	Yes	Wilding *et al.* (1992b)

Most of the groups conducting scintigraphic trials have provided useful reviews of the technique and the reader is referred to the following papers: Davis *et al.*, 1992; Hardy and Wilson, 1981; Meseguer *et al.*, 1994; Perkins and Frier, 1996; Wilding *et al.*, 1991, 1994a; Wilson *et al.*, 1982; Wilson and Perkins, 1992; Wilson and Washington, 1988

of the drug. One possibility is that the drug was absorbed through the mesenteric lymphatics. Absorption of an established hypolipidaemic agent in the rat occurs almost exclusively via this route (Palin and Wilson, 1984); the pharmacokinetic characteristics of slow time to peak and increase in availability in the case of a slowed gastric emptying are more consistent with this explanation. If the supply of drug is limited by dissolution or rate of release, then the ability to follow complete transit is extremely useful because it can help identify whether significant colonic absorption of the drug occurs.

The number of pharmacoscintigraphic studies which have appeared in the literature since the mid-1970s provide an indication of how much nuclear medical techniques have contributed to knowledge in the pharmaceutical sciences. Illustrations of the technique have been published by several groups as illustrated in Table 6.1.

6.5 Measurement of Gastric Emptying of Meals and Dosage Forms

6.5.1 Liquids

The most commonly used radiopharmaceuticals for the evaluation of the emptying

of aqueous liquid formulations are either ^{99m}Tc or ^{111}In diethylenetriaminepentaacetic acid (DTPA). DTPA is a water soluble chelating agent and is not absorbed from the gastrointestinal tract. Direct labelling of polymers with technetium-99m, including carboxymethylcellulose, has been described as a fibre marker for gastric emptying studies (Schade *et al*., 1991); however, its potential as a method of following polymeric excipients has yet to be explored.

Non-aqueous liquid phases are difficult to label and most lipophilic materials will undergo significant uptake in the liver following absorption into the portal system. The only alternative would be incorporation into a large polymer, as oil-soluble radiopharmaceuticals are well absorbed. Oil-phase labels are therefore only suitable to establish the early behaviour of materials, before significant intestinal processing of the fats has occurred. ^{99m}Tc-labelled (V)-thiocyanate olive oil has been used to label the fatty phase of meals for gastric emptying studies (Carney *et al*., 1995; Cunningham *et al*., 1991). Other workers have used selenium-75 glycerol triether incorporated into butter, and this demonstrated that intragastric layering of fat above non-lipids occurs after the first postprandial hour only, and the fact that lipids are emptied more slowly than any other component of an ordinary meal is not due only to layering of fat above water (Jian *et al*., 1982). Recently, our group has investigated the use of a ^{99m}Tc tertiary butyl isonitrile complex to label castor oil-based emulsions in preliminary studies for the evaluation of lipophilic drug delivery (Budisantoso *et al*., 1997). This label has good stability and a high partition coefficient, but may be absorbed from the gastrointestinal tract. The use of ^{111}In indium oxine for labelling very hydrophobic formulations is of limited value in situations where it will come into contact with a high concentration of protein in solution because the indium may dissociate from the oxine and bind to other ligands or active surfaces (Kaempfer, 1987).

6.5.2 Suspensions

The stomach cannot discriminate between small particles and liquids, hence suspensions generally behave in a manner similar to liquids. However, this is not always the case. In the study which compared the gastric emptying of a suspension of ^{99m}Tc-labelled micronized resin (20–40 and 90–125 μm) to water labelled with Tc-99m DTPA (Burton *et al*., 1995), the T_{50} for ^{99m}Tc-99m DTPA was 0.63 h, with emptying being complete within 1.75 h (Figure 6.2). Although a similar T_{50} value of 0.76 h was obtained for the micronized ion exchange resin, 20% of the label was still present in the stomach at 6 h indicating separation of resin from the water and gastric retention. In another study which investigated the gastrointestinal transit of a drug resinate formulation administered as an oral suspension together with a volume of water (Wilding *et al*., 1994c), the authors concluded that emptying of the suspension formulations from the stomach was independent of and slower than the concomitantly administered water wash. The assumption was made in this paper that the radiolabelled IRP-69 resin which was used to follow the formulation behaved in the same way as the IRP-64 which

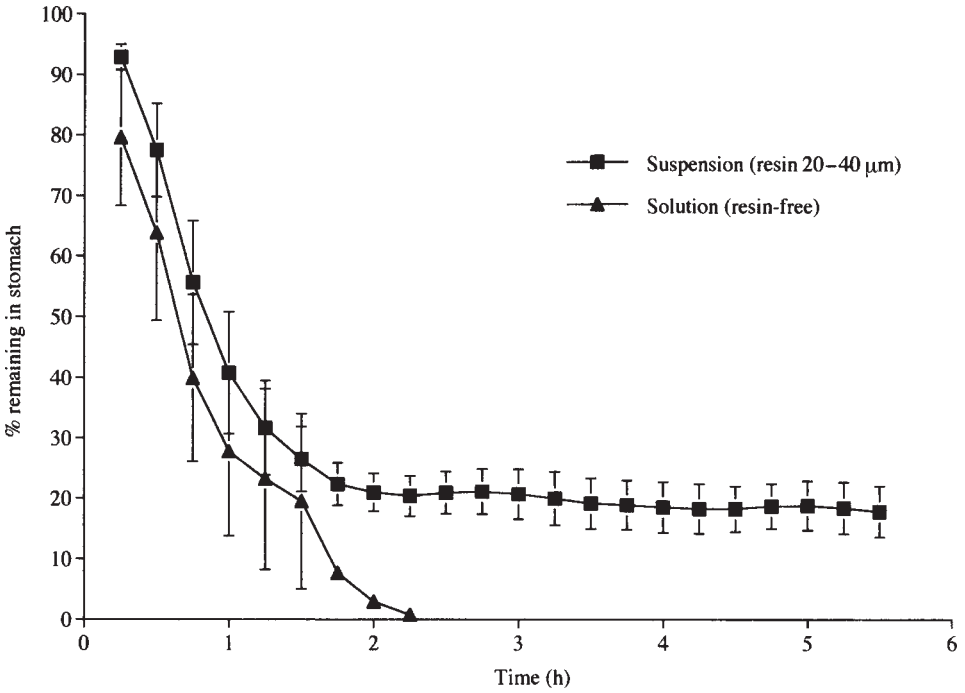

Figure 6.2 Comparison of the gastric emptying of an aqueous (^{99m}Tc-DTPA) label and particulate in suspension (micronized Amberlite resin) (modified from Burton *et al.*, 1995)

was used in the drug-resinate. The IRP-64 will not bind indium-111, indicating that its physicochemical properties are different to IRP-69 which binds indium strongly. A potential weakness of the use of different ion exchange resins is that they may not behave similarly in the stomach; because the gastric retention of the resin being studied is thought to be a function of surface charge. It is therefore important that adequate *in vitro* studies are carried out to ensure that the radiolabel and material to be tested are likely to perform in the same manner *in vivo*.

Antacids containing aluminium hydroxide can be labelled by forming a mixed hydroxide with ^{113m}In or ^{111}In by precipitation of the mixed chlorides with ammonium hydroxide. This approach was used in our laboratory to prepare radiolabelled antacid suspensions (Jenkins *et al.*, 1983; May *et al.*, 1984). These studies demonstrated that antacids do mix evenly with a meal, but generally are denser than the meal so a large proportion sinks to the bottom of the stomach (see Figure 6.3, colour section).

The most interesting suspension formulations are raft-forming materials such as alginates, which are used to treat gastro-oesophageal reflux (Washington, 1990) or as drug delivery platforms for H_2-antagonists (Stockwell *et al.*, 1986; Washington *et al.*, 1993) which float on the gastric contents. Alginates label with indium-111 readily, without significant changes to their properties and hence they are easy to study using scintigraphic techniques (Washington *et al.*, 1987a,b, 1988). These systems will be discussed in a later section.

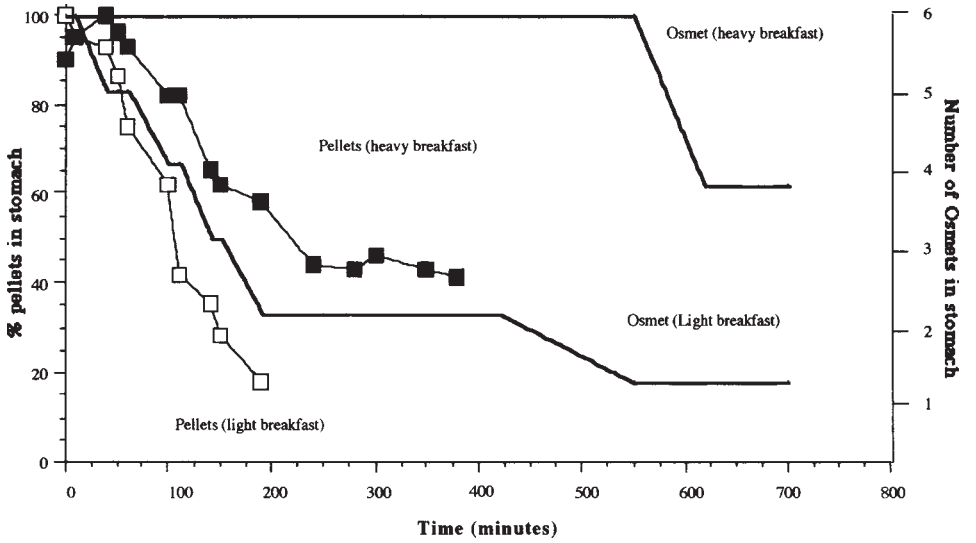

Figure 6.4 The effect of food on the gastric emptying of a large non-disintegrating single unit (Osmet®) and pellets (after Davis *et al.,* 1984)

6.5.3 Pellets and Tablets

Large tablets or capsules, whether intact or in large fragments, will be treated by the stomach as an indigestible material. The ability of the pylorus to discriminate between 'large' and 'small' tablets is a variable in man and no clear cut-off size has been established. Tablets of between 3 and 7 mm empty similarly from the stomach after light, medium and heavy meals, but it is the calorific value of the meal and not the tablet size which is the major influence on the rate of emptying (Khosla *et al*., 1989). However, the gastric emptying rate of single- and multiple-unit systems is high even when meal intake is controlled. Some studies have even suggested that the intrasubject variation of transit is less than the intersubject variation (Coupe *et al*., 1991) but this is inevitably true because it is difficult to visualize a situation in which a group of subjects show less variation than an individual from that population!

The gastric emptying rates of multiparticulate (e.g. pelleted) dosage forms are not as severely affected by the presence or absence of food as large single units, as shown in Figure 6.4 (Davis *et al*., 1984). An 'Osmet®' (Alza Corporation), which is a large non-disintegrating unit, and a pellet formulation were administered together to subjects after a light or a heavy breakfast in a crossover study. In a majority of subjects, when the Osmet® was administered with the light breakfast, emptying had occurred by 2 h. The heavy breakfast delayed the gastric emptying of the Osmet® to more than 9 h. The emptying of the pellets was also prolonged by the heavier meal, but not to the same extent as the Osmet®.

Gastric emptying of the pellet formulations does not follow a set pattern when given with food because it can occur either at a similar rate or slower than the

food (Coupe *et al*., 1993). The gastric emptying of pellets from the fasted stomach depends upon the nature of the capsule in which they were administered, the speed at which it disintegrates and the degree of dispersion of the pellets in the low volume of gastric contents (Hunter *et al*., 1983). In considering pellet formulations, Amberlite resins are predominantly used and lipophilic markers have been used in a few studies. Beten and colleagues described the use of particles based on Eudragit S which had been labelled with ^{111}In-indium oxine to form a co-evaporate particle (fraction size, 0.10–0.50 mm; density, 1.36 g cm^{-3}) (Beten *et al*., 1995).

6.6 Mechanisms to Increase Gastric Residence

As discussed earlier it is advantageous for certain drugs to remain in the stomach for prolonged periods. Usually this is a prerequisite for drugs with a high window of absorption or for local treatment of the gastric mucosa. Many strategies have been employed to improve the gastric residence of materials with varying degrees of success, the most common being floatation of drug delivery systems on the gastric contents, adhesion to the gastric mucosa and increasing the size of the dosage form to a size which is too large to pass through the pylorus (Moes, 1993).

6.6.1 Floatation

The effectiveness of floating systems can quite easily be established *in vivo* using dual label gamma scintigraphy (see Figure 6.5, colour section). This method is superior to ultrasound imaging and fluoroscopic imaging which were previously used to study gastric retention of enzyme-digestible hydrogels (Shalaby *et al*., 1992).

Many different approaches have been used to produce gastric floatation devices. Examples include tablets coated with a crosslinked polymer which expand to float on the gastric media (Agyilirah *et al*., 1991). Air and oil can be entrapped in an agar gel network to assist the floatation of tablets (Desai and Bolton, 1993). The term *hydrodynamically balanced systems* (HBS) has been used to describe sustained release oral dosage forms with a specific gravity lower than 1 (Ingani *et al*., 1987). Other systems rely on bicarbonate to produce bubbles of carbon dioxide gas in contact with gastric acid which confer the necessary buoyancy to the dose form. This approach has been used to float ion exchange resins (Atyabi *et al*., 1996) and anti-reflux agents (Moss *et al*., 1990).

Most of the information on floating systems has been gleaned from studies on anti-reflux agents and although these formulations are used for the treatment of gastro-oesophageal reflux, the lessons learnt from studying these products are directly applicable to any other floating technology. For floatation and consequently prolonged retention of these formulations to occur, it is essential that they are given after a meal (Agyilirah *et al*., 1991; Washington *et al*., 1990).

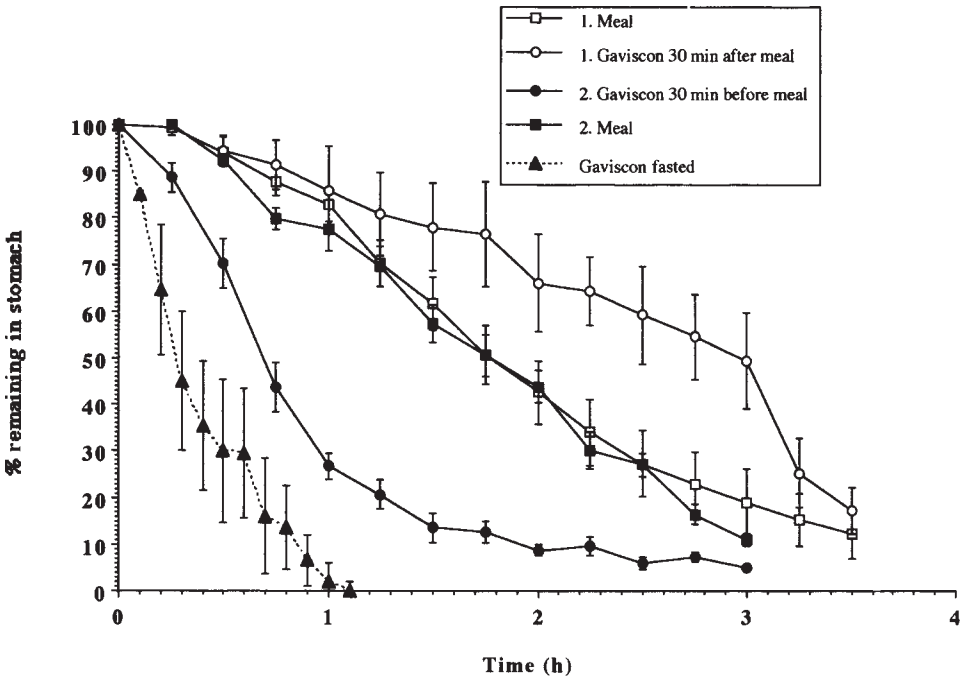

Figure 6.6 The effect of food on the buoyancy of an ^{111}In-labelled anti-reflux agent given to (a) fasted volunteers, (b) 30 min before a meal and (c) 30 min after a meal (after Washington, 1990)

In some cases, the necessity for the medications to be taken after food is ignored and clinical trials have been carried out on floating systems using fasting volunteers (Laitinen *et al.*, 1985). On an empty stomach, the formulation will sink to the base of the greater curvature and empty within minutes (Figure 6.6). When administered 30 min after a meal and whilst the subject is upright, the formulation is retained until the last of the meal is emptied, when it will generally leave the stomach (Washington *et al.*, 1990).

It has been speculated that anti-reflux agents do not form a buoyant raft, but only appear to float because they form a stratified layer on the food. When the gas-producing agent is omitted, the formulation mixes and empties with the meal and there is no localization of the material in the fundus (Moss *et al.*, 1990). Once a 'raft' or floating layer has successfully formed, then the intake of subsequent meals prolongs its residence in the stomach from 2.3 h to 4.3 h indicating that it is floatation and not stratification which is occurring. This was also demonstrated for a floating tablet in which the average gastric residence time was increased from 199±69 min after a single meal to 618±208 min after a succession of meals (Oth *et al.*, 1992). Unlike conventional single units, the size of a floating unit does not affect its gastric residence time (Moes, 1993).

Posture markedly affects the rate at which floating units empty from the stomach. Alginate rafts empty faster than food in subjects lying on their left

side, and slower in subjects lying on their right (Bennett *et al*., 1984). When the subject is lying on the right side, the raft is positioned in the greater curvature of the stomach where it would no longer provide protection to the oesophagus from the stomach contents. In the supine position the anti-reflux agents empty from the stomach at the same rate as a conventional antacid (Bennett *et al*., 1984).

Although there are many systems described which float on the gastric contents, there are surprisingly few pharmacokinetic comparisons with conventional dose forms. Floating systems result in a three-fold increase in absorption of an iron supplement (Cook *et al*., 1990).

6.6.2 Gastric Adhesion

With the current focus on *Helicobacter pylori,* there has been a sudden interest in topical drug delivery to the stomach. Conventional formulations are not optimized for this purpose because their coverage is not uniform and does not reach the fundus.

Polycarbophil was an initial candidate for gastric adhesion studies, but its retention was not dramatic (Harris *et al*., 1990). Scintigraphy has demonstrated that ion exchange resins can be used successfully to coat the stomach (Washington *et al*., 1989). In this study stomach images were divided into three regions, each of approximately the same pixel area. The resin was evenly distributed within these regions and the gastric residence of about 15% of the total dose administered was greater than 6 h (Figure 6.7) (Burton *et al*., 1995). Interestingly, a meal administered 4 h after dosing does not dislodge the resin embedded in the mucosa (Thairs *et al*., 1997).

6.7 Gastroresistant Coatings

For some drugs it is desirable to prevent release in the stomach. The drug may be irritant to the gastric mucosa, such as the non-steroidal anti-inflammatory drugs, or compounds may be degraded under acidic conditions, e.g. omeprazole. Generally, formulations containing these types of drugs are coated with a polymer which is insoluble in acid, but soluble at the higher pHs found in the small intestine. Gamma scintigraphy has been widely used to test the performance of these types of formulations because a radiolabel can be included in the interior of the formulation prior to coating with the gastroresistant polymer. Once the polymer has dissolved, the contents will be released. The behaviour of these types of formulation *in vivo* will be hard to predict because food intake will not only raise gastric pH, but will also delay the emptying of the formulation into the small intestine hence delaying onset of action of the drug. The performance of entericcoated starch capsules (Kenyon *et al*., 1994), naproxen tablets (Gamst, 1992; Hardy *et al*., 1987, 1991) and erythromycin pellets (Digenis *et al*., 1990) have been reported in the literature.

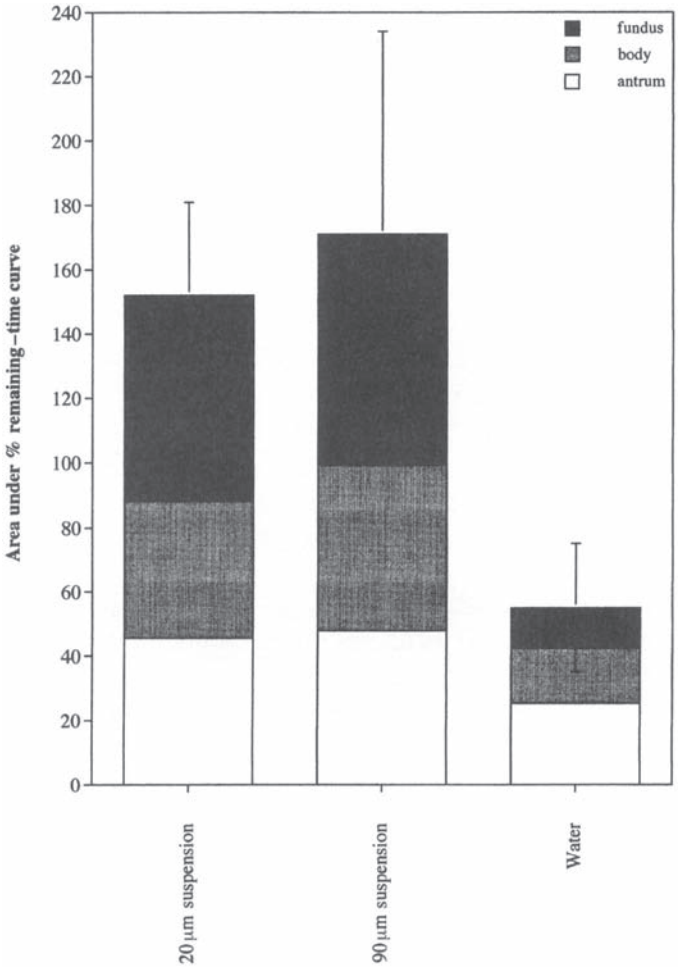

Figure 6.7 Distribution of Amberlite resin suspensions in the three major regions of the stomach compared with water. Note the increased retention compared to water (modified from Burton *et al.*, 1995)

6.8 Small Intestine

The main function of the small intestine is to mix the food with enzymes and intestinal secretions to facilitate digestion and it is the primary site of absorption of both food and drugs. Three morphological features increase the surface area of the small intestine: (1) mucosal folds, (2) the myriad finger-like projections, termed villi, which are lined with absorbing cells or enterocytes, and (3) projections called microvilli on the mucosal membrane of each enterocyte. It has been estimated that the potential epithelial surface available for absorption is about the size of a basketball court (463 m^2) and is far greater than in the stomach or colon. However, little of the epithelial surface area needs to be used for complete absorption of soluble nutrients.

The first part of the small intestine, the duodenum, is 20–30 cm long, the second 2.5 m is the jejunum and the final 3.5 m the ileum. These regions are not anatomically distinct, although there are differences in villus height, absorptive capability, specificity and secretion. There is no definite sphincter between the stomach and duodenum although in some studies a zone of elevated pressure between the two regions has been reported to exist.

6.8.1 Measurement of Small Intestinal Transit

The stomach and colon are planar organs and hence easy to visualize by gamma scintigraphy. The small intestine is a long tube which is highly folded and motile and consequently it produces an amorphous planar image. Although it is relatively simple to measure the complete transit time of a single non-disintegrating unit through the small intestine, it is harder to determine the small intestinal transit time (SITT) of materials which empty from the stomach over a period of time. In these cases, SITT is usually estimated from the difference between T_{50} arrival at the colon and gastric emptying T_{50}, although a group at the University of London have suggested that statistical moment analysis provides a more robust parameter (Podczeck *et al*., 1995).

6.8.2 Small Intestinal Transit Time of Dose Forms and Excipients

During fasting, both monolithic and multiparticulate dosage forms will be swept rapidly through the small bowel by the housekeeper sequence. The action is propulsive and not mixing in nature, thus a capsule containing pellets given on an empty stomach may leave the stomach and pass down the small intestine as a bolus with minimal dispersal (Hunter *et al*., 1982).

Pellets taken with a meal become mixed with the food in the stomach. As their particle size is small, pellets will continue to be emptied from the stomach as part of the chyme, thus increasing dispersal and prolonging their delivery to the small intestine. Monolithic tablets, on the other hand, depending upon their size, will empty erratically from the stomach after food and as the single unit traverses the small bowel, the presentation of the drug to the small intestinal mucosa will depend solely upon its dissolution characteristics in each area. The degree of spread of a formulation within the small intestine is particularly important for drugs with poor solubility or for drugs which are slowly transported across the epithelium. Microparticulate dosage forms show longer and more reproducible median transit times compared with single unit tablets, giving rise to more predictable and uniform blood levels. Microparticulates also reduce the risk of adhesion and possible mucosal damage once past the oesophagus.

It is only relatively recently that small intestinal transit time could be measured with any degree of accuracy, but many dosage forms were designed before the information gleaned from these studies was widely publicised. As a result, many sustained release dosage forms have been designed to release 90%

of their drug between 9 and 12 h after ingestion. This estimate was largely based on measurements of whole gut transit times, derived from X-ray contrast measurements or data collected from patients with ileostomies. X-ray contrast studies used in patients with terminal ileostomies give half-times for mouth-to-stoma transit of a radiolabelled solution and pellets 2 mm or larger in diameter from 1.3 to 7.8 h and from 3.5 to 5.8 h, respectively (Conrad and Robinson, 1982). The current general consensus in the literature is that small intestinal transit time for dose forms is around 3–5 h irrespective of formulation type (Abrahamsson *et al*., 1996; Davis *et al*., 1987, 1988). It was suggested by Read and Sugden that transit is influenced by whether the subject has eaten and the size of the meal (Read and Sugden, 1987). However, a study which measured the small intestinal transit times of meals matched for calorific value but with differing fat content (13% fat w/w supplying 66% of the total energy of the meal and 5% supplying 29% of the energy), reported that although the increased fat load significantly delayed SITT of the meal (6.1 h versus 8.1 h), it did not affect the transit of the co-administered pellets (5.7 h for both) (Washington *et al*., 1994).

The accumulation or bunching of dispersed material at the ileocaecal junction, prior to entry into the ascending colon as a bolus, has only been identified through scintigraphy. Non-disintegrating matrices may remain at this location for some time, with reported periods ranging from 2 to 20 h in both young and elderly volunteers (Marvola *et al*., 1987; Wilson and Washington, 1988). Stagnation at the ileocaecal junction may also cause problems for controlled release dosage forms which are designed to deliver drug over a period of 9–12 h, because the concentration of drug could build up within a localized area. This will have no effect on the absorption of most drugs, providing that the rate at which the drug is released from the dosage form is slower than the rate of uptake across the ileal epithelium and the drug is not degraded by ileal bacteria. Some drugs such as ibuprofen are absorbed from the proximal part of the colon, but this is not universal; for example, absorption of buflomedil and isosorbide-5-nitrate shuts off as soon as the unit enters the colon (Fischer *et al*., 1987; Wilson *et al*., 1991b). Drugs which are not absorbed well in the ileocaecal and colon regions of the gut should have much shorter delivery times, probably of the order of 5–7 h.

Pharmaceutical excipients such as sodium acid pyrophosphate (SAPP), mannitol and sucrose are generally regarded as ‘inert’. Mannitol is also frequently used as a marker to assess recovery in gastric intubation experiments. However, at concentrations of 0.755 g/200 ml, 1.509 g/200 ml and 2.264 g/200 ml, the mean SITT was reduced by 11%, 23% and 34% respectively, when compared to a control solution of 200 ml purified water. This demonstrates that mannitol has a concentration dependent effect on small intestinal transit and the notion that mannitol is physiologically inert is not correct (Adkin *et al*., 1995b). SAPP (1.1 g/200 ml) also reduces SITT by 39% when compared with the control solution (purified water = 240 min; SAPP = 147 min), however intestinal transit for the sucrose solution was similar to that for the control solution (sucrose = 229 min) (Adkin *et al*., 1995c). In practice, inclusion of some excipients in dosage forms

can significantly reduce the bioavailablity of certain drugs. For example, mannitol reduces absorption of cimetidine from a chewable tablet or a solution form compared to sucrose (Adkin *et al*., 1995a) and significantly less ranitidine absorption (54% based on AUC) occurred from the oral solutions containing SAPP when compared to those with this excipient omitted (Koch *et al*., 1993). Again, this was demonstrated to be due to a 56% decrease in SITT.

6.8.3 Density and Small Intestinal Transit

Studies carried out in the late 1970s indicated that pellet density affected small intestinal transit in ileostomy patients (Bechgaard and Ladefoged, 1978) although subsequent studies carried out in the early 1980s were unable to confirm this finding in normal subjects (Kaus *et al*., 1984). A further scintigraphic study eventually confirmed that small intestinal transit in patients with ileostomies was not affected by density in the range 0.94–1.96 g cm^{-3} (Bechgaard *et al*., 1985).

Small particles with densities close to that of a meal will be emptied continuously with the meal (Meyer *et al*., 1988; Mundy *et al*., 1989). This means that, for a well designed enteric-coated multiparticulate formulation, there is little delay in the onset of plasma levels even when the drug is given with food. As has been discussed previously, buoyant materials do show a slowed gastric emptying, which will of course affect the period for which they are presented to the small intestine. The situation is not as clear for dense materials. Gastric emptying studies on pellets of high (2.6 g cm^{-3}) and normal (1.5 g cm^{-3}) density demonstrate delayed gastric emptying and extended SITT of the denser dosage forms (Clarke *et al*., 1993). However, a study reported by the same group comparing 1 mm pellets of densities 1.5, 2.0 and 2.4 g cm^{-3} failed to reveal any differences in gastrointestinal transit of the three formulations (Clarke *et al*., 1995).

6.9 Regional Differences in Small Bowel Absorption

Does simply prolonging the contact time of the drug with the intestinal mucosa increase absorption when the small intestine shows considerable regional variability in absorptive capacity? The variation in absorptive capacity can, in part, be explained by the higher surface area per unit length in the upper intestine compared with the lower part. The permeability of the epithelium to small ions and water-soluble organic molecules is greater in the duodenum and jejunum than it is in the ileum, which would indicate that there are larger and more numerous water-filled channels high in the small bowel (Fordtran *et al*., 1965). Specific carriers for the transport of riboflavin and iron are found principally in the duodenum and jejunum, whereas carriers for bile acid and vitamin B_{12} are found mainly in the ileum. The acid microclimate is less obvious in the ileum, favouring the absorption of weak bases while discouraging the absorption of weak acids.

A few studies have been carried out to compare the absorption of drugs in the upper and lower small intestine which reveal that a proximal-to-distal small intestinal absorption gradient exists for some drugs, e.g. hydrocortisone, but not for others, e.g. triamcinolone (Schedl and Clifton, 1963). Digoxin (Beerman *et al*., 1972) and hydrochlorthiazide (Beerman and Grochinsky-Grind, 1978) were absorbed predominantly from the duodenum and upper jejunum. Although there is excellent absorption from the duodenum, transit through this area is extremely rapid, in the order of seconds (Kaus *et al*., 1984) and hence the actual amount of drug absorbed will be small. Finally, the ileum contains more commensal bacteria than the duodenum and jejunum. In elderly subjects, the bacterial population in the ileum may be high enough to metabolize certain drugs, thereby reducing their efficacy. Small-intestinal permeability is increased in subjects with small-intestinal bacterial overgrowth with colonic-type bacteria. This effect is independent of ageing and not mediated by vitamin B_{12} deficiency (Riordan *et al*., 1997).

6.9.1 Devices to Explore Windows of Absorption

The use of multilumenal tube and balloon systems to isolate segments of gut for drug absorption studies is well established within clinical pharmacology. The technique does have the potential for errors because mechanical stimulation of the gut during intubation has been shown to alter secretion and motility (Read *et al*., 1983). However, modern exponents of the technique claim that the measurement is benign. Lennernas and colleagues have explored the use of a patented intestinal isolating system (Loc-I-Gut®). It has been demonstrated that it is possible to determine the effective permeability for carrier-mediated transported compounds and hepatic extraction using this procedure (Lennernas, 1997; Lindahl *et al*., 1996).

One of the most important restrictions of intubation is that some subjects find the procedure uncomfortable or stressing. Other means of delivering drugs to specific sections of the gut which can be triggered to release drug at the required site can be swallowed; however, such devices are large (Wilson and Washington, 1995). In the early 1980s, Bieck and colleagues used a small device, the HF capsule, containing the drug as a solution in a small latex balloon (Bieck, 1993). Release of drug at the required part of the gastrointestinal tract was accomplished externally by a radiofrequency pulse which energized a relay within the device. In order to ascertain the position within the gastrointestinal tract, the subjects were fluoroscoped at regular intervals. The dosimetry associated with this X-ray procedure is relatively high and scintigraphic detection of location of the unit offers an obvious benefit. Gardner and colleagues have utilized an alternative device, the 'Intellisite®' capsule which can be filled with powders, suspensions or liquids (Figure 6.8). The system consists of a unit in which delivery ports are exposed by twisting of a section of the cap under the command of an externally applied radiofrequency pulse of around 120 watts (Gardner *et al*., 1997).

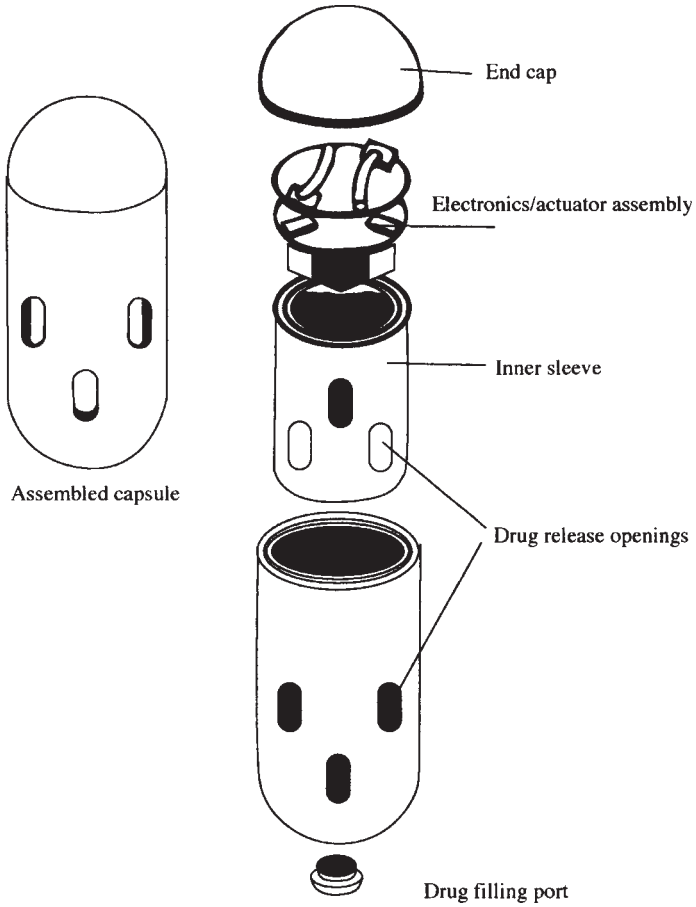

Figure 6.8 Intellisite® capsule showing the inner and outer sleeves. Actuation twists the inner sleeve to line up the three delivery ports and release drug (after Gardner *et al.*, 1997)

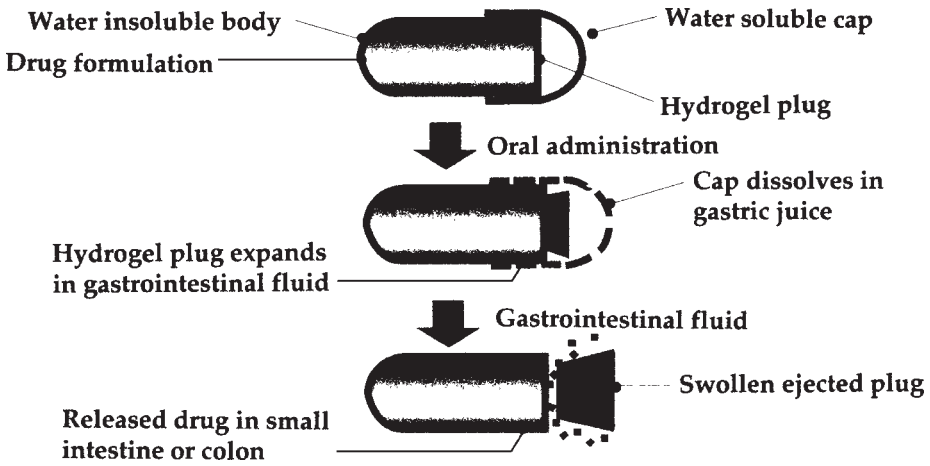

Figure 6.9 Schematic of Pulsincap® dosage form (after Wilson *et al.*, 1997)

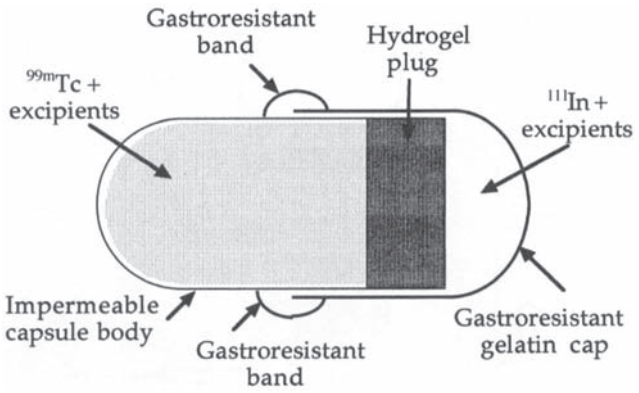

Figure 6.10 Configuration of the labelled Pulsincap® used to establish the effectiveness of the enteric coating

'Pulsincap®' is another time-delayed system (Figure 6.9) but this comprises of an impermeable capsule body containing the drug formulation sealed at the neck edge with a hydrogel polymer plug (McNeil *et al*., 1992). Application of a suitable enteric coat prevents dissolution until the pH rises above 5.5, i.e. until the unit has entered the intestine. On leaving the stomach, the capsule becomes exposed to intestinal fluid and the cap dissolves, allowing the hydrogel plug to hydrate. At a predetermined time point after ingestion, the swollen plug is ejected from the capsule body, thereby enabling the drug to be released. The time of plug ejection is controlled by the length of the hydrogel plug and its position relative to the neck of the capsule body.

During the design and optimization of the device, gamma scintigraphy was employed to demonstrate the integrity of the coat preventing preliminary release of the device in the stomach. This was accomplished by placing a ^{99m}Tc-fill in the body of the device and an ^{111}In marker in the cap (Figure 6.10). Following gastric emptying, spread of the ^{111}In-labelled contents was noted, although the ^{99m}Tc spread was much less than anticipated. Although the enteric coat performed as expected, there remained the question of whether body and plug had separated in the poorly stirred conditions in the terminal small intestine. A further configuration was prepared as illustrated in Figure 6.11. As noted in the figure, ^{111}In point sources were positioned on the interior of the body at the apex and on the bottom of the plug. Subjects were imaged in the anterior, posterior and lateral positions to determine separation of the point sources at intervals after ingestion. Subsequent studies revealed that body and plug clearly separated but that a large proportion of the encapsulated contents remained trapped in the body. This led to the final design in which the contents were fixed to the base of the plug as a tablet. Plug ejection then led to consistent pulsatile release of the contents (Wilson *et al*., 1997).

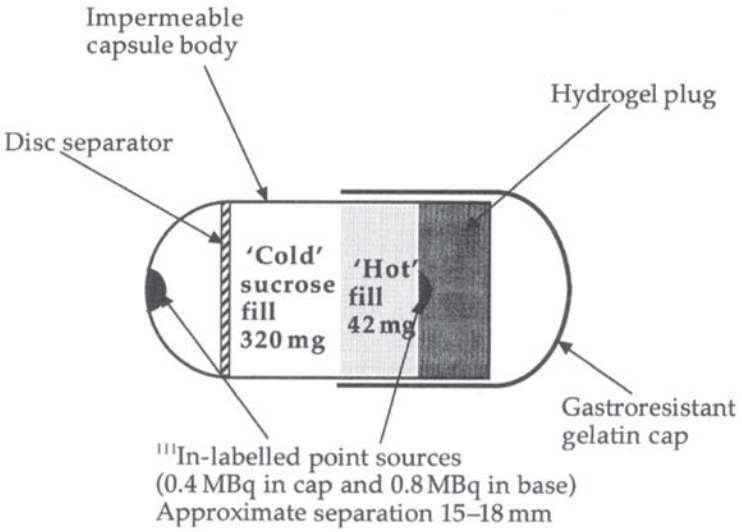

Figure 6.11 Configuration of the labelled Pulsincap® used to establish plug and body separation

6.10 Use of Gamma Scintigraphy to Examine the Performance of Novel Sustained Release Dosage Forms

Multilayer tablets comprising alternating active matrix and barrier layers applied during the tableting process enable the production of sophisticated drug release time kinetics. The Geomatrix® tablet (Skye-Jago Pharma) is an example which originated from the University of Pavia (Conte *et al*., 1993). The partial coating modulates the core hydration process and reduces the surface area available for drug release, resulting in an almost linear drug release profile. The device was mainly intended for soluble drugs; a time-dependent polymeric barrier was developed to control the release of sparingly soluble drugs. In the development of a diltiazem formulation based on the Geomatrix technology, scintigraphic studies using samarium and erbium oxide were used to follow the behaviour of the formulation and establish the role of the cap (Wilding *et al*., 1995). In the first formulation samarium-152 was used to label the core; in the second samarium-152 oxide was incorporated into the core and erbium-170 into the cap (Figure 6.12).

The 'Time-clock' system is a dose form which is reported to have fast and complete release of a drug after a predetermined lag time. This pulsed release system has the advantage of being manufactured using conventional film coating techniques and uses excipients normally present in pharmaceutical formulations. The *in vivo* behaviour of the preparation has been evaluated in three scintigraphic studies. The lag time for the system was found to be independent of normal physiological conditions, such as pH, digestive state of the subject and the anatomical position at the time of release. At the end of the lag time, disaggregation of the core was both rapid and complete. Scintigraphic evaluation was also used

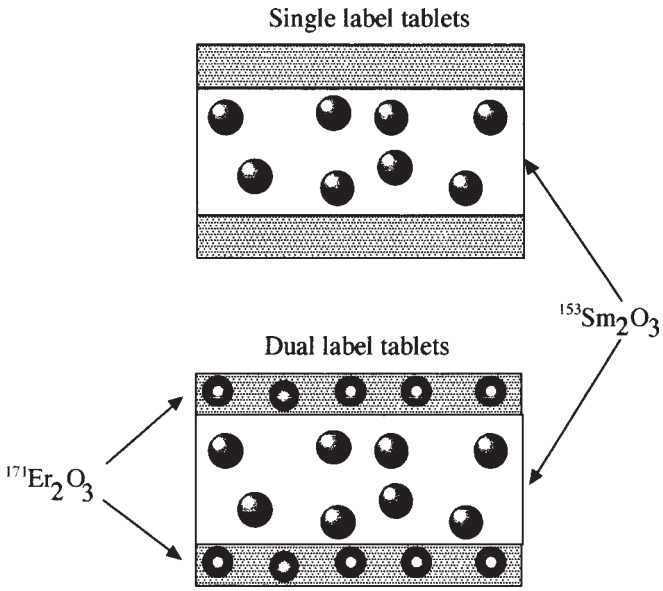

Figure 6.12 Use of erbium-171 and samarium-153 to monitor the behaviour of the release modifying cap in a Geomatrix® system

to establish *in vitro* methodology capable of predicting the subsequent *in vivo* performance of the 'Time-clock' system (Wilding *et al*., 1994b). Pharmacokinetic studies using salbutamol as a model drug demonstrated that its absorption was not influenced by the *in vivo* behaviour of the system (Pozzi *et al*., 1994).

6.11 Conclusions

In the past, there were few direct methods of assessing the behaviour of drug formulations within the body. Information was gleaned from *in vitro* dissolution tests and plasma concentration versus time curves for the drug and the true behaviour and relationship within the body of the two could only be speculated. Radiological contrast studies produce good definitions of anatomical structure, but are not well suited to measurements of transit and dispersion of pharmaceutical formulations because the radiation burden of multiple X-rays and/or repeated studies would be prohibitively high.

Future applications using multiple imaging modalities are being explored. In studies where the positioning of the sampling ports needs to be established, such as multiport pH monitoring, scintigraphy provides a useful adjunct. Magnetic resonance imaging (MRI) is still under development to study drug formulations. It does offer the promise that soft tissue structure can be directly visualized but unlike gamma scintigraphy, quantification is problematic. Combined MRI and scintigraphy is being explored in our laboratories and might offer interesting new possibilities in pharmaceutical and medical research.

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Scintigraphic Study of Colonic Release and Absorption

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7.1 Therapeutic Context and Objectives

Selective delivery of drug to a particular site in the colon maximizes the local concentration of therapeutic agent whilst minimizing exposure to the rest of the body, thus improving the efficacy of treatment and lessening potential side-effects.

Ulcerative colitis, an idiopathic inflammatory disease of the colonic mucosa, is a condition which is well suited to local topical drug delivery because steroid enema preparations can achieve very high local concentrations with minimal systemic effects. However, retrograde spreading of radiolabelled foam preparations administered rectally cannot reach beyond the splenic flexure (Wood *et al*., 1985), limiting enema treatment to distal disease. In addition there are often problems of compliance, and for these reasons attempts have been made to design oral preparations which target drug to the colon. The first such formulation working in this way was sulphasalazine, which consists of 5-aminosalicylic acid (5-ASA) linked to sulphapyridine by an azo bond (Peppercorn, 1984). The active moiety, 5-ASA, is released when the azo bond is split by the gut bacteria which reside in the colon (Khan *et al*., 1977), and hence colon-specific drug delivery is achieved. Another method of targeted colonic delivery of 5-ASA has been developed which involves coating the delivery capsule with a pH-sensitive polyacrylic resin (Asacol) which starts to dissolve as the pH changes from being acidic in the stomach to >pH 6 in the small intestine (Evans *et al*., 1988). The coat thickness is such that it takes 2–4 h for release to occur, by which time the capsole is usually in the terminal ileum or colon.

Steroids, the mainstay of treatment in moderate to severe flare-ups of colonic disease activity, may also be targeted to the colon. Site-specific delivery may be achieved by conjugation of steroids with glycosides; the latter being split from the parent pro-drug by bacterial glycosidases located primarily in the colon (Friend, 1991). As with Asacol, delayed release of steroid has been achieved by coating the formulation with an acrylic-based resin.

In addition to the topical treatment of inflammatory bowel disease, drug delivery to the colon may be useful for other reasons. Certain drugs, particularly those which are peptide or protein based may be unstable in the proteolytic environment of the upper gastrointestinal tract, and therefore a greater fraction may be absorbed if this region of the gut is bypassed. Currently, the feasibility of peptide hormone delivery by this route, for example insulin and calcitonin, is being studied. Finally, the colon may be exploited as a 'reservoir' allowing prolonged release and absorption of drug. The small intestine is designed to maximize absorption of nutrients by facilitated diffusion across a large absorptive area. Small intestinal transit time is usually of the order of 3–4 h and is sufficient to achieve complete absorption of nutrients. A similar fate for ingested drug formulation would result in large swings in peak and trough plasma levels, and usually require multiple daily dosing to achieve a smooth therapeutic profile. Delivery of formulation to the colon, through which material has a variable, but much slower, transit and absorptive rate, may be utilized to allow once-daily dosing of therapeutic agents. Moreover, delayed release may be advantageous in the treatment of diseases which show cyclical exacerbations, for example the early morning dips in peak flow rate seen in asthmatics and the morning stiffness and pain in rheumatoid arthritis. Medications taken at bedtime would be expected to reach the colon by early morning, and thus initiation of absorption would be coincident with exacerbations of disease activity.

7.2 Radiolabelled Controlled Release Formulations and Targeted Drug Delivery to the Right and Left Colon

Incorporation of a gamma-emitting radionuclide into a formulation provides a simple and convenient method for observing the transit through, and residence within, the gastrointestinal tract. It is of particular use in determining the time and site of release of delayed release formulations. Unlike radiological assessment it allows serial images to be taken without imposing an unacceptably high radiation burden on the subject, and therefore an accurate knowledge of site and release characteristics of the formulation can be gained.

The first objective in selective colonic drug delivery is to deliver the formulation through the stomach and small intestine, avoiding release or luminal degradation. In order to achieve this, a detailed knowledge of the physiology of the upper gastrointestinal tract is required. The most important variables are transit times (crucial to the success of timed release delivery systems) and the pH profile (determining the breakdown of the protective polyacrylic resins which constitute the 'enteric' coating of certain preparations).

After ingestion and passage down the oesophagus, the dosage form enters the stomach. Here the milieu is markedly acidic and protein or peptide based preparations must be protected from denaturation. Residence time within the stomach is dependent both on the physical characteristics of the dosage form, and on the presence or absence of food. The stomach is able to discriminate between liquids, digestible solids, and inert, indigestible solids. Thus to a

large extent, the gastric emptying time of a substance is determined by its physical form.

It is well established that liquids empty faster than solids (Minami and McCallum, 1984), with the latter being selectively retained until ground down to a suitable size in the antrum. In an elegant study in dogs, in which different sized spheres were added to a radiolabelled meal, emptying of the spheres became similar to emptying of the meal at a diameter of 1.6 mm; increasing and decreasing sphere diameter led to slower and faster emptying respectively (Meyer *et al*., 1985). Emptying of indigestible solids that cannot be broken down to a size less than 2 mm seems to depend upon resumption of the fasting motility pattern. Hence, 7 mm plastic spheres were found not to empty whilst food remained in the canine stomach (Hinder and Kelly, 1977), but were retained until resumption of the interdigestive migrating motor complex after the stomach had emptied all the food (Mrotz and Kelly, 1977).

More recently there have been several studies looking at the gastric emptying of non-disintegrating dosage forms in humans. In the fasted state, emptying of capsules ranging from 9.8×4.7 mm up to 25×9 mm was complete in the majority by 1–2 h (Park *et al*., 1984; Hardy *et al*., 1985), and was as rapid as that seen for small pellets, 0.5–1.8 mm in size (Davis *et al*., 1986). Although concomitant intake of food has relatively little influence on the gastric emptying of solutions and small pellets (less than 2 mm in size), there is a marked delay in the emptying of large capsules (Davis *et al*., 1986). These findings are consistent with canine gastric emptying studies (Hinder and Kelly, 1977; Mrotz and Kelly, 1977). Thus for dosage forms of less than 2 mm, gastric emptying will take place at the same rate as emptying of the food present, which in turn depends on such factors as energy density, fat and fibre content. However, for capsules and tablets which are greater than 2 mm in diameter, dosing needs to take place in the fasting state to avoid prolonged gastric retention.

On exiting the stomach the dosage form enters the small intestine, where there is a dramatic rise in pH due to bicarbonate secretion from the duodenal mucosa and pancreas. In contrast to the stomach, the transit time through the post-prandial human small intestine appears to be relatively constant and unaffected by either the dosage form (solutions, pellets or large capsules) or stomach contents (Davis *et al*., 1986), with a value of 3–4 h in the post-prandial state. This data is in agreement with other workers who have shown that the liquid and solid components of a meal traverse the small intestine at similar rates (Malagelada *et al*., 1984).

It can be seen therefore that dosing on an empty stomach should result in delivery of the formulation to the proximal colon approximately 4–5 h after ingestion. Indeed, several small studies have shown this to be the case using, variously: a large (25×9 mm) pressure-sensitive radiotelemetry delivery system and 0.5–1.8 mm diameter radiolabelled resin pellets (Hardy *et al*., 1985); capsules of differing densities and volumes (Parker *et al*., 1988); and 4×4mm non-disintegrating tablets (Hardy *et al*., 1988). These preliminary studies suggest that delivery to the proximal colon can be achieved reasonably reliably and predictably provided meal patterns are standardized, the dosage form is taken

in the fasting state, and thereafter a meal is ingested to encourage uniform passage to the colon.

The colon merits special consideration, not least because transit within this region is highly variable both between individuals and within the same individual (Degen and Phillips, 1996). In terms of distal colonic disease, such as left-sided colitis or diverticulosis, delivery of formulation to the distal colon would be preferential, whereas if systemic delivery of a drug over a prolonged period were required then release and retention within the proximal or right side of the colon may be more desirable, as will be discussed below.

7.3 Colonic Physiology and Absorption

It is clear that the colon is not a single homogeneously functioning organ, but is better thought of as being composed of two distinct units, the proximal and distal colon. These two regions are embryologically distinct, the former being derived from the midgut and the latter from the hindgut. It is therefore no surprise that each serves different functions, and indeed certain features of the proximal colon suggest that it may be better suited to drug absorption compared to the distal colon.

In recent years the most commonly used method for assessing whole gut transit has involved the ingestion of radio-opaque markers with subsequent radiographs of either the stool or the abdomen (Hinton *et al*., 1969). Using the time to excrete 80% of the administered pellets as a reflection of whole gut transit time, studies have shown that the mean whole gut transit time usually lies between 33 and 83 h (Hinton *et al*., 1969; Burkitt *et al*., 1972; Cummings *et al*., 1976, 1978). This range primarily relates to colonic transit time, as small bowel transit and gastric emptying (as mentioned earlier) make up only a small fraction of this time. A refinement of this method has been used to assess total and segmental colonic transit times. Using bony landmarks and gaseous outlines of the bowel on plain abdominal X-rays, the mean total colonic transit time has been shown to lie between 30 and 40 h with transit times through the right, left and rectosigmoid regions being similar (Arhan *et al*., 1981; Metcalf *et al*., 1987). There are however several problems with the radio-opaque marker studies:

- They impose a significant radiation burden, especially if they involve sequential abdominal X-rays.
- Accurate localization of pellets may be a problem, particularly in the case of a sagging transverse colon (see Figure 7.1, colour section).
- The radiographically dense markers may be treated differently to chyme and propagated at a different rate compared to native material. In an attempt to overcome these problems, attention has turned to the use of radionuclide imaging which has the advantages of low radiation exposure, the opportunity for sequential imaging without additional radiation burden, and allows more accurate quantitative analysis.

The use of radioisotope and gamma scintigraphic imaging for assessing colonic transit in humans was first reported in 1986 (Krevsky *et al*., 1986). The early scintigraphic studies employed orocaecal intubation for instillation of isotope, with a subsequent liquid diet. The main advantage of orocaecal intubation lies in the ability to deliver a discrete bolus of isotope to the ascending colon, allowing a precise starting point for measuring colonic transit. However, the presence of a tube within the lumen of the gut is both invasive and likely to alter normal physiology.

In order to create a more physiological situation, bolus delivery of material has been achieved by packaging particulates (0.5–1.8 mm diameter pellets) within a gelatin capsule coated with a pH sensitive polymer. This prevents release until arrival in the distal ileum or proximal colon (Proano *et al*., 1990). Studies using radiolabelled Amberlite resin (0.5–1.8 mm) report ascending colon transit times of 12–13 h (Proano *et al*., 1990; Barrow *et al*., 1992). Colonic transit times, however, appear to be shorter for larger objects (Hardy *et al*., 1985; Proano *et al*., 1990), with 80% of capsules reaching the splenic flexure within 10 h of entering the colon in one study (Parker *et al*., 1988). Thus, to achieve site-specific delivery to the left-sided colon, a 15 h delayed release system would seem appropriate.

One of the most important tasks of the colon is to absorb fluids and electrolytes, and it has been shown to be able to reclaim up to 61 of water per 24 h (Debongnie and Phillips, 1978). In order to accommodate the inflow of liquid chyme from the terminal ileum, the caecum and ascending colon are able to distend, showing a greater compliance compared to the left colon (Chauve *et al*., 1976; Waldron *et al*., 1989; Ford *et al*., 1995). Additionally, the elastance (the pressure increase induced by an imposed fixed volume) has been shown to be less (Waldron *et al*., 1989) for the proximal colon, consistent with a predominant storage function (Kamath *et al*., 1990; Proano *et al*., 1990). Compatible with this idea, the ascending and transverse colon are wide calibre, thin-walled structures, in contrast to the thick, more muscular, smaller bore sigmoid colon (Torsoli *et al*., 1968).

The proximal colon has a primary role in resorbing sodium and water before delivering the processed, dehydrated stool distally. Perfusion studies in the human colon have demonstrated larger net water and electrolyte movement in the proximal than the distal colon (Levitan *et al*., 1962), with insorption most rapid in the caecum and decreasing progressively in the transverse colon, descending colon and rectum (Devroede and Phillips, 1970; Devroede *et al*., 1971). Electrical potential difference measurements support these perfusion studies, showing the distal colon to be more impermeable to sodium than the proximal colon, as evidenced by the larger negative potential differences established distally (Rask-Masden, 1973; Davis *et al*., 1982).

7.4 Imaging and Presentation of Data

The radiometal indium-111 is produced by proton bombardment of cadmium-112. It tends to be the radionuclide of choice for whole gut or colonic transit studies,

chiefly because its half-life (67.5 h) is similar to whole gut transit time and therefore good quality images are obtained whilst minimizing the absorbed radiation dose. It is a gamma ray emitter with no charged particle emission but it does decay with the emission of auger electrons which add to the radiation absorbed dose. In the chemical form of ^{111}In-indium chloride it is easily adsorbed onto ion exchange resin to form a non-absorbable marker, ensuring compartmentalization within the gastrointestinal tract after ingestion.

One of the main advantages of radionuclide studies of the gastrointestinal tract is that they allow virtually an infinite number of images to be obtained without adding any extra radiation burden to the subject. In our colonic studies, we obtain high quality images of the delivery vehicles and subsequent release of their contents by adding 1 MBq of ^{111}In-labelled Amberlite resin to the dosage form. Thirty second anterior and posterior images are then taken at 30 min intervals throughout the study periods. Release and subsequent spread of the labelled Amberlite resin allow an image of the colon to be constructed and used to establish the exact site of release of marker probes within the distal gut. Concomitant plasma and urine samples then allow an estimate of absorption which can be correlated to the site of release.

In addition to information on the site of release of formulation, an idea of the spread or dispersion of material can also be obtained. The small intestine is uniquely adapted to maximize absorption by virtue of the large surface area afforded by its villous and microvillous lining. In an analogous manner, if is increased the dispersion or spread of a drug within a particular region of the intestinal tract, this is likely to facilitate absorption from that region. Currently there are no methods available for measuring dispersion of drug within the gastrointestinal tract. The addition of labelled Amberlite resin within the delivery vehicle, however, presents an opportunity to estimate the degree of dispersion of released formulation. In our studies on colonic absorption, we have divided the colon into eight regions of interest in a modification of the method described by Krevsky (Krevsky *et al*., 1986) (Figure 7.2). We have then counted the number of regions containing at least 10% of the total activity delivered by the vehicle at a set time point after the release of formulation, and used this number as a semi-quantitative index of dispersion.

7.5 Volunteer and Patient Studies

At University Hospital Nottingham, we have used a timed release delivery vehicle in an attempt to target permeability probes to the proximal and distal colon. Although timed release systems are dependent upon a uniform transit time to effect successful targeting of a specific region, they have the advantage of allowing a ‘bolus dose’ of the probes to be delivered to a specific region. In the case of pro-drugs which release active drug following bacterial cleavage, for example, there would be a gradual and unquantifiable release of agent over a variable length of the gut rather than in a specific region. Thus for the purposes of investigating regional permeability, the use of such a timed release delivery

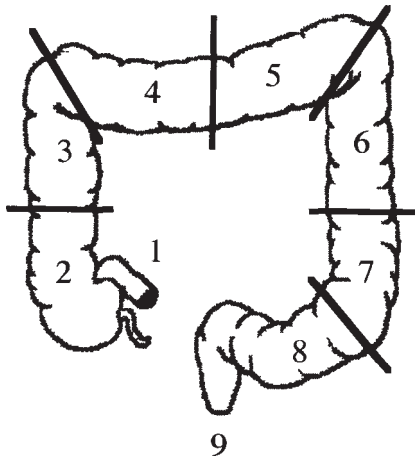
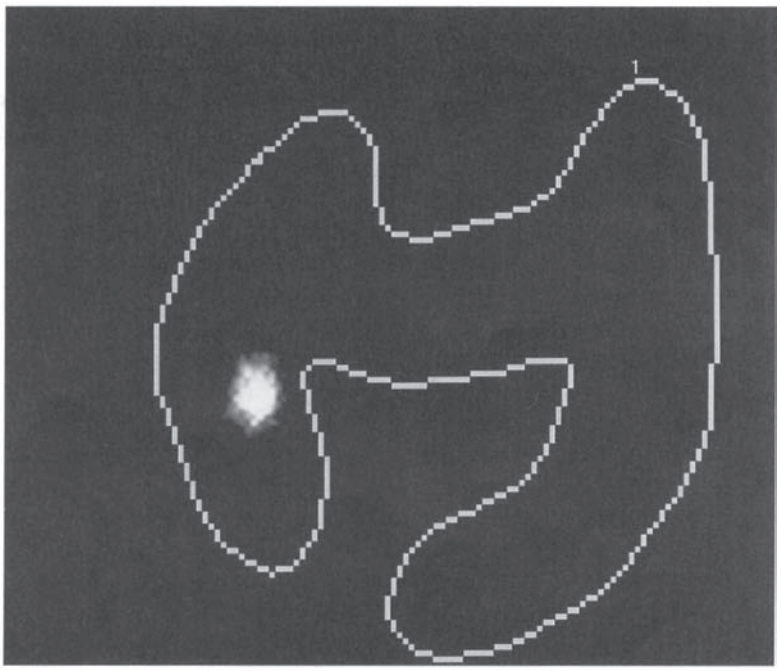
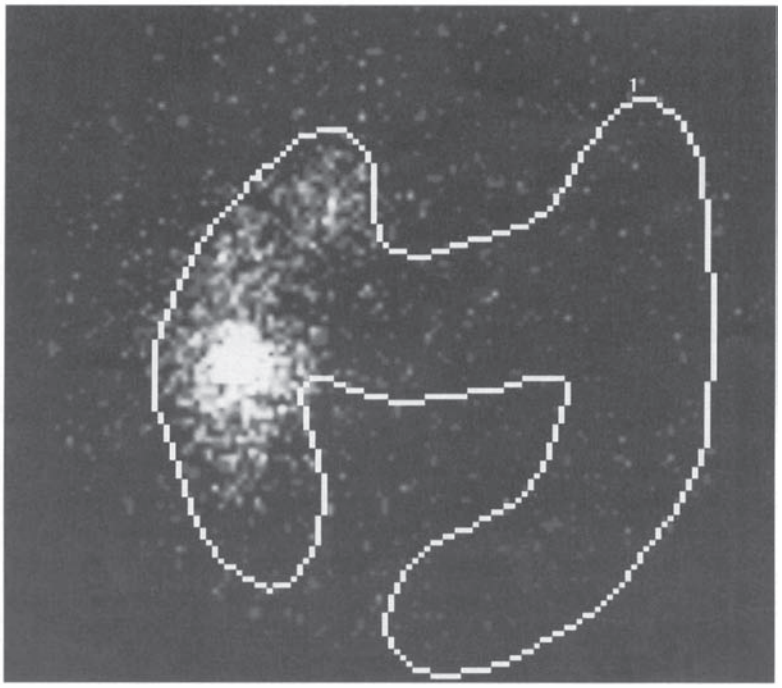


Figure 7.2 Colonic regions of interest. The colon was divided into regions of interest (ROI). The ROIs were numbered 1–8 with 9 equalling excretion. The ascending, transverse and descending colonic regions were defined by bisecting the hepatic and splenic flexures, and the junction between the descending and rectosigmoid colon was taken as the point where the image of the descending colon was seen to deviate medially

is advantageous. Our delivery system is described in Chapter 6. This contained ^{111}In-labelled Amberlite resin as well as permeability markers so that sequential gamma camera images could be obtained and the precise site of release of the permeability probes ascertained (Wilson *et al*., 1997). Release of contents from the delivery vehicle could be easily determined as judged by an increase in the area of the 'hot spot' and concomitant decrease in its intensity (Figure 7.3). In addition urine and plasma samples were obtained to assess the permeability following release from that site. Proximal colonic targeting was attempted using 5 or 6 h release delivery systems, whereas a 15 h release system was employed to preferentially target the distal colon. The 15 h system was ingested on the previous evening, so that release would occur around lunchtime the following day, facilitating imaging and ensuring equivalent conditions to the situation when dosing with the 5 h release system. We found however that the 15 h release systems were no further advanced at their anticipated release time compared to the 5 h release delivery systems, despite an extra 10 h within the gastrointestinal tract. A subsequent study demonstrated that colonic transit of both multiparticulates and large non-disintegrating capsules was significantly slowed during sleep compared to the daytime period (Hebden *et al*., 1995b), accounting for the retarded transit of our 15 h timed release systems. These data provided a valuable means of validating the subjective assessment of formulation release from the image data and showed good correlation of pulse time with the appearance of drug in the plasma (Figure 7.4).



(a)



(b)

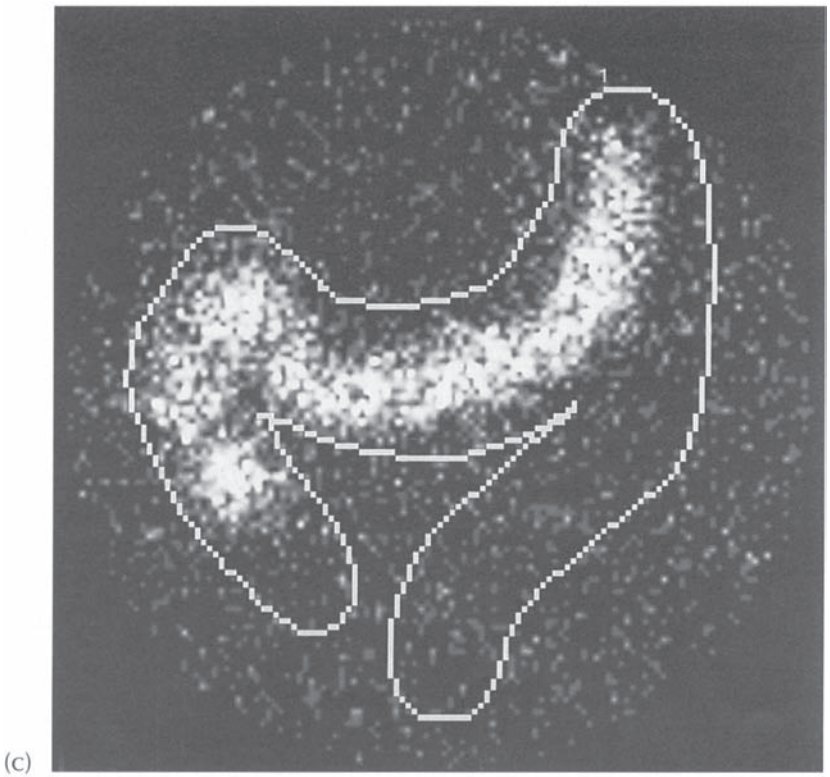


Figure 7.3 Release of contents (as shown by dispersion of ^{111}In) from the delivery vehicle, and subsequent spread around the colon, (a) Capsule intact in the ascending colon, (b) release and (c) dispersion of contents throughout the colon

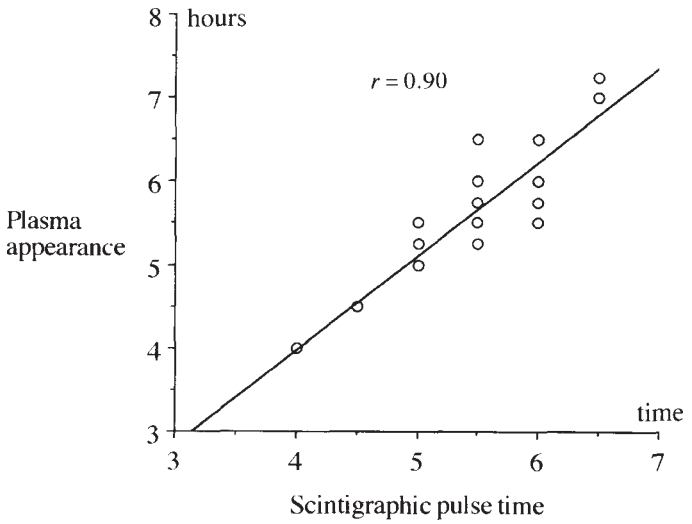


Figure 7.4 Scintigraphic pulse (release) time versus plasma appearance of quinine. There was good correlation between release of quinine as judged by scintigraphic imaging, and appearance in the plasma (n = 20)

Although we were not able to predictably target the proximal or distal colon, individual variations in intestinal transit times meant that initial release of the permeability probes from the 5 and 6 h timed release systems occurred in different regions of the distal gut. Twenty hour urine collections of quinine (a transcellular permeability probe) demonstrated progressively reduced absorption with more distal release within the gastrointestinal tract (Hebden *et al*., 1995c). Thus, we found that release of quinine within the distal small intestine resulted in 6.3% absorption, within the ascending colon 4.5%, and within the transverse colon 2.7%.

These findings gave rise to two possibilities: either the lining of the gut becomes progressively less permeable in the aboral direction, or dispersion of released contents is limited by the progressively dehydrated and viscous stool hence lessening the opportunity for mucosal contact and permeation. The use of gamma scintigraphy allowed us to explore these possibilities further.

We therefore went on to manipulate the fluidity of the stool within the colon by pre-dosing healthy volunteers with either lactulose or codeine to increase or decrease stool fluidity, and assess the influence of this intervention on dispersion and permeation of our probes. Using the method outlined earlier, we found that dispersion of the ^{111}In-labelled resin (and therefore by inference the dispersion of the permeability probes) was significantly increased by pre-dosing with lactulose compared to codeine. Furthermore, there was a clear trend for reduced quinine permeation as colonic stool water was reduced (Hebden *et al*., 1996).

The studies described above made use of a timed release delivery system and were concerned with a single dosing only. It is of interest however to look at the 'equilibrium' state within the colon which exists with daily dosing of drug. We addressed this by dosing healthy volunteers on 4 consecutive days with an enteric-coated capsule of ^{111}In-labelled resin, and then imaging throughout the fourth day. The results showed that significantly less material resided in the left colon (descending and rectosigmoid regions) compared to the right colon (ascending and transverse regions) (Figure 7.5), implying a relatively reduced exposure of the left colon to orally dosed formulations (Hebden, 1995a). Further studies in patients with active distal ulcerative colitis (affecting the left side of the colon) have shown the amount of material resident within the affected region is further reduced, effectively limiting the efficacy of oral topically acting agents within the colon. After 4 days of once-daily dosing, only 0.16 × daily dose was resident in the left side of the colon in patients with active distal ulcerative colitis, compared to 0.81 × daily dose in healthy controls (Hebden *et al*., 1997).

7.6 Value of Studies in Formulation Design

In addition to providing data on colonic physiology, employing radionuclides and gamma scintigraphy can therefore be seen to provide important data for the pharmaceutical industry in the formulation of their drugs. They provide tools for assessing:

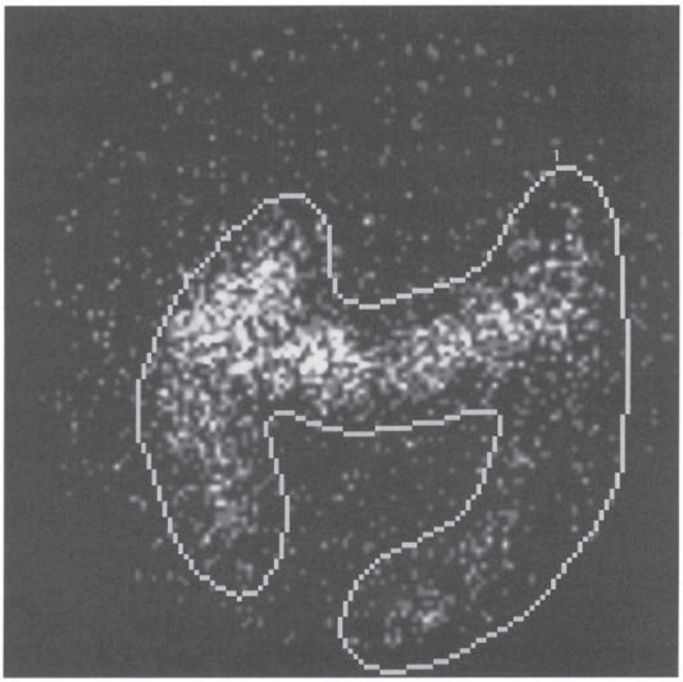


Figure 7.5 Scintigraphic image of a healthy volunteer (on fourth day of daily dosing), showing paucity of material in the left side of the colon

- the ability to target the release or delivery of a formulation to the colon, and assess the effects of time of dosing (morning or night-time) on delivery;
- the effect of formulation size on delivery (smaller particulates for example have slower transit through the colon; large capsules show markedly delayed gastric emptying in the fed state which may affect the timing of colonic delivery);
- regional permeability within the colon, which has implications for bioavailability and the plasma profile of the drug;
- the dispersion characteristics of a formulation which are likely to influence absorption characteristics;
- the residence of material within the colon in the equilibrium state and therefore assess the likely exposure of different regions of the colon to the formulation.

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8

Scintigraphic Study of Ocular Drug Delivery

C.G.WILSON, Y.P.ZHU and M.FRIER

8.1 Introduction

The topical route is the most commonly utilized method of administering a medication to the eye. Theoretically, it is adequate for the treatment of most external conditions of the eye and those of the anterior segment, which includes the iris and ciliary muscle. Introducing the drug directly to the conjunctival sac should in theory localize the drug effect, facilitate drug entry which is otherwise hard to achieve by systemic delivery and avoids first-pass metabolism. In practice, topical application frequently fails to establish a therapeutic drug level for a desired length of time within the target ocular tissues and fluids. Inefficient ocular treatment is a result of many factors, including the rapid precorneal clearance, the highly selective absorptive properties of the corneal barrier, the unproductive drug loss via the conjunctival route and the difficulty that many people, particularly the elderly, have in dosing eyedrops to the eye. Typically, less than 5% of the instilled dose reaches the aqueous humour (Jarvinen and Jarvinen, 1996).

8.2 Problems with Ocular Drug Delivery

8.2.1 The Clearance Mechanism

The eye has an efficient drainage system which removes excess lacrimal fluid and cell debris from the precorneal area. The action of blinking spreads the tear fluid over the surface of the eye, preserving the acuity of the ocular apparatus. When the upper lid approaches the lower lid, the tear fluid is forced medially towards the lacrimal puncta, which are connected to the lacrimal sac via the canaliculi. Tears from the lacrimal sac drain into the nasolacrimal duct which leads to the nose, where it empties laterally to the inferior nasal meatus, as shown in Figure 8.1. This drainage pattern is commonly observed for substances dissolved

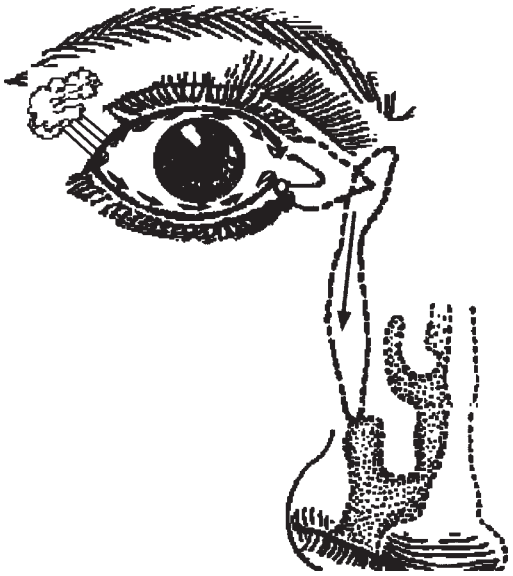

Figure 8.1 Tear drainage (flow of tears)

in the tear film; and clearance is augmented by high tear turnover and lacrimation. Irritant substances are therefore cleared rapidly from the eye surface.

Under normal conditions the volume of the human tear film is about 7–9 µl and is relatively constant. The maximum amount of fluid that can be temporarily accommodated in the lower lacrimal lake is 25–30 µl, but only a small proportion of a solution can be incorporated in the precorneal film without causing it to destabilize. When eyedrops are administered, the tear volume is suddenly increased which can cause rapid reflex blinking. Most of the eyedrop is pumped through the lacrimal drainage system into the nasolacrimal duct, and excess fluid is spilled on the cheeks and splashed on the eyelashes. The drainage rate of the solution is related to the instilled volume; the smaller the volume the slower the drainage rate. Several workers have hypothesized that the instilled drop should have a volume of around 8 µl and be no larger than 15 µl (Edman, 1993). However, the typical drop size delivered by commercial eyedroppers is in the range 25–56 µl (Brown and Lynch, 1986). Only a small proportion of the drug dosed in the eyedrop can therefore be retained by the eye. Formulations usually disappear from the cul-de-sac within 5–10 min following instillation in rabbits and 1–2 min in humans (Wilson *et al*., 1983; Robinson, 1993). The rapid loss of material is of consequence because systemic side-effects may result from rapid absorption of drug through the mucous membrane of the nasolacrimal duct.

8.2.2 The Local Effect/Systemic Effect Balance

Tears and drugs contained in tears are drained into the nasal lacrimal duct by the zipper-like action of the eyelids pumping excess fluid into the drainage system.

The nasal meatus is a highly vascular area and drugs in the tears are readily absorbed into the systemic circulation through this region. Together with the conjunctiva/scleral route, these systems are effective in allowing the systemic absorption of ocular drugs (see Figure 8.2, colour section), which proceeds at a rate typically one order of magnitude greater than their ocular absorption (Urtti, 1994).

For the purposes of ocular treatment, systemic absorption is undesirable as it reduces the ocular bioavailability and leads to systemic side-effects due to the potent action of many ophthalmic drugs, including ß-blocking agents, atropine, steroids, epinephrine and pilocarpine on other body tissues (Salminen and Huupponen, 1989). However, over the past two decades, studies have suggested that systemic absorption via the ocular route might be very useful for the systemic delivery of peptides and several patents have been filed for delivery of glucagon and insulin. Clearly, the ability to quantify the ocular residence time provides a powerful method of selecting the appropriate drug delivery vehicle.

8.3 *In Vivo* Methods of Assessment of Ocular Retention

Drug levels in the aqueous humour or other interior tissues in man are difficult to ascertain directly and a number of indirect methods have been developed to estimate ocular bioavailability. Methods employed include tear sampling, the dye dilution test, pharmacodynamic evaluation, reflectance spectrophotofluorimetery, gamma probes and lacrimal scintigraphy.

Typical applications of the tear sampling method have been described to follow the precorneal kinetics of 0.3% ofloxacin and 0.3% tobramycin ophthalmic solutions to healthy volunteers (Limberg and Bugge, 1994; Tang-Liu *et al*., 1997). The method involves the removal of a small volume of tear fluid from the eye. The process of sampling requires great care as the removal of volumes as small as 1 µl can disturb the dynamics of the tear film which represents more than 10% of the basal tear volume.

Bach and co-workers utilized a dye dilution technique to evaluate the ocular residence times of hydroxypropylmethyl cellulose and polyvinyl alcohol solutions. Argyrol marker was applied to stain the corneal and conjunctival epithelium (Bach *et al*., 1972). Similar methods employing fluorescein/rose bengal are described in the literature. These techniques provide a limited ability to measure turnover and more quantitative tests are needed. A non-invasive technique based on an administered radiolabel and an external gamma probe was first described in 1973 by Chrai and colleagues. The technique was used to measure the residence time of ophthalmic formulations labelled with technetium-99m in the rabbit (Chrai *et al*., 1973). In our laboratories, we have found the probe technique useful to perform measurements in the Macaque monkey, where the use of a gamma camera is impractical (Greaves, 1994). An important limitation of this technique is that it is not possible to accurately resolve the distribution of the activity over the eye surface and drainage system.

Reflectance fluorometry is a non-invasive technique first described by Maurice and colleagues. The technique has been extensively used to measure intra-ocular kinetics and the behaviour of some drug vehicles, for example the residence of gellan gums in the eye (Maurice and Srinivas, 1992). The application is limited to transparent structures and linearity is fairly restricted. Lacrimal scintigraphy, using a radiolabelled probe and a gamma camera, sometimes fitted with a pinhole collimator, is an attractive alternative technique. The technique was first described by Rossomondo as a method of assessing blockages of the nasolacrimal duct (Rossomondo *et al*., 1972). In comparison to fluorometry, lacrimal scintigraphy provides a quantitative measure of the precorneal distribution and a simultaneous measurement of the proportion of the dose drained down the lacrimal duct.

As described elsewhere in this volume, gamma scintigraphy has found wide application in the measurement of formulations in man and over the past 30 years has become an established technique in pharmaceutical research. For human ophthalmic use, the isotope of choice is technetium-99m, in order to restrict dosimetry to the radiation-sensitive lens. The usual procedure involves the incorporation into the formulation of a small amount of sterile fluid containing technetium-99m at high specific activity. Deposition and clearance of the material is followed by serial measurements of the eye to determine the precorneal activity-time profile. The technique is associated with a low dosimetry and therefore can be used on healthy male and female volunteers. Table 8.1 gives typical examples of ophthalmic formulations tested using this technique.

8.4 Imaging and Analysis

8.4.1 Imaging Procedure

For the test, the subject is encouraged to arrive early to allow sufficient time to acclimatize to the procedure. The subject is seated in front of a gamma camera fitted with a 3 mm aperture pinhole collimator and the head supported by an ophthalmic table positioning the eye 5 cm from the collimator. 25 µl of the radiolabelled formulation is then placed in the lower fornix according to a randomized allocation schedule. Precise time of the completed instillation of the test formulations is recorded. Clearance is normally monitored for 8 min using dynamic imaging (96×5 s frames) followed by a series of static images over a period of 30 min to 2 h. Data are stored on optical disk for subsequent analysis. A suitable control such as saline should be used to allow for a clear interpretation of the data. Subjects sometimes stare into the collimator and the blinking pattern is abnormal. This is evident in the analysis, when a distinct ‘day 1’ effect is noted. It is therefore important to ensure that the subject is comfortable and familiar with the technique. Contact lens wearers or subjects receiving ocular drug therapy or any other form of drug therapy on a regular basis would normally be excluded from this type of trial unless this were the object of the study.

Table 8.1 Ophthalmic formulations evaluated using lacrimal scintigraphy

Formulations	Species	Sources
Methylcellulose	man	Hardberger *et al.* (1975)
Polyvinyl alcohol	rabbit	Zaki *et al.* (1986); Wilson *et al.* (1983); Davies *et al.* (1991); Fitzgerald *et al.* (1992)
	man	Hardberger *et al.* (1975); Trueblood *et al.* (1975); Zaki *et al.* (1986); Greaves *et al.* (1992); Fitzgerald *et al.* (1992); Snibson *et al.* (1992)
Hydroxyethylcellulose	rabbit	Greaves *et al.* (1990); Meseguer *et al.* (1993a,b)
	man	Kitazawa *et al.* (1977); Greaves *et al.* (1990); Meseguer *et al.* (1993a,b)
Hydroxypropylmethylcellulose	rabbit	Zaki *et al.* (1986)
	man	Trueblood *et al.* (1975); Kitazawa *et al.* (1977); Zaki *et al.* (1986); Snibson *et al.* (1992)
Xanthan gum	rabbit	Meseguer *et al.* (1993b)
	man	Meseguer *et al.* (1996)
Gelrite®	rabbit	Greaves *et al.* (1990); Meseguer (1993b)
	man	Greaves *et al.* (1990)
Thermoreversible gel	rabbit	Gurny *et al.* (1987)
pH-reversible latex	rabbit	Gurny *et al.* (1987); Ibrahim *et al.* (1987)
18F-imirestat	rabbit	Vaidyanathan *et al.* (1990)
Ointment	man	Greaves *et al.* (1993)
Hyaluronate	rabbit	Gurny *et al.* (1987)
	man	Gurny *et al.* (1990); Snibson *et al.* (1992)
Timolol solutions	rabbit	Lee *et al.* (1996)
Liposomes and nanoparticles	rabbit	Fitzgerald *et al.* (1987); Davies *et al.* (1989)
W/O microspheres	rabbit	Durrani *et al.* (1995a,b)
Carbopol	rabbit	Davies *et al.* (1991, 1992)
	man	Wilson (1998)

8.4.2 Data Analysis

The stored data are recalled and the dynamic sequence summed to give an image of the eye and the drainage apparatus. This image is then analysed by creating five regions of interest: (i) cornea, (ii) inner canthus, (iii) lacrimal duct, (iv) whole

eye, (v) background (see Figure 8.3, colour section). The total activity in the three anatomical ROIs (cornea, inner canthus, lacrimal duct) in the first frame is assumed to be 100% of the instilled dose, calculated from the first frame. The remaining activity in the ROI at each time point is calculated as a percentage of the initial activity. The count rates from the regions of interest are corrected for background and isotope decay using a validated spreadsheet document. Percentage remaining in each ROI as a function of time is plotted for each test solution in each subject. Data sets are then combined to produce mean plots (±s.d.) for each solution and for each region of interest.

8.4.3 Quantitative Endpoints

There are four analytical methods commonly used for the evaluation of the dosage forms, including (i) measure the time necessary for eliminating 50% (T_{50}) or 63.2% (MRT) of the dose administered; (ii) measure the proportion of the dose administered remaining after a given time; (iii) calculate the values of the rate constants for the elimination of the drug in the two sections of the profile; or (iv) calculate the values of AUC (areas under the % remaining vs. time curves).

8.5 Scintigraphic Studies of Various Ophthalmic Formulations

Simple non-viscous aqueous solutions have the disadvantage that most of the instilled solution is lost within the first 15–30s post-instillation due to rapid drainage (Shell, 1982). In an attempt to prolong ocular contact times of drugs, a variety of viscosifying agents, including hydroxyethylcellulose, polyvinyl alcohol, sodium hyaluronate, gellan gum and carbomer, and other novel systems have been employed (Greaves and Wilson 1993; Meseguer *et al*., 1993a and b; Kumar and Himmelstein 1995; Zignani *et al*., 1995). The ocular pharmacokinetics of these formulations have been widely investigated using gamma scintigraphy.

8.5.1 Hydroxyethylcellulose (HEC)

Cellulosic excipients can be conveniently labelled with diethylenetriaminepentaacetic acid labelled with Tc-99m (^{99m}Tc-DTPA), available in kit form (Pententate®, Amersham Radiopharmaceuticals, UK). Sodium pertechnetate is prepared at high specific activity by elution of an Elumatic III technetium-99m generator (CIS International) using a vacuum vial to yield a target of around 2500 MBq in 1 ml.

Test materials are conveniently provided as sterile 5 ml ampoules or unopened material passed for human use. In a typical investigation, samples are radiolabelled on the day of the trial by the addition of sterile ^{99m}Tc-DTPA to give an activity of 1 MBq per dose at the time of administration. The labelled formulations are used within 3 h of preparation.

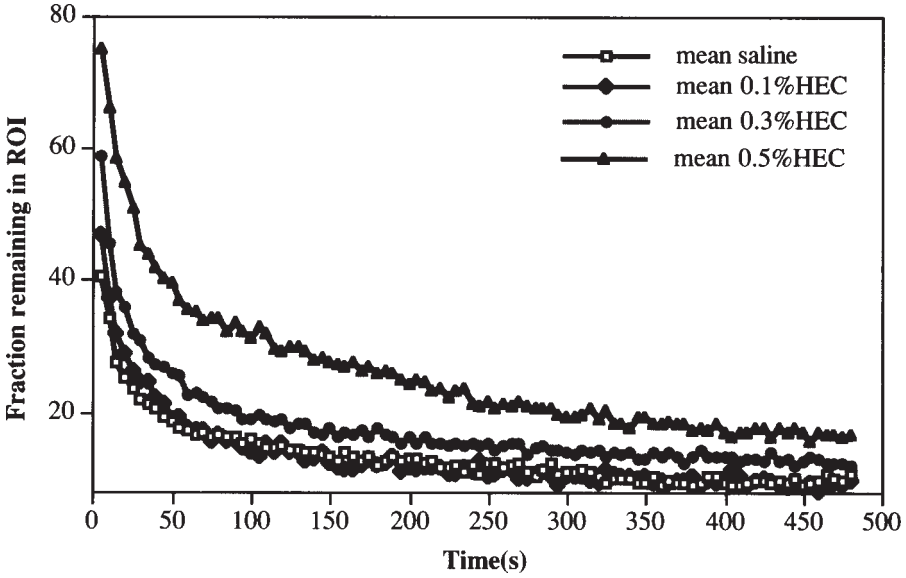

Figure 8.4 Mean corneal clearance of ^{99m}Tc-labelled HEC or saline formulations in man (data generated from dynamic images, n = 11)

Table 8.2 The mean AUC values in the corneal ROIs and mean residence time for HEC (0, 0.1, 0.3 and 0.5% w/v) formulations in man (mean±s.d., n = 11)

Formulations	Saline	0.1% HEC	0.3% HEC	0.5% HEC
AUC_{0-30}(% min)	258.8 ± 140.0	295.6 ± 160.1	391.4 ± 169.7	505.4 ± 256.1
MRT(s)	9.9 ± 6.8	15.5 ± 15.1	19.7 ± 15.8	109.7 ± 60.1

Work by Professor Gurny's group at Geneva confirm these findings. HEC at 0.325 and 0.5% w/v were tested against a non-viscous control. A similar behaviour in ocular clearance was shown by HEC solutions, i.e. the precorneal retention of 0.5% HEC was significantly greater than that of 0.325% HEC, which, in turn, was greater than that of a reference solution (Meseguer *et al.*, 1993a,b)

The mean activity-time profile generated from dynamic study following administration of the HEC solutions is shown in Figure 8.4. As expected, the ocular residence of the HEC formulations is related to the viscosity of the vehicles. 0.1% HEC formulation is an almost non-viscous solution (4 cps), which would not be expected to increase retention. This is in agreement with the observed short precorneal residence time. The experimental data indicates that 0.5% HEC provides superior ophthalmic residence compared with the diluted HEC solutions or saline. (See Table 8.2.) The relatively inexpensive vehicle is therefore the viscosifier of choice in many ophthalmic formulations.

The results suggest that the ocular residence is improved when the thicker solution is applied, because the precorneal contact time is related to the ocular drug bioavailability (Patton and Robinson, 1975). This is confirmed when the solutions are compared as a function of the area under the curve (AUC) or mean residence time (MRT).

8.5.2 Polyvinyl Alcohol (PVA) Inserts

Aseptic production of inserts based on polyvinyl alcohol containing pilocarpine nitrate and ^{99m}Tc-DTPA has been carried out in our laboratory (Greaves *et al*., 1992). The polyvinyl alcohol used in the preparation of inserts was a highly soluble form of the polymer (Gohsenol GH-7) with a median molecular weight of 98000 with a 87–89 mol% degree of hydrolysis. The casting solutions were prepared at a concentration of 2.58% w/w to allow for dilution of the formulation to 2% w/w with the radioactive marker.

A stainless steel hand-spreader was used to cast a PVA/pilocarpine nitrate film onto a Melinex backing strip. A film approximately 35×8 cm was cast and left to dry in a ventilation cupboard for approximately 1 h, and cut into 25 mm^2 sections using a sharp scalpel. The inserts were then stored in a sterile petri dish until required.

Ocular residence of the test formulations were compared in 12 healthy volunteers (8 males and 4 females, aged 19–20 years). The PVA gel insert (5 mm^2) was placed in the low fornix of the right eye with sterile forceps. The radiolabel was found to remain on the ocular surface significantly longer when delivered by a film insert than when delivered in a solution, with a mean half-life of 406±217 and 2.9±1.5 s, respectively. Pilocarpine delivered in the insert produced significantly decreased intraocular pressure in the test eye compared with the control eye. This effect did not occur after delivery of pilocarpine as a solution. The novel system showed considerable promise for prolonged drug delivery because vision is minimally affected by the presence of an insert positioned on the sclera.

8.5.3 Gellan Gum

Gellan gum is a multifunctional hydrocolloid for use in a wide variety of food products as a gelling, texturizing, stabilizing, film-forming, suspending and structuring agent. It can also be used, like other gelling agents, to prepare structured liquids, sometimes referred to as ‘fluid gels’. The gelling properties of gellan gums are related to the ability to form a double-helix structure in spite of the presence of substituents and side chains. Association between double helices is facilitated by ions and water molecules and a colloidal suspension environment of low ionic strength is relatively fluid. In the presence of the cations provided in the normal tear film, interactions between the polymer helices lead to entanglement and a rapid rise in viscosity.

To label gellan gum, the material is prepared as an over-strength solution (0.75% w/v). Using the conventional radiolabelling method described previously, ^{99m}Tc-DTPA was added to 2.0 ml of 0.75% Gelrite® to give a final concentration of 0.6% w/v Gelrite®. Ocular distribution of the material was examined in rabbits and in volunteer subjects.

In man the 0.6% w/v Gelrite® solution was found to remain on the cornea longer than the saline solution (Greaves *et al*., 1990). Detectable quantities of gellan gum remained on the cornea or at the site of application for up to 100 min, with a mean half-time of 18.1±24.7 min.

Greaves and co-workers found that the ocular behaviour of the Gelrite® in rabbit differs markedly from that in man. In the rabbit the vehicle drained rapidly from the cornea into the inner canthus with a mean half-time of 1.1±0.6 min. However, the vehicle gelled immediately in the inner canthus where more fluid is available. The physiological and anatomical differences between the two species may cause the differences in ocular behaviour of the vehicle between the rabbit and man. Basal tear turnover is higher and reflex tearing is much more pronounced in man than in rabbits. Both factors lead to a dilution of viscous solutions but provide a large volume, hence more cations for gelation of the Gelrite® in the precorneal area in man. Once the gel has formed on the surface of the eye, the cation concentration in the tears ensures that the vehicle does not dissolve. The formulation is subsequently dispersed by the shearing action of the eyelids which fragments the gel.

8.5.4 Mucus Glycoproteins

Because mucins secreted by the periocular apparatus have an important function, various studies have been proposed to follow the kinetics of mucins in the tear film. A novel method of radiolabelling mucus glycoproteins was described by Greaves (1994), based on an established method for labelling antibodies. Disulphide bridges within the glycoprotein are cleaved by the use of the reductant 2-mercaptoethanol. Following a subsequent purification the labelling is performed via Sn++ reduction of the pertechnetate in the presence of an excess of a weak competing chelating ligand; these conditions are provided by the use of a radiopharmaceutical methylene diphosphate (MDP) bone-scanning kit.

To label the material, mucus glycoprotein suspension was mixed with 2-mercaptoethanol solution and left to reduce for 30 min. The mucus glycoprotein sample was passed through the Sephadex G-5 column and the column washed with phospate buffered saline (PBS). The washings were combined and 0.5 ml of the sample, 5 ml saline and 1 ml Tc-99m sodium pertechnetate (approximately 500 MBq) added to an MDP kit. The reconstituted kit was then left for 2 min.

The radiolabelled mucus glycoprotein was combined with a sample of unlabelled mucus glycoprotein to produce a 0.5% mucus glycoprotein suspension. The precorneal residence of the radiolabelled mucus glycoprotein suspension was studied in New Zealand white (NZW) rabbits.

The scintigraphic study showed that approximately 70% of the ^{99m}Tc-labelled mucus glycoprotein suspension cleared from the ocular surface within the first minute following administration. However, 30% of the mucin glycoprotein remained associated with the ocular surface up to 10 min and 15% was detected 30 min after instillation. In contrast, 90% of the Tc-99m MDP solution had cleared from the ocular surface within the first minute, with less than 5% remaining 10 min post-dose. The Tc-99m MDP solution cleared rapidly into the inner canthus and then drained into the nasolacrimal duct; the ^{99m}Tc-labelled mucus glycoprotein suspension which drained rapidly from the ocular surface appeared to become attached to the nictitating membrane. Over 50% of the mucus suspension remained attached to the nictitating membrane for 30 min post-instillation.

In this study, it was difficult to differentiate between the inner canthal region and the nictitating membrane on the scintigraphic images due to the size and position of this membrane. Ideally, the test materials should be administered to a species without a nictitating membrane and several investigators have described the surgical removal of the nictitating membrane of the rabbit before ophthalmic dosing, making the rabbit a more suitable animal model for the prediction of the ocular behaviour in man. This would establish whether the mucus glycoprotein is binding to the nictitating membrane, being trapped between the membrane and the ocular surface or whether a considerable portion of the mucus glycoprotein is adhering to the inner canthal region.

8.5.5 Liposomes and Particulates

Liposomes are microscopic vesicles composed of membrane-like lipid bilayers surrounding aqueous compartments. This unique feature enables liposomes to encapsulate both lipophilic and hydrophilic compounds. Consequently liposomes have been investigated extensively as the basis for drug carrier systems by various routes of administration. One of the more recent applications is the concept of employing liposomes as drug carriers in ophthalmology.

The preformed small unilamellar vesicles may be labelled with technetium-99m pertechnetate using a stannous chloride complex (Fitzgerald *et al*., 1987). The method is outlined as follows: a dilute solution of stannous chloride (2.5 mg/ml) is prepared in deoxygenated distilled water and the pH reduced to 1.0 by the addition of 0.1 N HCl. The liposome suspension (1 ml) is then shaken with 0.1–0.5 ml stannous chloride solution followed by the addition of 0.5 ml technetium-99m pertechnetate (75–100 MBq/0.5 ml) in sterile saline. The mixture is briefly vortexed and allowed to incubate at room temperature for 1 h. Excess activity is then removed by an ion-exchange method. Pre-washed Dowex Ag 1-X8 resin was added to each suspension and shaken. The suspension was decanted from the resin, which was then washed with two further fractions of saline. The washing fractions were combined and centrifuged to remove any tin colloid that may have formed. The liposomes in the supernatant are then centrifuged at 140000 *g* (4°C) for 1 h to concentrate the sample. Aliquots (10 μl) are removed and assayed

at each stage of the labelling process to determine the efficiency of labelling. The target efficiency is >70%.

8.5.6 Nanoparticles

Particulate carriers in theory offer the opportunity to saturate the tear film and sustain drug concentrations on the cornea and conjunctiva for longer. Large particulates are irritant and there has been much interest in sub-micron particles: nanoparticles and nanocapsules. Nanoparticles can be surface labelled with [^{111}In] oxine. A complex is freshly prepared from [^{111}In] indium chloride and 8-hydroxyquinoline, and added dropwise to the neutralized filtered nanoparticle suspension. Using this method, an average binding capacity of 95±1.3% was achieved (Fitzgerald *et al*., 1987).

The clearance of nanoparticles and liposomal preparations was tested in a group of six NZW rabbits. 25 µl of the material was instilled directly onto the corneal surface and the animal positioned in front of the pinhole collimator of the gamma camera.

The ocular clearance of liposomes and nanoparticles from the cornea was multiphasic in nature, with an initial rapid phase (150 s) followed by a slower phase. Clearance of all tested colloids in the inner canthal region was shown to be monophasic and exponential and was significantly slower than the reference solution.

It was found that the particle size and surface charge are important factors in determining ocular residence times. Positively charged small unilamellar vesicles were retained significantly longer than negatively charged or neutral small unilamellar vesicles on the corneal surface due to the electrostatic attraction of small positively charged particles to the negatively charged epithelium. Particles were found to be retained mainly in the inner canthal and extra-ocular regions of the eye and not on the corneal surface where significant drug absorption takes place.

8.5.7 Carbopols

Carboxyvinyl-based polymers including Carbopol 940 have proved to be extremely useful in the formulation of sustained release gels. Schoenwald and Boltarik demonstrated a four to ten-fold increase in the corneal and aqueous humour concentration of prednisolone acetate or prednisolone phosphate suspended in Carbopol 940 compared to the reference aqueous suspensions (Schoenwald and Boltarik, 1979). Investigators have shown that the addition of 0.05% sodium fluorescein or 0.01% w/v disodium edetate caused a significant change in the rheological properties of carbomer formulations (Unlu *et al*., 1991). Therefore, the ocular contact times of the gels would be underestimated if using sodium fluorescein as a tracer for *in vivo* fluorophotometric studies.

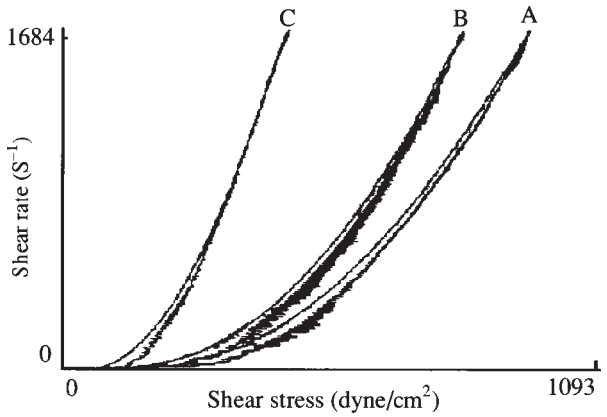

Figure 8.5 The effect of sodium ions on flow curves of GelTears. A: GelTears, h' = 1.93 cps; B: GelTears with low sodium ^{99m}Tc-DTPA, h' = 1.77 cps; C: GelTears with isotonic sodium ^{99m}Tc-DTPA, h' = 0.72 cps, where h' is apparent viscosity at shear rate 1684 s^{-1}

In our pilot studies, the incorporation of ^{99m}Tc-sodium pertechnetate into isotonic saline at concentrations of 4–10% v/v into carbomer gels caused visible viscosity changes in the vehicle. In order to accurately evaluate the precorneal residence of the carbomer gels, it was necessary to develop a novel method to reduce the concentration of sodium added in the labelling procedure.

Tc-99m was prepared in the form of ^{99m}Tc-sodium pertechnetate by elution in sterile saline from a Mo-99/Tc-99m generator (CIS UK Ltd). To concentrate the pertechnetate, an aliquot of the eluate (0.25 ml, 3 GBq) is extracted into an equal volume of n-butanone. The organic supernatant is then transferred to a clean vial and dried under a stream of warm dry air in a laminar flow cabinet. Finally, the residue is redissolved in 100 µl DTPA solution (Technescan, Mallinckrodt Medical) reconstituted with sterile water. This modified low sodium ^{99m}Tc-DTPA adduct is then used for radiolabelling the carbomer 940 gel.

A comparison of the apparent viscosities of GelTears labelled with ^{99m}Tc-DTPA in isotonic sodium chloride solution, low sodium ^{99m}Tc-DTPA and the unlabelled product showed that the rheological behaviour of the labelled GelTears was altered on addition of the label (see Figure 8.5). However, the rheogram was closer to the original product in the case of the low sodium ^{99m}Tc-DTPA. The use of the n-butanone extraction step significantly reduced the sodium concentration added to the gel, thereby maintaining the gel strength. We have found this method is suitable for the labelling of all carbomer-based ophthalmic formulations studied to date.

8.5.8 Labelled Micronized Carbon

Frequently, an acceptable solid to mimic the behaviour of suspended solids is required to model the behaviour of ophthalmic suspensions. In the past, finely

ground Amberlite ion-exchange resin has been used, although the particle size range is usually broad. Particles larger than 15 microns may be uncomfortable and cause the sensation of grittiness, leading to lacrimation. Recently, we have explored the use of charcoal as an alternative. This material has several advantages:

- it can be heat sterilised;
- it is available as a particulate with a mean size of around 5 microns;
- it is inert;
- it adsorbs ^{99m}Tc-DTPA with great avidity.

In the labelling process, sterile carbon (~50 mg) is mixed thoroughly using a sterile spatula with ^{99m}Tc-DTPA solution (~10 µl) and dried. The radiolabelled carbon is then added to the formulation. The method is also suitable for preparing a formulation containing low levels of sodium.

The clearance of radiolabelled carbomer 940 products labelled in the solute phase and with the carbon suspension have been compared by Wilson *et al*. (1998). Saline was used as the control in this study, in which 12 subjects received the three ophthalmic formulations in a randomized sequence.

It was shown that the residence times on the eye of the gel formulations were significantly greater than that of the saline control. At 8 min post-dosing, the label levels retained (mean±s.d.) were: saline, 7±7%; ^{99m}Tc-DTPA gel, 42±27% and ^{99m}Tc-carbon gel, 42±20%.

8.5.9 Labelling Emulsions

Emulsions are defined as a mixture of two immiscible liquids, one of which is dispersed uniformly in small droplets throughout the other. The dispersed liquid is called the internal or discontinuous phase, and the dispersed medium the external or continuous phase. The system is stabilized by the addition of an emulsifying agent. Emulsions, particularly those based on castor oil, are used as vehicles in ocular delivery systems. Radiolabels can be incorporated into either the aqueous phase or the oil phase. The aqueous phase presents few problems, because the majority of technetium-99m radiopharmaceuticals exist in the form of hydrophilic, polar molecules, and therefore provide models for water-soluble drugs. For the study of emulsion formulations containing lipophilic drugs, alternative models must be considered (Wilson *et al*., 1997). Some lipophilic radiopharmaceuticals have been examined by Budisantoso *et al*. (1997), to assess their suitability as drug models in an emulsion comprising castor oil and water. Radiopharmaceuticals investigated were ^{99m}Tc-exametazime (Ceretec, Nycomed Amersham), ^{99m}Tc-hexakis-methoxyisobutyl isonitrile (Cardiolite, Du Pont), ^{111}In-indium oxine (Nycomed Amersham), and three other ^{99m}Tc-hexakis-isonitrile derivatives (*t*-butyl, *n*-butyl and *cyclo*hexyl) synthesized in the laboratory. Rates and extents of partitioning in an emulsified system comprising castor oil and water were assessed. Radiochemical purity determinations on the radiopharmaceuticals were performed

where appropriate by HPLC using a reversed phase column. In order to achieve some simulation of the *in vivo* conditions encountered by formulations on administration, serum albumin was incorporated into the aqueous phase, at 0.5% and 5% concentrations.

All compounds showed a high degree of lipophilicity. Active compounds introduced into the castor oil phase generally remained associated with the oil although Ceretec did show some losses into the aqueous phase. HPLC analysis showed this activity to be present as ^{99m}Tc-pertechnetate, with no evidence of the primary or secondary complex. Indium oxine demonstrated a high affinity for the oil phase, but in the presence of serum albumin 5.0% in the aqueous phase, some loss from the oil phase did occur. None of the technetium compounds behaved differently in the presence or absence of serum proteins. Active compounds introduced into the aqueous phase migrated at different rates into the castor oil. Ceretec demonstrated the fastest rate but again was shown to be unstable and ^{99m}Tc-pertechnetate was detected on HPLC analysis. The stability was improved by increasing the tin (II) concentration of the formulation by the addition of stannous pyrophosphate. Of the isonitriles, *n*-butyl showed the highest affinity.

All compounds studied have potential as models for lipophilic drug formulations. The use of Ceretec is limited by instability problems to studies of short duration (<30min). The use of ^{111}In indium oxine may be limited in situations where formulations come into contact with high concentrations of protein in solution. This is probably caused by a disassociation of the indium from the oxine in the presence of other ligands on the protein molecule with higher affinity constants for indium (Kaempfer, 1987). Isonitriles appear to be highly stable chemically, and remain associated with the oil phase for long periods of time (>2 h). Tert-butyl and methoxy*iso*butyl isonitriles both have an established use in myocardial imaging and have therefore been evaluated toxicologically. The other isonitrile compounds have similar properties, but have not previously been used in human subjects. Isonitriles offer great potential as lipophilic drug models but toxicological evaluation of some derivatives is necessary.

8.5.10 Non-aqueous Vehicle Systems

The high surface tension of an aqueous formulation, 72.75 dynes/cm for water at 25°C, does not permit the drop size to be reduced much below 25 µl. Clearly, to reduce the drop size to a more acceptable 8–15 µl, a liquid with a lower surface tension is needed. Recently, the use of ultra-pure perfluorodecalin (PFD) as a vehicle has been investigated. With suitable modification of the applicator tip, drops of 5–8 µl can easily be dispensed because the surface tension is only 19.3 dynes/cm. Perfluorocarbons (PFCs) have been used as a vitreous replacement during retinal surgery for the repair of giant retinal tears and the manipulation of intraocular foreign bodies such as dislocated intraocular lenses.

One problem with these materials is that they are totally inert: they are extremely poor solvents for both hydrophilic or hydrophobic drugs. The material

must therefore be delivered as a suspension using colloidal silicon dioxide to suspend the particulate. Using the method previously described to prepare charcoal labelled with ^{99m}Tc-DTPA chelate, samples were freeze-dried overnight under carefully monitored conditions. Just before use, sterile colloidal silicon dioxide is added to PFD and the suspension sonicated for 15 min to make a suspension ready for administration.

The clearance of PFD-based suspensions and a similar charcoal formulation prepared in saline were examined in rabbits and in volunteers using lacrimal scintigraphy. Each formulation was studied in one eye only; the other eye was untreated. A drop of 8 µl of the ^{99m}Tc-DTPA (1 MBq) labelled saline or PFD formulation was instilled onto the cornea using a positive displacement pipette fitted with a sterile tip. Clearance of the formulation from the eye was followed for 10 min by dynamic imaging using a gamma camera.

The PFD/carbon system was observed to be retained on the surface of the eye for at least 2 h in both rabbit and man. Little activity was detected in the lacrimal duct after dosing with the PFD formulation. The mean data from both rabbit and man showed that an increased corneal residence of particulates is obvious following administration of the PFD formulation. In contrast, carbon particles suspended in the aqueous formulation demonstrated a very short residence in the eye. More than 60% of the carbon particulates remained on the corneal surface in both rabbit and man at 10 min following dosing with the PFD vehicle, while particles in the saline formulation were cleared away rapidly from the cornea. Uniform distribution along the conjunctival margin was seen with the perfluorocarbon, whereas carbon agglomerated and ejected over the medial canthus following administration of the aqueous formulations.

8.6 Conclusions

Over the past 15 years, lacrimal scintigraphy has proved of great value in the assessment of ophthalmic drug delivery systems. Further developments in this field are limited by the availability of suitable markers, particularly (a) as lipophilic probes and (b) as small molecular weight peptides. The technique of conventional ophthalmic delivery is very ineffective and more than 20% of patients cannot use dropper bottles effectively. The use of gamma scintigraphy to evaluate novel delivery devices and new polymers will no doubt afford researchers valuable insights into better ophthalmic therapy in the future.

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9

Scintigraphic Study of Drug Carriers and Conjugates

M.V.PIMM

9.1 Introduction—Nature of the Problem

Because the biodistribution of drugs—particularly novel therapeutic agents— is essentially unpredictable, uncontrollable and governed by poorly understood physiological processes, the idea of attaching such drugs to carriers, with more controllable biodistribution characteristics, has long been of interest (Duncan, 1992; Duncan *et al*., 1996; Matthews *et al*., 1996).

9.1.1 Nature of Drug Carriers

Generally drug carriers will be of high molecular weight, compared to the low molecular weight of most therapeutic agents, and can even be particulate rather than soluble, e.g. liposomes, micelles or emulsions (Davis *et al*., 1996; Hagan *et al*., 1996; Mizushima, 1996), although the present review will concentrate on soluble, mainly polymeric, carriers. Such carriers may simply modify, in a passive way, the biodistribution of a drug, but they also offer scope for manipulation of that biodistribution, for example by changes in molecular weight, charge, etc. Alternatively they may have linked to them ligands for specific receptors. These can be, for example, hormones (O'Hare *et al*., 1993; Mezo *et al*., 1996; Vincze *et al*., 1996), sugars (Seymour *et al*., 1991; Nishikawa *et al*., 1993; Pimm *et al*., 1993, 1996b), peptides (Fransenn *et al*., 1994), or antibodies or their fragments such as Fab and Fv. (Rihova *et al*., 1989; Torchilin *et al*., 1993; Ulbrich *et al*., 1996). Antibodies in particular theoretically provide exquisite specificity for the target organ or tissue.

9.1.2 Studying the Biodistribution of Drugs and Drug Carriers

Free drugs

In studying the biodistribution of free drugs, their pharmacokinetics is clearly paramount. Gamma scintigraphy is, in theory, a possible way of studying the biodistribution of drugs, but it is not usually possible to introduce into them radionuclides of the appropriate characteristics for scintigraphy (see below) and their biodistribution is usually studied by assays of drugs and their metabolites on blood, tissues, cells and tissue and cell fractions. These are usually chemical assays, for example by HPLC, but may be immunological using anti-drug antibodies (Pimm *et al*., 1988b,c), which also have potential for use in immunohistochemistry.

Drug-carrier conjugates

In studying the distribution of possible drug carriers, techniques for examining their biodistribution are much less straightforward than methods of determining the biodistribution of free drugs, mainly due to chemical reasons. At their simplest such carriers may be normal body or blood components, for example serum proteins for drug conjugation (Tanaka *et al*., 1993; Swart *et al*., 1996) or lipids, for example in lipid microspheres (Mizushima, 1996), so an assay of the carrier itself is virtually impossible against normal tissue backgrounds of these materials. At their most complex, carriers may be synthetic polymers, such as polypeptides (Hudecz *et al*., 1992a; Torchilin *et al*., 1993; Sgouras and Duncan, 1994; Hudecz, 1995; Duncan *et al*., 1996; Vincze *et al*., 1996), with no unique chemical moiety to allow assay in body tissues.

However, unlike simple drugs, drug carriers *do* lend themselves much more to radiolabelling with those radionuclides appropriate for scintigraphy. Bear in mind also that unlike the biodistribution of a 'simple' drug, when studying the biodistribution of a drug-carrier conjugate we often need to assess the *in vivo* characteristics of a range of possible carriers and be able to determine the effect of such parameters as charge, size, biodegradability, etc. Thus techniques to visualize how small changes in the characteristics of potential carriers may affect their biodistribution will be valuable.

This chapter will review work on such radiolabelling of drug carriers, but will aim to illustrate general principles rather than comprehensively review the growing literature in this field. Specific examples will usually be taken from the work of the author and his colleagues at Nottingham and their other collaborators.

9.2 Imaging Methods

9.2.1 Gamma Cameras

Gamma cameras are generally found in hospital nuclear medicine departments, and their basic instrumentation, image display and analysis are described in Chapter 3.

Clinical trials on the scintigraphic study of drug carriers and conjugates will have to be carried out in collaboration with nuclear medicine specialists, using cameras in clinical departments.

Although essentially designed for clinical use, such cameras are also suitable for animal studies. Animals as large as rats, or even rabbits, can be placed on the face of the camera collimator—akin to the use with patients. With smaller animals such as mice the use of pin-hole collimators, which essentially expand the image of the animal to the full diameter of the camera collimator, allows imaging of the whole animal with sufficient precision to discern and quantify uptake of radiotracers in organs such as the liver, lungs, kidneys and tumours. By drawing regions of interest (ROI) on the computer images, the radionuclide count rate can be quantified in particular organs. For example, early studies at Nottingham with a gamma camera fitted with a pin-hole collimator showed its precision in determining the whole body count rate of ^{131}I-labelled monoclonal antibody present in human tumour xenografts growing subcutaneously in immunosuppressed mice, in comparison to data obtained by sacrifice and dissection of the animals and counting of the radiolabel in tissues (Perkins *et al*., 1987a). Furthermore, even with an animal as small as a mouse, the whole animal does not have to be imaged and regions within the animal, such as the abdomen, can be made to fill the whole image by moving the pin-hole collimator closer to the animal (Pimm *et al*., 1996b).

While it is not generally possible, and indeed would be inappropriate, to use the gamma cameras in clinical departments for studies on animals, it is feasible to set up such a camera and its associated equipment in animal units. The major consideration here of course is cost, because a gamma camera set-up costs in the region of £125000 to £150000. Although a pharmaceutical company might consider purchasing a gamma camera especially for this type of work, it would be outside the reach of most grant-funded research in academic departments. However, perfectly satisfactory animal studies can usually be achieved with older gamma cameras taken out of service from clinical departments because they are being replaced by new equipment. Such second-hand gamma cameras may cost about £10000 to £15000, comparable to the cost of a conventional well-type gamma counter. All of the animal studies at Nottingham, some of which are described below, were carried out on such ‘clinically obsolete’ cameras removed to the animal facility.

9.2.2 Kinetic Studies

A major advantage of gamma scintigraphy, in both humans and animals, is that it allows repeat studies, at appropriate time intervals as short as a few minutes or even seconds, allowing construction of time-activity curves.

The value of this, particularly in experimental studies in animals, cannot be overemphasized. Ethical considerations and cost dictate that the greatest amount of data should come from the smallest number of animals. In particular areas, for example the growth of human tumours as xenografts in immune-incompetent

animals such as Nude mice or SCID (severe combined immunodeficiency disease) mice the cost of animals is a major, indeed often prohibitive, consideration.

As emphasized by Perkins *et al*. (1997), a time course study with analysis of biodistribution of, for example, a drug carrier, at say six time points (over a period of hours or days, as appropriate) would require 30 mice if five had to be killed for dissection analysis at each time point to ensure adequate statistical meaningfulness. However, only five mice in total would be needed if the same animals could be examined at each time point in a non-destructive manner such as gamma scintigraphy. There is a significant saving here, as such animals may cost £30 or more each (in addition to labour costs for their maintenance, etc.). The calculation is simple: about £900 for dissection analysis, about £150 with a gamma camera. However, this calculation assumes that appropriate times of analysis of biodistribution by sacrifice and dissection can be predicted, but this is often not the case, and acquisition of images at intervals of a few seconds over several minutes or even hours following, say, the intravenous administration of a drugcarrier conjugate can often be very valuable in showing hitherto unexpected changes. Sometimes these may be happening too quickly to be detected by sacrifice and dissection of the animals at intervals of a few minutes or even seconds.

Gamma scintigraphy is not only useful in imaging biodistribution over short time intervals, it can also be used for repeat imaging on the same small groups of animals weeks, or even months, apart. This is valuable for determining if repeated exposure to a carrier has produced an immune response to it sufficient to alter, detrimentally, its biodistribution. This has clearly been shown in the case of a monoclonal antibody, of mouse origin, repeatedly administered to rats, in which it evokes an antibody response (Pimm *et al*., 1990). It was possible to quantify the extent of rat antibody mediated clearance of the mouse antibody (to the liver) as repeated doses of the mouse antibody were given at intervals of several weeks for up to 9 months. It is known that some other potential drug carriers, based on poly(L-lysine) backbones, can evoke antibody responses in mice (Hudecz *et al*., 1992a), and gamma scintigraphy could clearly be of value in assessing the impact of such responses on biodistribution of repeatedly administered doses.

9.3 Choice of Radiolabel

The choice of radiolabel for any imaging study, not only those with drug carriers, is dictated essentially by the nature of gamma cameras. This because such cameras detect most efficiently and precisely within a narrow range of gamma energies (see Chapter 3), and there are only a few radionuclides with such energies which are also readily available, and would not pose problems of radiation doses to patients/human volunteers if experimental studies led to clinical evaluation. The chemistry of attaching such radionuclides to drugs and/or carriers also has to be taken into consideration (see below).

Table 9.1 Radionuclides of most value and interest for labelling drugs or drug carriers for gamma scintigraphy

Radionuclide	Gamma energy (keV)	Physical half-life	Availability	Usable time range for *in vivo* use
^{153}Sm	103	47 h	Available from some radiochemical suppliers. Can be produced from neutron activation of ^{152}Sm. Possible to carry out labelling with ^{152}Sm before its activation to ^{153}Sm	Up to 10 days
^{99m}Tc	141	6 h	As pertechnetate (TcO_4^-) from technetium generators used in every nuclear medicine unit	Up to 24 h
^{111}In	145, 171	2.8 days	As [^{111}In]$InCl_3$ from most radiochemical suppliers	Up to 10 days
^{123}I	159	13 h	As [^{123}I]NaI from some radiochemical suppliers	Up to 4 days
^{131}I	364	8 days	As [^{131}I]NaI from most radiochemical suppliers	Up to 3 weeks

Table 9.1 lists those radionuclides which have proved most appropriate for these purposes. The nature of the gamma camera means that it works most efficiently and with greatest resolution with gamma energies around that of ^{99m}Tc (140 keV) (see Chapter 3), and so the radionuclides are listed in order of their principal gamma energies. Although early pilot imaging studies with agents such as monoclonal antibodies and drug carriers were carried out with ^{131}I (because of its cheapness and ready availability), its high gamma energy meant that there was soon a move to more suitable radionuclides, and ^{111}In, ^{99m}Tc and ^{123}I are now more commonly used, with ^{153}Sm (which also has potential for radiotherapy) also being explored, although somewhat limited by its availability.

Table 9.2 Examples of the few drugs which can be labelled with gamma-emitting radionuclides

Drug[a]	Radionuclide	Site of radiolabel	Biodistribution assessed in	Reference and comments
Idoxifene (pyrrolidino-4-iodotamoxifen)	^{131}I, ^{123}I	Substitution for iodine in iodotamoxifen	Rats with mammary carcinoma	Trivedi *et al.* (1995)
DOX	^{57}Ni	Complexation with drug	–	Zweit *et al.* (1994). ^{57}Ni is a positron emitter but similar labelling of DOX with copper, iron and gallium has been described (Greenway and Dabrowiak, 1982; Beraldo *et al.*, 1985; Yotsuyanag *et al.*, 1990)
DAU	^{125}I	Oxidative incorporation	Mice	Rihova *et al.* (1989)
OXY	^{99m}Tc	?	Mice	Rebello *et al.* (1994). Oxamniquine is an antischistosomal drug
MTX	^{125}I	Iodinated histamine conjugated to the MTX	–	Kamel and Gardner (1978) Primarily intended for *in vitro* use in radioimmunoassays. *In vivo* characteristics could differ from those of drug itself

[a] DOX = doxorubicin; DAU = daunomycin; OXY = oxamniquine; MTX = methotrexate

Note that these are all drugs for chemotherapeutic purposes. Labelled compounds intended only as radiopharmaceuticals are not included

9.4 What to Label in Drug Carriers and their Conjugates

Clearly when aiming to alter the biodistribution of a drug by attaching it to a carrier, it is the biodistribution of the drug, rather than that of the carrier, which is of prime importance. Of course the biodistribution of the carrier *is* important—it will usually have been assessed in preliminary studies (including scintigraphic studies) and selected on that basis for attachment of the drug. It is important to ascertain that attachment of the drug has not altered the biodistribution of the carrier (either because the carrier has been denatured by the chemical reactions involved in drug conjugation, or because the biodistribution of the drug dictates the distribution of the carrier, instead of *vice versa*). But at the end of the day it is the distribution of the *drug* which is vitally important.

Where the drug is a peptide or protein, for example a ribosomal inhibiting protein (RIP, see below), it is feasible to label it (after or perhaps before conjugation) with virtually all of the radionuclides listed in Table 9.1, because their attachment to proteins, peptides, and even individual amino acids, is chemically feasible and well established (see below).

Unfortunately there are only a limited number of instances where it is feasible to label a synthetic chemical drug with a gamma-emitting radionuclide. This is because such drugs usually do not contain atoms of those elements listed above whose radionuclides are suitable for gamma scintigraphy, so radionuclide cannot be substituted for the stable nuclide, or because of chemical difficulties in otherwise attaching such a radionuclide directly to the drug. There are some instances where drugs do contain such elements (e.g. Idoxifene, Trivedi *et al*., 1995) or it is possible to incorporate the radionuclide into the drug (Table 9.2) and indeed imaging studies have been reported with them, although as yet not in carrier conjugate form.

In some instances it is possible to label a drug by chemical linkage to it of another small moiety which it is feasible to label. An example that can be given here is methotrexate. This has been conjugated to carriers, particularly anti-tumour monoclonal antibodies, by a number of workers, in an attempt to achieve sitespecific (tumour) targeting. Methotrexate can be labelled with radioiodine by conjugation to it of pre-labelled histamine (Kamel and Gardner, 1978). Although such labelling was developed for use of ^{125}I-labelled methotrexate in competitive drug assays such as radioimmunoassay binding assays (e.g. Pimm *et al*., 1988b), it would be feasible to label methotrexate with ^{131}I, or even ^{123}I. It should however be borne in mind that any drug will be considerably changed chemically by conjugation to it of an iodinated material (for example with methotrexate its molecular mass will have increased from 450 Da to about 690 Da by conjugation to iodinated histamine), and thus the biodistribution of such a labelled drug may differ considerably from that of the free drug, although such labelled drugs do not seem to have been used in *in vivo* studies.

It should be mentioned here that its is much easier to label drugs with positron emitters, because positron emitters of the elements making up the basic structure of most drugs (carbon, hydrogen, nitrogen, etc.) are available. The use of positron emitters is outside the scope of this chapter, but is dealt with in Chapters 3 and in the Note in Proof.

9.5 Labelling Methods for Carriers and Protein Drugs

It is notable that many of the chemical methods for labelling polymeric drug carriers with radionuclides were originally designed for labelling proteins, either for use in radioimmunoassays, or biodistribution studies, or later with antibodies for scintigraphy—immunoscintigraphy (Perkins and Pimm, 1991). Thus these methods have been used virtually unmodified for labelling peptides or protein drugs [e.g. hormones (O'Hare *et al*., 1993; Mezo *et al*., 1996), RIPs (Byers *et al*., 1987)] attached to carriers. Alternatively they can be adapted to label those carriers which have some of the same reactive groups as proteins. For example amino groups, which can be reacted with the succinimidyl group of N-succinimidyl 3-(4-hydroxy 5-[^{125}I]iodophenyl propionate (Bolton and Hunter reagent, see below) or anhydride derivatives of chelating agents such as diethylenetriaminepentaacetic acid (DTPA) for subsequent labelling with radiometals such as ^{111}In (Pimm *et al*., 1992a) or ^{153}Sm (Awang *et al.*, 1994; Awang, 1995). Even if the carrier has no suitable reactive groups it may be feasible to modify its structure slightly to contain suitable constituents, for example tyrosine (Seymour *et al*., 1991), for radioiodine labelling.

9.5.1 Radioiodine

There are basically two simple methods of labelling with radioiodine—direct oxidative incorporation of the iodine atom into tyrosine amino acids, or attachment of chemical groups which have been pre-iodinated, or which can be subsequently iodinated.

Direct incorporation

In direct labelling, radioiodine, available from many suppliers as a sodium iodide solution, is treated with a mild oxidizing agent in the presence of the antibody. Cationic iodine (I^+) is presumed to be formed under these oxidizing conditions and there is an electrophilic aromatic substitution ortho to the hydroxyl group in the phenolic ring of tyrosine, giving predominantly monoiodotyrosine (Figure 9.1).

Many different reagents and methods have been described for oxidative radioiodination but the two major reagents are chloramine-T and iodogen.

Chloramine-T is the sodium salt of N-chloro-p-toluene sulphonamide. In aqueous solution it slowly produces hypochlorous acid and is therefore a mild oxidizing agent. For this method of labelling, antibody in solution buffered at pH 7–8 (the optimum for incorporation of iodine) is mixed with the radioiodine, and an aqueous solution of freshly prepared chloramine-T added. Because only 10–20 μg of chloramine-T are needed for each 37 MBq of radioiodine, only a very small volume of solution need be used. The reaction is usually considered to be complete within a few minutes, after which the continuing oxidative effect of the chloramine-T can be stopped by the addition of an appropriate amount of

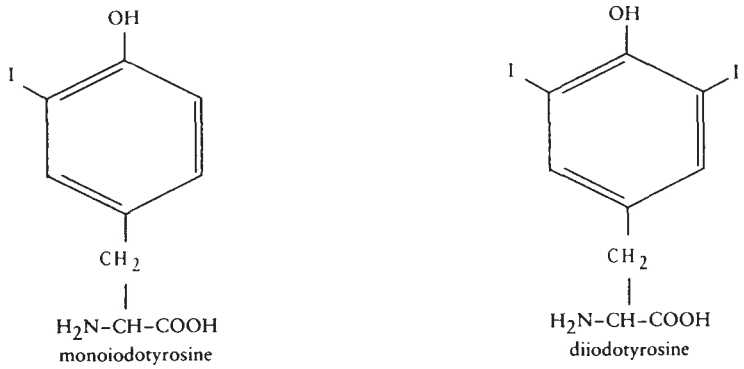


Figure 9.1 The structure of monoiodotyrosine and diiodotyrosine. Radioiodines such as ^{123}I, ^{125}I and ^{131}I can be introduced into proteins and peptides by oxidative incorporation into their tyrosine amino acids, giving predominantly the monoiodo form. For polymeric drug carriers lacking any tyrosine it may be possible to incorporate it into the polymer to facilitate labelling (see Figure 9.5). (Reproduced with permission of Amersham Life Science Ltd, from their handbook *Guide to Radioiodination Techniques*)

reducing agent (often sodium metabisulphite). Unreacted radioiodine is usually removed by gel permeation chromatography, for example on Sephadex G-25, or by dialysis.

Iodogen was introduced by Fraker and Speck (1978) as an alternative to chloramine-T to minimize exposure of proteins to soluble oxidizing agents during radioiodination and this reagent is probably now more widely used than chloramine-T. Chemically the material is 1,3,4,6,-tetrachloro-3a,6a-diphenylglycoluril. It is virtually insoluble in water, and is used as a solid phase. For example it can be coated on the inner surface of a small reaction vessel, by evaporation under a stream of nitrogen of a solution of the material in chloroform or methylene chloride. Iodination reactions are then carried out by adding the appropriate volume of solution of the material to be labelled buffered to pH 7–8 and radioiodine to the coated tube, mixing and leaving, usually at room temperature, for 2–5 min. The reaction is then stopped simply by removing the reaction solution from the tube. An alternative is to prepare a fine suspension of iodogen by adding a concentrated solution in acetone to phosphate-buffered saline solution in which it will come out of solution as a fine suspension to which can be added material to be labelled and the radioiodine. Gel permeation chromatography, used to remove unreacted radioiodine, also removes the particulate iodogen.

For these labelling reactions, ^{131}I is widely available as a solution in sodium hydroxide at pH 7–11, free from reducing agent and specifically produced for iodination reactions. However, ^{123}I is less readily available than ^{131}I, and is not always supplied in a radiochemical form specifically designed for iodination, so it may require adjustment of pH, etc. For example, when labelling an N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-doxorubicin conjugate by oxidative incorporation of ^{123}I into tyrosine introduced into the polymer backbone

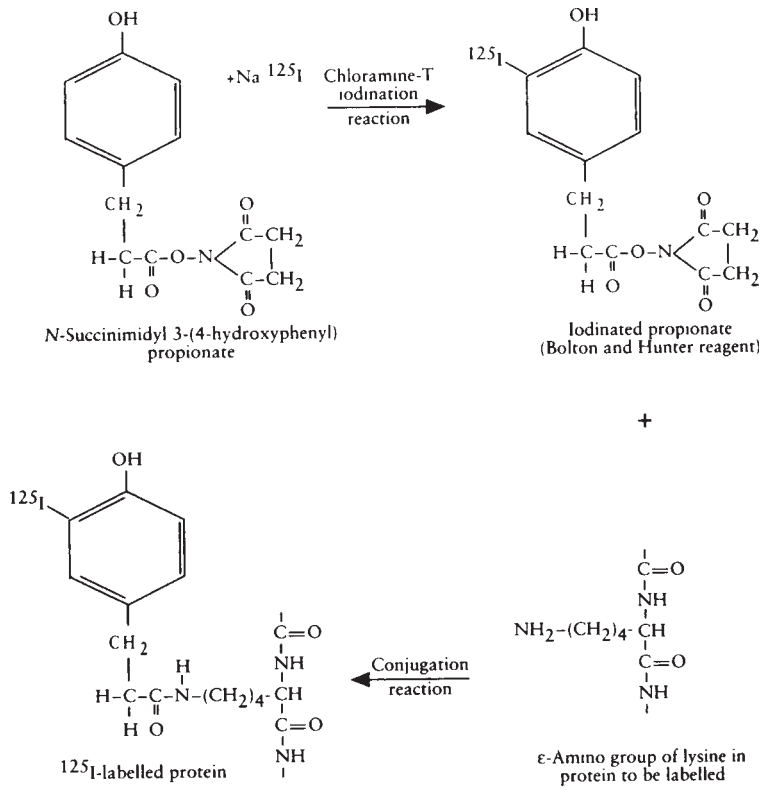


Figure 9.2 Radioiodine labelling of a protein's lysine epsilon amino groups by reaction with pre-iodinated (^{125}I) Bolton and Hunter reagent, forming a stable amide bond. Terminal amino groups of synthetic polypeptides with short side chains can be similarly labelled. Alternatively, non-labelled Bolton and Hunter reagent may be conjugated first, and subsequently labelled with ^{123}I or ^{131}I. (Reproduced with permission of Amersham Life Science Ltd, from their handbook *Guide to Radioiodination Techniques*)

as tyrosinamide, Pimm *et al.* (1996b,c) found that in view of the high specific activity of [^{123}I]sodium iodide it was advisable to add stable carrier iodide.

Indirect labelling

One of the most widely used methods of indirect radiolabelling, and one which still bears their name, is that proposed by Bolton and Hunter in 1973. As first introduced, N-succinimidyl 3-(4-hydroxyphenyl)propionate (now known as Bolton and Hunter reagent) was first iodinated with ^{125}I to N-succinimidyl 3-(4-hydroxy 5-[^{125}I]iodophenyl) propionate, using the chloramine-T method, purified from the reactants, and then reacted with proteins, peptide, antibody, etc. (Figure 9.2). During the reaction an amide bond is formed with lysine epsilon amino groups of the protein. ^{125}I-labelled Bolton and Hunter reagent subsequently became available commercially, particularly for protein iodination. Such ^{125}I-labelled Bolton and Hunter reagent was

used by Clegg *et al*. (1990) to label a series of branched polypeptides with a poly(L-lysine) backbone which had free amino groups at the side chain terminal positions, and which were being examined as potential drug carriers. However, such labelling is not limited to the use of preiodinated (^{125}I) Bolton and Hunter reagent, because it was subsequently shown that such drug carriers can be reacted with non-iodinated Bolton and Hunter reagent, and then iodinated (e.g. with the iodogen method) with ^{131}I or ^{123}I (Pimm *et al*., 1995b).

9.5.2 Radiometals

The search for techniques for radiometal labelling of macromolecules was encouraged in the early 1980s by the need to radiolabel the then newly available monoclonal antibodies (particularly against tumour-associated antigens). Such labelling is now generally achieved by pre-conjugation to the antibody of a chelating agent. The most widely used chelating agent is the bicyclic anhydride of DTPA introduced by Hnatowich *et al*. in 1983, and which subsequently became commercially available. Stable amide bonds are formed when the anhydride reacts with epsilon amino groups of the protein's lysine amino groups. The negatively charged carboxyl groups of the DTPA will subsequently bind positively charged trivalent metal ions (Figure 9.3). Essentially the same method (although alternative chelators have been introduced) has been used for protein labelling for: scintigraphy with ^{99m}Tc (e.g. Childs and Hnatowich, 1985); scintigraphy and radiotherapy with ^{153}Sm (e.g. Boniface *et al*., 1989), ^{186}Rh (e.g. Breitz *et al*., 1992) and ^{67}Cu (e.g. Smith *et al*., 1993); and magnetic resonance enhancement with gadolinium (e.g. Gohr-Rosenthal *et al*., 1993).

A major consideration with such metal labelling is the form of presentation of the cationic metal to the chelating agent. For example ^{111}In is available as a solution of indium chloride, but this is not suitable for addition directly to the DTPA conjugate of the material to be labelled. Chelation of the radioindium takes place most efficiently at a pH below 5, but to prevent the formation of colloidal

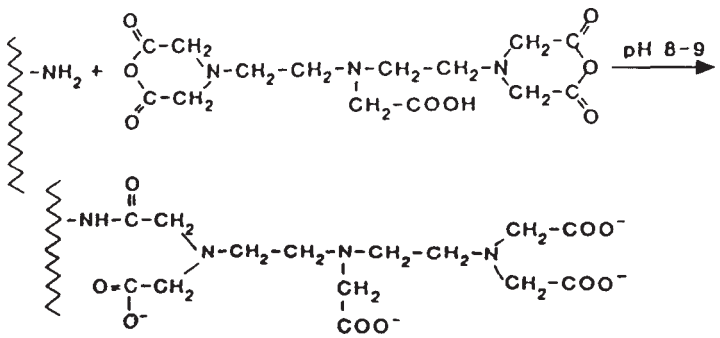


Figure 9.3 Reaction of a protein's lysine epsilon amino groups with the cyclic anhydride of DTPA to form a stable amide bond allowing chelation of radiometals such ^{111}In. Similar methods can be used for labelling with ^{51}Cr, ^{111}In and ^{53}Sm

forms of indium it is necessary to add acetate or citrate to the reaction mixture (as a transchelating agent). With ^{99m}Tc, it is in the form of pertechnetate from technetium generators and needs to be reduced to a cationic form (Tc^{4+}) to be chelated, a reduction often carried out with Sn(II) salts.

This chelation labelling method was subsequently adapted for radiometal labelling of drug carriers which had available amino groups to react with the anhydride form of chelators, or into which they could be introduced. These carriers include plasma proteins and styrene-maleic acid/anhydride copolymer-lysine conjugates labelled with ^{51}Cr (Matsumura and Maeda, 1986), and branched polypeptides with a poly(L-lysine) backbone labelled with ^{111}In and ^{51}Cr (Pimm *et al*., 1995b), ^{153}Sm (Awang, 1995) and ^{99m}Tc (Frier *et al*., 1995).

9.6 Some Practical Examples of Drug Carriers which have been Radiolabelled and their Biodistribution Studied Scintigraphically

Clearly, drug-carrier conjugates intended to improve the therapeutic index of a drug can be designed to achieve one of four broad aims:

- A drug-carrier conjugate which relies for its efficacy on reduced toxicity of conjugated compared with free drug and/or prolonged survival of the drug with its possible slow release.
- A drug-carrier conjugate with some propensity to accumulate at the desired target site by normal physiological processes.
- A conjugate in which the drug carrier has an intrinsic targeting moiety (e.g. it is an antibody).
- A conjugate in which the carrier has chemically linked to it a specific targeting moiety (e.g. drug-polymer-antibody or drug-polymer-hormone conjugate).

It is of course feasible that any one conjugate may be effective by a combination of these methods of increased efficacy, and indeed it may be difficult to identify which is the major mechanism.

Based on the possible methods of radiolabelling, some of which have been outlined above, it is clear that the possibilities for potential pharmacokinetic studies and gamma scintigraphy of drug-carrier conjugates are three-fold:

- A conjugate of drug and carrier in which the carrier is labelled with a radionuclide.
- A conjugate in which the drug is labelled (the drug is most likely to be a protein or peptide). The carrier may also be radiolabelled with the same or a different radionuclide.
- A conjugate in which the carrier has an additional targeting moiety (e.g. antibody or hormone) in which either or all components are labelled with the same or different radionuclides.

The second and third of these offer particular advantages because if carrier, drug and targeting moiety can be labelled separately it is possible to compare the biodistribution of the components of the conjugates and thus to know something about the *in vivo* stability and metabolic fate of the conjugate. Clearly if different components can be labelled simultaneously with different radionuclides this offers tremendous advantages for the study of biodistribution of the conjugate.

Because this chapter is concerned principally with the methodology of radiolabelling and scintigraphy with drug-carrier conjugates, the specific examples which are given below are based on the three possible different sites of radiolabelling rather than the design of drug-carrier conjugates *per se*.

9.6.1 Drug-carrier Conjugates in which only the Carrier is Labelled

Table 9.3 lists some drug-carrier conjugates whose biodistribution has been assessed following radiolabelling of the carrier component. These have not all been used in imaging studies, but even where they have not, such imaging would certainly be possible. The carriers are either monoclonal antibodies or synthetic polymers.

Monoclonal antibodies

One of the earliest classes of potential drug-targeting agents to be examined scintigraphically were monoclonal antibodies. Monoclonal antibodies directed against disease-associated antigens have been used in what is now called immunoscintigraphy in a number of disease conditions including tumours (where the antigen may be tumour-specific) or non-malignant pathologies such as thrombus formation, etc. These monoclonal antibodies, particularly those against tumourassociated antigens, have been the subject of intensive investigation for the targeting of anti-tumour drugs. For example, at Nottingham such monoclonal antibodies were conjugated to methotrexate, vindesine and daunomycin. The biodistribution of these drug-antibody conjugates, in comparison to that of their parent antibodies, was assessed by radioiodinating the antibody moiety of the conjugates (Table 9.3). Generally the biodistribution of the conjugates was found to be virtually identical to the parent antibodies, and when drug assays were carried out on blood and tissue extracts the distribution of the drug was virtually identical to that of the antibody.

With one such conjugate clinical imaging studies were conducted with an antibody-methotrexate conjugate labelled in the antibody moiety by direct incorporation of ^{131}I using the iodogen oxidation method (see Figure 9.4, colour section).

As an alternative to direct conjugation of drug to antibody, the use of intermediate carriers has also been explored. The idea here is that each intermediate carrier molecule can carry (for chemical reasons) more molecules of drug than the antibody molecule itself. If conjugates can be prepared in which the carrier

Table 9.3 Examples of drug-carrier conjugates in which only the carrier is radiolabelled

Carrier and approximate molecular mass[a]	Drug[b]	Type and site of radiolabel	Method of radiolabelling	Biodistribution assessed in	Reference and comments
Mab 791T/36 (150 kDa)	MTX	(^{131}I-antibody)–MTX	Incorporation into tyrosine residues of antibody	Mice with human tumour xenografts	Pimm *et al.* (1988c). Biodistribution of drug assessed by radioimmunoassay
				Patients with colorectal carcinoma	Ballantyne *et al.* (1988). Scintigraphic study (see Figure 9.4). Biodistribution of drug assessed by radioimmunoassay
(Mab 791T/36)–HSA (300 kDa)	MTX	Antibody–(^{125}I-HSA)–MTX	Incorporation into tyrosine residues of HSA	Mice with human tumour xenografts	Pimm *et al.* (1988a). Parallel studies carried out with (^{125}I-HSA)–MTX
	VDS	(^{125}I-antibody)–VDS	Incorporation into tyrosine residues of antibody	Mice with human tumour xenografts	Rowland *et al.* (1985)
	DAU	(^{125}I-antibody)–DAU	Incorporation into tyrosine residues of antibody	Mice with human tumour xenografts	Pimm *et al.* (1988c). Biodistribution of drug assessed by radioimmunoassay
HPMA (25–90 kDa)	DOX	(^{125}I-HPMA)–DOX	Incorporation into tyrosine introduced into HPMA backbone as tyrosinamide (see Figure 9.5)	Mice with melanoma	Seymour *et al.* (1994). Biodistribution of drug assessed by HPLC

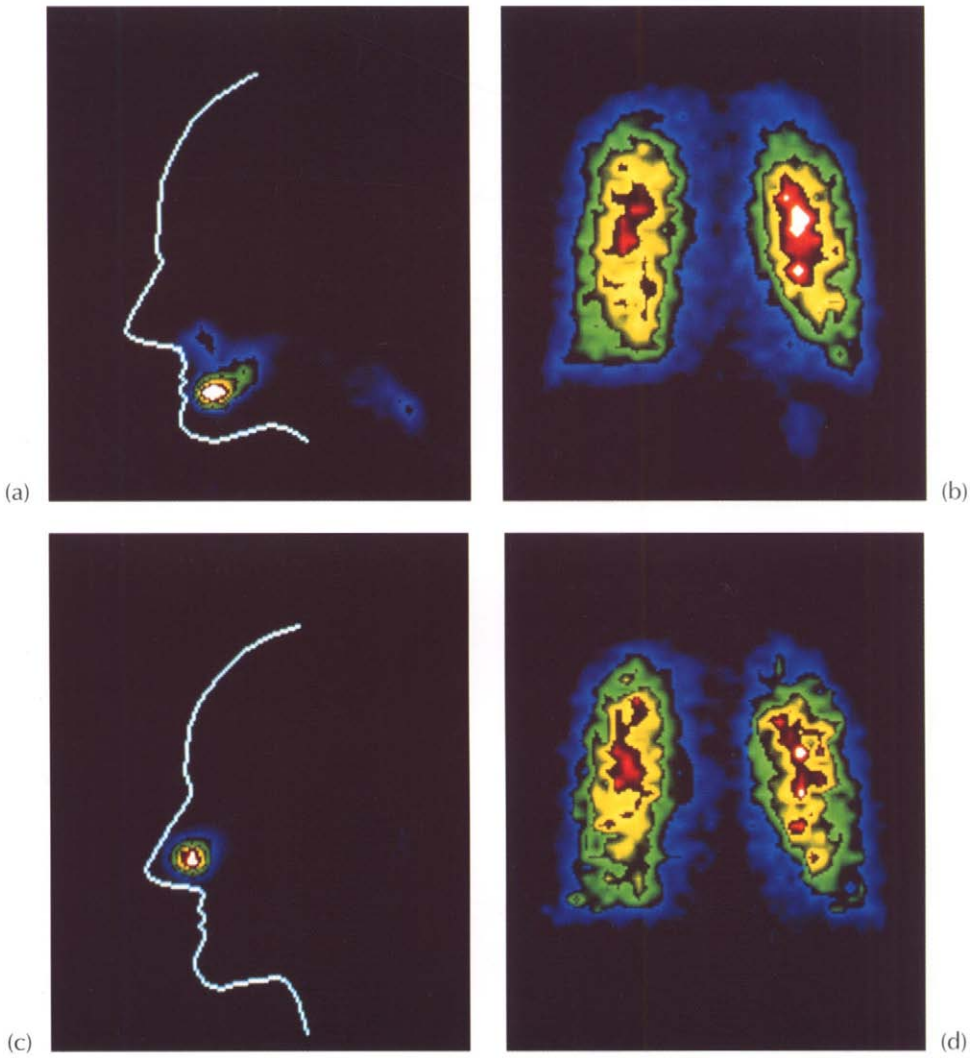


Figure 4.2 Tracer deposition following inhalation of nebulized solution via (a and b) the mouth and (c and d) the nose.

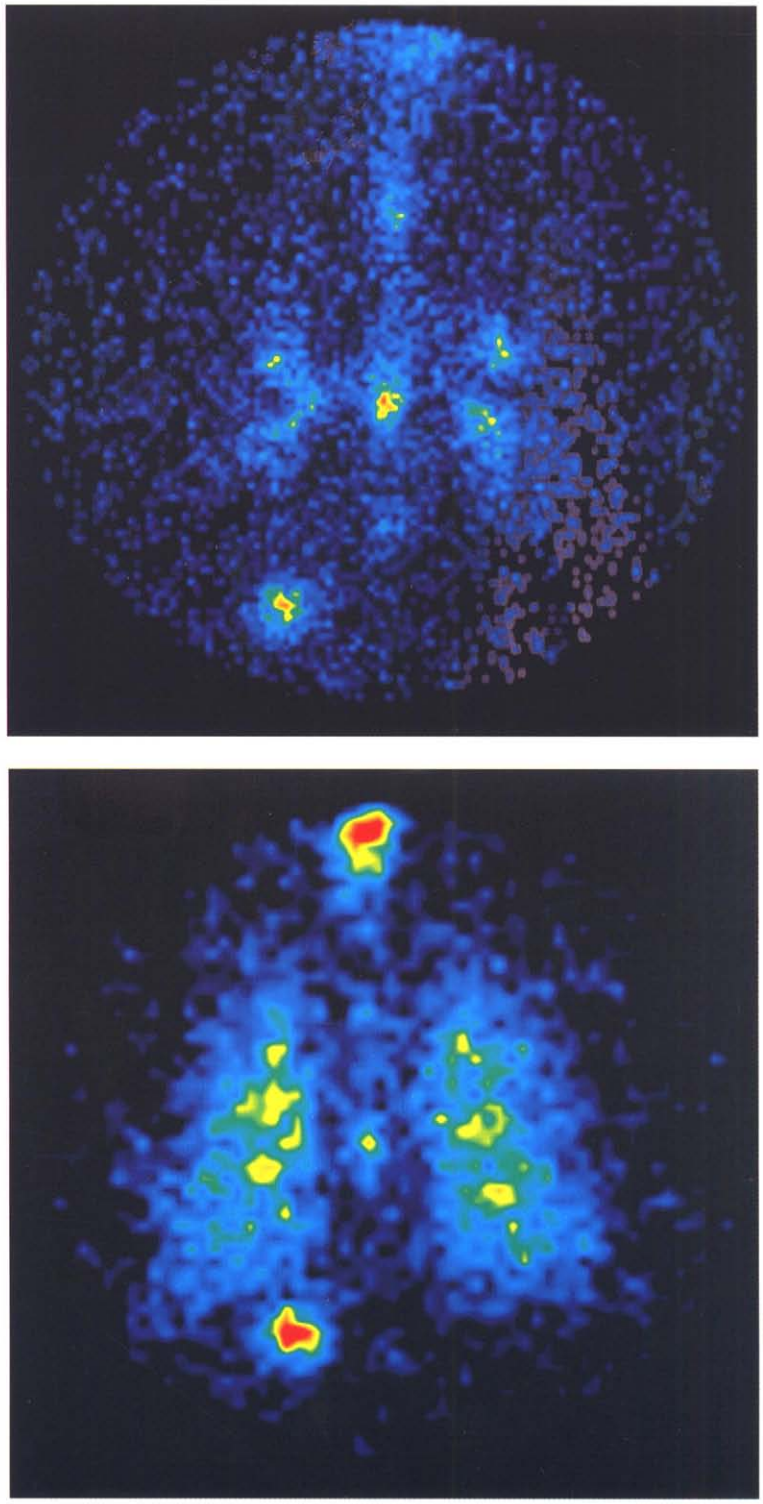


Figure 4.3 Drug delivery from a pressurised metered dose inhaler, showing more centra lung deposition in (a) an asthmatic patient than in (b) a healthy subject. Posterior gamma camera images are shown

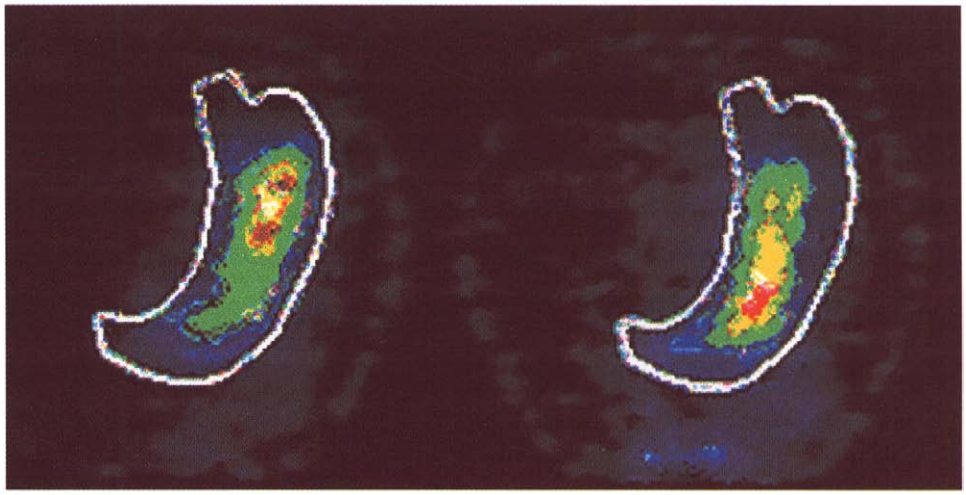


Figure 6.3 Gastric distribution of an ^{111}In-labelled antacid (right) and a ^{99m}Tc-labelled meal (left) administered 30 min before the antacid

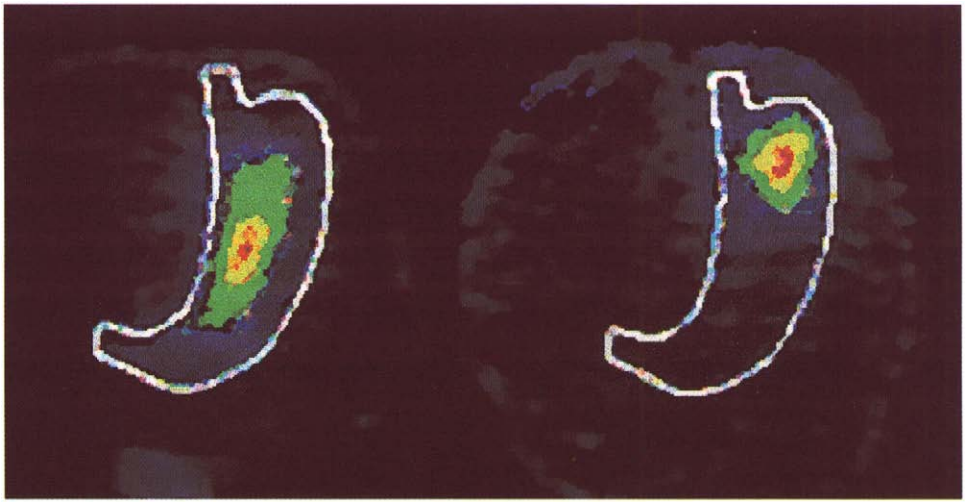
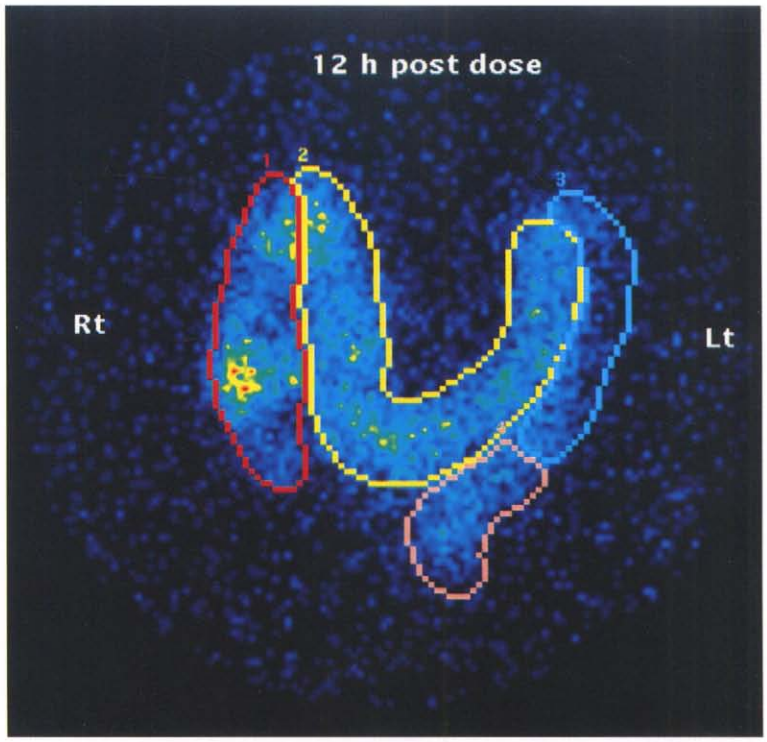
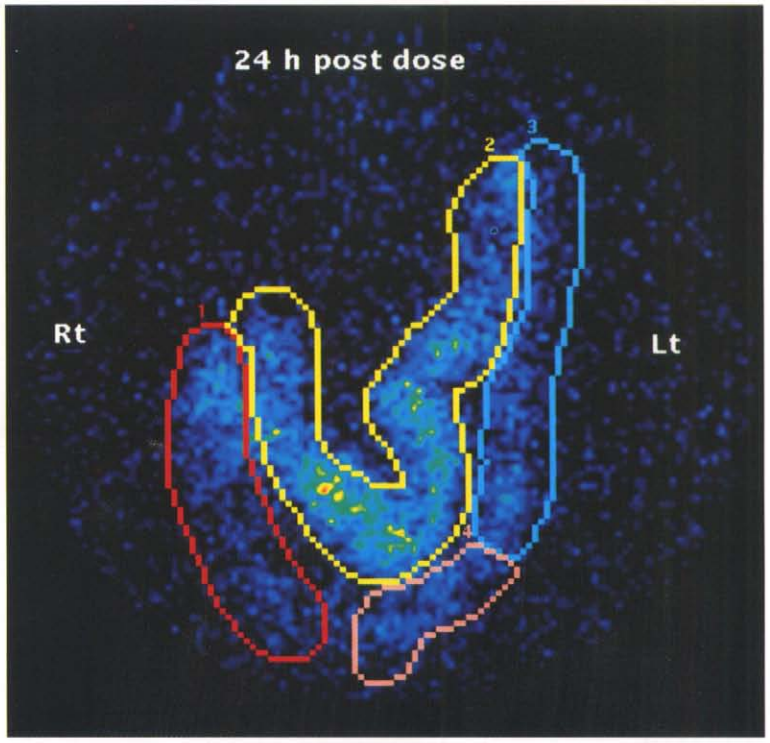


Figure 6.5 The use of dual radiolabelling to follow the behaviour of an anti-reflux agent (Gaviscon) in the presence of food. The ^{99m}Tc-labelled food is shown in the left panel and the ^{111}In-labelled anti-reflux agent in the right panel



(a)



(b)

Figure 7.1 Scintigraphic images following oral administration of a targeted dose form containing ^{111}In-resin, showing movement of a sagging transverse colon at (a) 12 h and (b) 24 h post-dose

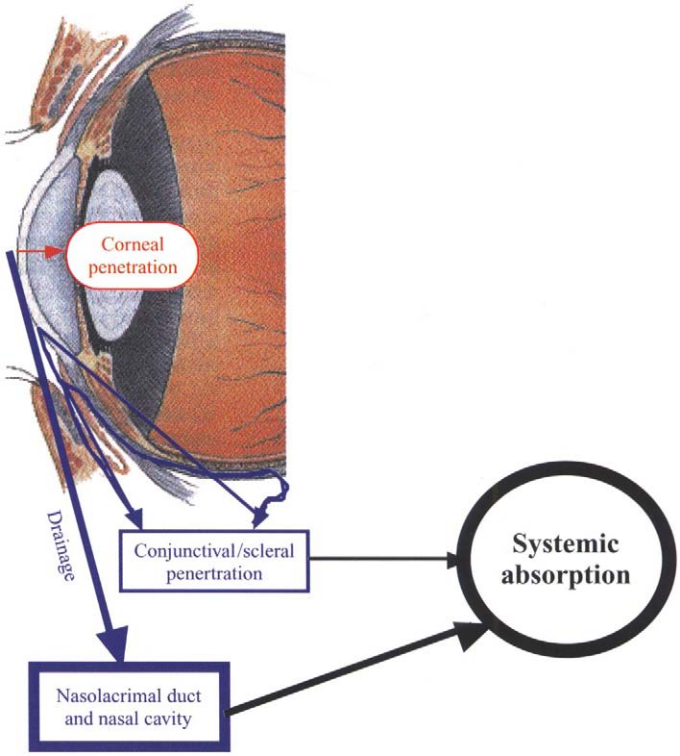


Figure 8.2 Ocular/systemic absorption routes

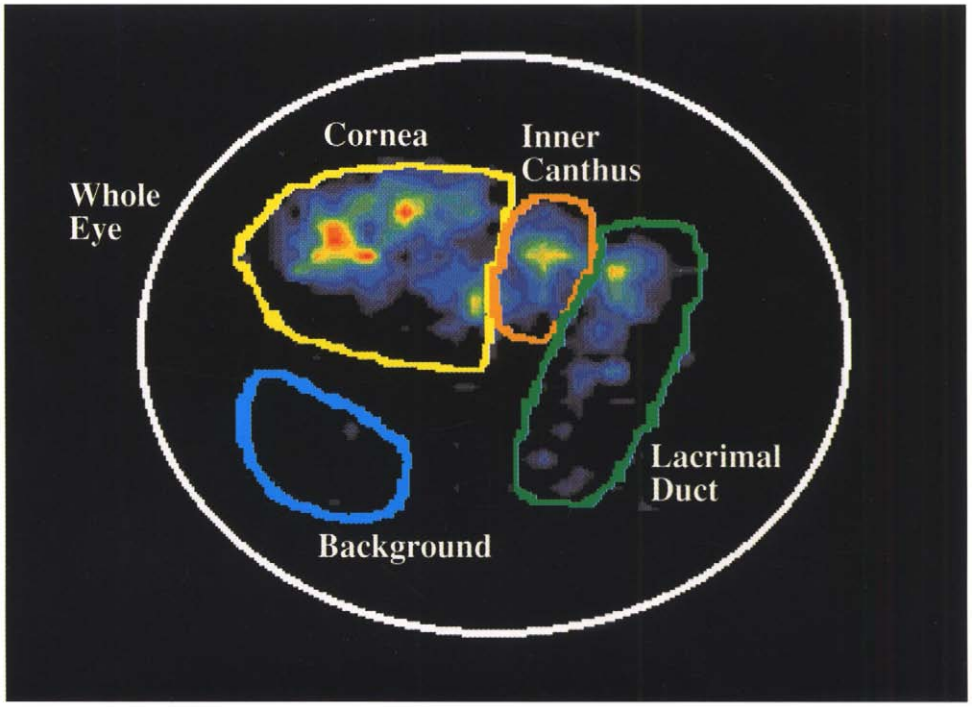


Figure 8.3 The generation of five main regions of interest (ROIs) in the eye: cornea, inner canthus, lacrimal duct, whole eye and background

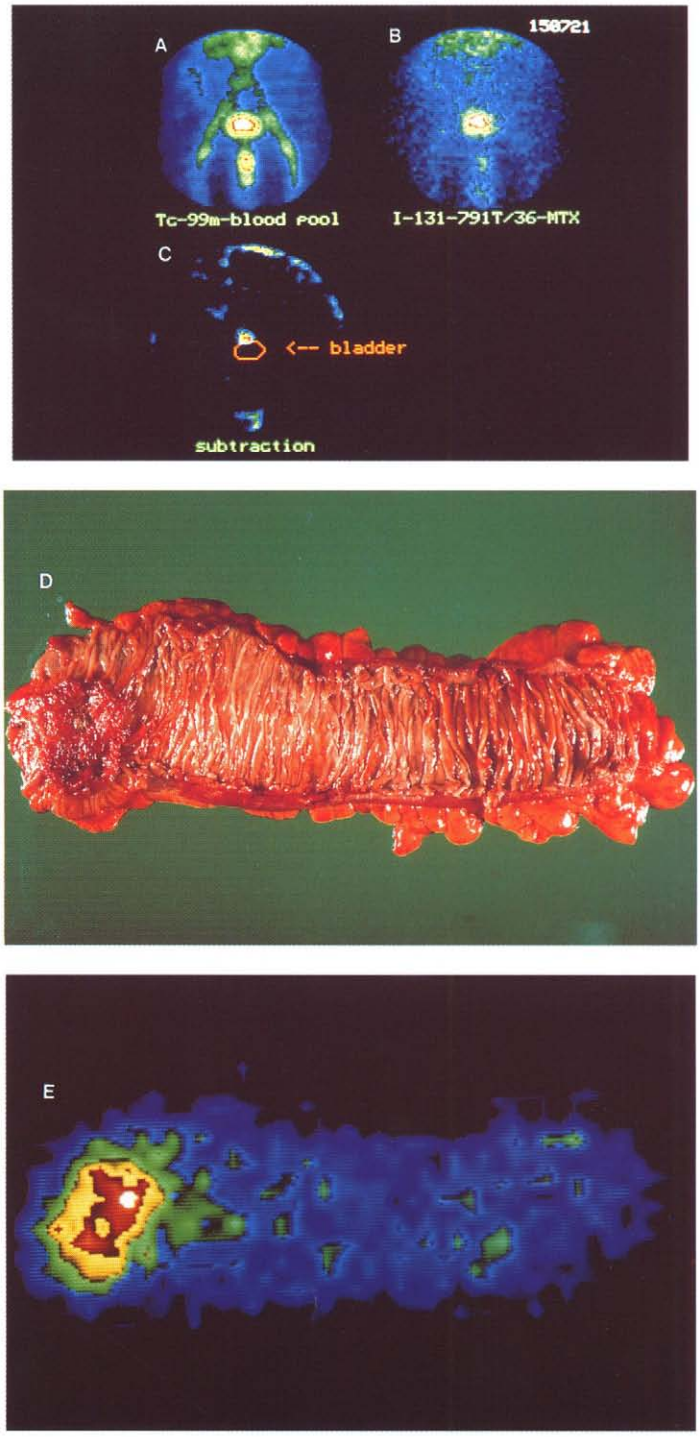


Figure 9.4 A patient with a primary rectal tumour and who had been injected 48 h previously with about 70 MBq ^{131}I-labelled monoclonal antibody 791T/36 conjugated to methotrexate. Anterior gamma camera images of the pelvic region of (A) ^{99m}Tc blood poo image, (B) ^{131}I image, (C) subtraction image showing tumour site and region of interest around bladder, (D) photograph of resected specimen, (E) image of the resected specimen showing concentration of ^{131}I within the tumour. (Reproduced, with permission of John Wiley & Sons Inc., from Ballantyne *et al.*, *International Journal of Cancer,* © 1988, John Wiley & Sons Inc.)

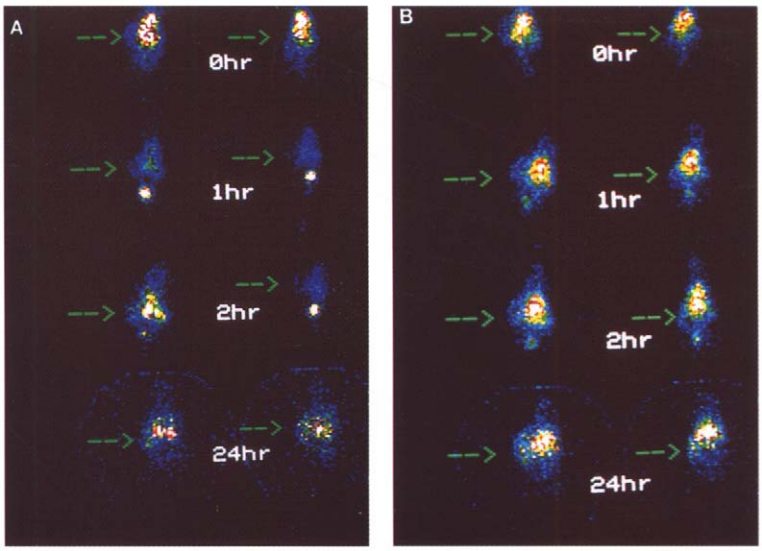


Figure 9.6 Gamma camera images (pin-hole collimator) of pairs of mice with subcutaneous grafts of a transplanted mouse mammary carcinoma and which had been injected intravenously with about 2 MBq ^{123}I-labelled HPMA-doxorubicin conjugates of (A) low molecular mass (25 kDa), or (B) high molecular mass (94 kDa). ^{123}I labelling was by oxidative incorporation into tyrosinamide in the polymer backbone (Figure 9.5). The positions of tumours are arrowed. Note the appearance of radiotracer in the urinary bladders of mice within an hour of injection, but particularly in those given the lower molecular mass copolymer. Although the sites of tumours can be discerned on these images, tracer is present at as high or higher concentrations in normal tissues. (Reproduced, with permission of Harwood Academic Publishers, from Pimm *et al.*, 1996c)

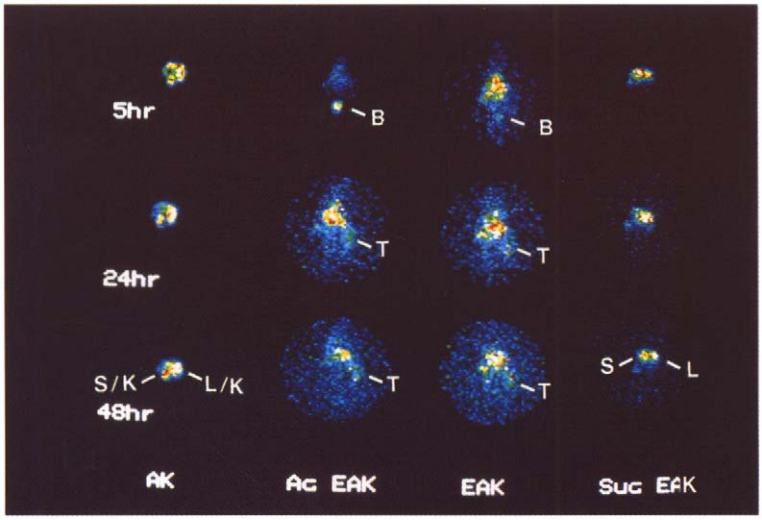


Figure 9.8 Typical serial gamma camera images (1–2 minutes per image; pin-hole collimator) of mice with a subcutaneously transplanted mammary carcinoma injected intravenously with about 1 MBq ^{111}In-labelled cationic branched chain polypeptide AK, amphoteric EAK, anionic acetylated EAK (ACEAK), or highly anionic succinylated EAK (SucEAK). Some tumour uptake of ^{111}In is seen at 24 and 48 h with EAK and ACEAK, while AK and SucEAK are rapidly cleared to kidney, liver and spleen, and to liver and spleen respectively. B = urinary bladder, T = tumour (each about 1.5 cm diameter), L = liver, K= kidney, S = spleen. (Reproduced, with permission of the Japanese Society of Nuclear Medicine, from Pimm *et al.*, 1995c)

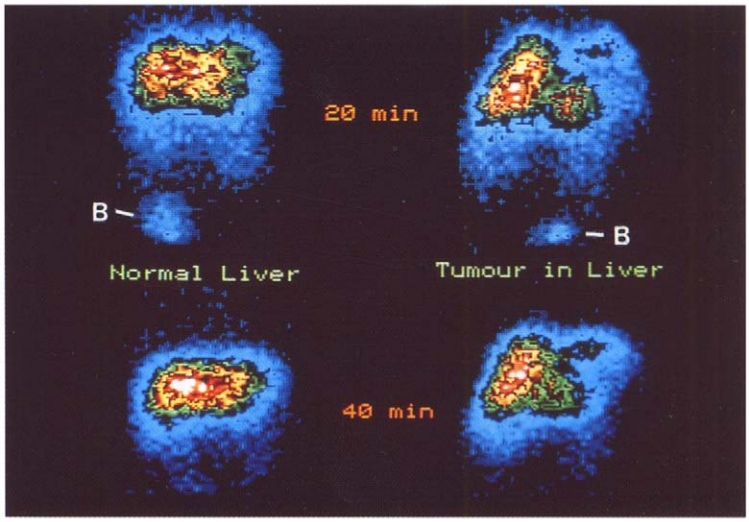


Figure 9.11 Gamma camera images acquired with a pin-hole collimator positioned to view only the abdominal region of Nude mice, either normal or with hepatic growth of a human colon carcinoma, following intravenous injection of 1 MBq ^{123}I-labelled HPMA-doxorubicin-galactose conjugate. Some ^{123}I is visible in the urinary bladders of both mice in the 20 min views (B = urinary bladder). The shape of the liver in the normal mouse is discernable at 20 min and more precisely delineated after 40 min. But in the tumour-bearing mouse there is clearly a defect/distortion of the liver uptake, and this was confirmed on imaging the resected liver *ex vivo* (Figure 9.12). (Reproduced, with permission of Harwood Academic Publishers, from Pimm *et al*., 1996b)

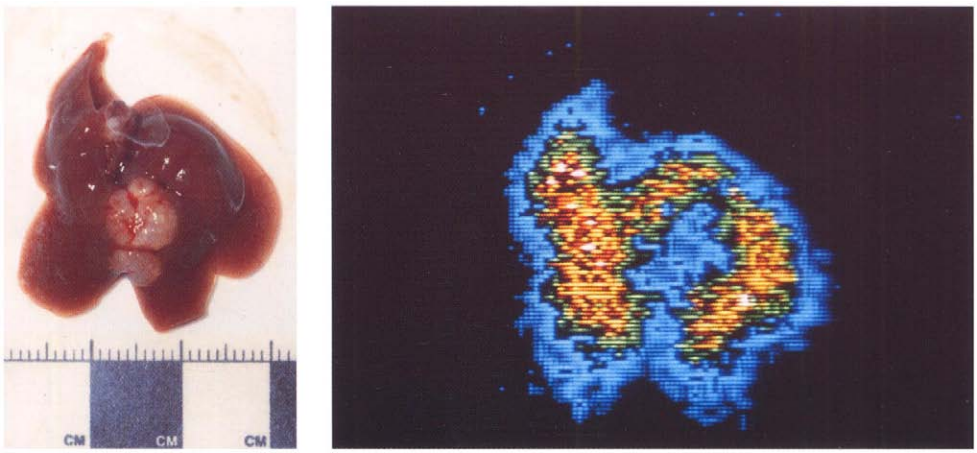


Figure 9.12 (A) Photograph of the resected liver of a Nude mouse with hepatic growth of a human colon carcinoma, 40 min after the injection of 1 MBq ^{123}I-labelled HPMA-doxorubicin-galactose conjugate, and (B) its gamma camera image. There is clearly an absence of ^{123}I coincident with the position of the tumour. (Reproduced, with permission of Harwood Academic Publishers, from Pimm *et al*., 1996b)

		(^{131}I-HPMA)–DOX		Mice	Pimm *et al.* (1993). Scintigraphic study
		(^{123}I-HPMA)–DOX		Patients with malignant disease	Vasey *et al.* (1996)
				Mice with mammary carcinoma and melanoma	Pimm *et al.* (1996c). Scintigraphic study (see Figure 9.6)
Alginate (61–250 kDa)	DAU	(^{125}I-alginate)–DAU	Incorporation into tyrosine introduced into backbone as tyrosinamide	Mice with B16 melanoma	Al Shamkhani and Duncan (1995a,b)
EAK (40–100 kDa)	MTX	(^{125}I-EAK)–MTX	Bolton and Hunter reagent reacted with side-chain terminal amino acids (pre-iodinated or labelled after conjugation)	Mice	Hudecz *et al.* (1993)
	DAU	(^{125}I-EAK)–DAU		Mice	Hudecz *et al.* (1992b)
	MTX	(^{111}In-EAK)–MTX	Chelation to DTPA reacted as anhydride with side-chain terminal amino acids	Mice	Pimm *et al.* (1992a,b). Scintigraphic study
SMANCS (15 kDa)	NCS	(^{51}Cr-SMA)–NCS	Chelation to DTPA reacted as anhydride with amino group of lysine conjugated to SMANCS	Mice with sarcoma	Matsumura and Maeda (1986)

[a] Mab = monoclonal antibody; HSA = human serum albumin; HPMA = hydroxypropylmethacrylamide; EAK = branched polypeptide of polylysine with side chains of alanine with terminal glutamic acid; SMANCS = styrene-maleic acid/anhydride copolymer conjugated to neocarzinostatin

[b] MTX = methotrexate; VDS = vindesine; DAU = daunomycin; DOX = doxorubicin; NCS = neocarzinostatin

can be radiolabelled separately from the antibody, biodistribution studies can examine the stability of the conjugate. An example that can be given here is the use of human serum albumin (HSA) as carrier. Work at Nottingham used monoclonal antibody conjugated to HSA at a ratio of three HSAs per antibody, but in which each molecule of HSA had up to 30 molecules of methotrexate. It was possible to prepare conjugates in which both protein moieties (antibody and HSA) had been labelled with ^{131}I or ^{125}I (using oxidative incorporation with iodogen) or in which only one had been labelled before chemical conjugation to the other (Pimm *et al*., 1988c). The biodistributions of such labelled conjugates could then be compared with those of labelled antibody and labelled HSA.

Synthetic polymers

Although site-specific targeting of drugs directed by antibodies is at first sight the most appropriate way to achieve accumulation of the targeted drug at the site of the pathological lesion, there is one situation in which simple passive accumulation of macromolecules may be sufficient to give increased target levels of drug. Thus tumours show a phenomenon of enhanced permeability and retention (EPR) of macromolecules (Matsumura and Maeda, 1986; Duncan, 1992; Yuan *et al*., 1995; Duncan *et al*., 1996; Matthews *et al*., 1996). Such a phenomenon has been known for many years with normal plasma proteins, but the more recent availability of synthetic macromolecules and the chemistry to attach drugs to them has awakened interest in this phenomenon. The physiology of this process is poorly understood, but it seems to depend on tumours having leaky vasculature, allowing entry into the interstitium of macromolecules, but poor lymphatic drainage to carry them away. The involvements of nitric oxide (Maeda *et al*., 1994) and a permeability-controlling vascular endothelial factor (Steyger *et al*., 1996) in this process have also been implicated.

Examples of four such macromolecular drug carriers examined for this EPR effect by radiolabelling of the polymer, are given in Table 9.3.

First, the conjugation of HPMA copolymer to doxorubicin and its therapeutic effect in mouse tumour models has been extensively described (Duncan, 1992; Seymour *et al*., 1994, 1995). This copolymer was not immediately amenable to radiolabelling, but later synthesis with only a small amount (about 1 mol%) of tyrosinamide in its backbone (Figure 9.5) meant that although it was unaltered in other respects, it could then be labelled with radioiodine (^{125}I) by oxidative incorporation into that tyrosine. Such radiolabelling with ^{131}I and ^{123}I has permitted imaging studies in mice with transplanted tumours (Pimm *et al*., 1993, 1996c; Figure 9.6, colour section) and the initiation of clinical trials including such imaging (Vasey *et al*., 1996). Secondly, a similar introduction of tyrosinamide into the polymer's backbone has enabled the radioiodine labelling of alginate (Al Shamkhani and Duncan, 1995a).

A third example of a polymeric drug-carrier conjugate labelled in the polymer is that of the series of branched chain polypeptides based on a backbone of poly(L-lysine) developed by Hudecz and his colleagues (Hudecz *et al*., 1992a; Hudecz, 1995). These polymers contain a polylysine backbone in which to the epsilon

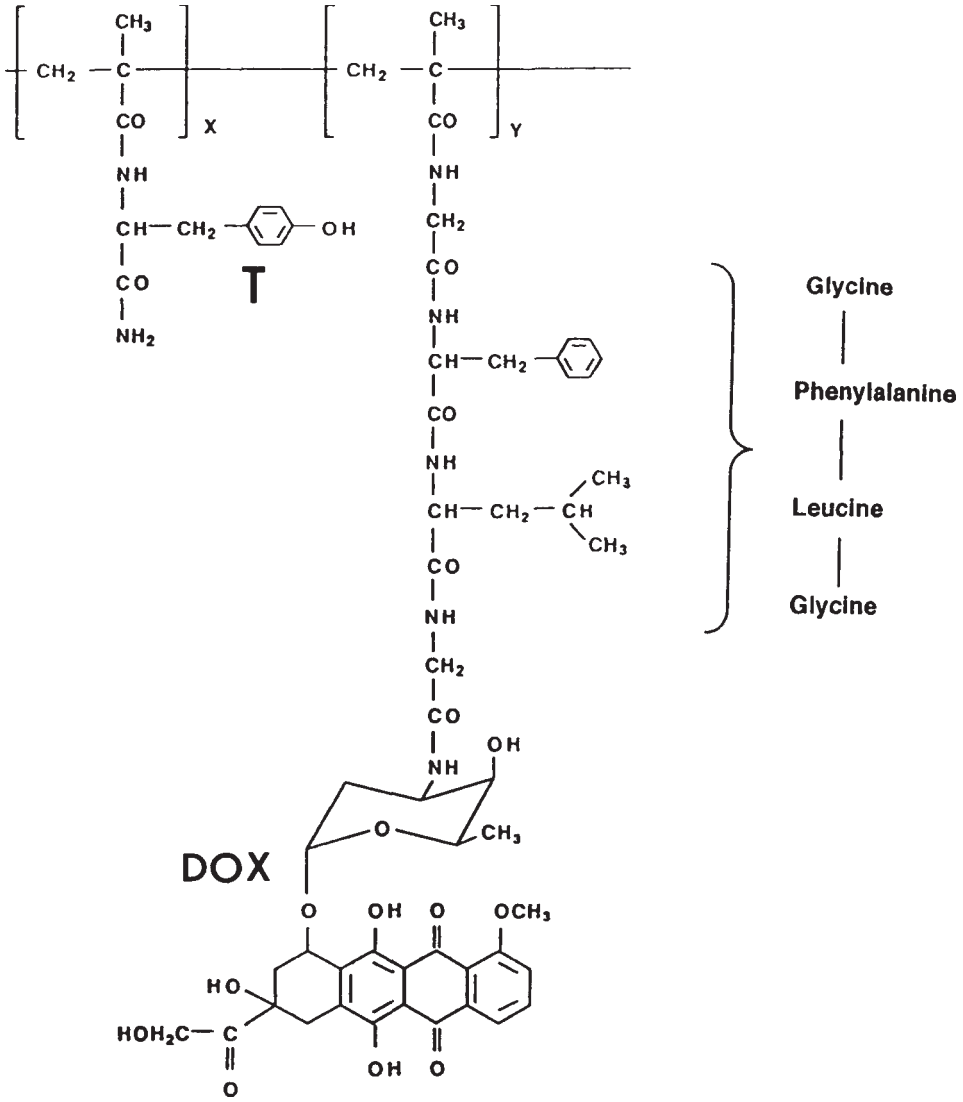


Figure 9.5 Structure of HPMA-doxorubicin conjugate containing tyrosinamide (T) in its backbone to allow its iodination. Such radiolabelling with ^{131}I and ^{123}I has permitted imaging studies in mice with transplanted tumours (Pimm *et al.*, 1993, 1996c; Figure 9.6) and the initiation of clinical trials including such imaging (Vasey *et al.*, 1996). The drug (DOX) is attached to the backbone via a glycine-phenylalanine-leucine-glycine tetrapeptide designed to limit drug release in plasma, but to allow its enzymatic cleavage intracellularly in lysosomes

amino group of each lysine is attached a short side chain composed of three DL-alanine residues with or without one other amino acid, either at a position next to the backbone [poly(Lys-(DL-ala-X)] or at the end of the branches [poly(Lys-(X-DL-ala)]. These polypeptides are designated by the conventional single letter

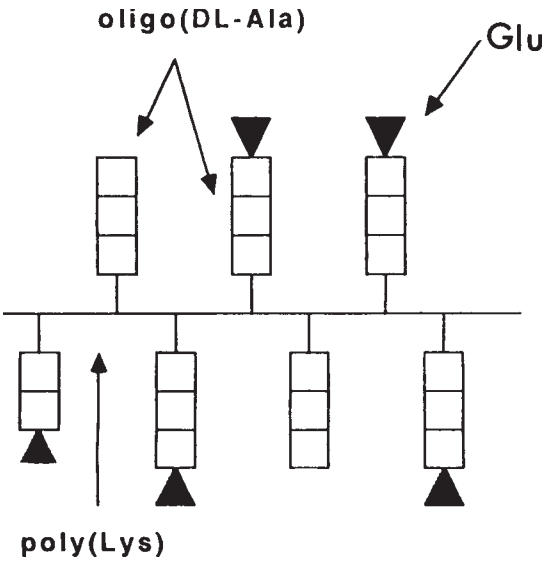


Figure 9.7 Schematic representation of the branched chain polypeptide designated EAK. This has a poly(L-lysine) backbone and each lysine has linked to its epsilon amino group a short chain of about 3 DL-alanine amino acids, each of which has an additional terminal amino acid, in this case glutamic acid. It is designated EAK from the conventional single letter abbreviation of its constituent amino acids (E = glutamic acid, A = alanine, K = lysine). These types of polypeptides have been labelled with radioiodines by reaction of the terminal amino group with Bolton and Hunter reagent (Figure 9.2), or with radiometals by chelation to DTPA following reaction of the amino groups with DTPA anhydride (Figure 9.3) (Pimm *et al.*, 1995b). Conjugates of EAK with methotrexate and daunomycin have been similarly radiolabelled and their biodistributions studied in comparison to the antibody alone

abbreviation of their constituent amino acids (E = glutamic acid, A = alanine, K = lysine, etc.). Thus poly(L-lysine) with alanine side chains is AK. One of the most widely studied of these polymers has glutamic acid as the side chain terminal amino acid (which renders the polymer amphoteric under physiological conditions), and thus is designated EAK (Figure 9.7). Variations in the type of additional amino acid in the side chain has a dramatic effect on the biodistribution of the polymers, principally due to changes in charge on the molecules (Clegg *et al.*, 1990; Pimm *et al.*, 1995a,b, 1996a). These polypeptides have been conjugated to anti-cancer drugs, particularly daunomycin (Hudecz *et al.*, 1992b) and methotrexate (Hudecz *et al.*, 1993).

It proved possible to label these polypeptides with radioiodine and a range of radiometals. Radioiodination could be carried out by reaction of the side chains' terminal amino groups with ^{125}I-labelled Bolton and Hunter reagent (Clegg *et al.*, 1990). Alternatively, reaction with non-labelled Bolton and Hunter reagent could be carried out first, and the product purified and stored for subsequent labelling

with ^{131}I or ^{123}I (Pimm *et al*., 1995b). Radiometal labelling was accomplished by reaction of the side chains' amino groups with DTPA anhydride, prior to chelation of ^{111}In (Pimm *et al*., 1992b, 1995a,b), ^{51}Cr (Pimm *et al*., 1995a,b), ^{99m}Tc (Frier *et al*., 1995) or ^{153}Sm (Awang, 1995). It is possible to modulate the biodistribution of any one such polymer by acetylation or succinylation of side chain terminal amino acids. In the case of EAK this will make it polyanionic and highly polyanionic respectively. It was found to be possible to conjugate EAK to Bolton and Hunter reagent or DTPA prior to acetylation or succinylation, so that such modified EAK preparations could still be labelled with radioiodines or radiometals (Pimm *et al*., 1995a). Gamma camera imaging of the biodistribution of ^{111}In-labelled AK, and EAK and its succinylated and acetylated variants, has been carried out in mice with transplanted melanoma (Pimm *et al*., 1994) or mammary carcinoma (Pimm *et al*., 1995c) in an attempt to visualize any EPR effects. At least with the mammary carcinoma, tumours were discernible on images with EAK and its acetylated derivative, indicating the feasibility of drug targeting with these polypeptides (see Figure 9.8, colour section). Imaging showed rapid clearance of the cationic AK to liver, spleen and kidneys, and to spleen and liver with highly anionic succinylated EAK.

One interesting observation to arise from imaging studies with such polymers is that imaging can show characteristics of the polymers hitherto unexpected, and difficult to assess except by dynamic imaging. Unlike monoclonal antibodies, which are very defined in terms of molecular mass and configuration, synthetic polymers are heterogenous, as a result of variation in the polymerization process which produced them. In imaging the biodistribution of EAK preparations in mice, Pimm *et al*. (1992a) showed an early (i.e. within minutes) unexpected clearance of about 30% of ^{111}In-labelled material through the kidneys and into the urinary bladder (Figure 9.9). By a few hours after injection this had stopped. Further analysis of this phenomenon showed that this was due to rapid renal filtration of a subcomponent of the EAK preparation. Thus gel permeation chromatography studies on Sephacryl S-300 of ^{111}In-labelled EAK showed that it eluted in a broad peak. Gel permeation chromatography of the material surviving in the blood of mice showed the loss of the later eluting material. That is, the later eluting components had been cleared from the circulation, while earlier eluting components survived in the circulation. Such unpredictable findings on gamma camera imaging, with their subsequent explanation by *in vitro* analysis of serum samples, illustrates clearly one of the advantages of imaging and its role in the overall development of drug carriers.

A fourth example of a synthetic polymer designed to have an EPR effect in tumours and thus potential for targeting anti-cancer drugs and where radiolabelling and imaging is feasible is that of a styrene-maleic acid/anhydride copolymer (SMA) conjugated to neocarzinostatin (NCS) to give the agent SMANCS (Matsumura and Maeda, 1986). This has been labelled with ^{51}Cr by chelation to a DTPA conjugate of SMANCS. SMANCS has no free amino groups for reaction with DTPA anhydride, and so was first conjugated with L-lysine (3–4 residues per molecule of SMANCS) using water-soluble carbodiimide. Such conjugates could be labelled with ^{51}Cr from $[^{51}Cr]CrCl_3$, and although not used by these

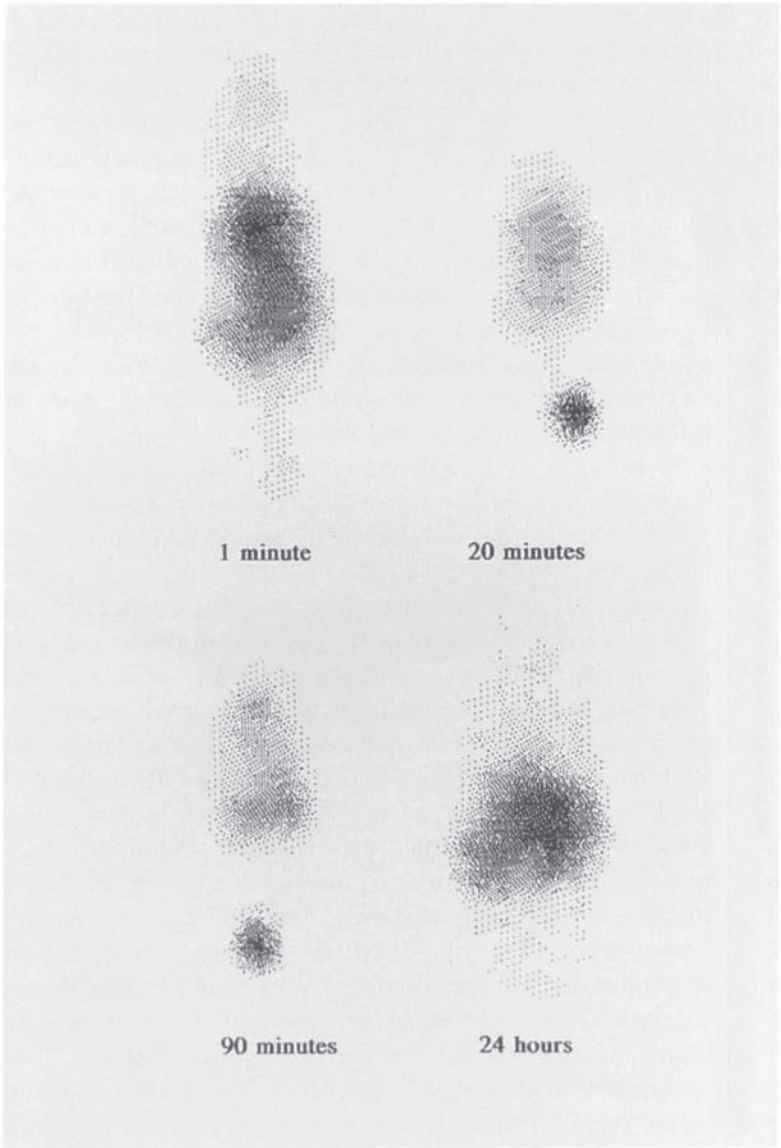


Figure 9.9 Gamma camera images (pin-hole collimator) of a mouse injected intravenously with about 0.5 MBq ^{111}In-labelled EAK branched chain polypeptide conjugated to daunomycin. Dynamic images were acquired at 1 min intervals for 20 min, followed by later static images. The majority of radiolabel remained in the blood pool, but some started to appear in the urinary bladder within minutes of injection, reaching about 33% of the total count rate within 1.5 h. Subsequently excretion was slower, and at 24 h there was no significant level of radioactivity in the bladder

authors it should be equally possible to label with other metallic radionuclides such as ^{111}In.

9.6.2 Drug-carrier Conjugates in which the Drug is Labelled

There are some circumstances in which it is possible to label a drug attached to a carrier independently of any label on the carrier itself. Often in these circumstances the drug is a protein or peptide. Some examples are summarized in Table 9.4.

The concept of labelling different parts of a drug-protein conjugate with different radionuclides can be well exemplified by studies on the biodistribution of conjugates of antibodies with ribosomal inhibiting proteins (RIPs). These are proteins (of plant or bacterial origin) which have the ability to inhibit protein synthesis in ribosomes. They act enzymatically, in the sense that each RIP molecule can function repeatedly in this inhibitory manner, and in theory only one molecule is needed to kill a cell. These RIP molecules often consist of two parts, one, the so-called B chain which binds to the cell surface so that subsequent internalization can take place, and an A chain which is the actual ribosomal inhibiting component. By separating the two chains, and conjugating only the A chain to an antibody (usually by a disulphide bridge), the exquisite specificity of the antibody is used to target the tremendously potent toxicity of the RIP.

Because both antibody and RIP A chain are proteins they would both be labelled if such a conjugate were put through a radioiodination procedure, or reacted with a chelating agent for radiometal labelling (e.g. Byers *et al*., 1987). But it is chemically possible for one of the components to be labelled independently of the other. Thus Perkins *et al*. (1987b) labelled sulphydryl-protected ricin toxin A chain (RTA) with ^{131}I (by oxidative incorporation), deprotected the sulphydryl groups and conjugated the ^{131}I-labelled RTA to a monoclonal antibody. Such a conjugate was used to image the rapid hepatic uptake of the immunotoxin in mice (Perkins *et al*., 1987b) (a feature of RTA because of its degree of natural mannosylation causing uptake by liver Kupffer cells and which was later overcome by using deglycosylated RTA or RTA produced by recombinant technology to lack any glycosylation). The same labelling procedure was used to prepare a conjugate between ^{131}I-labelled antibody and ^{125}I-labelled RTA (Byers *et al*., 1987), so allowing simultaneous evaluation of the components of the conjugate.

A similar procedure was later used to prepare an RTA-antibody conjugate in which the RTA had been reacted with DTPA anhydride prior to its coupling to antibody, so that the final product could be labelled with ^{111}In for use in imaging studies in Nude mice with human tumour xenografts reactive with the monoclonal antibody (Perkins *et al*., 1990).

Morgan *et al*. (1996), in using insulin B chain as a model, have used an ^{125}I-labelled conjugate of it and HPMA (iodination of such a conjugate would label only the peptide, because HPMA lacks sites for iodination unless synthesized with tyrosinamide in its backbone).

Table 9.4 Examples of drug-carrier conjugates in which the drug and/or carrier are radiolabelled

Carrier and approximate molecular mass[a]	Drug[b]	Type and site of radiolabel	Method of radiolabelling	Biodistribution assessed in	Reference and comments
Mab 791T/36 (150 kDa)	RTA	Antibody–(^{131}I-RTA)	Incorporation into tyrosine residues of RTA before conjugation to antibody	Mice	Perkins *et al.* (1987b), scintigraphy used to assess rapid hepatic clearance of conjugate, Byers *et al.* (1987), used to assess influence of hepatic blocking agents on liver clearance
		Antibody–(^{125}I-RTA)	Incorporation into tyrosine residues of RTA before conjugation to antibody	Mice	Byers *et al.* (1987), used to assess influence of hepatic blocking agents on liver clearance
		(^{125}I-antibody)–RTA	Simultaneous incorporation into tyrosine residues of antibody and RTA already conjugated	Mice	Byers *et al.* (1987), used to assess influence of hepatic blocking agents on liver clearance
		(^{131}I-antibody)–(^{125}I-RTA)	Separate incorporations into tyrosine residues of antibody and RTA before their conjugation	Mice	Byers *et al.* (1987), used to assess influence of hepatic blocking agents on liver clearance
		Antibody–(^{111}In-RTA)	Chelation to DTPA reacted as anhydride with lysine amino groups of RTA before conjugation to antibody	Nude mice with human tumour xenografts	Perkins *et al.* (1990), scintigraphy used to assess extent of tumour targeting of RTA by antibody
HPMA (20 kDa)	DAU	HPMA–(^{125}I–DAU)	Oxidative incorporation into DAU before its conjugation to HPMA	Mice	Rihova *et al.* (1989)
HPMA (23 kDa)	Insulin B chain	HPMA–(^{125}I–B chain)	Incorporation into tyrosine residues of B chain	–	Morgan *et al.* (1996)

[a] Mab = monoclonal antibody; HPMA = hydroxypropylmethacrylamide
[b] RTA = ricin toxin A chain; DAU = daunomycin

Recombinant technology is promoting the availability of a wide range of potentially therapeutic proteins and peptides and these pose a myriad of challenges in their pharmacokinetics and pharmacodynamics (Modi, 1994). Appropriate drug delivery will play a critical role in pharmacodynamics and prevention of adverse reactions. Clearly gamma camera imaging could play a useful role in this area, especially as such proteins and peptides should be simple to label with suitable radioiodines and radiometals before or after conjugation to potential drug carriers, and indeed recombinant proteins such as interferon and interleukin-2 have been labelled with ^{123}I for imaging studies for some time (e.g. Signore *et al*., 1992).

9.6.3 Drug-carrier Conjugates with an Additional Targeting Moiety

Although inert carriers may be able to alter the therapeutic index of drugs in a non-specific way, by reducing toxicity, allowing slow release, or by passive accumulation at targets such as tumours, there is also the possibility of site-specifically targeting them by attachment of hormones, sugars, etc. If one or more components of such conjugates could be appropriately radiolabelled, imaging not only of the carrier but of the targeting moiety, and possibly even the drug, might be possible (Table 9.5).

Hormones

It can be envisaged that a possible therapeutic construct will be a drug-carrier conjugate to which a hormone has been further added. Radiolabelling either or all of these components will enable their biodistribution collectively or independently to be assessed by scintigraphy.

The HPMA copolymer referred to above has been conjugated to melanocyte stimulating hormone (MSH), and HPMA-doxorubicin-MSH conjugates constructed for targeting doxorubicin to melanoma (O'Hare *et al*., 1993). By using a conjugate constructed with HPMA not incorporating tyrosinamide, labelling with ^{125}I or ^{123}I by oxidative incorporation has been used to label only the MSH for *in vivo* studies. It should be possible to label such a conjugate which does have tyrosinamide in its backbone, in which case both the MSH and HPMA would be labelled, or even to conjugate MSH pre-labelled with one radioiodine to HPMA labelled with another. It should also be quite feasible to label MSH in such a conjugate with ^{111}In via a DTPA chelation method, and indeed ^{111}In-labelled MSH has been used for clinical imaging of malignant melanoma (Wraight *et al*., 1992).

Another example that can be given here, and one in which labelling of different components of drug-carrier conjugates has been carried out, is that of polylysine backbone polymer EAK conjugated to a potent gonadotrophin releasing hormone (GnRH) antagonist. Such conjugates were prepared with EAK to which DTPA had been conjugated prior to its being linked to GnRH. Thus subsequent iodination by oxidative incorporation labelled only the GnRH peptide, while only the EAK was labelled by chelation of ^{111}In (Mezo *et al*., 1996).

Table 9.5 Examples of drug-carrier conjugates which have an additional targeting moiety and in which one or more components is radiolabelled

Carrier and approximate molecular mass[a]	Drug[b]	Targeting moiety[c]	Type and site of radiolabel	Method of radiolabelling	Biodistribution assessed in	Reference and comments
AcEAK (40 kDa)	None in this study, but MTX and DAU previously conjugated to this carrier	GnRH analogue	AcEAK–(^{125}I-GnRH)	Incorporation into tyrosine residues of GnRH within the conjugate	Mice	Mezo *et al.* (1996)
			(^{111}In-AcEAK)–GnRH (would be possible to prepare dual labelled conjugate)	Chelation to DTPA reacted as anhydride with side-chain terminal amino acids of AcEAK before its conjugation to GnRH	Mice	Mezo *et al.* (1996)
HPMA (25–90 kDa)	DOX	MSH	HPMA–(^{125}I-MSH)	Incorporation into tyrosine residues of MSH within the conjugate	Mice with B16 melanoma	O'Hare *et al.* (1993)
			HPMA–(^{123}I-MSH)	Incorporation into tyrosine residues of MSH within the conjugate	Mice with B16 melanoma	Pimm *et al.* (unpublished)
HPMA (25–90 kDa)	DOX	GAL	(^{125}I-HPMA)–DOX–GAL	Incorporation into tyrosine introduced into HPMA backbone as tyrosinamide	Mice	Seymour *et al.* (1991), Wedge *et al.* (1991) Conjugate with ^{3}H-labelled drug also used to follow drug moiety
			(^{123}I–HPMA)–DOX–GAL	Incorporation into tyrosine introduced into HPMA backbone as tyrosinamide	Nude mice with human colon carcinoma xenograft in liver	Pimm *et al.* (1996b). Imaging showed kinetics of liver uptake around tumour deposits (see Figures 9.11 and 9.12)

					Patient with primary liver cancer	Seymour *et al.* (1997). Imaging showed uptake into normal liver, but not tumour or pulmonary metastasis
CMD (80–100 kDa)	araC	GAL	([^{14}C]CMD)–GAL CMD–GAL–([^{3}H]araC)	Components labelled before conjugation	Mice	Nishikawa *et al.* (1993). GAL conjugates shown to target drug to liver parenchymal cells, MAN conjugates to non-parenchymal cells
		MAN	([^{14}C]CMD)–MAN CMD–MAN–([^{3}H]araC)	Components labelled before conjugation	Mice	
Biotinylated CMD (40 kDa)	CDDP	TNP-modified streptavidin	^{125}I-(TNP–streptavidin)–biotinylated CMD	Incorporation into tyrosine residues of streptavidin within its conjugate with TNP. (Biotin and streptavidin spontaneously complex with very high avidity.)	Mice	Schechter *et al.* (1996). Liver targeting of CDDP monitored by flame atomic absorption spectrometry of levels of platinum
Poly(L-lysine) (3 kDa)	Plasmid DNA	Mab to thrombomodulin	(^{125}I-DNA)–(polylysine-antibody)	Pre-labelled DNA spontaneously formed complex with polylysine conjugated to Mab	Mice	Trubetskoy *et al.* (1992). DNA was targeted to lungs via cell-surface thrombomodulin
Negatively charged polylysine (10 kDa)		$F(ab')_2$ of mouse/human chimeric anti-atherosclerotic plaque Mab (100 kDa)	$F(ab')_2$–(^{111}In-polylysine)	Chelation to DTPA reacted as anhydride with polylysine before its conjugation to $F(ab')_2$	Rabbits with atherosclerotic lesions	Narula *et al.* (1995). Charge modification of antibody fragment favourably influenced target discrimination. Although used here for atherosclerotic imaging, the application to drug targeting is obvious

[a] AcEAK = acetylated branched polypeptide of polylysine with side chains of alanine with terminal glutamic acid; HPMA = hydroxypropylmethacrylamide; CMD = carboxymethyl-dextran

[b] MTX = methotrexate; DAU = daunomycin; DOX = doxorubicin; araC = cytosine arabinoside; CDDP = cis-dichlorodiammineplatinum

[c] GnRH = gonadotrophin releasing hormone; MSH = melanocyte stimulating hormone; GAL = galactose; MAN = mannose; TNP = trinitrophenol; Mab = monoclonal antibody

Sugars for lectin targeting

Many mammalian cells have on their surface proteins called lectins which can bind sugar molecules. A number of different lectins with specificities for different sugars have been identified. Thus sugars may be used as specific recognition signals to deliver drugs to particular cell types in the body. A major example which will be discussed here is the galactose receptors on hepatocytes. These are the only cells in the body with lectins for this particular sugar. Thus hepatocyte-specific targeting might be possible using galactose-carrier-drug conjugates. A disease of major importance here is hepatitis B (the world's most important chronic viral infection), in which hepatocyte targeting of interferon-alpha, antiviral nucleoside analogues and possibly antisense oligonucleotides, are being investigated with galactosylated carriers such as neoglycoproteins, carboxymemyl-dextran and galactosyl-terminating glycoproteins such as asialofetuin and lactosaminated albumin (Nishikawa *et al*., 1993; Fiume *et al*., 1994; Mosigny *et al*., 1994; Meijer *et al*., 1996; Rensen *et al*., 1996).

This galactose receptor targeting approach also offers hope for the treatment of liver cancer. For example Duncan and her colleagues (Duncan *et al*., 1986; Seymour *et al*., 1991; Wedge *et al*., 1991) have described constructs of the copolymer HPMA with the anticancer drug doxorubicin but which also has galactose attached to the polymer backbone. As detailed above, if HPMA is constructed with a low level of tyrosinamide in its backbone it is possible to label it with radioiodine by oxidative incorporation into the tyrosine, and thus an HPMA copolymer-doxorubicin-galactose conjugate could be constructed which could be labelled with radioiodine (Figure 9.10). Using ^{125}I-labelled HPMA-doxorubicin-galactose conjugates it was shown that these were indeed captured by galactose receptors in the livers of rats and mice.

Using ^{131}I as the label it was possible to image this hepatic uptake in mice, and to show by quantification of count rates in ROIs on the computer images that uptake was about 70 to 80% of the whole body count rate within 10–20 min of intravenous injection (Pimm *et al*., 1993). Later, studies were carried out with ^{123}I-labelled HPMA-doxorubicin-galactose in Nude mice which had growing in their livers xenografts of a human colon carcinoma (Pimm *et al*., 1996b). Imaging was carried out with a gamma camera with a pin-hole collimator positioned first to include the whole body in the field of view, and then lowered closer to the animal to include only the upper abdomen. Using this method it was possible to again show hepatic clearance of virtually the whole of the conjugate within minutes of injection, but to show a defect in uptake believed to be coincident with the areas of tumour growth within the liver (see Figure 9.11, colour section). This latter interpretation was confirmed by imaging livers *ex vivo* after sacrifice of some of the animals, and showing that the defect in uptake of radiotracer agreed precisely with sites of intra-liver growth of tumour(s) (see Figure 9.12, colour section). (Human colon carcinomas such as the one used in the experimental study will not express galactose receptors and thus it is not surprising that there was no significant uptake of the tracer into the tumour

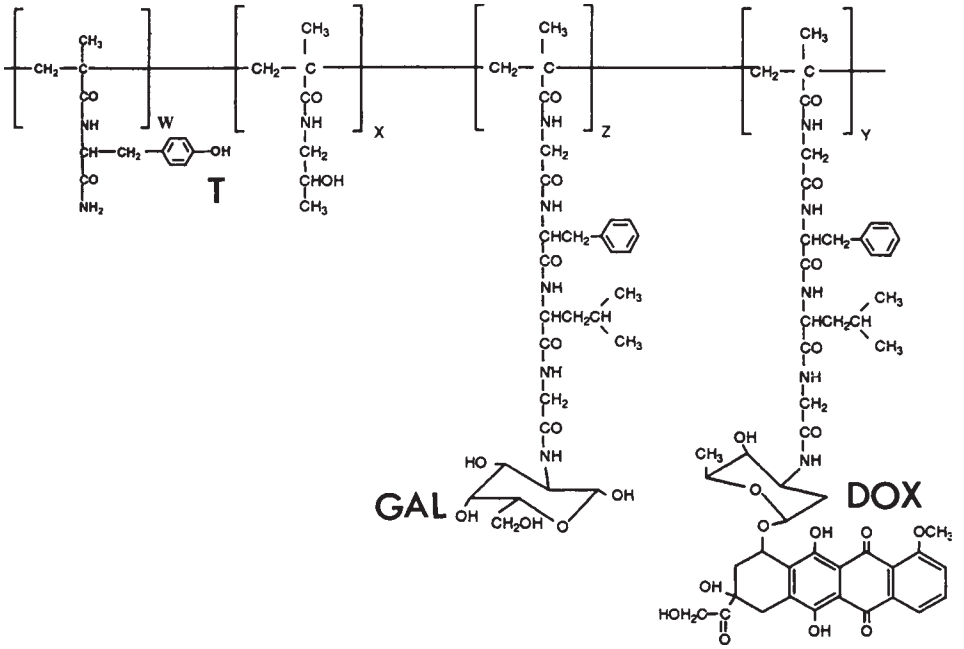


Figure 9.10 Structure of HPMA-doxorubicin conjugate with galactose (GAL) attached to its backbone for targeting to galactose receptors on hepatocytes. The copolymer contains tyrosinamide (T) in its backbone to allow its iodination. Such radiolabelling with ^{123}I has permitted imaging studies of hepatic uptake in mice, including Nude mice with hepatic xenografts of human colon carcinoma (Pimm *et al.*, 1996b; Figure 9.11) and the initiation of clinical trials including such imaging. Both drug (DOX) and GAL are attached to the backbone via a glycine-phenylalanine-leucine-glycine tetrapeptide designed to limit their release in plasma, but which allows enzymatic cleavage for release of DOX in lysosomes

sites. This study is described here only to illustrate the precision of the imaging method.)

Following development of the ^{123}I-labelling procedure for HPMA-doxorubicingalactose, clinical studies of its biodistribution by gamma camera imaging have now been initiated, and are validating findings from mice. For example, in a patient with primary hepatocellular carcinoma, imaging showed rapid, but transient, excretion of some ^{123}I into the urinary bladder, and substantial deposition of tracer in the liver. Correlation with CT scans showed that this coincided with an area of apparently normal liver, with a level of ^{123}I three to four times higher than in intra-hepatic tumour tissue (Seymour *et al.*, 1997). (Hepatomas frequently lose expression of galactose receptors, which would explain this finding, and in this patient a single pulmonary metastasis also showed no significant uptake of ^{123}I.)

Antibodies

The interaction of an antibody with its appropriate antigen offers great potential for the targeting of drugs. However, there are some limitations to this technique which might be overcome by attachment of the antibody to a polymer, to which drug can also be attached.

On the one hand conjugation of a polymer to an antibody might favourably alter the distribution of the antibody. For example cell surface and intercellular matrices contain acidic residue, making them negatively charged. However antibodies are basic, positively charged glycoproteins encouraging non-specific ionic interactions with normal tissues, increasing normal tissue levels of the targeted drug and reducing site-specific targeting. Modification of the antibody with negatively charged modified polymers may increase target discrimination (Narula *et al*., 1995).

On the other hand conjugation of an antibody to a polymer might favourably alter the distribution of the polymer (to which drug has been attached), achieving some site-specific targeting of that drug. The drug may be a small conventional therapeutic agent, chemically linked to the carrier. Another possible application of this approach is for targeting of anti-sense oligonucleotides or targeted gene delivery. For example Ferkol *et al*. (1993) and Trubetskoy *et al*. (1992) used poly(L-lysine) carrier chemically conjugated to antibodies to carrier plasmids into mouse lung epithelial cells and human colon carcinoma and epithelial tracheal cells. In this system the DNA spontaneously formed complexes with the poly(L-lysine) chemically linked to the antibodies. Trubetskoy *et al*. (1992) used ^{125}I-labelled DNA to show its lung uptake in mice after such complex formation with antibody-poly(L-lysine). The possibility of gamma camera imaging in this and similar situations with DNA labelled with ^{131}I or ^{123}I is obvious, and in addition Hnatowich *et al*. (1996) have recently described a technique for labelling oligonucleotides with ^{99m}Tc.

9.7 Polymers as Radiopharmaceuticals

Although the present chapter is about the role of scintigraphy in the development of drug-carrier conjugates rather than radiopharmaceuticals *per se,* it should also be recognized that some features of synthetic polymers which may make them suitable as drug carriers may also in some circumstances make them suitable for development as straightforward radiopharmaceuticals.

Thus those that show prolonged survival in the blood may be adapted to blood pool imaging agents (e.g. Bogdanov *et al*., 1994; Frier *et al*., 1995), or following labelling with gadolinium as magnetic resonance imaging (MRI) contrast agents (e.g. Adam *et al*., 1994; Frank *et al*., 1994; Loubeyre *et al*., 1996). Non-specific accumulation, for example at the sites of tumours, makes some polymers suitable for examination as tumour imaging agents, either by scintigraphy (e.g. Pimm *et al*., 1995c) or MRI (e.g. Orang Khadivi *et al*., 1994; Adam *et al*., 1996), while others may be suitable for imaging sites of inflammation or infection (e.g. Gupta

et al., 1995; Frier *et al*., 1995). Clearly site-specifically targeted polymers could be used not only to target therapeutic agents but also for imaging. For example galactosylation of polymers or proteins to allow targeting of hepatocytes can also be turned to imaging functional hepatic mass (Virgolini *et al*., 1993).

Clearly there is potential here for two-way interaction. Scintigraphic evaluation of agents suitable as drug carriers may aid in the identification of possible radiopharmaceuticals, and conversely agents developed as radiopharmaceuticals for identification of specific lesions, and whose pharmacokinetic characteristics have been fully evaluated by scintigraphy, may then be considered as agents for possible targeting of therapeutic agents to that type of lesion.

9.8 Conclusions

A major requirement with drugs of the conventional type and the newer generations of therapeutic agents (e.g. peptides, genes, antisense oligonucleotides) is to target them to their intended sites of action, and the development of carrier molecules, and ways to study their biodistribution will be paramount.

Although there are many examples of drug formulation studies in which a radionuclide is incorporated into the dosage form for gamma scintigraphy (Meseguer *et al*., 1994 and chapters in the present volume), the use of the technique for soluble drug carriers seems to have been more limited, and relatively few examples have been given in this chapter. This may in part be due to the limited availability of gamma camera equipment, but cannot be explained by any intrinsic difficulty of the technique, because the methods for radiolabelling soluble (usually polymeric) drug carriers, peptide drugs and oligonucleotides described and illustrated in this review are chemically and technically simple to perform, certainly relative to the original design and synthesis of the drug carrier itself. Indeed there are numerous studies with materials labelled with radionuclides not suited for gamma camera imaging (e.g. the radioiodine ^{125}I, and the radiometal ^{51}Cr), but which could easily be replaced by those that are (i.e. ^{131}I or ^{123}I, and ^{111}In respectively).

In summary the technique has the following clear advantages:

- In phase I clinical trials and in any fuller, therapeutic, studies, it is virtually the only way to evaluate targeting to the tissue/organ of interest.
- In clinical and experimental studies time course studies can be carried out, even at intervals of a few seconds. In animal studies this results in savings in the numbers of animals used, which is important for both ethical and financial considerations. It also allows large numbers of potential materials to be studied rapidly, even simultaneously if labelled with different imaging radionuclides.
- Numerous repeat studies can be carried out in the *same* patients or experimental animals, thus allowing visualization of targeting to disease sites as the disease progresses or perhaps as it is controlled by the drug-carrier conjugate.

- Repeat studies will allow visualization of the development of any adverse responses such as immunogenicity which may have a detrimental effect on biodistribution and/or target discrimination.
- It is feasible to label drug, carrier and any targeting moiety with different radionuclides, allowing simultaneous imaging of their biodistributions.

This area seems ripe for further development, and gamma scintigraphy is advocated for wider consideration in both experimental and clinical situations.

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Note in Proof

Positron Emission Tomography in Drug Research

Positron emission tomography (PET) has emerged as one of the most powerful tools for understanding basic metabolic function in man. The ability of PET to image molecular pathways and molecular interactions *in vivo* has led to its increased use in both biological research and medical diagnosis. PET studies are capable of the measurement of specific processes in tissue, such as tissue perfusion, metabolic rate, protein synthesis and fatty acid metabolism. Whereas clinical pharmacology is generally based on the measurement of drug concentration in body fluids, PET imaging provides the opportunity for the *in vivo* study of tissue-based pharmacokinetics of appropriately radiolabelled drugs.

PET imaging has two basic strengths. Firstly, by virtue of the physical process of coincidence detection of dual 0.5 Mev photons (see Chapter 2) the spatial resolution of the images is superior to that of single photon emission tomography (SPECT) performed on a gamma camera. Secondly, and more importantly, the positron emitters which are available for clinical and experimental use are based on the "organic" elements of carbon, nitrogen, and oxygen which are the basic constituents of most drugs. The other main positron emitter which is in routine use is fluorine, which is essentially an analogue for hydrogen. These positronemitting tracers are of greater biological significance than radiometals such as Tc-99m or In-111 which are single photon emitters used for gamma scintigraphy. Other less commonly used positron emitters include potassium-38, selenium-73, bromine-75 and bromine-76.

Some examples of the use of clinical PET imaging are given in Table 1. It can be seen that this technique offers wide scope for use by the pharmaceutical industry. The main disadvantage in the use of positron emitting radionuclides is that they require a cyclotron for production and that they generally have short physical half-lives, which reduces the time available for radiolabelling and manufacturing of

Table 1 Some examples of PET imaging in pharmacological research

Radionuclide	Physical half-life (min)	Tracer	Measured parameter
C-11	20	Nomifensine	Dopamine uptake
		Raclopride	Dopamine D_2 receptors
		SCH 23390	Dopamine D_1 receptors
		Diprenorphine	Opiate receptors
		Flumazenil	Benzodiazepine receptors
		Deprenyl	Monoamine oxidase B sites
		Methionine	Amino acid metabolism
O-15	2	Oxygen	Oxygen metabolism
		Carbon dioxide	Blood flow
		Water	Blood flow
		Carbon monoxide	Blood volume
N-13	10	Cisplatin	Biodistribution
F-18	110	Levodopa	Dopamine storage capacity
		Deoxyglucose	Glucose (energy) metabolism
I-124	4.18 days	Idoxifene	Oestrogen receptor binding

the material under test. Because of the short physical half-life PET radiochemistry has to be performed rapidly. Chemical separation from the target material and radiolabelling of the test compound is usually performed using remote automated techniques. This is obviously a critical stage when considering a PET study since radiolabelling a novel drug or compound with a positron emitter requires careful preparation and planning.

Clinically PET imaging is becoming of increasing importance in the fields of neurology, oncology and cardiology. These are also the main areas of interest in pharmaceutical development. The requirements for PET studies of drug delivery are similar to those for gamma camera studies. However, the highly specialized nature of the equipment required for the production of positron emitters and PET imaging has in the past severely restricted the use of this technique in clinical research. The prospects are encouraging as technical and engineering developments have increased the availability of imaging instrumentation. It is now technically possible to modify a dual head gamma camera for positron imaging and a number of manufacturers have produced software for coincidence detection of the dual 0.5 MeV photons on such systems. Widespread clinical imaging is envisaged using the longer-lived positron emitting radiopharmaceutical F-18 fluorodeoxyglucose (FDG). It is therefore apparent that PET imaging will become more commonplace both as a tool for clinical diagnosis and in drug development.

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