THIRD EDITION

SWINE in the LABORATORY

Surgery, Anesthesia, Imaging, and Experimental Techniques

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To my family, Paula, Katelyn, Ashley, Matt, Doug, and Seth

Contents

Preface

This book is an expanded and updated version of *Surgery, Anesthesia, and Experimental Techniques in Swine*, 2nd edition, which was published in 2007. Over the years, I have kept a file of questions and communications concerning the use of swine as animal models that arose from readers of that publication. We also hold annual training classes for swine users, which have involved discussion and demonstration of many models and surgical techniques that have not yet been published in another format. Answers to all of the questions I have been asked from these sources are included in this edition. The purpose of the book is to provide a practical technical guide for the use of swine in biomedical research. The primary target audience is investigators, veterinarians, and technicians using swine for experimental procedures. Alison C. Smith, DVM, has been added to the cover since she provided valuable editorial comments. This new edition will become part of the series of textbooks sponsored by the American College of Laboratory Medicine (ACLAM), and all royalties will be donated to the ACLAM Foundation which funds research projects.

Predominantly this book provides information on models produced by surgical or other invasive procedures. There is a presumption that physicians and scientists will have at least a rudimentary knowledge of the fundamental principles of surgery. It is impossible to fully describe all the models that can be developed in this species; however, there should be enough detail to provide basic principles of performing experiments with an organ or system of interest.

There are new chapters on toxicology, transgenics, cancer, necropsy techniques, and an overview of porcine models that were not included in the previous edition. The number of tables of normal values has been greatly expanded both in the chapters and in the appendix. Rajesh K. Uthamanthil, DVM, PhD, from the Fred Hutchinson Cancer Center and Ramon Duran-Struuck, DVM, PhD, led the efforts to submit two new chapters. Michael Sturek, PhD, and colleagues from Indiana University have updated a chapter with information on the unique Ossabaw pig along with scanning images that are included on the DVD. Niels Christian-Ganderup, MS, of Amplexa Genetics in Denmark submitted a new chapter providing an overview of the use of swine in research.

Dr. Guy Bouchard, Derek Brocksmith, and Andrew Henning from Sinclair Research Center supplied much of the information for Yucatan, Hanford, and Sinclair minipigs as well as important collaboration in developing the DVD. The staff of Ellegaard Göttingen Minipigs in Denmark were very helpful in supplying me with the information on their minipig. Anatomical and physiological details have also been expanded. Much of this information has never been published in any other format. The sections on anesthesia and perioperative care have been updated substantially. In particular, I have attempted to provide practical information concerning postoperative care for the more complex models. The issues concerning anesthesia and perioperative care have been the primary source of questions I have received over the years.

The DVD attached to this book is greatly expanded. New in this edition are normal data on farm and minipig breeds, a colored atlas of histology, videos on training swine, videos on surgical procedures, intubation, and a file of short articles on various research and technical procedures. I owe a great deal of gratitude to my colleagues from the PET Center and Aarhus University Hospitals in Aarhus, Denmark, for this contribution. The imaging effort was led by Aage Kristian Olsen Alstrup, DVM, PhD, and his colleagues. Jan Duedal Rölfing, MD, PhD, from Aarhus collaborated with information on the orthopedic chapter. I would also like to express my appreciation to Frederik Dagnaes-Hansen, DVM, PhD, and Birgitte Koushholt, DVM, PhD, for continuing to invite me to participate in their training activities in Aarhus. This continued collaboration has been very helpful to me.

Most of this book was written by me as a sole author; however, I received input from my fellow faculty members at the Medical University of South Carolina. In particular, Katherine Morgan, MD, from the Department of Surgery added a great deal of detail in the section on endoscopy.

Joseph Sistino, PhD, collaborated with information on cardiopulmonary bypass. Other faculty and staff members in the Department of Comparative Medicine were also helpful to me in this endeavor, with advice and editorial comments. Kris Helke, DVM, PhD, contributed histological images of normal tissues and two chapters, Mary Ann McCrackin, DVM, PhD, also contributed to two chapters. Other colleagues have supplied me with photos from their work, and they are credited in the photo captions they supplied. Technical staff from the Surgical Research Lab were responsible for producing many of the videos on the DVD concerning training and perioperative care. I thank Roxanna Swagel, Erica Hussey, Clayton Craft, Tyrique Brown, and Jennifer Hendrick. Dr. McCrackin and Marissa Wolfe, DVM, also helped with the effort to produce the videos.

My primary interest in publishing this textbook is to decrease the learning curve associated with using swine as models. The default terminology and names of structures are based upon veterinary terminology rather than human, which may initially cause some confusion with some, but the meaning should be clear. It is hoped that my ideas and suggestions will contribute to the appropriate and humane use of swine in biomedical research.

M. Michael Swindle

Editors

M. Michael Swindle, DVM, is a professor emeritus and retired chairman of the Department of Comparative Medicine at the Medical University of South Carolina. He received BS and DVM degrees from Texas A&M University (1969) and is a diplomate of the American College of Laboratory Animal Medicine (1982) and the European College of Laboratory Animal Medicine (2001). He was in the U.S. Army from 1969 to 1972, in private veterinary practice from 1972 to 1979, and at Johns Hopkins Medical School from 1979 to 1985.

He is the recipient of research awards from the American Heart Association, the Academy of Surgical Research, the American Association for Laboratory Animal Science, and the American Society of Laboratory Animal Practitioners. He was honored as an outstanding alumnus of the College of Veterinary Medicine. His many publications and presentations are mainly in the areas of experimental surgery, anesthesia, and swine as animal models. Dr. Swindle is a private consultant but continues to be active in research and teaching activities using porcine models.

Alison C. Smith, DVM, is the interim director, Division of Laboratory Animal Resources, and interim chair, Department of Comparative Medicine at the Medical University of South Carolina (MUSC). She is a professor in the Department of Comparative Medicine and an associate professor in the Department of Surgery. She has been employed at MUSC since 1987. Dr. Smith received her undergraduate education from Illinois Wesleyan University in Bloomington, IL, and BVSc and DVM degrees from the University of Illinois in 1979 and 1983, respectively. She was in private veterinary practice from 1983 to 1987, first in a traditional small animal practice and then in a busy small animal emergency practice. She was trained in laboratory animal medicine at MUSC and is a diplomate of the American College of Laboratory Animal Medicine (1991). Her publications and presentations are primarily in the area of swine as large animal surgical models, anesthesia and analgesia of swine, and perioperative care of swine.

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1 Biology, Handling, Husbandry, and Anatomy

Mary Ann McCrackin and M. Michael Swindle

CONTENTS

INTRODUCTION

Swine are used extensively in biomedical research with a significant increase in recent decades. They are replacing other mammalian species such as the dog as animal models, while also being developed as a model on their own merit. Swine have become accepted as a general surgical model. This increased use of swine in the laboratory would not have occurred if technical procedures for handling, husbandry, and anesthetizing the species were not refined during the same time period.

Major symposia on the use of swine in biomedical research have been organized in the last few decades, and their proceedings have resulted in books providing a vast amount of baseline information on the species (Swindle, 1992; Tumbleson, 1986; Tumbleson and Schook, 1996). Much of the discussion in these books is based on particular laboratory models and may not provide enough detailed information overall for persons not familiar with the species.

Other reference books on swine provide information on specific experimental fields (Lindberg and Ogle, 2001; Pond and Mersmann, 2001; Stanton and Mersmann, 1986; Swindle, 1983) in addition to the general references on anatomy and biology given in this chapter. Technical guidelines and health-care programs for managing swine in research have also been published (Bollen et al., 2000; Laber et al., 2002; Swindle et al., 2003).

This textbook is an updated and expanded edition of *Swine in the Laboratory: Surgery, Anesthesia, Imaging, and Experimental Techniques* (Swindle, 2007). The purpose of the book and this chapter, in particular, is to provide concise technical data on the use of swine in the laboratory,

with an emphasis on surgically produced models. Tables of normal values are included in the appendix as well as within the various system chapters.

BREED SELECTION

All domestic and miniature swine are *Sus scrofa domestica*, as are many of the feral strains of swine found throughout the world. However, they differ substantially from each other in appearance, behavior, and size. Within this textbook, all commercially available breeds of swine raised mainly for meat production are referred to as domestic farm breeds, unless the breed is of particular importance to the model being described when the breed is mentioned. The most common breeds of domestic swine found in the literature are Yorkshire, Landrace, Duroc, and crossbred animals. Breeds of miniature swine are either naturally occurring or commercially raised for research or pet keeping. Miniature swine are referred to by their particular breed if it is mentioned in the references. Miniature and micro pigs are distinguished by size, with miniature pigs being the larger of the two. Consider Yucatan™ miniature male and female pigs at 6 months of age, weighing about 46 and 44 kg (Sinclair, 2009b) compared to smaller, micro Yucatan miniature male and female pigs at 6 months of age, weighing about 20 and 19 kg, respectively (Sinclair, 2009a).

The most commonly used breeds of miniature swine that are commercially available and which appear in recent biomedical literature, are the Yucatan miniature (Figure 1.1) and micro varieties; the Hanford (Figure 1.2), the Göttingen (Figure 1.3), the NIH, and the Sinclair S-1 (Hormel; Figure 1.4). Other breeds of miniature pigs are available in limited markets and are not widely cited in the literature currently. These include the Ossabaw (Figure 1.5), Banna, Ohmini, Pitman-Moore, Chinese Dwarf, Meishan, Vietnamese potbellied (Figure 1.6), and the Panepinto, to name a few. Some of these, such as the Hanford, Sinclair, and Göttingen, were the foundation stock for miniature breeds currently in use. Panepinto (1986) has written a summary of the derivation of various breeds of miniature swine. Of the breeds currently important to biomedical research, as listed previously, only the Yucatan and Ossabaw are naturally occurring miniature breeds.

The main difference between commercial farm breeds and miniature breeds is size at sexual maturity. Miniature breeds were purposely developed to provide a slower-growing porcine model that would be manageable at sexual maturity. Domestic swine exceed 100 kg body weight (BW) at sexual maturity and will continue to grow at an accelerated rate that is breed and diet related. It is not unusual to have a domestic pig at 12 months of age exceed 200 kg. In contrast, most breeds of miniature pigs weigh 12–45 kg at sexual maturity (Figure 1.7; Fisher, 1993; Swindle et al., 1994).

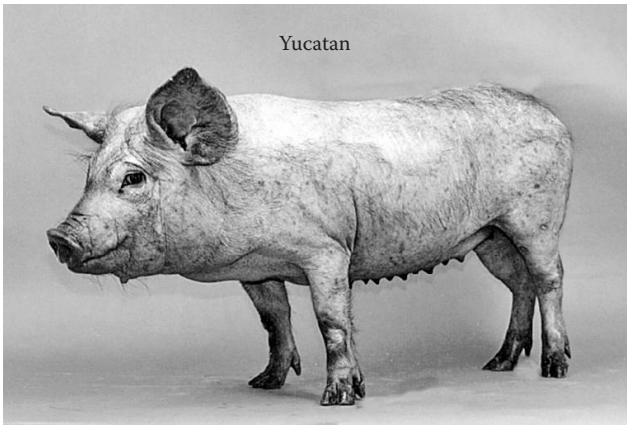


FIGURE 1.1 Yucatan™ miniature pig. (Courtesy of Sinclair Research Center.)

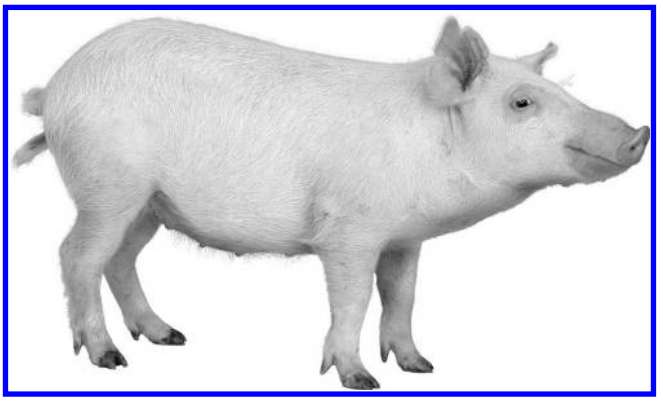


FIGURE 1.2 Hanford miniature pig. (Courtesy of Sinclair Research Center.)

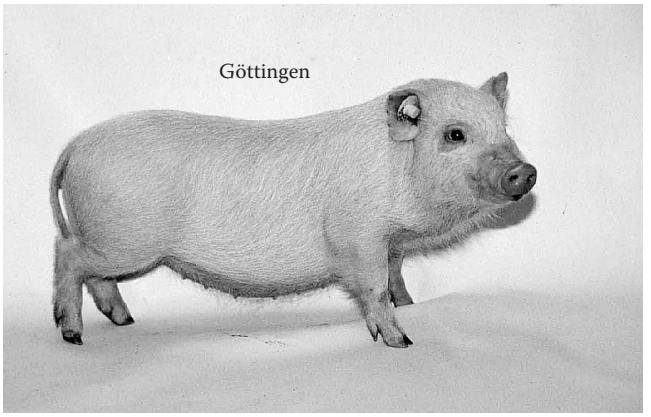


FIGURE 1.3 Göttingen miniature pig. (Courtesy of Ellegaard Minipigs Denmark.)

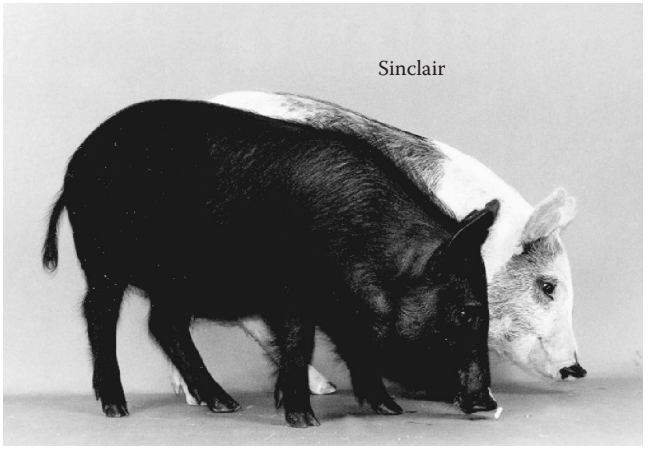


FIGURE 1.4 Sinclair S-1 miniature pig. (Courtesy of Sinclair Research Center.)

FIGURE 1.5 Ossabaw miniature pig. Obese (left) and thrifty (right) phenotypes. (Courtesy of Michael Sturek, PhD, University of Indiana.)

FIGURE 1.6 Vietnamese potbellied miniature pig.

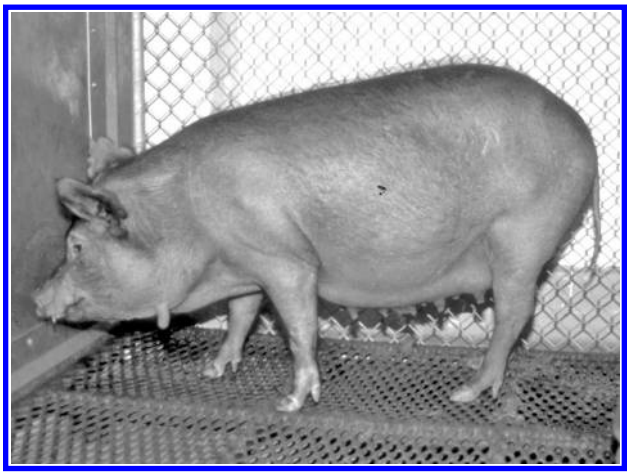
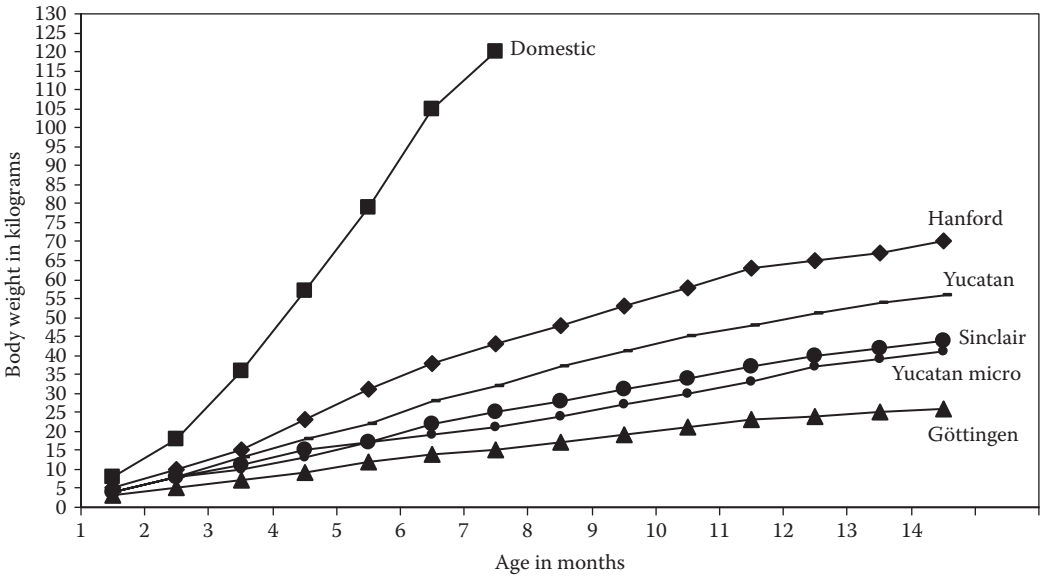


FIGURE 1.7 Sexually mature Yucatan™ pig on a plastic coated expanded metal floor.

TABLE 1.1
Relative Body Weights and Growth of Domestic and Miniature Pigs



It is neither cost-effective nor user friendly to attempt to use domestic breeds as chronic models merely to save money on the purchase price. The higher cost of feed, husbandry considerations, and personnel safety issues quickly negate any perceived savings. Also, the rate of growth of domestic swine on long-term projects scientifically skews the results unless growth is part of the hypothesis. Rather, the breed of animal should be selected on the basis of biological characteristics necessary to conduct the experimental study. Table 1.1 represents a growth chart of estimated weights for various breeds of swine. Variations from this chart due to individual genetics, nutrition, gender, health status, and environmental conditions are possible. Species-specific growth charts are included in the appendix.

All investigators should describe accurately the swine used in their particular research in the materials and methods section of manuscripts. Essential information for meaningful comparison to other studies includes the anesthetic methods, breed, weight, age, and gender. Information that may be important in particular research protocols or for some journals includes the source of the animal, its health status, a description of the housing environment and diet, and pertinent genetic information. A recent attempt has been made to standardize the description of animal studies in cardiopulmonary resuscitation research because of the problems associated with making meaningful comparisons of results between laboratories (Idris et al., 1996). This document may be helpful as a blueprint for other areas of research that require comparisons between laboratories.

BIOLOGY

In the appendix, Tables A.1 through A.9 and Tables A.31 and A.36 provide biological values for growth, development, BWs, and organ weights of miniature swine used in research. If a particular breed of pig is not listed in the tables, then the comparison to the normals should be based on age. Within this chapter, only the general biological characteristics of swine that should be of practical importance to biomedical researchers and laboratory animal personnel are discussed. A complete reference book on the biology of domestic swine has been published (Pond and Mersmann, 2001),

and information on the biology and diseases of domestic swine with an emphasis on data important to commercial production of food animals is also available (Straw et al., 1999). Reference books have been written on the Vietnamese potbellied pig for pet owners and veterinary practitioners (Boldrick, 1993; Reeves, 1993). Additional information is provided in the system chapters and the appendix of this book.

TAXONOMY AND NOMENCLATURE

Swine are even-toed ungulate (hoofed) mammals (Etnyre et al., 2011). The taxonomic classification of the species used in research is as follows:

Phylum: Chordata
  Subphylum: Vertebrata
Class: Mammalia
Order: Artiodactyla
  Suborder: Suiforme
Family: Suidae
Genus: *Sus*
Species: *scrofa*
Subspecies: *domestica*

Agricultural terminology is typically used in the literature and may cause some confusion to investigators not familiar with the terms. Some common definitions are listed as follows:

Swine	Refers to the whole species or multiple animals.
Pig	Newborn animal; sometimes used interchangeably with swine, or to denote a single animal.
Shoat	Weaned animal.
Gilt	Immature female.
Sow	Sexually mature female.
Barrow	Castrated male.
Boar	Sexually mature male.
Farrowing	Parturition, giving birth to piglets.
Porcine	Adjective used to describe anything pertaining to swine.
Sounder	Herd of wild swine.

REPRODUCTION

The sow has a bicornuate uterus and is polytocous. Its chromosome number is 2N = 38. The gestation period is typically 114 d (range, 110–116 d) for the larger breeds, with that of miniature breeds usually shorter by a few days. Swine are weaned around 4–6 weeks of age (range, 3–8 weeks) and may start to eat solid food (creep feed) as early as 2–3 weeks of age.

The average estrous cycle is 21 d (range, 18–24 d), with estrus being typically 2 d (range, 1–5 d). In commercial operations, sows are usually mated twice when they are showing vulvar swelling and willingly accept the boar. Ovulation occurs 30–36 h after the onset of estrus.

Sexual maturity ranges from 3 to 7 months, most miniature breeds being sexually mature at 4–6 months of age. Sows will rebreed as soon as 3–9 d after parturition and may have approximately two litters per year for 5–6 years (Frandsson, 1981). The litter size varies greatly between breeds and may range from 4 to 20. However, attrition of the newborn may substantially reduce the number of animals weaned. In general, litter size is reduced with miniature swine.

In the appendix, Tables A.37 through A.46 provide reproductive values for miniature swine, fetal skeletal diagnoses and their frequencies of occurrence, and fetal population data.

GROWTH AND DEVELOPMENT

The birth weight of pigs depends on the breed, nutritional status of the sow, and size of the litter, and ranges from 0.5 kg in smaller miniature pigs to 1–2 kg in domestic swine (Fisher, 1993; Swindle et al., 1994).

Newborn pigs require an external heat source such as a heat lamp to provide an ambient temperature that is close to normal body temperature to prevent cold stress. They do not have brown fat, nor do they metabolize glycogen and lipid stores for thermal control. The ability to regulate temperature gradually improves with physiological maturity over the first few days of life (Committees to Revise the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 2010). Pigs are mobile shortly after birth. They nurse almost hourly in the first few days of life. In large litters, they may have to compete with littermates for adequate nutrition, depending upon the number of nipples on the sow.

The growth rate of domestic swine is significantly different from that of miniature breeds. Domestic swine are bred and managed so as to grow to 100–110 kg of BW within 150–200 d of age. Their daily weight gain can be between 0.2 and 1.0 kg depending upon husbandry and nutritional circumstances within a particular age group. The weight range of 12–45 kg for miniature breeds at the same age provides a vivid contrast of the differences in growth rates (Fisher, 1993; Swindle et al., 1994).

The epiphyses of the long bones are not completely closed in domestic swine until approximately 3.5 years. Miniature swine vary depending upon the stature of the animal. For example, Yucatan microswine have epiphyseal closure at 1.5–2.0 years, whereas standard Yucatans have closure at 3.0–3.5 years.

Even though the commercial life span of domestic swine is less than 6 months for meat production and less than 5 years for breeding stock, their general life span may be 15–25 years depending upon the breed and environmental circumstances.

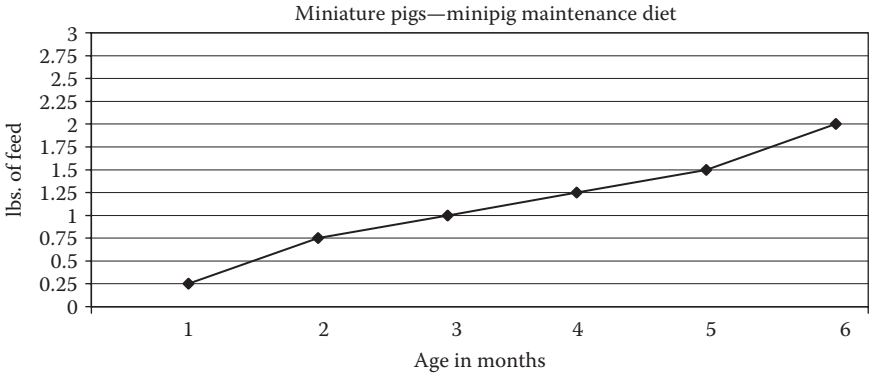
NUTRITION

Swine are true omnivores and will consume a wide variety of diets and test substances. Specific test diets, such as those for inducing atherosclerosis, are discussed with model descriptions in this book when appropriate.

Commercial pig food is designed to enhance the growth rate of domestic swine and may contain food additives such as antibiotics or growth stimulants. The feeding of various rations to swine within particular weight groups is a science based on optimum muscle growth. As a general guideline, commercially raised swine require metabolizable energy (ME) intake for the indicated weights as follows (in kcal/day): 3265 (10–20 kg), 6050 (20–50 kg), 8410 (50–80 kg; National Research Council, 1998).

Ad libitum feeding of swine for long-term projects may produce obesity without any particular gain in nutritional value. Because this rapid weight gain is undesirable in miniature swine, commercial feed manufacturers have developed diets specifically for these breeds, which can also be fed to farm pigs used in research. The diets are designed to limit weight gain without causing nutritional deficiencies, which may occur if regular commercial diets are restricted only on a weight or volume feeding basis. Vitamin E and selenium content are of importance to swine and deficiencies can lead to cardiac and hepatic pathology. Experience has shown that the feeding of miniature swine diets to domestic swine does not cause nutritional disorders; however, chronically housed animals should have their BW and general condition monitored (Bollen, 2001; Fisher,

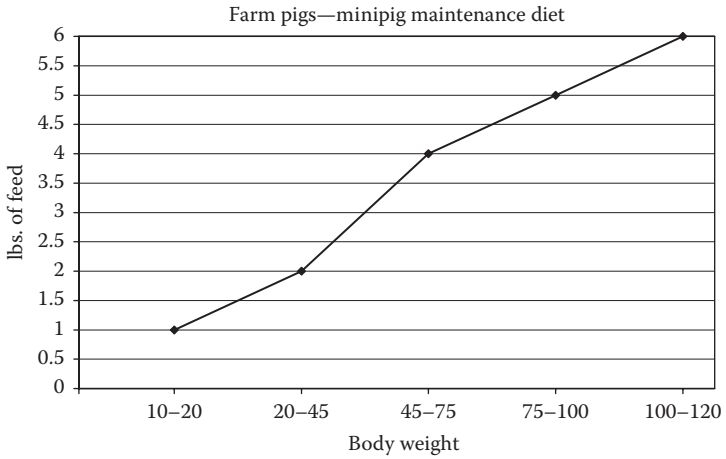
TABLE 1.2
Sample Feeding Chart for Minipigs



1993; Swindle et al., 1994). Generally, miniature pig diets are lower in protein and higher in fiber than commercial diets designed for farm pigs. Usually, the diets are made in the following three formulations: starter diet, grower diet, and breeder and lactation diet. Starter feeds (creep feed) are usually fed free choice within the first few weeks of life. The other diets, rations containing 3–3.2 kcal/g of ME, are then usually fed at a rate of 2%–3% of BW per day. The formula used for calculating ME for growth in BW is ME = 2.52 × BW 0.63 for swine (Bollen et al., 2000). For most feeds, this calculates to approximately 0.2 to 2.5 kg of feed per day for swine in a weight range of 5–55 kg. Tables 1.2 and 1.3 give two examples of how to chart the amount of ration to be fed for the guidance of animal caretakers. The chart values will differ between manufacturers of rations. The manufacturer's recommendations should be followed. Swine can be fed either the entire day's ration in a single feeding, or the calculated ration can be split into two feedings a day (National Research Council, 1998).

Gender differences in minipigs have been described in a study in which females were found to have a higher percentage of nitrogen retention and a higher percentage of body fat than males in

TABLE 1.3
Sample Feeding Chart for Farm Pigs



the Göttingen breed (Bollen, 2001). In the appendix, Table A.3 provides guidelines for feeding and nutrition of miniature swine.

Swine require water of approximately 2.5 l/1.0 kg of feed consumed. This is best supplied with an automated watering system. If bowls are utilized, they should be attached to the side of the cage to prevent spillage and soiling. In this situation, water should also be mixed with the food to ensure adequate hydration. Automatic watering devices need to be checked daily for function. Water restriction can lead to sodium toxicosis (salt poisoning), which is characterized by encephalitic symptoms due to development of eosinophilic encephalomyelitis (Laber et al., 2002; Swindle et al., 2003).

Newborn swine are also susceptible to development of microcytic and hypochromic anemia when raised indoors without access to soil. This is prevented by routinely administering 100–200 mg of iron dextran intramuscularly (IM) within 48 h of birth.

Presurgical fasting of 8–12 h will empty the stomach and small intestine, and the colon usually requires 48–72 h. If a prolonged fast is required for some procedures, hydration and energy may be maintained by providing flavored oral electrolyte and glucose solutions commercially available from grocery stores (i.e., Gatorade®; Gatorade, Chicago, IL). These liquids do not interfere with endoscopic or laparoscopic procedures because they do not leave residue. Bedding and chewable objects should be removed from the cage of an animal being fasted for surgery (Swindle, 1983; Swindle et al., 1994).

HEMATOLOGY AND BIOCHEMISTRY

Hematologic and plasma biochemical values for swine vary to some degree with environmental conditions, health status, breed, age, and gender. However, normal values are generally comparable, and tables of values from various breeds of swine are included in the appendix. Social status has been purported to create chronic stress conditions in both dominant and subordinate female growing farm pigs (Landrace × Yorkshire) housed in recently formed but stable social groups between 9 and 14 weeks of age, resulting in increased neutrophil–lymphocyte ratios in dominant pigs and increased percentages of neutrophils, decreased CD4+ and CD8+ cell counts, and increased *ex vivo* tumor necrosis factor α (TNF-α) reactivity in subordinate pigs (Hjarvard et al., 2009). It is not clear whether the relatively small differences between dominant and subordinate pigs reported in this study reflect clinically relevant immune compromise. However, studies of an immunological nature conducted in pigs may benefit from considering this possible confounder and ensuring that each pig serves as its own control for comparison, where possible.

Several general statements may be made concerning these values in swine. The hematocrit is physiologically low in neonatal swine, which requires the administration of iron dextran injections (100 mg IM) during the nursing period. Swine generally have a higher percentage of neutrophils than lymphocytes, but this can be variable (Pond and Mersmann, 2001). The blood volume of adult swine is 61–68 mL/kg. Their blood clotting pathway is similar to that of humans. They are considered to have 16 blood group antigens, A-P (erythrocyte antigen A and P (EAA-EAP)), but the antigens are generally weak (Feldman et al., 2000). Swine have approximately 80% homology with the human erythropoietin amino acid sequence but have low levels of erythropoietin at birth (David et al., 2002).

The health and welfare of swine can be monitored using results from serial blood work. Clinical chemistry findings have been linked to behavioral changes in a well-characterized model of *Sarcocystis miescheriana* in Pietrain-Meishan pigs (Reiner et al., 2009). The principles of serial monitoring of both pig behavior and blood work may be applied to other chronic studies, including surgical models, in order to develop scoring systems for establishing objective humane end points.

In the appendix, Tables A.10 through A.30 provide hematology and serum chemistry values for miniature swine.

BEHAVIOR

Swine are social animals and prefer to be in contact with other members of their own species. Recognizing the need for swine to have social interactions with conspecifics, recent regulatory updates in the United States now synchronize with the European standards in stressing the best practice of providing pair or group housing as the default situation for social animals except where incompatibility, scientific justification, or veterinary medical concerns are documented (Council of Europe, 2006; National Research Council, 2011). Swine can be housed in socialized groups of similar health status, age, and gender; however, dominance fighting will transiently occur when new animals are introduced into the group until social hierarchy has been reestablished. Careful monitoring of animals after modifying social groups is suggested by both U.S. and European regulations (Council of Europe, 2006; National Research Council, 2011). The Council of Europe (2006) promotes purchasing socially compatible farm animals, including minipigs, and the *Guide for the Care and Use of Laboratory Animals* encourages raising animals together from a young age (National Research Council, 2011).

Exceptions to social housing are permissible in certain situations, as described in the previous paragraph. An example of social incompatibility is adult boars that tend to prefer solitude (Committees to Revise the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 2010; Council of Europe, 2006). Scientific justification of single housing may include situations such as behavioral studies requiring uninterrupted task performance. Swine tend to be cannibalistic if a sick or injured animal is housed with them, so a veterinary medical concern supports single housing during the postoperative period until incisions are well healed. Both U.S. and European regulations state that single housing should last for the shortest duration possible, and single-housed animals should be able to see, hear, smell, and touch animals in other pens whenever possible (Council of Europe, 2006; National Research Council, 2011). This can be accommodated by using vertically slatted cage sides to allow them close, but protected, social contact with each other. Increased interactions with familiar caretakers may also be beneficial for swine (Committees to Revise the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 2010).

Pigs rely heavily on smell, touch, and sound for sensory input and interactions with conspecifics and the environment. Pigs often touch each other with their noses, primarily on the nose, head, and body. In 8-week-old crossbred pigs (Large White × Landrace) × Pietrain, Camerlink and Turner (2013) found that only 2.5% of all social nosing was associated with injurious behavior (e.g., belly nosing) compared to a previous study that linked social nosing to tail biting (Beattie et al., 2005). In addition, social nosing did not appear related to dominance hierarchy and was interpreted as a positive affiliative behavior (Camerlink and Turner, 2013). Swine have relatively poor vision as demonstrated by measured visual acuity scores of 150–1000 times the threshold of humans (Zonderland et al., 2008a). Visual cues were no better distinguished by 4-month-old gilts singly housed indoors at a light intensity of 40 compared to 12 lux (Zonderland et al., 2008a). Consideration of the relatively poor vision of swine should guide the distance of mirror placement from pens when used for enrichment of singly housed animals. If the mirror is placed too far away, pigs may not see their reflections easily and gain little benefit from the mirror.

Tail biting is a behavioral and animal welfare problem in domesticated swine, particularly in intensive indoor housing systems with barren environments (Taylor et al., 2010) and has been thoroughly reviewed (Schrøder-Petersen and Simonsen, 2001; Taylor et al., 2010). In general, tail biting characteristics have included the following: white breeds most commonly (e.g., Yorkshire, Landrace), males having higher risk for receiving tail biting, thermal stress, high stocking densities, and ill health (Taylor et al., 2010). More recently, changes in gene expression in the brains of pigs conducting and receiving tail biting linked the behavior, in part, to a lean meat phenotype (downregulation of pyruvate dehydrogenase kinase, isozyme 4 [*PDK4*] gene), genotype associated with hypersociability in humans and mice (downregulation of general transcription factor IIi [*GTF2I*]

gene), and explorative behavior, similar to novelty seeking in humans (upregulation of epidermal growth factor [*EGF*] gene; Brunberg et al., 2013). In a separate study of stress associated with tail biting, tail biters and victims showed behavioral and biochemical evidence of chronic stress compared to neutral pigs that were housed in the same pens as tail biters and victims but did not engage in either aspect of the behavior. Neutral pigs were interpreted to have developed better coping strategies that resulted in lower stress levels (Munsterhjelm et al., 2013).

Tail biters will bite the tails and ears of victim pigs particularly at feeding time (Schrøder-Petersen and Simonsen, 2001). This can be minimized by housing problem animals separately and by providing longer or separate feeding troughs (Committees to Revise the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 2010; Fisher, 1993; Panepinto, 1986; Swindle et al., 1994). Provision of twice-daily straw or straw racks resulted in the greatest reduction in tail wounds and bite marks when studied by Zonderland et al. (2008b), with chains and rubber hoses showing no effect on tail biting behavior. In a 23-week longitudinal study of tail biting in pigs, the behavior was first observed preweaning. After weaning, pigs were housed in barren (chain with ball, hanging jute sack) or enriched (barren provisions plus daily wood shavings and straw) environments, and weaner-, growing-, and finishing-stage pigs showed a lower incidence of tail biting in the enriched pens (Ursinus et al., 2014). Tail biting was easiest to predict at the pen rather than individual level. Telkänranta et al. (2014) found that providing piglets with suspended sisal ropes and newspaper from birth to weaning resulted in lower incidence of tail damage (9.8%) compared to controls (32.1%). The authors concluded that chewable materials during preweaning may assist piglets to cope with stressors such as the weaning experience (Telkänranta et al., 2014). When working with pigs in a research setting, it may not be possible to provide straw or bedding, depending on the structural design of the pens and the drains serving the area. However, it may be possible to work with swine breeding operations to include additional preweaning enrichment to piglets in an attempt to reduce tail biting later in life when they are group housed in a research setting. Swine develop a dunging pattern and will defecate at the opposite end of the cage from where they are fed. The cage should therefore be designed to have their food and water separate from the area where they are supposed to defecate.

Swine primarily are sedentary animals and will seldom exercise on their own. Generally, they will only move when aroused by activity such as feeding or the introduction of personnel or new swine. They tend to move around the perimeter of their area of confinement rather than in the center, and they will scratch themselves against the side of the cage since they cannot bend to groom themselves.

Pigs are highly intelligent. One- to two-month-old male and female farm pigs (Large White × Landrace) with only 5 h of exposure to a mirror were later able to gain information from a mirror related to the location of a food bowl placed behind a solid barrier. Seven of eight pigs took a direct and intentional path around the barrier to the food bowl in a mean of 23 seconds on their first try (Broom et al., 2009).

Swine are easily habituated to humans, particularly when positive reinforcement is used, but their natural tendency as a prey species is to become startled and to shy away from humans (Lorentsen, 2014). A recent behavioral study demonstrated that the routine presence of a familiar, but purposely non-interactive, caretaker in the room affected the choice of enrichment selected by male weaner pigs (Yorkshire × Landrace). Selection of a pen with a mirror was increased while a human was present, and this behavior was interpreted by the authors as a choice for social support during the presence of a perceived threat (DeBoer et al., 2013). Despite these natural behavioral tendencies, the trust of young pigs, in particular, can be gained quickly, allowing them to be trained to perform complex tasks that facilitate the conduct of research. For instance, at the authors' institution, both young farm pigs and adult minipigs have been trained to walk up a ramp, stand on a stationary platform, and drink or eat for fluoroscopic examination of swallowing (see DVD). Intensive training of a young pig was successful within 2–3 weeks, while older minipigs took 2–3 months.

ENRICHMENT

Like social housing, environmental enrichment has received attention in recent years and is emphasized in current regulatory documents in the United States and Europe (Committees to Revise the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 2010; Council of Europe, 2006; National Research Council, 2011). Van de Weerd and Day (2009) suggested in a review article that environmental enrichment should meet four success criteria as follows: (1) increases species typical behavior, (2) maintains or improves health, (3) is economical, and (4) is practical. Although intended in the context of swine production, these success criteria apply well to the research environment. Studies of various enrichment options have repeatedly shown that the novelty of new items fades quickly (Van de Perre et al., 2011; Van de Weerd et al., 2006) and that enrichment objects, also called point source enrichment, should be rotated in a non-repeated sequence to maintain effectiveness (Van de Perre et al., 2011). Physical characteristics of popular enrichment items included "ingestible, chewable, flexible and destructible" (Van de Perre et al., 2011). Van de Weerd et al. (2006) found that straw bedding was the most effective enrichment at engaging pigs and reducing tail biting among pen mates, but this may not always be feasible in a laboratory setting, especially when swine must be fasted for anesthetic and surgical procedures. Interestingly, they also found that less complex but highly popular objects increased tail biting behavior, so caution and careful monitoring should be employed when trying new enrichment.

Swine have a rooting behavior and like to use their snouts to dig through bedding or pastures. In artificial environments, such as those likely to be found in research institutions, it is best to provide them with toys. Large Teflon balls can be used to provide objects that can be rooted and thrown, and they are easily sanitized. Swine also like to pull on objects such as chains hung from the ceiling or roof of the cage. However, these objects for psychological well-being (Figures 1.8 and 1.9) must be carefully selected to minimize damage to the cage or animal (Panepinto, 1986; Swindle et al., 1994). Five-month-old, singly housed female Yucatan minipigs have been shown to spend more time interacting with soft, flexible rubber cones compared to a hard plastic ball or apple at first introduction and in subsequent sessions (Smith et al., 2009).

FIGURE 1.8 Yucatan™ pig in a cage with automatic water, attached feeder, and enrichment toys.

FIGURE 1.9 Yucatan™ pig in a typical pen.

HUSBANDRY

In the United States, the following two sets of regulatory guidelines exist for laboratory swine: one for biomedical research (National Research Council, 2011) and another for agricultural research (Committees to Revise the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 2010). These guidelines are contradictory for specified cage size. The cage sizes recommended are listed in Tables 1.4 and 1.5. Guidelines from other countries are generally close to these two standards, and the Council of Europe revised their standards in 2006 (Table 1.6).

No consensus exists on the best type of caging to use for swine in a laboratory setting. Most research facilities are not dedicated to swine housing and require flexibility in housing other types of large animals in the same facility. Some general standards related to research housing are discussed next.

Swine require flooring that provides for secure footing and a surface for wearing down their hooves. The flooring also needs to be easily sanitized. Swine can become stressed with slippery flooring and develop symptoms such as stress ulcers. Several types of flooring have been used with different degrees of success. Concrete or seamless epoxy floors should have grit added to provide secure footing. These types of floors are best utilized with deep bedding of wood shavings or straw. These floors plus the bedding provide an outlet for the rooting behavior of the species. Plastic-coated metal grid floors provide good sanitation, especially if they are raised above the floor level (Figure 1.7). However, swine will pull the plastic off the metal with the first sign of a tear. In the authors'

TABLE 1.4
Space Requirements: Institute of Laboratory Animal Research (ILAR) Guide

		Floor Area per Animal	
Animals in Enclosure	**Weight (kg)**	**ft²**	**m²**
1	<15	8.0	0.72
	Up to 25	12.0	1.08
	Up to 50	15.0	1.35
	Up to 100	24.0	2.16
	Up to 200	48.0	4.32
	>200	≥60.0	≥5.40
2–5	<25	6.0	0.54
	Up to 50	10.0	0.90
	Up to 100	20.0	1.80
	Up to 200	40.0	3.60
	>200	≥52.0	≥4.68
>5	<25	6.0	0.54
	Up to 50	9.0	0.81
	Up to 100	18.0	1.62
	Up to 200	36.0	3.24
	>200	≥48.0	≥4.32

Source: Adapted from National Research Council, 2011. *Guide for the Care and Use of Laboratory Animals*: 8th ed., Washington, DC: National Academies Press. With permission.

TABLE 1.5
Minimum Floor Area Recommendations for the Animal Zone for Swine Used for Agricultural Research and Teaching

	Individual Pigs (per Pig)		Groups of Pigs (per Pig)	
Stage of Production	**m²**	**ft²**	**m²**	**ft²**
Litter and lactating sow, pen	3.15	35	—	—
Litter and lactating sow, sow portion of crate	1.26	14	—	—
Nursery, 3–27 kg (7–60 lb) of body weight	0.54	6	0.16–0.37	1.7–4.0
Growing, 27–57 kg (60–125 lb) of body weight	0.90	10	0.37–0.56	4.0–6.0
Finishing, 57–104 kg (125–230 lb) of body weight	1.26	14	0.56–0.74	6.0–8.0
Late finishing, 105–125 kg (231–275 lb) of body weight	1.26	14	0.74–0.84	8.0–9.0
Mature adults	1.26	14	1.49	16.0

Source: Adapted from Committees to Revise the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 2010. *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*, 3rd ed., Champaign, IL: Federation of Animal Science Societies (FASS). With permission.

TABLE 1.6
Pigs and Minipigs: Minimum Enclosure Dimensions and Space Allowances

	Minimum Enclosure Size[a]		Minimum Floor Area per Animal		Minimum Lying Space per Animal (in Thermoneutral Conditions)	
Weight (kg)	**m²**	**ft²**	**m²**	**ft²**	**m²**	**ft²**
Up to 5	2.0	22.2	0.20	2.2	0.10	1
Over 5–10	2.0	22.2	0.25	2.7	0.11	1.2
Over 10–20	2.0	22.2	0.35	3.8	0.18	1.9
Over 20–30	2.0	22.2	0.50	5.4	0.24	2.6
Over 30–50	2.0	22.2	0.70	7.5	0.33	3.6
Over 50–70	3.0	32.3	0.80	8.6	0.41	4.4
Over 70–100	3.0	32.3	1.00	10.8	0.53	5.7
Over 100–150	4.0	43.0	1.35	14.5	0.70	7.5
Over 150	5.0	53.8	2.50	26.9	0.95	10.2
Adult (conventional) boars	7.5	80.7			1.30	14.0

Source: Adapted from Council of Europe, 2006. European Convention for the protection of vertebrate animals used for experimental and other scientific purposes, ETS 123, Strasbourg, France, http://conventions.coe.int/treaty/en/treaties/html/123.htm. With permission.

[a] Pigs may be confined in smaller enclosures for short periods of time, for example, by partitioning the main enclosure using dividers, when justified on veterinary or experimental grounds, that is, for monitoring individual food consumption.

experience, diamond-shaped grids of approximately 5/8 in. openings provide best all-around conditions for housing swine of multiple sizes. Newer fiberglass-slatted-rail types of flooring can be used in the same manner as expanded metal floors (Figure 1.8). They provide good sanitization and are not easily damaged. They are also lighter and can be removed easily for sanitization. In the authors' experience, the slats should have a width of 1.75 in. (4.4 cm) with a space of 0.25 in. (0.64 cm) between slats. When housing single pigs weighing more than 100 kg in a pen measuring 61 in. (1.53 m) by 72 in. (1.83 m), the floor of this housing system (Figures 1.8 through 1.10) will bow if the pig loads all of its weight on one floor panel (61 × 36 in.; 1.53 m × 0.91 m). These grids can also be manufactured to provide a light–medium gritty surface to provide for hoof wear. Rails and grids can be manufactured from aluminum and wood; however, care must be taken to make sure that the floors will support the weight of large animals and that they can be easily sanitized. Wood is inappropriate except for some agricultural settings. If the flooring does not cause wear on the hooves of chronically housed animals, then they will have to be trimmed with hoof nippers approximately every 3–6 months. A typical caging system for swine and other large animal species is depicted in Figures 1.8 through 1.10.

Swine do not climb, but in some circumstances they may stand on their rear legs and lean against the cage to see other animals. They also use the sides of the cage to scratch themselves. If they cannot see other animals, they will use their snouts to try to open the cage. Also, if there is any loose-fitting area of the cage, swine will manipulate it with their snouts; consequently, the cage should be sturdy and free of any surface that can be manipulated or torn by the animals. Chain-link fencing can be used for small swine, but care should be taken to ensure that the sides of the cage meet the flooring securely to prevent animals from catching their hooves. If aluminum or stainless steel sides

FIGURE 1.10 Farm pigs in a divided pen providing social contact.

are used, the edges should be rounded and the bars sturdy. Vertical bars are preferred for swine and ruminants; however, these will not be as satisfactory for dogs. Cages can be manufactured to provide solid side panels that can be used to replace slotted panels when required.

Food bowls should be secured to either the side of the cage or the floor. Easily sanitized stainless steel or Teflon feeders are preferable to the rubber feeders frequently used in agricultural settings. When the feeders are attached to the sides of the cage, guillotine-type closures should be avoided. In its excitement to eat, the pig may slam it shut and injure the caretaker when food is being delivered.

Swine readily utilize automated watering systems, which are preferred to the use of water bowls because they provide a constant source of water without contamination and the water cannot be spilled. If bowls are utilized, they should be secured to the side of the cage. Water deprivation can result in dehydration and a clinical presentation of sodium toxicosis, which may be fatal (Straw et al., 1999). For this reason, water is not withheld presurgically in swine except in cases when it is absolutely essential for gastric procedures. Placement of food and water sources should be considered in relation to the dunging pattern of the swine in the cage.

Cages without bedding are best washed with a hose once or twice daily to eliminate odor. Additional targeted scrubbing of the pen floor and sides is necessary to achieve optimal cleanliness. The animals should be removed from the cage during the hosing procedure to avoid being wetted and chilled. However, they readily accept baths and can be periodically washed with mild soap and warm water. They also develop dry skin in some housing conditions and can be rubbed with

moisturizing oils or ointments to prevent scaling and flaking of the skin. If deep bedding is used, the cage can be spot cleaned daily because of the dunging pattern, and the bedding changed once or twice weekly. Swine tend to keep cleaner in bedding than in cages that are hosed. Drains should be large and easily flushed. If bedding is used, then the drains should be covered with solid caps to avoid blockage. The main disadvantage of using bedding in surgical protocols is that swine will eat it when fasted (Fisher, 1993; Panepinto, 1986; Swindle et al., 1994).

If modular units are utilized, they may be taken apart and cleaned in a cage washer to sanitize the cage between uses by different animals or every 1–2 weeks with chronically housed animals. Pressure washers may be used to clean caging units in place.

The recommended dry-bulb temperature range for housing laboratory swine is 16°C–27°C (61°F–81°F) in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011). The Committees to Revise the Guide for the Care and Use of Agricultural Animals in Research and Teaching (2010) provide preferred ranges of temperatures depending upon the age of the animal. Their standards recommend 26°C–32°C (79°F–90°F) for animals of 3–15 kg, 18°C–26°C (64°F–79°F) for swine of 15–35 kg, 15°C–25°C (59°F–77°F) for swine weighing 35–70 kg, and 10°C–25°C (50°F–77°F) for swine larger than 70 kg. Increased environmental temperatures are appropriate for postsurgical care and other stressful situations and may be provided by suspended heat lamps. Normal swine are capable of surviving temperatures outside this range, especially at the lower end of the scale; however, environmental control is an essential part of the research environment and should be provided.

Standard laboratory animal practice is to provide 10–15 air changes per hour with 100% fresh outside air. Relative humidity ranges of 30%–70% are also standard. This type of ventilation greatly aids in reducing odor and minimizing ammonia levels, which can contribute to respiratory disease. The location of air ducts will depend upon the design of the facility; however, care should be taken to ensure that temperature is controlled at the floor level to prevent the pigs from becoming chilled (National Research Council, 2011).

The photoperiod for swine is not as critical as for other species, and they may be provided with up to 16 h of light in the laboratory setting (Committees to Revise the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 2010). Recommendations of the Council of Europe are more conservative, with 8–12 h of light or reproduction of ambient light cycles preferred in the absence of natural lighting (Council of Europe, 2006). Specific research topics may require particularly close attention to photoperiod and other environmental variables such as temperature or humidity. An example is the demonstration of circadian temperature rhythms in 4.5- to 5-month-old domestic farm pigs by Hanneman et al. (2005) that required strict attention to detail in ensuring consistent environmental parameters for the duration of the study.

DISEASE PREVENTION

Vaccination of swine depends upon the source of the animals, the length of time they are housed, and the protocol. Most of the vaccinations are given to neonatal and weanling animals, except those in breeding programs. The attending veterinarian should use professional judgment to establish the vaccination protocol, taking into account the source of the swine and the diseases prevalent in the area. Vaccination injections are conventionally given in the neck. The agents against which prophylaxis may be provided include *Erysipelothrix*, *Pasteurella*, *Bordetella*, *Escherichia*, *Clostridium*, *Parvoviridae* (Parvovirus), and *Reoviridae* (Rotavirus) (Laber et al., 2002; Straw et al., 1999; Swindle et al., 2003). Porcine epidemic diarrhea virus (PEDv), a highly contagious coronavirus that causes severe diarrhea and high mortality in young piglets, has significantly impacted swine production in the United States since June 2013, causing an estimated 7 million pig deaths in the year after identification of PEDv in the country for the first time (Davis and Waters, 2014). A federal order mandating reporting of both PEDv and porcine delta coronavirus (PDCoV) infections to either the U.S. Department of Agriculture (USDA) or state animal health officials was released on June 5, 2014 (USDA, 2014).

Two vaccines for use in pre-farrowing PEDv-infected sows received conditional approval from the USDA in 2014, with a possible third pending (Strom, 2014). Some breeding programs may benefit from these vaccines as research continues into combatting the disease.

Swine require the trimming of needle teeth during the first day of life to prevent damage to the nipples of the sow and to their siblings. Newborn animals also should have the umbilicus cleaned with iodine solution. An iron dextran injection is given to 3-day-old animals to protect against the physiological anemia that occurs in animals not housed on soil. Swine are susceptible to vitamin E and selenium deficiencies, and care must be taken to ensure that an adequate level is provided in the feed (Fisher, 1993; Straw et al., 1999; Swindle et al., 2003).

In agricultural settings, castration and hernia repair are performed on neonates. However, it is not routine for miniature swine from commercial producers. If these surgeries are performed in an agricultural setting, it is not unusual to see incisional infections. Observation of animals upon receipt from the supplier should include an inspection for problems related to these surgeries.

All animals should be examined for health problems upon receipt. To avoid stress and potential disease transmission in the laboratory setting, swine should not be mixed with other species. It is a good practice to isolate and quarantine animals upon arrival away from animals already on the premises. It is also a good practice to house animals from different suppliers in separate rooms. Animals purchased from auctions are probably from mixed sources and should be considered to have an increased risk of exposure to infectious diseases, similar to that of dogs from municipal pounds. When purchasing animals, it is best to limit suppliers to those who have been determined to have high standards of husbandry and disease control. *Specific pathogen free* (SPF) is a specific proprietary term used in swine breeding (National SPF, 2000; Saffron and Gonder, 1997). This means that the herd of animals has been certified by veterinary and gross necropsy examinations to be free of the specific diseases of atrophic rhinitis, pneumonia, swine dysentery, lice, mange, pseudorabies, and brucellosis. Although this certification does not guarantee that swine will be free of all diseases, it is a gold standard among swine producers and provides the best assurance that swine will be of a suitable condition for surgical protocols. Higher standards are required for animals used in xenographic procedures (Swindle, 1996). Zoonotic diseases are discussed in Chapter 14. European standards are summarized in the appendix (Table A.54). Dosages of antimicrobial agents that have been utilized in laboratory swine are included in the appendix (Table A.55).

RESTRAINT AND HANDLING

Agricultural methods of restraining swine are inappropriate for laboratory settings. These methods include snout tying, hog tying, and suspending animals by their rear legs. Such methodologies are stressful and make chronically housed animals timid and potentially aggressive toward their handlers. These animals are easily trained and can be restrained in sling apparatuses, such as the Panepinto sling or modifications of the original design (Panepinto et al., 1983; Figures 1.11 and 1.12). Small animals can be supported in the handler's arms (Figure 1.13), as with other species such as the dog. Larger animals may be herded and restrained against the side of the cage with handheld panels (Figure 1.14). Agricultural squeeze chutes may be appropriate in some circumstances for very large animals if care is taken to avoid trauma to the animal. Swine can also be trained to walk with a leash and harness (Houpt, 1986; Panepinto, 1986; Swindle et al., 1994).

Swine respond well to food treats for training, and food may be used to calm them during long-term restraint in slings. Foods that have been used successfully include dog biscuits, carrots, candy, doughnuts, and cookies.

If complete restraint is required, it is best to utilize short-term anesthetics and chemical restraint agents (Chapter 2). In addition, hydraulic lift devices may have to be utilized for ergonomic reasons, and their use is depicted in Chapter 2.

FIGURE 1.11 Restraint sling.

FIGURE 1.12 Pig resting in a humane restraint sling.

FIGURE 1.13 Manual restraint of a small pig.

FIGURE 1.14 Herding a pig into a transport cart with a handheld panel.

ADMINISTRATION OF MEDICATIONS AND INJECTIONS

Preoperative handling and socialization of research pigs that will receive medications and injections is a key to improving research outcomes and reducing stress on pigs and research personnel. Positive reinforcement training is recommended for achieving optimal results from swine and may increase animal caretaker job satisfaction and morale (Sørensen, 2010). Pre-study training to voluntarily participate in experimental procedures may also reduce animal stress through the development of coping mechanisms (National Research Council, 2011). Socialization to human contact preoperatively was demonstrated to result in ease of applying topical eye medications post-corneal

transplant in swine leukocyte antigen (SLA)[cc] NIH minipigs (Nicholls et al., 2012). Investigators must budget appropriately for extended acclimation and training as the mean housing duration before surgery in the study by Nicholls et al. (2012) was 41 d, incurring significant per diem cost. However, the reduction in stress on the animals and avoidance of adverse events from restraint for otherwise difficult procedures such as applying topical ophthalmic medication is arguably worth the expense.

It should be noted that swine, which are in the human food chain, are restricted from receiving certain antibiotics (Table A.55). These are, generally speaking, antibiotics such as vancomycin, which are considered to be lifesaving for humans. Judicious use of antibiotics in research animals is recommended, even though it is unlikely that they will become food for humans. Antibiotic resistance is a serious issue and veterinarians should take this factor into consideration when utilizing antimicrobial agents in research swine (Payne et al., 1999).

Oral medications can be administered by multiple methods. The easiest method is to mix the medication with food. Administration of oral medications can be made easier by determining food and liquid preferences preoperatively and habituating pigs to receive these treats before any medications are added that may alter the taste. In the case of substances with foul tastes that cannot be masked, they may be placed inside gelatin capsules. Medications can be mixed with chocolate syrup or canned cat food or dog food. Swine will consume these substances quickly and without substantial chewing; thus, breaking capsules and tablets is seldom necessary. Balling guns have been utilized in agricultural settings (Figure 1.15). If these devices are required, then it is best to restrain the animals in a sling and utilize equipment with flexible necks to avoid trauma to the pharynx and larynx. Swine can also be readily medicated using stomach tubes if the animal is restrained in a sling. Stomach tubes should be approximately the size of the trachea and have rounded tips (Figure 1.16). The tube should be lubricated and passed slowly through the side of the mouth. Medication should be administered quickly, and the tube removed. If precautions are not taken, swine may sever the tube with their incisors. Mouth gags may be utilized in smaller swine; however, larger swine are too powerful for the handler to hold the mandible closed around it (Swindle et al., 1994).

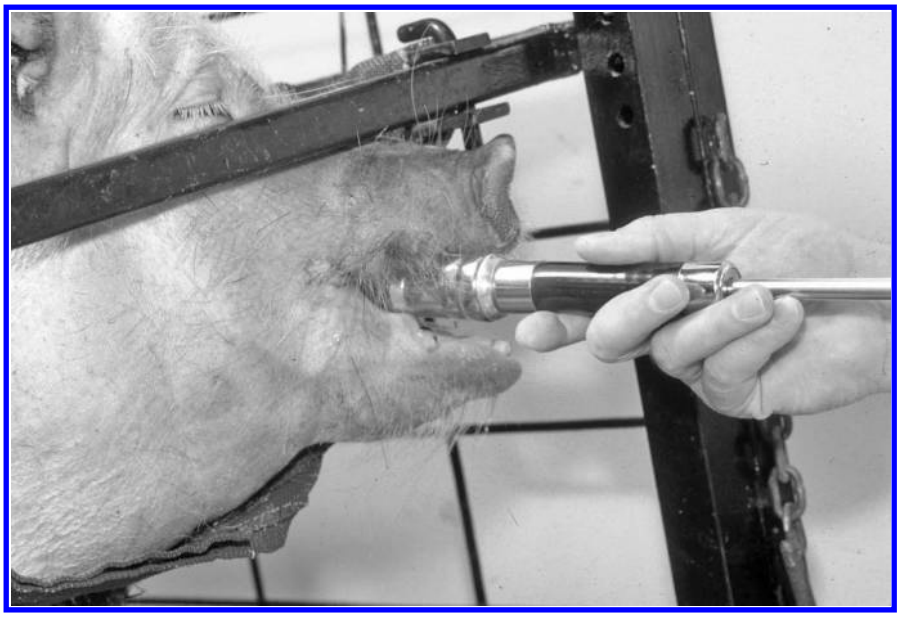


FIGURE 1.15 Administering a tablet to a pig in a sling, using a flexible tipped balling gun.

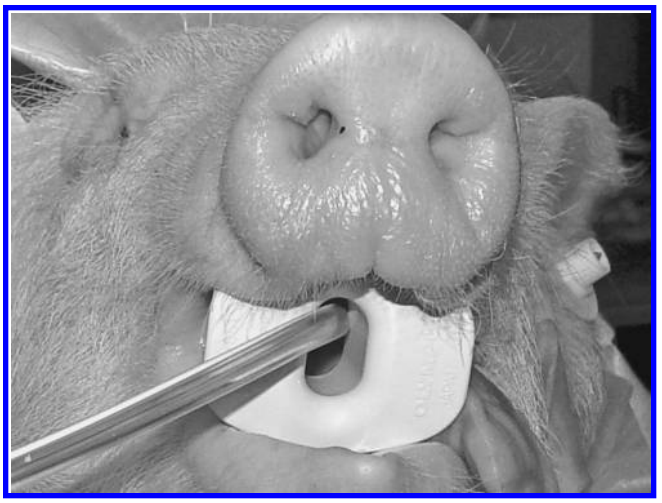


FIGURE 1.16 Administering medication with a stomach tube passed through a mouth gag.

IM and subcutaneous (s.c.) injections may be given in the rear legs or neck. In the rear leg, the gluteal, semimembranosus, and semitendinosus muscles may be used (Figure 1.17). Care should be taken to avoid the sciatic nerve. The flank may be utilized for s.c. injections; however, s.c. injections require that the pigs be larger and have a layer of fat to separate the skin from the underlying muscles (Figure 1.18). Muscles on the sides of the neck or behind the ears may be utilized in small swine; however, injections given in this area in larger swine will be s.c. rather than IM because of the layers of fat that develop (Figure 1.19). Experience has shown that this does not present a problem for injectable anesthetics, and this area is preferred because larger-volume injections can be given with less pain to the animal than IM injections in the rear legs. Animals should be restrained in a sling or with panels when given injections with a hypodermic needle. It is seldom necessary to use a needle larger than 20 gauge (ga). A method that does not require restraint is to use a slapping motion to instill a butterfly catheter into the side of the neck and allow the animal to recover from the insertion. The medication can then be administered through

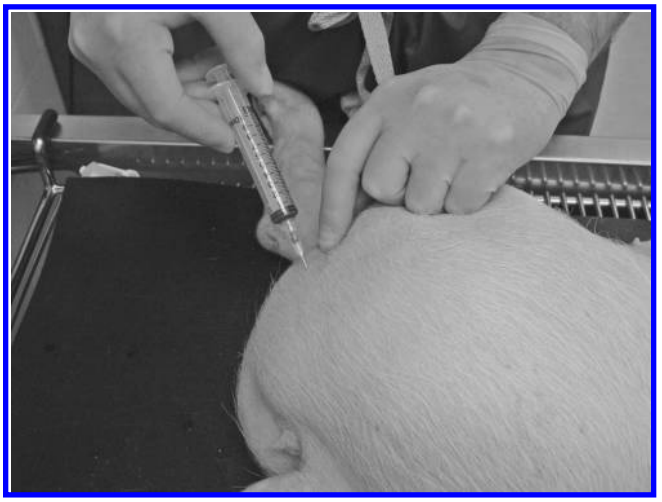


FIGURE 1.17 Intramuscular (IM) injection in the rear leg.

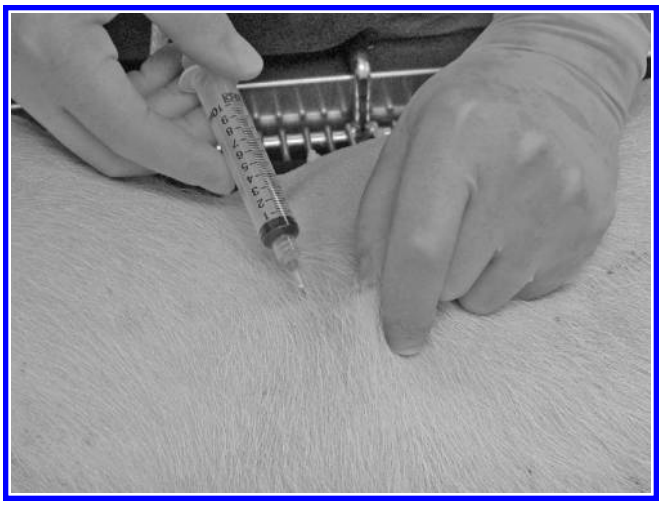


FIGURE 1.18 Subcutaneous (s.c.) injection in the flank.

the butterfly catheter (Figure 1.20) while the animal is walking around the cage (Swindle, 1983; Swindle et al., 1994).

Intraperitoneal injections (Figure 1.21) may be given in the lower quadrants of the abdomen off the midline. However, they are rarely used except in neonates. Intrathoracic injections (Figure 1.22) should be given in the dorsocaudal intercostal spaces if they are required by the research protocol. Methodologies for giving injections and taking samples in the subarachnoid space and cisterna magna are discussed in Chapter 11.

Intravenous injections and venipuncture for sampling or implanting catheters can be performed in a variety of peripheral locations (Figures 1.23 through 1.33). Procedures for chronic catheterization and percutaneous catheterization for cardiovascular procedures are discussed in Chapters 2, 9, and 12. Arterial pulse sites are illustrated in Chapter 2. The standard methodology

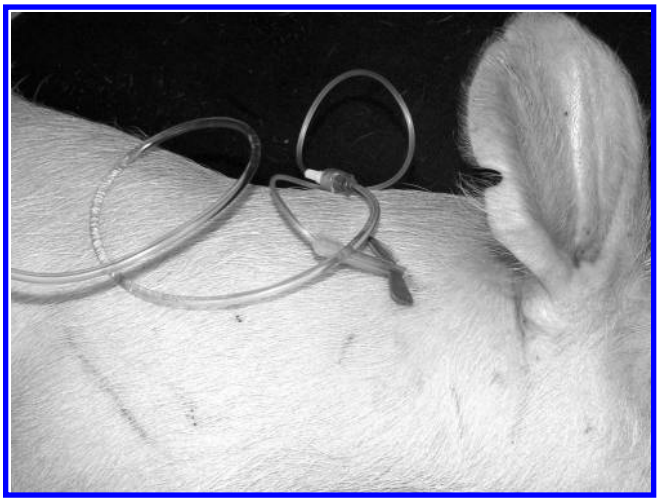


FIGURE 1.19 Subcutaneous (s.c.) injection in the neck. This is the preferred site and method of administering injections in swine.

FIGURE 1.20 Administering a subcutaneous (s.c.) injection in the neck using a butterfly catheter and extension tube. Note that the animal is not physically restrained.

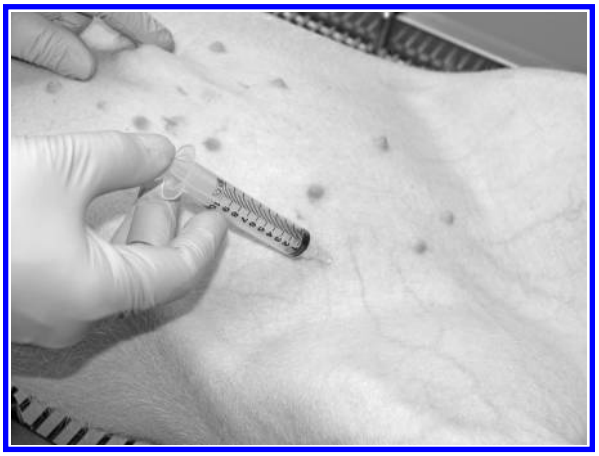


FIGURE 1.21 Intraperitoneal injection.

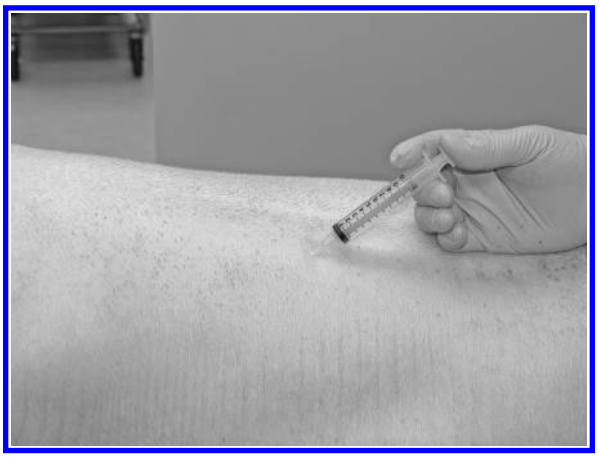


FIGURE 1.22 Intrathoracic (i.t.) injection.

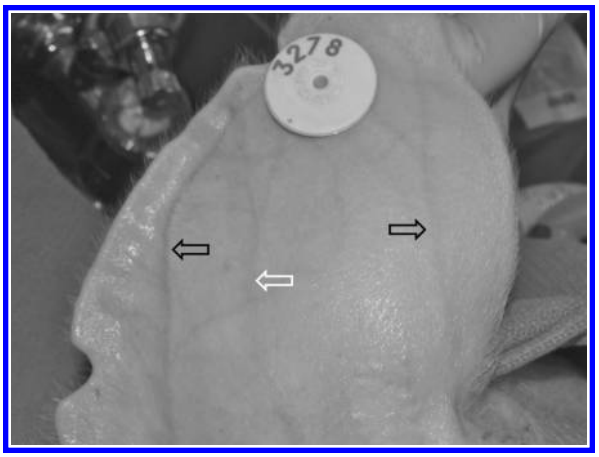


FIGURE 1.23 Auricular artery (white arrow) and auricular veins (black arrows).

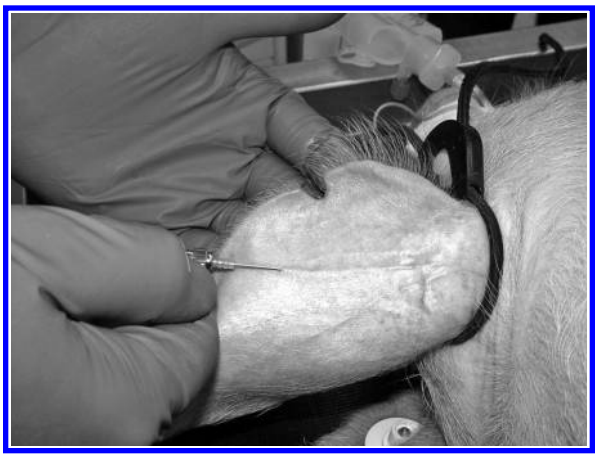


FIGURE 1.24 Intravenous injection in auricular (ear) vein.

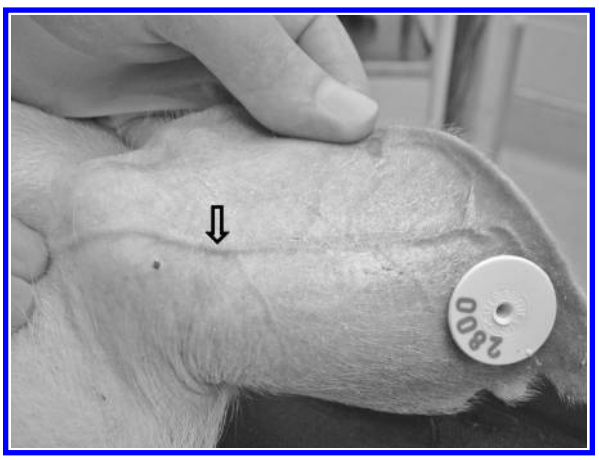


FIGURE 1.25 Anatomy of this auricular vein is conducive to using it to catheterize the external jugular vein from the ear.

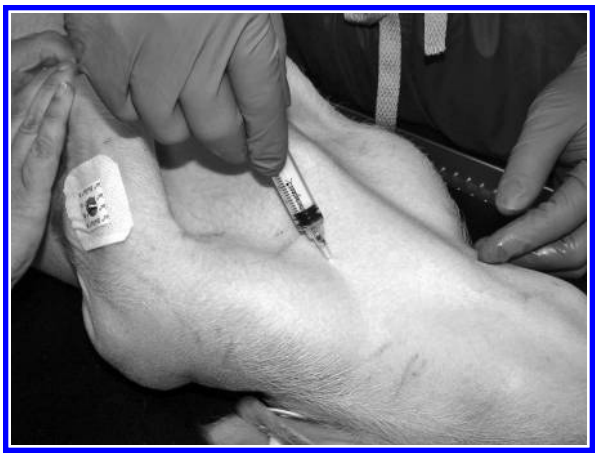


FIGURE 1.26 Venipuncture site for the external jugular vein.

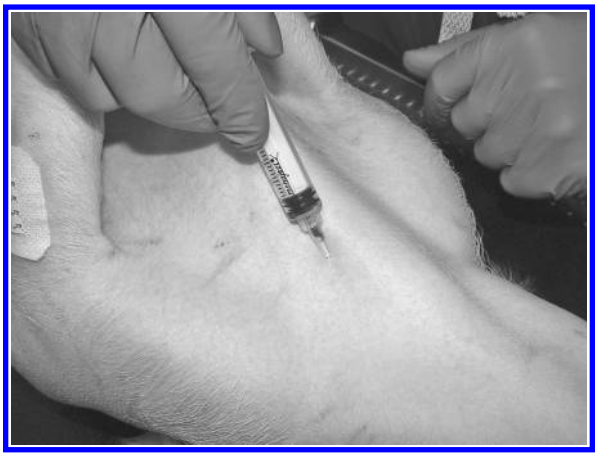


FIGURE 1.27 Access site for the internal jugular vein and carotid artery.

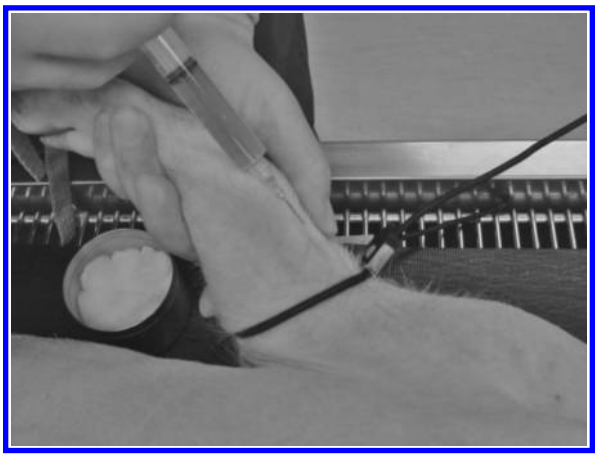


FIGURE 1.28 Cephalic venipuncture on the foreleg.

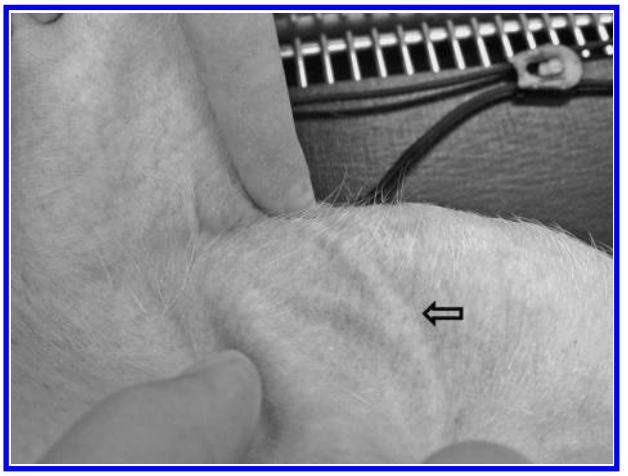


FIGURE 1.29 Cephalic vein (arrow) as it crosses the neck superficially at the thoracic inlet.

FIGURE 1.30 Venipuncture site for the superficial cranial epigastric vein.

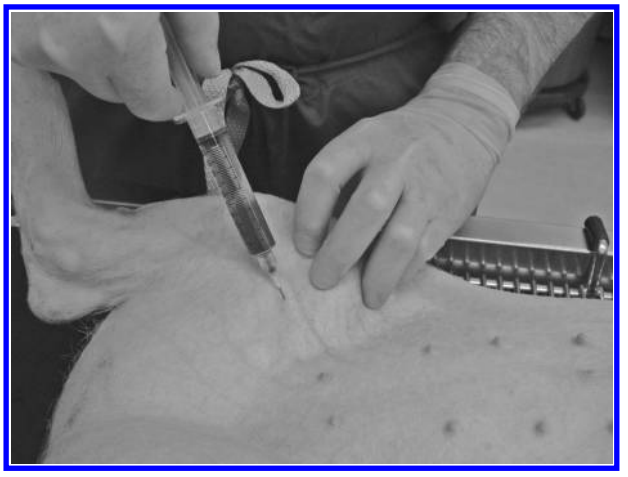


FIGURE 1.31 Access site for the femoral artery and vein.

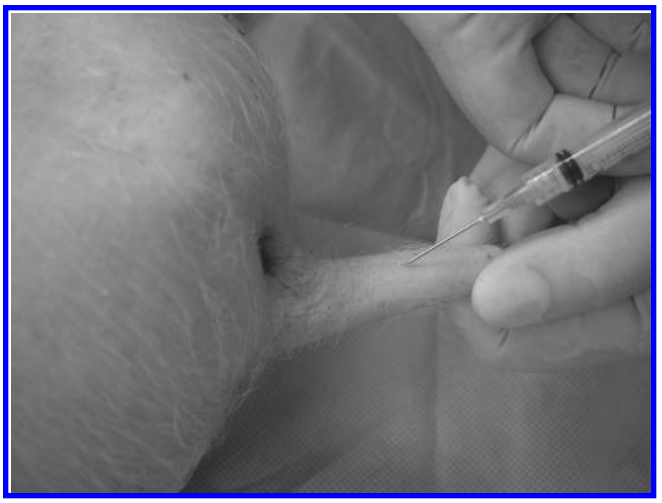


FIGURE 1.32 Venipuncture site for the coccygeal vein.

for withdrawing blood samples in agricultural settings utilizes the precava (Figure 1.33). The use of large-gauge (l4–16 ga) 3- to 5-in. "hog needles" is unnecessary except in large breeding stock. For swine less than 50 kg, the largest needle size that is required is 20 ga 1.5 in. Animals may be restrained in a sling or on their backs with the forelegs retracted caudally. In order to avoid injury to the vagus nerve, the needle is inserted into the right side of the neck, lateral to the manubrium sterni, and directed at an angle of 30° to 45° toward the left shoulder. A popping sensation will be felt when the needle enters the vein, and then blood can be readily withdrawn. This method can also be utilized for sequential venipuncture, but hematomas form in the area after the needle is withdrawn; therefore, it is best reserved for procedures that do not require withdrawal more often than weekly (Panepinto et al., 1983; Swindle, 1983; Swindle et al., 1994). Both restraint and interaction with humans is stressful for awake pigs when blood samples are collected. Sedation may be used to reduce the stress reaction as long as the sedatives do not interfere with the scientific study. Another reported alternative is a unique housing system called the PigTurn® that allows centrally

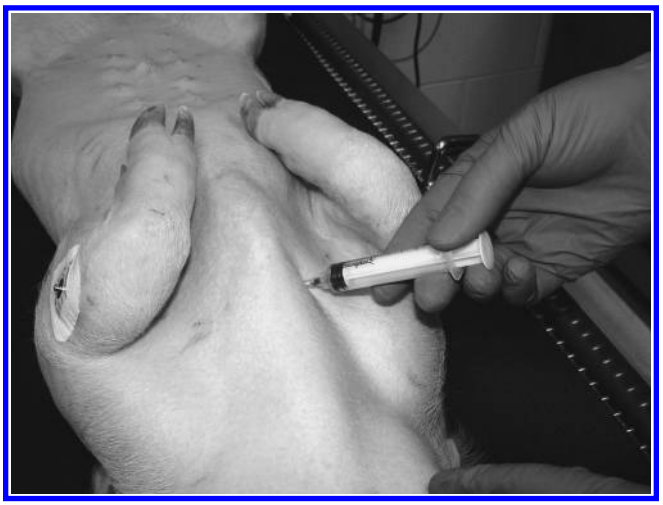


FIGURE 1.33 Venipuncture of the cranial vena cava at the right side of the thoracic inlet.

catheterized pigs to move freely in a rotating pen during automated sample collection. Cortisol and noradrenalin plasma levels collected from male crossbred (Yorkshire × Landrace) pigs using the PigTurn were significantly lower than those of pigs manually restrained for jugular venipuncture (Marchant-Forde et al., 2012).

GENERAL ANATOMY

Several textbooks (Getty, 1975; Gilbert, 1966; Popesko, 1977; Sack, 1982) and review articles (Sasaki et al., 2010) describing the detailed anatomy of swine are available. A review details the anatomy and physiology of swine applicable to research animals (Swindle and Smith, 1998). This section provides an illustrated introductory overview of the gross anatomy of swine. Specific aspects of anatomy important to surgical procedures are included in the introduction to the various system chapters.

The vertebral formula is C7, T14–15, L6–7, S4, Cy20–23. Some miniature breeds may have one fewer of the thoracic or lumbar vertebrae or of both. There are seven sternal and seven asternal ribs. If a 15th rib is present, it is usually a floating rib rather than one attached to the cartilage of the costal arch. The clavicle is absent. In the forelimb, there are eight carpal bones, four metacarpal bones (2–5), and three phalanges; proximal and distal sesamoid bones are present. In the hind limb, there are eight tarsal bones, four metatarsal bones, and phalanges with sesamoid bones present (Figure 1.34).

The musculature of the pig is massive, as would be expected of an animal that has been bred predominantly for meat production (Figure 1.35).

The unique features of the gastrointestinal tract include the torus pyloricus in the pyloric region of the stomach, the mesenteric vascular arcades, and the spiral colon. The torus pyloricus is a muscular and mucoid glandular structure adjacent to the pylorus, which is involved in the functional closure of the orifice. The small intestine is arranged in a series of coils from the stomach to the pelvis, dorsal and lateral to the spiral colon. The bile duct and pancreatic ducts enter the duodenum separately in the proximal portion in the right upper quadrant. The mesenteric vascular arcades form in the subserosa of the intestine rather than the mesentery, giving a fanlike appearance. The spiral colon contains the cecum, the ascending, transverse, and main portion of the descending colon arranged in a series of centrifugal and centripetal coils in the left upper quadrant of the abdomen, caudal to the stomach. The descending colon continues caudally on the left side to become the rectum (Figures 1.36 through 1.40).

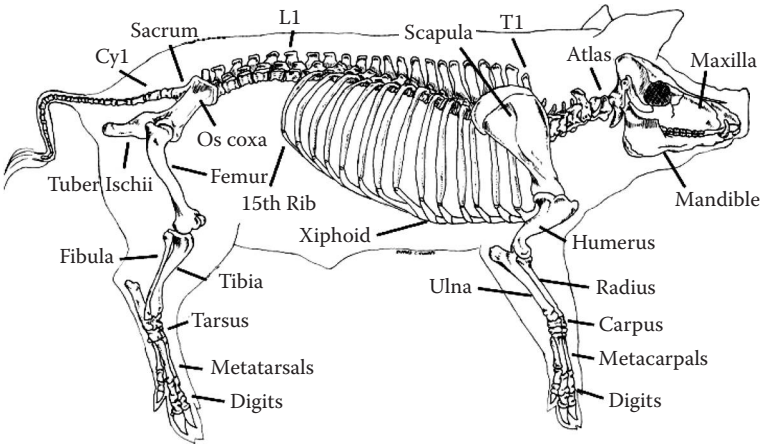


FIGURE 1.34 Skeleton of the pig.

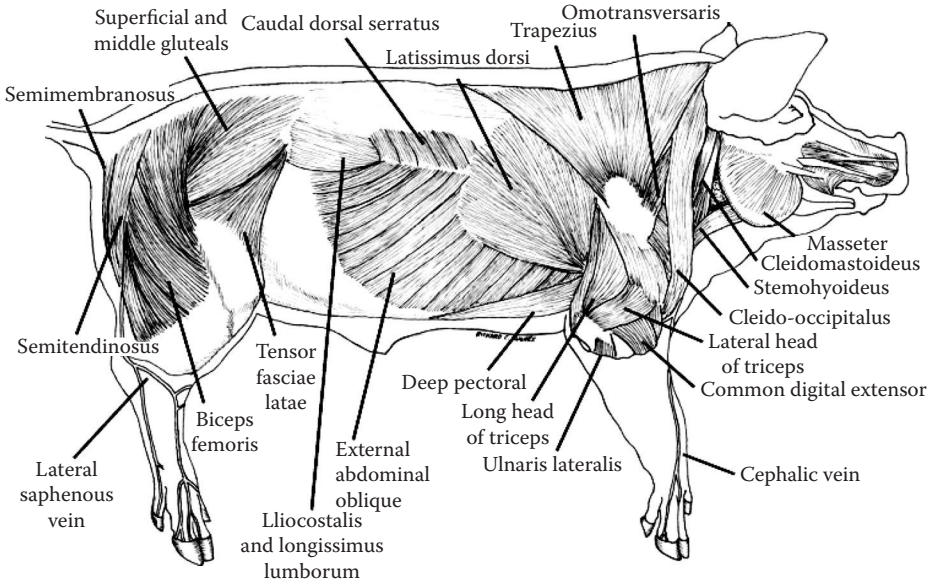


FIGURE 1.35 Superficial musculature of the pig.

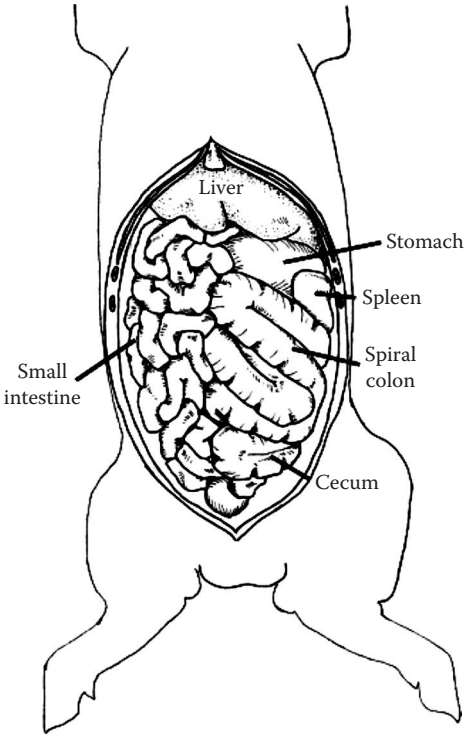


FIGURE 1.36 Ventral view of the abdominal viscera.

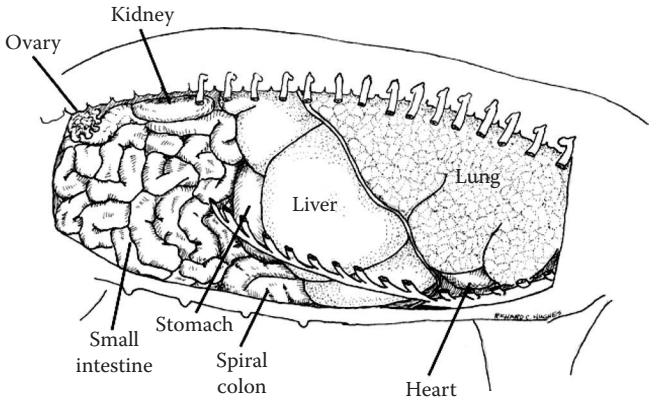


FIGURE 1.37 Right lateral view of the thoracic and abdominal viscera.

The kidneys are usually ventral to the transverse processes of the first four lumbar vertebrae with the left kidney usually more cranial than the right one. The adrenal glands are craniomedial to the hilus of the kidneys. The right gland is attached to the vena cava (Figure 1.41).

The male urogenital system contains large vesicular glands, a prostate, and bulbourethral glands as accessory genital glands. The scrotum and testicles are ventral to the anus and more caudal than ventral on the perineum. The fibromuscular penis has a sigmoid flexure and terminates in a corkscrew-shaped tip in the preputial diverticulum, caudal to the umbilicus (Figure 1.42). The female urogenital tract has long, flexuous fallopian tubes and a bicornuate uterus with a small body. The urethra enters the vagina on the ventral floor within the pelvic cavity, cranial to the vestibule (Figure 1.43). There are usually 10–12 paired mammary glands on the ventral abdomen.

The larynx is prominent with a large vestibule that narrows caudally because of internal compression of the cricoid cartilage. There are middle and lateral ventricles that have to be avoided during intubation (Chapter 2).

The thyroid gland is located on the ventral midline of the trachea at the level of the thoracic inlet. A pair of parathyroid glands can be found associated with the craniomedial portion of the thymus in the neck (Figure 1.44). The lymph nodes have an inversion of the cortex and medulla. Histologically, this presents a unique appearance with the more central location of the germinal centers (Figure 1.45).

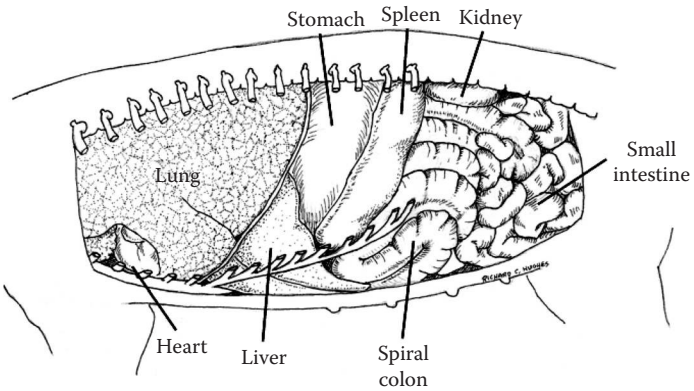


FIGURE 1.38 Left lateral view of the thoracic and abdominal viscera.

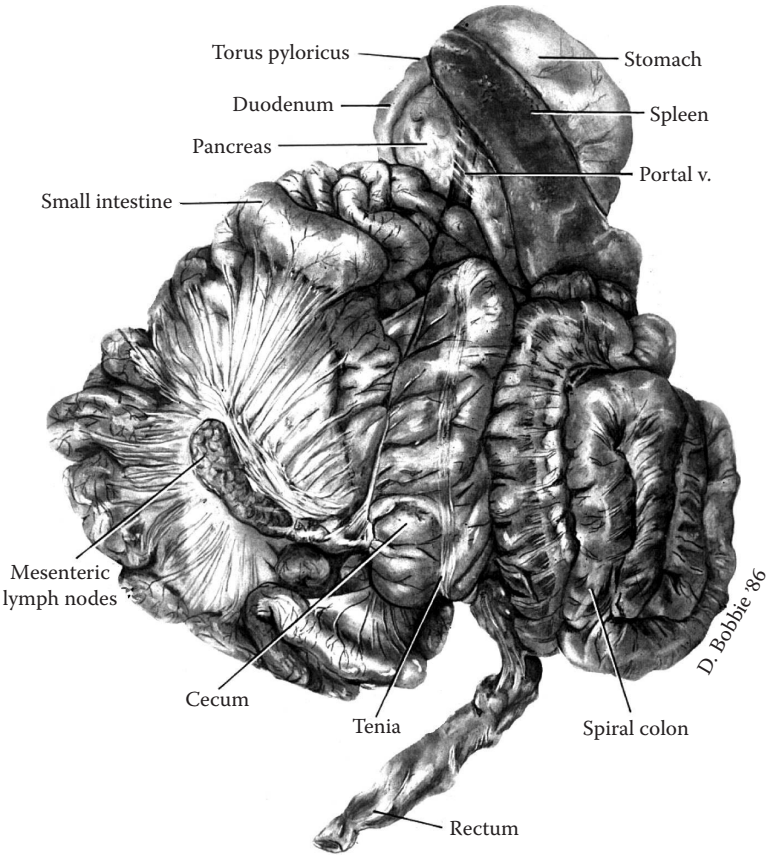


FIGURE 1.39 Schematic of the intestinal viscera showing the details of the spiral colon.

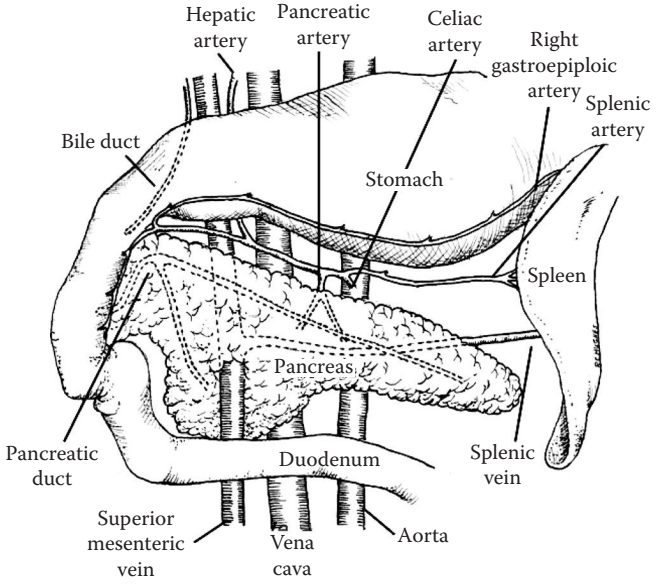


FIGURE 1.40 Schematic of the pancreatic blood supply and ductal system.

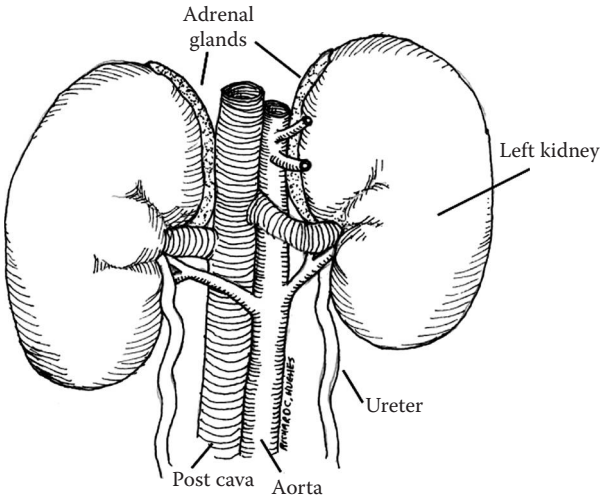


FIGURE 1.41 Ventral view of the kidneys and adrenal glands.

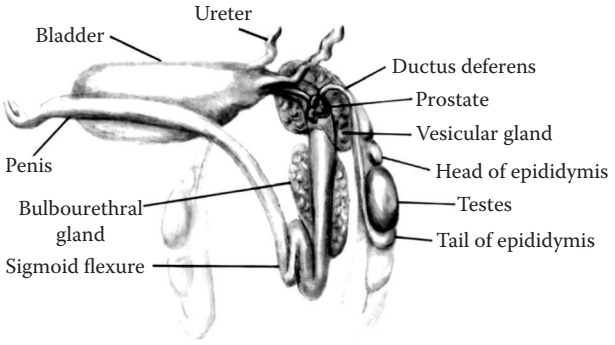


FIGURE 1.42 Left lateral view of the male urogenital system.

The trachea extends from C4–5 to T5, where it bifurcates into the bronchi. At T3, it provides a bronchus to the apical lobe of the right lung. The lungs are composed of apical, middle, and diaphragmatic lobes, with an accessory lobe to the right lung. The intralobular fissure is incomplete between the left apical and middle lobes.

The heart extends from T2 to T7 and has sternal contact over most of the caudal distance (Figure 1.46). The left azygous vein is the most unique feature of the anatomy. It curves caudoventrally from the dorsal thorax across the dorsal surface of the heart to enter the right atrium in the coronary sinus.

Unique aspects of the vasculature and the other visceral organs are discussed in the various system chapters.

MODEL SELECTION

The principles for selecting swine as a model are similar to those of other species, and the references on symposia proceedings cited in the introduction to this chapter contain descriptions of many diverse biomedical models developed in this species. This textbook details many of the surgical procedures utilized in the development of induced porcine models.

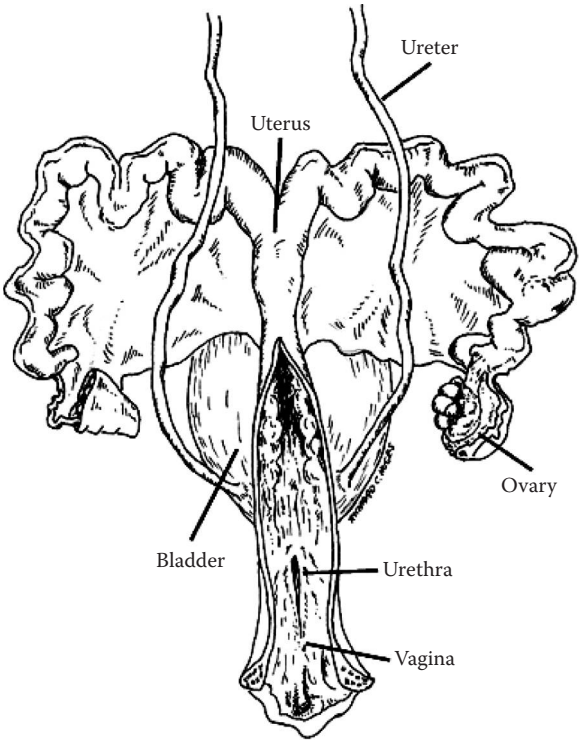


FIGURE 1.43 Dorsal view of the female reproductive tract.

One of the principal concerns when considering the use of swine is the age of the animal, because swine grow rapidly. If small domestic pigs are used as biological models, the physiological process being studied may be defined as that of a pediatric rather than an adult model. Therefore, the animal selected should be either a miniature pig or a larger domestic animal if maturity is a factor to be considered in the experiment.

Likewise, if a chronic experiment is planned, then the growth of the animal is a consideration, especially if biomaterials are implanted. As a general rule, the growth of sexually immature

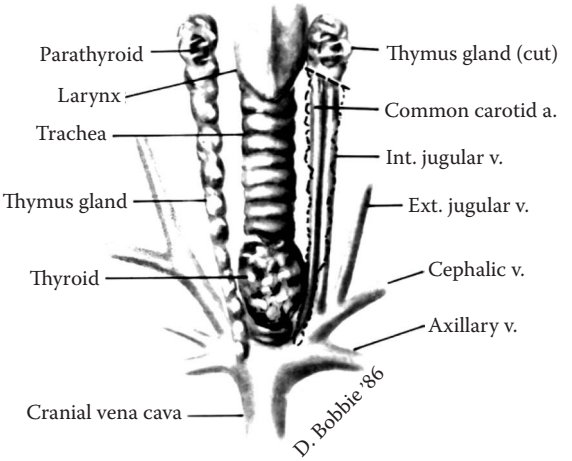


FIGURE 1.44 Schematic of the ventral aspect of the trachea and glandular structures of the neck.

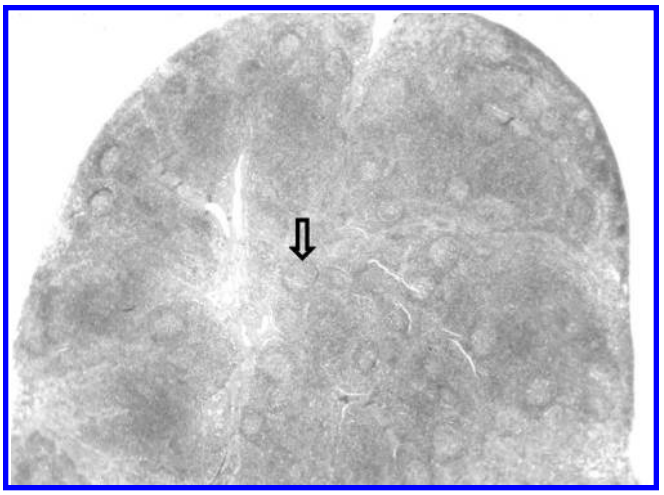


FIGURE 1.45 Cross section of a lymph node demonstrating the inversion of the cortex and medulla. A germinal center is illustrated with an arrow. H&E, ×100.

domestic swine over a 3-week period will be significant enough to have an impact on the physiological parameters of the study. Consequently, the use of miniature breeds should be considered for experiments in which growth could be a factor.

Also, as a general rule, miniature pigs are more mature at a given BW than are domestic swine, and this should be considered if maturity of the system or wound healing characteristics are a factor in the experiment.

When comparing experiments between laboratories, animals should be matched for age, weight, gender, and breed. Because of the line breeding that occurs in herds of commercial animals raised

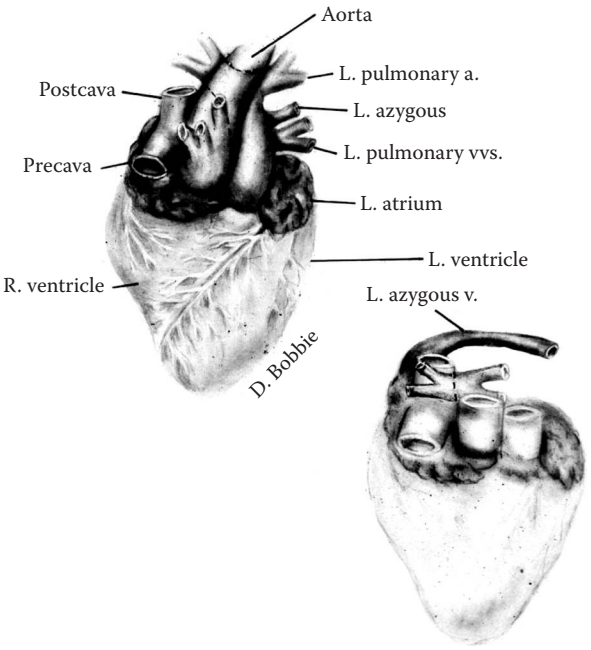


FIGURE 1.46 Gross anatomy of the heart.

for food, there may even be differences between animals of the same breed from different herds. Animals bought from the same supplier may be siblings or otherwise closely related; therefore, the relatedness of the animals should be considered if it is a potential factor in the experiment. Consequently, if differences are noted in experiments between laboratories, the genetic factors should be considered.

Most of the porcine models are selected because of the close similarity of the physiology of the various organ systems to those of humans. In the literature, most of the models involve either the cardiovascular or the digestive system, a fact reflected in the disparity in the size of various system chapters of this textbook. The reasons for the selection of models of different body systems are discussed in those chapters.

A list of general references, which may be useful in providing information on the use of swine as animal models in biomedical research, is provided in the appendix.

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2 Anesthesia, Analgesia, and Perioperative Care

M. Michael Swindle and Joseph J. Sistino

CONTENTS

INTRODUCTION

The purpose of this chapter is to provide practical advice on anesthetic procedures in swine. An emphasis on the physiological effects of injectable anesthetics and cardiopulmonary bypass (CPB) procedures is included in this publication. Recent veterinary publications (Riebold et al., 1995; Thurmon and Benson, 1996) provide an overview of anesthesia in swine with an emphasis on agricultural and pet pig procedures. Reviews of agents used in cardiovascular research (Thurmon and Tranquilli, 1986; Tranquilli et al., 2007) and laboratory animal medicine (Fish et al., 2008; Flecknell, 2009; Hawk et al., 2005; Kohn et al., 1997; Swindle et al., 2002) are also available.

Anesthetic protocols for experimental procedures should be selected with consideration of the potential physiological complications on the experiment. For example, no anesthetic protocol has been developed that is without effects on cardiovascular hemodynamics; however, the effects may be minimized by judicious selection of the agents. This chapter discusses the most commonly used protocols in research and makes specific recommendations for their selection. Because most of the pharmaceuticals are not marketed as approved for swine, in our laboratories we use the human pediatric dosage as the trial dose when testing a new pharmaceutical agent. Physiological effects of the agents discussed in this chapter are dose dependent.

ANESTHETIC INDUCTION AND ANIMAL PREP ROOM ACTIVITIES

Animals may be induced under anesthesia in a prep room after transport from the housing area (Figure 2.1) or in their cage as described with the neck butterfly technique (Figure 2.2) in Chapter 1. For ergonomic protection of personnel, devices to minimize lifting should be utilized. Minimizing stressful manipulation of animals also aids in prevention of animal discomfort. If tranquilizers are to be used as part of the anesthetic protocol, it is best to administer them in the cage before the other anesthetic agents. Animals may be transported in transport carts (Figure 2.1) or lifted by personnel using a hammock (Figure 2.3). For larger swine, use of hydraulic lifting devices may be developed as illustrated in Figures 2.4 through 2.6. In those illustrations, a hydraulic engine lifter has been modified with a sling attachment to manipulate and transport large swine. The hydraulic lift table in the prep room also acts as a scale.

The prep room should be separate from the operating room, and preliminary aseptic preparation should be performed in that area. Animal prep room activities should include shaving, preliminary skin scrubbing, protection of the eyes with bland ophthalmic ointment (Figure 2.7), securing an intravenous (i.v.) catheter (Figure 2.8), placement of electrodes for electrocardiogram (ECG)

FIGURE 2.1 Pig being moved from a cage into a transport cart.

FIGURE 2.2 Subcutaneous injection in the neck using a butterfly catheter.

monitoring (Figure 2.9), and endotracheal intubation (described in the following text). The techniques and theories for aseptic skin preparation in the operating room are discussed and illustrated in Chapter 3.

ENDOTRACHEAL INTUBATION

Endotracheal intubation should be performed on all swine when they undergo general anesthesia. The procedure is easily performed when the species-specific anatomic considerations are understood. The laryngeal passage is narrow, and the vocal cords and blindfolds are easily traumatized if too large a tube or too much force is used during intubation. The lateral folds of the larynx can be easily ruptured and the tube passed into the subcutaneous (s.c.) tissues.

FIGURE 2.3 Anesthetized pig being moved with a transport hammock.

FIGURE 2.4 Large pig being hoisted with a hydraulic lift and hammock.

FIGURE 2.5 Close-up view of a large pig in the hammock.

FIGURE 2.6 Transfer of a large pig from the hydraulic lift onto a hydraulic procedure table.

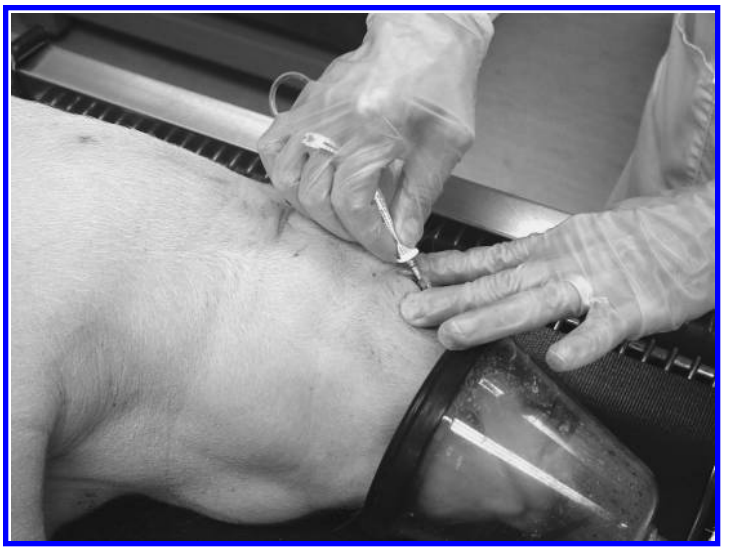

FIGURE 2.7 Protective ophthalmic ointment being administered into the eye of an anesthetized pig.

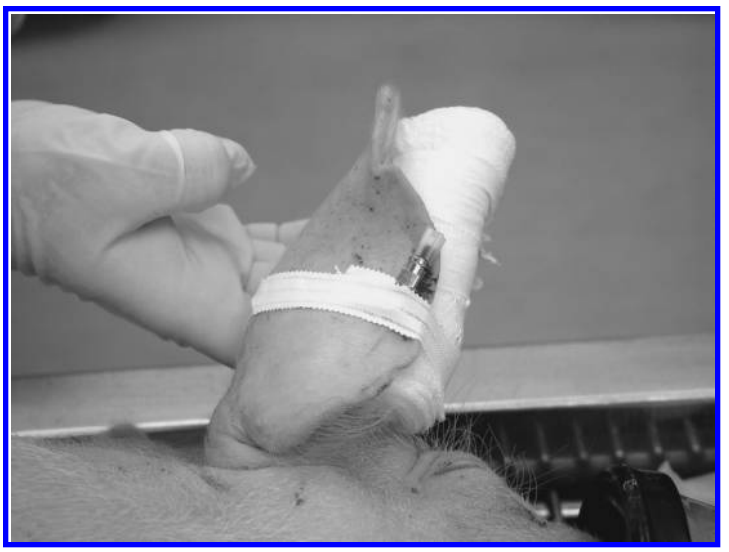

FIGURE 2.8 Ear vein catheter properly taped into place in the marginal ear vein.

Swine may be intubated from any position. The most common positions are dorsal, lateral, and sternal recumbency. The dorsal recumbency position tends to be easier for swine less than 50 kg when personnel are used to human intubation. Straight laryngoscope blades with a curved tip are the best for swine. Standard laryngoscope blades, 195 mm or longer, are sufficient for swine less than 50 kg. For larger animals, modified blades with 3- to 5-cm extensions are optimal.

When intubating swine in dorsal recumbency, assistance with holding the jaws open is not necessary; a simplified atraumatic method of intubation has been published (Swindle, 1991). When the pig is placed in sternal recumbency, an assistant must hold the jaws open with gauze strips, or a mouth gag should be used. The method of unassisted intubation in dorsal recumbency is pictured (Figures 2.10 and 2.11) and illustrated (Figure 2.12). The positioning for intubation in lateral recumbency is depicted in Figure 2.13.

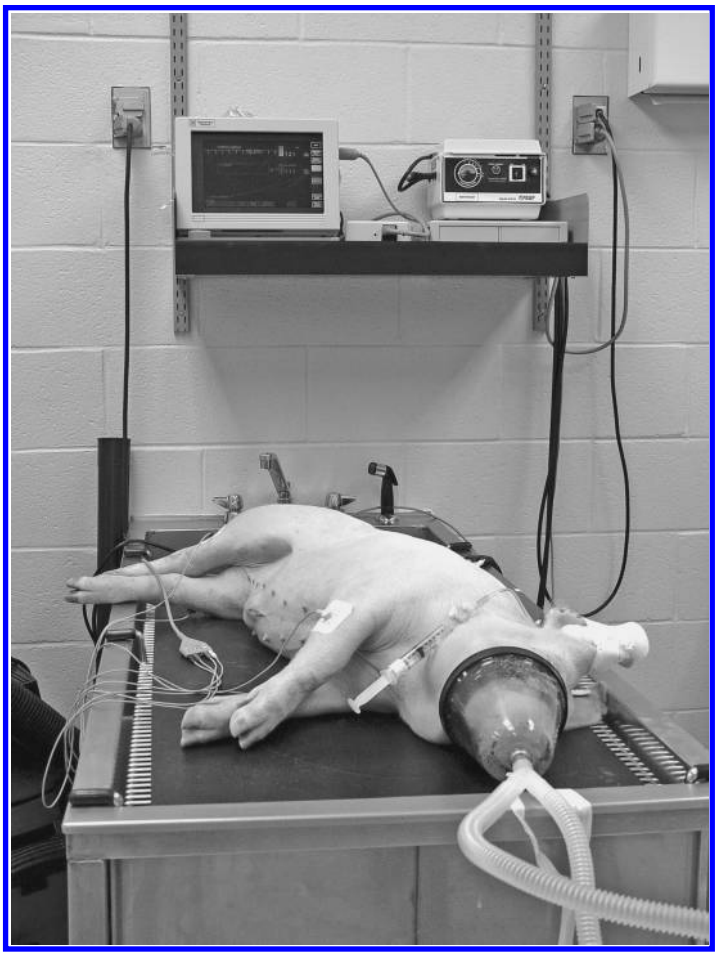

FIGURE 2.9 Anesthetized pig in the prep room with ECG electrodes attached.

FIGURE 2.10 Correct position of the laryngoscope for intubation of a pig in dorsal recumbency.

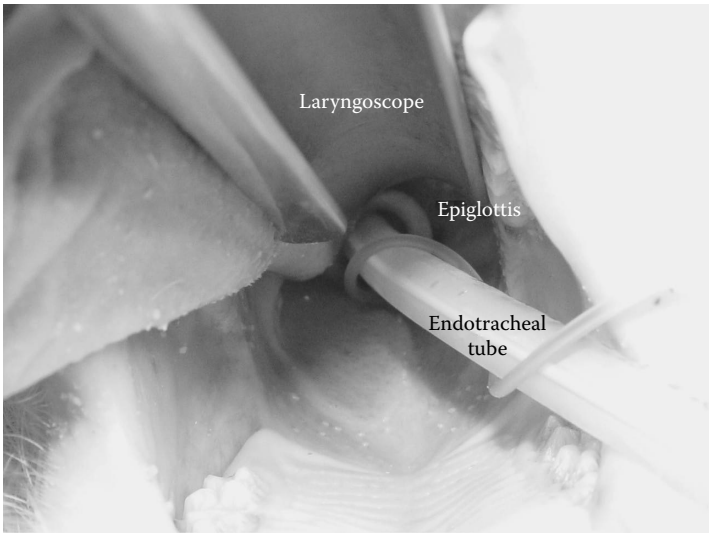

FIGURE 2.11 Oral view of the placement of a laryngoscopy tube in a pig in dorsal recumbency.

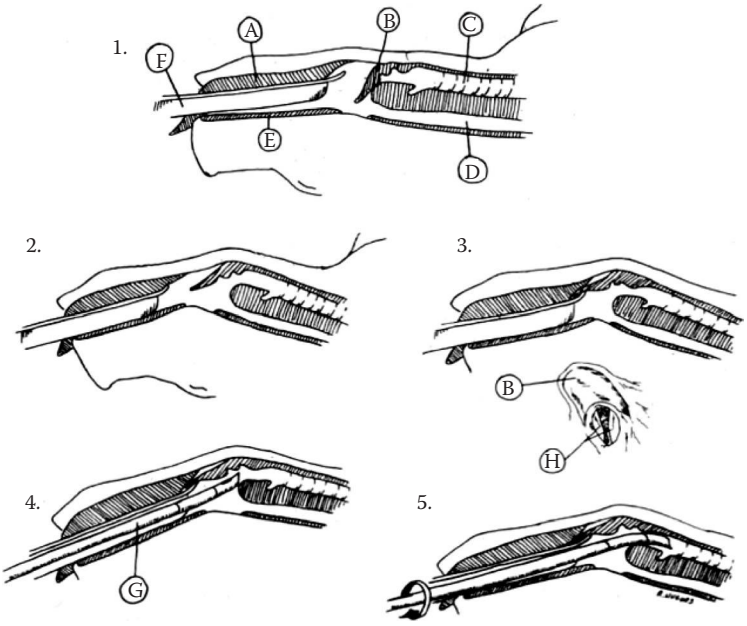

FIGURE 2.12 Schematic drawing of the steps involved in intubation of a pig. A—tongue, B—epiglottis, C—trachea, D—esophagus, E—hard palate, F—laryngoscope blade, G—endotracheal tube, H—vocal cords.

After the laryngoscope is passed into the pharyngeal cavity, the tip is used to displace the epiglottis from the soft palate. After this procedure, the epiglottis and laryngeal aperture are easily seen, and the larynx is sprayed with a topical anesthetic, such as lidocaine, to prevent laryngospasm. The tip of the epiglottis is caught with the tip of the curved laryngoscope blade and displaced ventrally against the tongue. At this point, the vocal cords can be observed moving with each breath. The handle of the laryngoscope is tilted toward the operator at approximately a 45° angle. This will

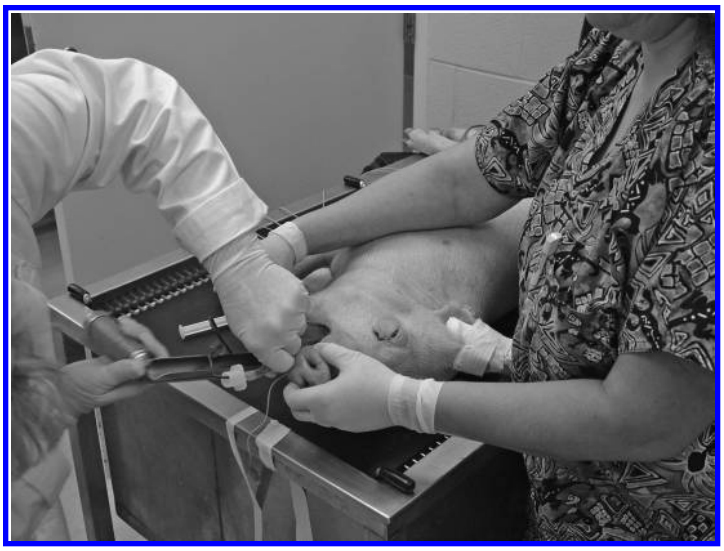

FIGURE 2.13 Correct position for intubating a pig in lateral recumbency.

result in the larynx being flattened against the ventral surface of the neck; this can be seen readily from the outside. This maneuver will result in the laryngeal passage being straightened. The tip of the endotracheal tube is placed into the laryngeal cavity from the side of the oral cavity while watching the positioning of the tube along the laryngoscope blade. After the tip of the tube is in place, it is passed into the trachea while being simultaneously rotated in a screw-like fashion to facilitate passage through the aperture. Resistance should not be felt if the angle of the laryngoscope blade is correct and the size of the endotracheal tube is not too large.

When using this method, the use of stylets or other assist devices is unnecessary. Endotracheal tubes ranging from 4.5 to 8.0 mm in outside dimension are sufficient for most swine used in biomedical research. The size of the tube required can be estimated by palpation of the trachea prior to intubation. As a general rule, most 20- to 30-kg swine can be intubated with 6.5–7.5 mm tubes.

With practice, small swine can be intubated without a laryngoscope. The technique involves placing the pig in dorsal recumbency and pinching the dorsum of the larynx between the thumb and forefinger of one hand. This pinching movement should elevate the larynx and close the esophageal passage. A popping will be felt when the epiglottis is displaced ventrally from the soft palate. The endotracheal tube is then passed blindly through the oral cavity with the other hand. This methodology can best be utilized in small swine in which the larynx can be easily manipulated from the outside.

Regardless of the methodology utilized, a free passage of air should be felt and heard when the pig is properly intubated. The two most common problems are inadvertent closure of the laryngeal opening with the laryngoscope and placement of the tube in the esophagus. With traumatic methodologies, the larynx may also either be ruptured through one of its membranes or traumatized sufficiently to produce laryngeal edema. Any gasping or cyanosis is a sign of improper tube or laryngoscope placement. Cyanosis can easily be observed in the snout or nipples of pigs with light-colored skin. The technique of tracheostomy is described in Chapter 9, should it be indicated.

Single lung ventilation is sometimes required for thoracic surgery. In humans, there are double lumen endotracheal tubes which are designed for their anatomy and tracheal sizes. These do not work well for pigs. Some groups have designed tubes specific for pigs in their projects (Schwarzkopf et al., 2010). If there is no access to such a tube, then single lung ventilation is most easily performed in the left lung and the anatomy has been detailed (Nakakuki, 1994). There are several methods that can be used with a standard endotracheal tube. A flexible vascular wire can be passed through the tube

following passage of the tube beyond the larynx. The wire can be manipulated to pass into the bronchus of choice followed by passage of the tube into the structure. This visualization may be aided by echo or fluoroscopy. Alternatively, a bronchial endoscope can be passed through the tube to visualize the opening of the bronchus. It can also be placed blindly after familiarizing the operator with the anatomic sites of the bronchus. Confirmation of single lung inflation can be made using a stethoscope.

SPECIAL PERIOPERATIVE CONSIDERATIONS

Consideration should be given to several issues concerning perioperative care and monitoring of swine while under anesthesia. These issues include methodologies to provide homeostasis and prevent other potentially fatal complications. The general principles of proper anesthesia and monitoring are applicable to swine, but some issues are species specific (Smith and Swindle, 1994, 2008; Smith et al., 1997).

Preoperative Fasting

Swine should be conditioned for 5–7 d in the facility prior to undergoing survival surgery. They tend to dehydrate and lose weight during shipping. Acute procedures may be performed on newly arrived animals, but i.v. fluids should be administered to maintain hydration. Swine have rapid intestinal transport times in the upper gastrointestinal (GI) tract and require only a few hours to empty the stomach. Consequently, a fast of solid food for 6–8 h preoperatively is sufficient for most surgical procedures. Water may be provided up until the time of surgery. Swine will readily consume most liquid diets and flavored drinks if they are required to prevent hypoglycemia from prolonged fasts, as for colonic procedures. Bedding should be removed from the cages of swine being fasted, because they will readily consume it if not provided food. Some bedding materials such as straw can cause intestinal impaction if a fasted pig eats too much of it presurgically. Issues of fasting specifically applicable to GI procedures are discussed in Chapter 4.

Intravenous Fluid Administration

The sites for administration of i.v. fluids are discussed in Chapter 1. Once i.v. access is obtained, maintenance fluids should be administered for all anesthetic procedures requiring more than short-term chemical restraint. The rate of administration should be 5–10 mL/kg/h with isotonic solutions, unless a problem or specific indication for a different rate of administration is observed. A flow rate of approximately 3–5 mL/h is required to maintain patency of i.v. catheters.

Thermal Support

The relatively hairless skin, the common use of alcohol in skin preparation for surgery, and administration of chemical restraint agents that induce peripheral vasodilation make swine more susceptible to hypothermia than most other animal species. Rectal temperature should be continuously monitored and not allowed to decrease below 36°C (normal, 38–39.5°C). The use of circulating hot water blankets and warm air blankets, and complete draping of the anesthetized animal are usually sufficient to prevent hypothermia. More intense methods, such as administration of warm i.v. fluids and increasing the room temperature, may be necessary for some highly invasive procedures or if hypothermia occurs.

Cardiac Monitoring and Arrhythmias

Swine are susceptible to anesthetic-induced cardiac arrhythmias and, as a minimum requirement, should be monitored by ECG during anesthesia. For prolonged and invasive procedures, especially

those involving the cardiothoracic system, blood pressure and blood gases should also be monitored. Pulse oximetry is useful, and finger cuffs can be placed either on the ears, tail, tongue, or the dewclaws (Figures 2.14 through 2.17). Peripheral blood pressure cuffs are most reliable for the coccygeal and medial saphenous arteries. Palpation for a peripheral pulse is most reliable on the medial saphenous artery or the radial artery (Figures 2.18 and 2.19). Peripheral arterial access in the medial saphenous artery or using the methodologies described for arterial access in Chapters 9 and 12 can be utilized to implant catheters and measure blood gases.

Cardiac arrhythmias are more likely to be a problem during manipulation of the heart or central vessels or when using anesthetics such as tiletamine/zolazepam, xylazine, or halothane, which have a proarrhythmic effect on the myocardium. Farm animals appear to be more susceptible than miniature breeds. Most of the fatal instances of cardiac arrhythmias can be prevented by

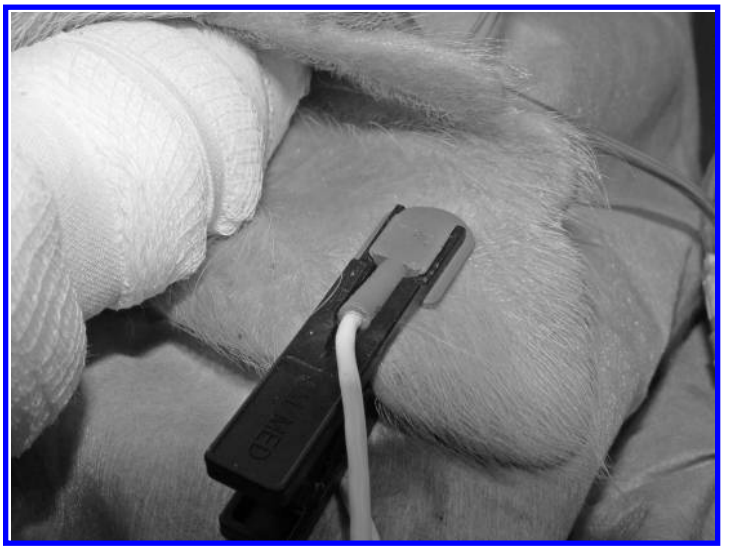

FIGURE 2.14 Pulse oximetry sensor in place on the ear.

FIGURE 2.15 Pulse oximetry sensor in place on the tail.

FIGURE 2.16 Pulse oximetry sensor in place on the tongue.

FIGURE 2.17 Pulse oximetry sensor in place on the dewclaw.

the administration of bretylium (3–5 mg/kg i.v.) given by slow injection every 30 min during cardiac manipulation (Horneffer et al., 1986; Schumann et al., 1993). Bretylium is not currently being manufactured, and amiodarone (10–12 mg/kg i.v.) followed by 0.5 mg/kg/h i.v. infusion has been utilized as a substitute for arrhythmia prevention. It is effective, but the infusion rate needs to be closely monitored because it can rapidly lead to hypotension in swine. Lidocaine (2–4 mg/kg i.v.) can be administered as a continuous i.v. infusion 0.3 mg/kg/h (50 µg/kg/min) as an antiectopic and antivasospasmotic agent. Other cardiovascular support agents can be administered as indicated; the dosages are included in Table 2.1.

If ventricular fibrillation occurs, then defibrillation should be attempted with 10 J countershock for internal paddles or 200 J countershock for external paddles. The principles of treatment of this potentially fatal condition are the same as for other species.

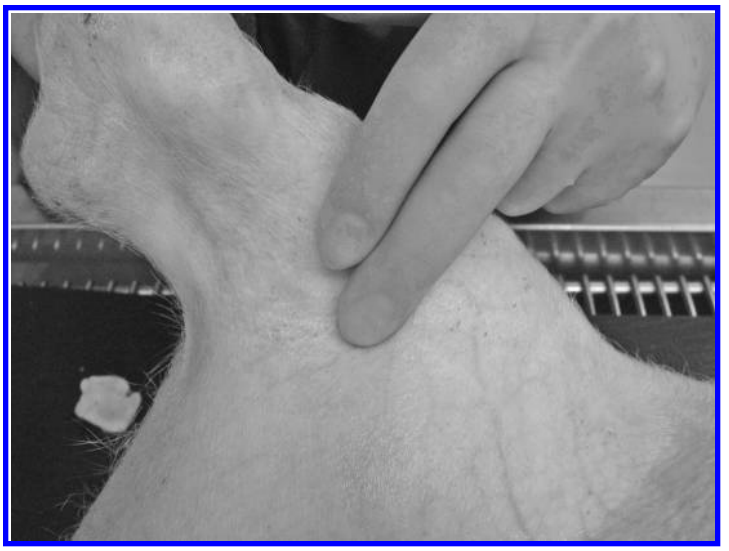

FIGURE 2.18 Palpation of the saphenous pulse on the medial aspect of the stifle (knee) joint.

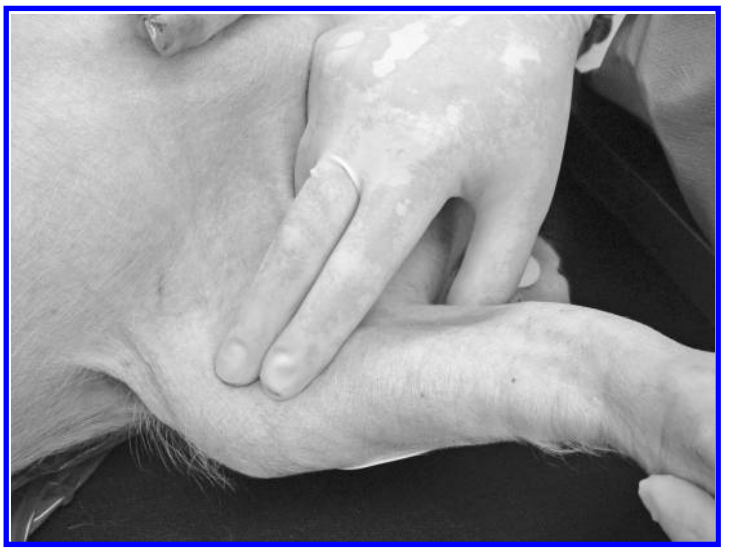

FIGURE 2.19 Palpation of the radial pulse on the medial aspect of the ulna.

Specialized Intraoperative Monitoring

Bispectral index (BIS) monitoring, which is a processed electroencephalogram (EEG) index, has been evaluated in swine using various combinations of sevoflurane, isoflurane, desflurane, propofol, fentanyl, and atacurium (Greene et al., 2004; Martin-Cancho et al., 2003, 2004, 2006). BIS was determined to be reliable for identification of light versus deep anesthesia but was not reliable for discrimination in the mid ranges of anesthesia with isoflurane. Misinterpretation of EEG burst suppression by the program in that anesthetic range is theorized to be a species-specific difference. Decrease in blood pressure and heart rate correlated with anesthetic depth with isoflurane (Greene et al., 2004) but not sevoflurane during BIS monitoring (Martin-Cancho et al., 2004). Without additional research to aid interpretation of results, BIS should be considered an adjunct research tool and not a replacement for

TABLE 2.1
Cardiopulmonary Emergency Drugs

Agent	Dosage	Indication
Aminophylline	5.0 mg/kg i.v.	Produces bronchodilation
Amiodarone	10.0–12.0 mg/kg, followed by 0.5–3.5 mg/kg/h i.v.	Antiarrhythmic
Atropine	0.05 mg/kg i.v.	Counteracts bradycardia, heart block
Bicarbonate Na	1.0 mEq/kg bolus, followed by 0.5–1.0 mEq/kg/h	Counteracts acidosis
Bretylium	3.0–5.0 mg/kg i.v.	Antiarrhythmic
Calcium chloride	5.0–7.0 mg/kg slow i.v. infusion	Increases contractility
Digoxin	0.01–0.04 mg/kg i.v.	Counteracts supraventricular arrhythmias; decreases conduction; increases contractility
Dopamine	2.0–20.0 μg/kg/min i.v.	Counteracts hypotension, cardiogenic shock
Dobutamine	2.5–10.0 μg/kg/min i.v.	Counteracts hypotension, cardiogenic shock
Epinephrine	0.5–2.0 mL of 1:10,000 solution i.v. or i.c. (30 μg/kg)	Counteracts asystole, decreased contractility
Isoproterenol	0.01 μg/kg/min i.v.	Induces bronchodilation; counteracts AV block, sinus bradycardia
Lidocaine	2.0–4.0 mg/kg bolus followed by 50 μg/kg/min i.v.	Antiarrhythmic, antiectopic
Nitroprusside Na	0.5–0.8 μg/kg/min i.v.	Reduces hypertension
Neosynepherine	0.5–1.0 mg/kg i.v.	Increases blood pressure by vasoconstriction
Propanolol	0.04–0.06 mg/kg i.v.	Counteracts tachycardia

monitoring of hemodynamics for depth of anesthesia in swine. A similar form of monitoring, cerebral state index (CSI), has been developed, which uses a different algorithm to calculate a numerical value (Bollen and Saxtorph, 2006). Likewise, this particular technique is in development.

Similarly, auditory-evoked potentials (AEP) have been used for monitoring depth of anesthesia with propofol, isoflurane, and sevoflurane (Bollen et al., 2004). The values obtained did not directly correlate with data from humans; consequently, AEP should also be considered to be an experimental adjunct until further research validates the methodology.

MALIGNANT HYPERTHERMIA

Malignant hyperthermia is a genetic condition in certain breeds of domestic swine, such as the Landrace, Yorkshire, and Pietrain. The condition is transmitted as an autosomal dominant gene (Hal genotype). The ryanodine receptor gene (ryr-1 locus) is the probable site (Geers et al., 1992; Houde et al., 1993). The condition has not been reported in miniature swine.

The condition is induced by stress, that is, porcine stress syndrome (PSS), or by many anesthetic and paralytic agents. Susceptible animals can be screened genetically or by testing for abnormal creatine phosphokinase serum levels. The condition may also be prevented by the prophylactic intramuscular (i.m.) or i.v. administration of dantrolene (5 mg/kg) (Anderson, 1976; Ehler et al., 1985; Smith et al., 1997).

If malignant hyperthermia is encountered, it is associated with elevated rectal temperatures and skeletal muscle rigidity. Elevated CO_2 levels and associated cardiovascular responses such as tachycardia occur rapidly at the onset. The condition is rapidly fatal in susceptible animals. If encountered, it may be treated by discontinuing the triggering agent, cooling of the animal, and administrating dantrolene; however, it is best to avoid sources of animals that have the condition rather than using screening methods or pharmaceutical interventions to prevent the disease.

There is another postoperative condition in swine in which hyperthermia occurs intraoperatively or during the recovery period, which is apparently not the same condition as malignant hyperthermia. The symptoms are similar and rapidly elevating temperatures can lead to death if

untreated with cooling interventions such as ice and cold i.v. solutions. It is uncertain if dantrolene is effective as a treatment; however, methylprednisolone (1–5 mg/kg i.v.) has some effectiveness for shock, and diazepam (0.5–1 mg/kg i.v.) may be useful for the muscular tremors. The pathogenesis of the condition has not been described, but it has been associated with an increase in lactate levels (>2.5 mmol/L, normal = <2.2 mmol/L) and pH (Ayoub et al., 2003). It tends to occur in groups of farm animals that may be closely related. The incidence is sporadic and unpredictable. It is not related to changes in the anesthetic or surgical protocol and tends to disappear from the herd without any intervention on the part of the breeder. It may occur suddenly in a group of animals from a supplier who has been used for long periods of time without ever experiencing the condition before, and whose herd has not experienced PSS or malignant hyperthermia. If the condition is noted in a research facility, the best prevention is to request unrelated swine from the same or a different breeder, because anecdotal information suggests a genetic predisposition. Administration of a tranquilizer as a preanesthetic using the butterfly neck injection technique 10 min prior to administration of other anesthetics provides a nonstressful induction, which some laboratories have found to prevent the occurrence of the condition.

PARALYTIC AGENTS (NEUROMUSCULAR BLOCKADE)

Paralytic agents may be indicated for some procedures either to paralyze the diaphragm during cardiac surgery or to provide increased muscle relaxation. Paralytic agents should not be administered during surgery until it is established that surgical analgesia has been obtained and baseline hemodynamic measurements have been recorded. For example, the use of paralytic agents is helpful to paralyze the diaphragm during cardiac surgical manipulations. The thoracotomy can be performed without the administration of paralytic agents to ensure that analgesia levels are sufficient. As a minimum requirement, at least the skin incision should be performed before the paralytic agent is administered to observe the animal's reactions. It is unnecessary to administer these agents throughout the surgical procedure in most cases.

Paralyzed animals should be monitored for a sustained increase in heart rate or blood pressure as an indication of inadequate analgesia. The initial administration of pancuronium (0.02–0.15 mg/kg, 0.003–0.030 mg/kg/h i.v. infusion) is associated with a physiological increase in heart rate. Vecuronium (1 mg/kg; 0.01–0.05 mg/kg/h i.v. infusion) does not affect the heart rate (Smith et al., 1997). Rocuronium (1–1.5 mg/kg, 2.0–2.5 mg/kg/h i.v. infusion) or atracurium (0.001 mg/kg; 0.4 mg/kg/h i.v. infusion) may be preferred for some protocols and they are similar to vecuronium in their cardiovascular effects.

Anesthetic Monitoring

Anesthetic monitoring can be performed by monitoring heart rate and blood pressure as described in the preceding text (Figure 2.20). This methodology is more sensitive than the muscular or ocular reflexes that are commonly used in veterinary medicine. Ocular reflexes are difficult to observe in swine because of the depth of the orbit and the frequently used combinations of drugs in porcine anesthesia that can make meaningful observations obscure. Muscular reflexes can be induced by pinching at the coronary band of the hoof, the tip of the ear, or the tail, or by observing mandibular jaw tone. Jaw tone seems to be the most reliable and is the preferred method of observing muscular reflexes. Rigidity of the mandibular muscles should be taken as an indication that the anesthetic level is light (Swindle, 1983).

Ventilation Rates

The pulmonary tissue of swine is sensitive to overventilation and may rupture and cause emphysematous bullae, pneumothorax, and pneumoperitoneum. Pneumatosis intestinalis may occur in

SURGICAL PROCEDURE / ANESTHESIA MONITORING

INVESTIGATOR _____ AR # _____

SURGERY DATE _____ ANIMAL # _____ WEIGHT_____KG

DESCRIPTION OF PROCEDURE _____

_____

_____

COMPLICATIONS _____

SURGEON _____ ASSISTANT _____ ANESTHETIST _____

SURGERY START TIME _____ SURGERY END TIME _____

Time																		
Oxygen (Liters)																		
Nitrous Oxide (Liters)																		
Isoflurane (%)																		
Pulse (HR)																		
Oxygen saturation																		
Respiratory rate																		
Tidal vol. (cm water)																		
Blood Pressure																		
End tidal CO_2																		
Esophageal Temp																		
Rectal Temp																		
Lactated Ringers ml/hr																		
NaCl ml/hr																		
1% Nembutol ml/hr																		

SURGICAL NOTES

FIGURE 2.20 Sample anesthesia and surgical procedure monitoring form.

severe cases. The ventilatory pressure for swine should be 18–22 cm H_2O. The tidal volume for swine is 5–10 mL/kg. End tidal CO_2 is generally adjusted to 40–50 mmHg. Positive end-expiratory pressure (PEEP) may be adjusted with the ventilator to ensure proper pulmonary pressure. The respiratory rate varies depending upon the anesthetic and individual animal characteristics. For swine 20–40 kg on inhalational anesthesia, a rate of 12–15 breaths per minute is usually sufficient; however, monitoring with pulse oximetry, arterial blood gases, or end tidal CO_2 should be performed to ensure proper ventilation (Smith and Swindle, 2008; Smith et al., 1997; Swindle, 1983).

POSTOPERATIVE MONITORING

The postoperative period should include monitoring of temperature, pulse, and respiration at least every 15 min until extubation and recovery of the righting reflex. For major procedures, monitoring of ECG and pulse oximetry should be included (Figures 2.20 and 2.21). Perioperative care procedures specific to particular procedures are discussed in the various organ and system chapters in this book.

Procedures for continuous monitoring in an intensive care setting (Figures 2.22 and 2.23) for up to 7 d have been described (Hanneman et al., 2004a,b). In this setting, sedation, analgesia, and parenteral nutritional support, as well as continuous intensive monitoring for homeostasis and infection, are required. Likewise a neonatal intensive care unit for piglets which allows 24 h monitoring and sample collection has been described in detail (Lennon et al., 2011). Other groups have

MUSC Department of Laboratory Animal Resources
Post-Operative Observations for Lab Animals

Please check the following daily for each animal that has undergone surgery or an invasive procedure. Space is available for 7 days of observations. Use Progress Note form for observations beyond 7 days. (N = normal)

Species _____ Procedure _____ ARC # _____
Animal # _____ Date of Procedure _____ PI _____

DAY 1	Incision		Urine		TPR	
	Attitude		Eating		Pain score	
	Feces		Drinking		Initials/Time	
DAY 2	Incision		Urine		TPR	
	Attitude		Eating		Pain score	
	Feces		Drinking		Initials/Time	
DAY 3	Incision		Urine		TPR	
	Attitude		Eating		Pain score	
	Feces		Drinking		Initials/Time	
DAY 4	Incision		Urine		TPR	
	Attitude		Eating		Pain score	
	Feces		Drinking		Initials/Time	
DAY 5	Incision		Urine		TPR	
	Attitude		Eating		Pain score	
	Feces		Drinking		Initials/Time	
DAY 6	Incision		Urine		TPR	
	Attitude		Eating		Pain score	
	Feces		Drinking		Initials/Time	
DAY 7	Incision		Urine		TPR	
	Attitude		Eating		Pain score	
	Feces		Drinking		Initials/Time	

For animal emergencies, questions regarding animal health evaluation and/or post-op care, contact DLAR office.

Phone:
Pagers:

Pain Assessment:
Use the following number designations, as appropriate, to reference no pain perception or reaction as if painful.

1. Deep palpation of the surgical site and immediate surrounding tissue does not provoke a response. (Remember that freshly opened tissue is susceptible to infection and palpation should be done with a gloved hand).
2. Deep palpation of the surgical site and immediate surrounding tissue provokes a response but a similar response can be seen on the contra-lateral side or limb, suggesting a hyperesthetic and/or hyperreflexive state.
3. Deep palpation of the surgical site and immediate surrounding tissue that provokes a response much greater than a similar stimulus on a non-surgical part of the body. Probably indicative of some pain and appropriate analgesic should be administered.
4. Deep palpation of the surgical site and immediate surrounding tissue that provokes a response much greater than a similar stimulus on a non-surgical part of the body and accompanied by vocalization in an otherwise quiet patient. Requires analgesia.

FIGURE 2.21 Sample postoperative observation and pain scoring form.

developed intensive care monitoring and treatment unique to their particular protocol, such as for transplantation of the trachea (Murison et al., 2009).

Following recovery from anesthesia, monitoring should be performed at least once a day until the sutures are removed or the incision is healed. The monitoring should include state of the incision, attitude and behavior, urination, appetite, feces, water consumption, temperature, pulse, and respiration. Use of a pain score is also helpful in evaluating the need for analgesic administration.

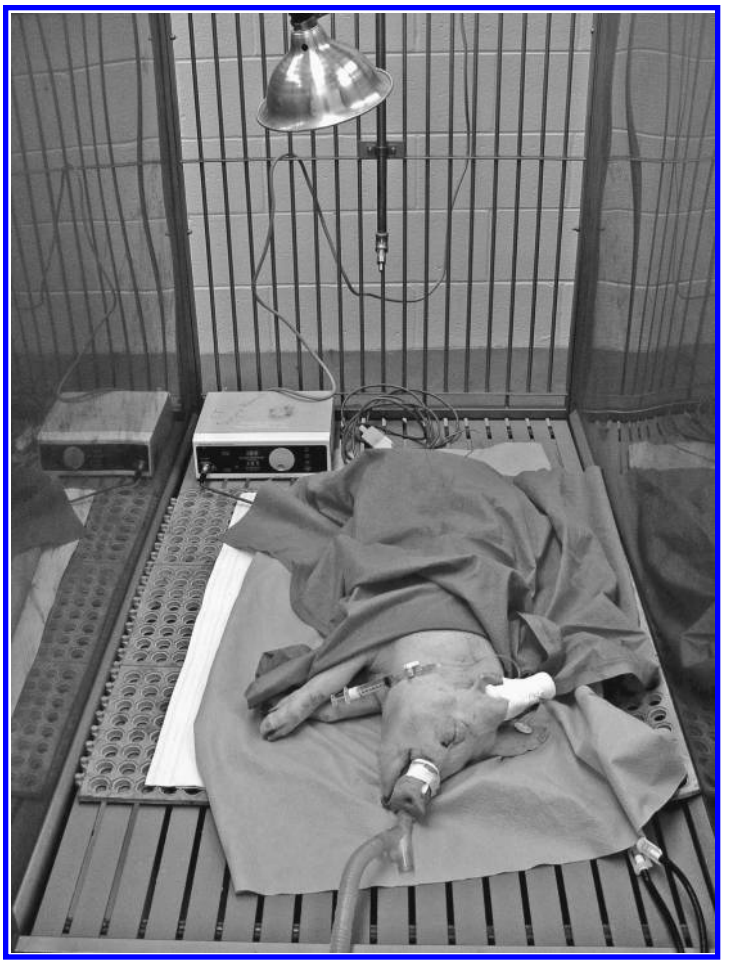

FIGURE 2.22 The pig is in a recovery cage with heat support and padding.

Administration of systemic analgesics on a preset schedule without evaluation of the individual's condition is not recommended. Professional judgment is essential in the decision on whether analgesics should be administered. It may be harmful to the animal's recovery to be administered pharmacological agents when they are not necessary. The following pain assessment score is an example of such an evaluation system.

Pain Assessment

Use the following number designations, as appropriate, to reference no pain perception or reaction as if painful.

1. Deep palpation of the surgical site and immediate surrounding tissue does not provoke a response. (Remember that freshly opened tissue is susceptible to infection and palpation should be done with a gloved hand.)
2. Deep palpation of the surgical site and immediate surrounding tissue provokes a response, but a similar response can be seen on the contralateral side or limb, suggesting a hyperesthetic or hyperreflexive state.

FIGURE 2.23 An intensive care cage with oxygen support.

3. Deep palpation of the surgical site and immediate surrounding tissue that provokes a response much greater than a similar stimulus on a nonsurgical part of the body. Probably indicative of some pain and appropriate analgesic should be administered.
4. Deep palpation of the surgical site and immediate surrounding tissue that provokes a response much greater than a similar stimulus on a nonsurgical part of the body and accompanied by vocalization in an otherwise quiet patient. Requires analgesia.

Long-term monitoring may have to be performed for animals in which debilitating conditions, such as heart failure, are produced. This can involve daily monitoring for homeostasis for months. In this situation, the protocol and potential complications should be examined in advance and an appropriate monitoring system with endpoints should be developed by the investigator in collaboration with the veterinary staff. Daily examination can be aided by providing food treats during the examination (Figure 2.24).

Nonpharmacological Control of Pain and Distress

Pain and distress can be largely prevented by nonpharmacological preventive measures in surgical protocols. Included in this is provision of environment and husbandry practices conducive to an animal's well-being (Chapter 1). The skill of the surgeon is an important element in this equation. Attention to the basic principles of surgery (Chapter 3) and the species-specific requirements will be helpful in a shortened recovery period after surgery.

FIGURE 2.24 Socialization of a pig with a food treat during auscultation.

Chronic Pain and Distress

Individual behavior variations may result in an animal that develops anxiety or chronic pain and distress on a protocol in which other individuals respond favorably. Some procedures, such as orthopedic surgery, may also produce chronic pain, which is significantly harder to control than soft-tissue surgery.

Veterinary monitoring of the animals may identify these individuals by observation of hyperesthesia, hyperreflexia, pain facilitation, stereotypic behavior patterns, abnormal vocalizations, and attitude abnormalities.

In these cases, systemic analgesic administration may not be sufficient to correct the problem. Adjuncts to systemic analgesics, such as opioids, can include anxiolytic tranquilizers or sedatives, local nerve blocks, and anti-inflammatory agents.

PREANESTHETIC AGENTS

Preanesthetic agents are useful to relieve anxiety, abolish the vagal reflex, decrease the amount of general anesthetic required, and facilitate handling. These agents fall into the categories of anticholinergics, sedatives, hypnotics, and tranquilizers. Giving these agents in the cage before inducing anesthesia may make the induction and transport of the animals less stressful and easier for the technical staff. For example, if the induction is to be performed with ketamine and acepromazine, administration of the acepromazine first calms the animal for the larger volume and more irritating injection with ketamine. The use of preoperative and intraoperative analgesics is discussed in the section on analgesics in this chapter.

Anticholinergics

Atropine (0.05 mg/kg i.m., s.c. or 0.02 mg/kg i.v.) and glycopyrrolate (0.004–0.01 mg/kg i.m., s.c.) are used preoperatively to dry bronchiole secretions and abolish the vagal reflex during endotracheal intubation or suctioning. It is also useful to counter the bradycardia that is associated with the use of some anesthetic agents. Routine use of these agents is not required, and the physiological effects of tachycardia and vagal blockade should be considered when designing the protocol (Smith and Swindle, 2008; Smith et al., 1997; Swindle, 1983).

Tranquilizers and Sedatives

The phenothiazine, benzodiazepine, and butyrophenone tranquilizers are the most commonly used agents for preanesthesia. All these agents have been combined with dissociative agents to induce anesthesia and are discussed in that context in the following text. Of the phenothiazines, acepromazine (1.1–2.2 mg/kg i.m., i.v., or s.c.) is the most commonly used agent. It is associated with peripheral vasodilation and α-adrenergic blockade in higher dosages. Its effects as a sole agent last for 8–12 h (Benson and Thurmon, 1979; Riebold et al., 1995; Swindle, 1983).

The two most commonly used benzodiazepine tranquilizers in porcine anesthesia are diazepam, which is fat soluble, and midazolam, which is water soluble. Diazepam (0.5–10 mg/kg s.c., 0.44–2 mg/kg i.v., 1 mg/kg/h i.v. infusion, or 2–10 mg/kg p.o.) provides good hypnosis and sedation for up to 6 h (Benson and Thurmon, 1979; Thurmon and Tranquilli, 1986). Midazolam (0.1–0.5 mg/kg i.m., s.c. or i.v., 0.6–1.5 mg/kg/h i.v. infusion) provides complete sedation for 20 min with minimal hemodynamic depression and can be used safely on a daily basis for prolonged periods of time (Ochs et al., 1987; Smith et al., 1991). However, the decrease in cardiovascular parameters at the higher dosage range (0.5 mg/kg i.m. or s.c.) is significant as compared to unsedated minipigs using peripheral cuff measurements (Goodrich et al., 2001). A combination of azaperone (4 mg/kg i.m. or s.c.) and midazolam (1 mg/kg i.m. or s.c.) has been used as a preanesthetic prior to propofol induction and inhalation anesthesia (Svendsen and Carter, 1997).

Butyrophenones are usually found in combination with other agents, except for azaperone (2–8 mg/kg i.m. or s.c.). It has minimal cardiovascular effects, but provides relatively short immobilization of about 20 min (Portier and Slusser, 1985; Riebold et al., 1995; Thurmon and Tranquilli, 1986).

INJECTABLE ANESTHETIC AGENTS

Injectable anesthetic agents used in swine include the dissociative anesthetics, barbiturates, opioids, and miscellaneous hypnotic agents. Most of these agents are used in combination with tranquilizers or other agents to provide surgical anesthesia. Experience in our laboratories has shown that these agents can be administered s.c. in the neck or flank to provide the same effect as i.m. injections, usually cited in the literature. The s.c. route is preferred because it is less traumatic and less painful for the animal when administering large volume or irritating substances. Unless these agents are meant to provide short-term chemical restraint, they should be administered as continuous i.v. infusions rather than repeated i.m. or i.v. injections in the research setting. Experience has shown that continuous i.v. infusions provide more stable hemodynamics than repeated injections. The i.v. dosages are variable depending upon the characteristics of the individual animal and the protocol. The dosages cited in this text are guidelines, and careful anesthetic monitoring should be used to determine if an adequate plane of anesthesia has been achieved. Agents commonly combined with dissociative agents, such as benzodiazepines, hypnotics, and α-2-adrenergic agonists, are discussed with the dissociative agents because they have little value as sole agents in swine.

Dissociative Agents and Combinations

Ketamine and the combination agent tiletamine/zolazepam (Telazol®, Ft. Dodge Animal Health (Wyeth), Overland Park, KS) are the two most common injectable anesthetic agents utilized in swine (Table 2.2). Usually they are combined with other agents to produce surgical anesthesia and only used as sole agents to provide up to 20 min of chemical restraint. They provide poor muscle relaxation but have minimal cardiovascular effects with a single i.m. injection in clinically normal animals (Benson and Thurmon, 1979; Cantor et al., 1981; Smith and Swindle, 2008; Smith et al., 1997).

Ketamine (11–33 mg/kg i.m., 3–33 mg/kg/h i.v. infusion) does not provide visceral analgesia at any dose. It may be combined with other agents to provide muscle relaxation and analgesia for

TABLE 2.2
Ketamine Combinations

Drug	Dosage	Route of Administration
Ketamine	11–33 mg/kg	i.m., s.c.
	3–33 mg/kg/h	i.v. infusion
Ketamine	33 mg/kg	
Acetylpromazine	1.1 mg/kg	i.m., s.c.[a]
Ketamine	20 mg/kg	
Xylazine	2 mg/kg	i.m., s.c.
Ketamine	11 mg/kg	
Fentanyl-Droperidol (Innovar-Vet)	1 mL/14 kg	i.m., s.c.
Ketamine	2 mg/kg	i.v.
Xylazine	2 mg/kg	
Oxymorphone	0.075 mg/kg	(2× dose for i.m., s.c.)
Ketamine	15 mg/kg	
Azaperone	2 mg/kg	i.m., s.c.
Ketamine	20 mg/kg	
Diazepam	2 mg/kg	i.m., s.c.[a]
Ketamine	33 mg/kg	
Midazolam	500 µg/kg	i.m., s.c.
Ketamine	33 mg/kg/h	
Midazolam	1.5 mg/kg/h	Continuous i.v. infusion[a]
Ketamine	20 mg/kg	
Climazolam	0.5–1 mg/kg	i.m., s.c.
Ketamine	1 mg/mL	i.v. bolus followed by 1 mL/kg/h[a]
Xylazine	1 mg/mL	
Glyceryl guiaconate 5% in 5% dextrose	1 mL/kg	
Ketamine	1 mg/kg	i.m., s.c.[a]
Dexmedetomidine	0.1 mg/kg	
	5 mg/kg/h	Continuous i.v. infusion[a]
	10 µg/kg/h	
Ketamine	5–19 mg/kg/h	
Pentobarbital	6.5–20 mg/kg/h	Continuous i.v. infusion[a]
Ketamine	10–20 mg/kg	i.m., s.c.
Xylazine	2.0 mg/kg	
Midazolam	0.25 mg/kg	

[a] Most highly recommended agents and combinations.

minor procedures (Benson and Thurmon, 1979; Boschert et al., 1996; Smith and Swindle, 2008; Smith et al., 1997; Swindle, 1983). The most commonly used i.m. combinations in research are as follows:

Ketamine (33 mg/kg) and acepromazine (1.1 mg/kg)
Ketamine (15 mg/kg) and diazepam (2 mg/kg)
Ketamine (10 mg/kg) and flunitrazepam (0.2 mg/kg)
Ketamine (33 mg/kg) and midazolam (0.5 mg/kg)
Ketamine (15 mg/kg) and azaperone (2 mg/kg)
Ketamine (20 mg/kg) and xylazine (2 mg/kg)
Ketamine (10 mg/kg) and dexmedetomidine (0.2 mg/kg)

Only the combination with midazolam provides longer than 20–30 min of restraint; however, this combination is profoundly hypothermic. It may last 45–60 min and provides sufficient relaxation to perform intubation. Nevertheless, the side effects outweigh the advantages of this combination. The combination of ketamine and medetomidine has a wide range of dosages reported with the dosage of ketamine varying from 1 to 10 mg/kg and the dosage of medetomidine varying from 0.08 to 0.2 mg/kg. The dosage in the list in the preceding text is used by the author. In general, if you increase ketamine, you decrease medetomidine proportionately. Ketamine (5 mg/kg/h)/medetomidine (10 μg/kg/h) infusions provide a stable plane of anesthesia. Medetomidine has now been replaced by dexmedetomidine but the dosage remains the same.

One group studied a variety of ketamine combinations to provide effective sedation and anesthesia for domestic and Yucatan pigs with and without various forms of cardiovascular compromise (Linkenhoker et al., 2010). Pigs were sedated for transport and imaging procedures prior to being placed under inhalant anesthesia. The combination which demonstrated the greatest success for their procedures was ketamine 27 mg/kg and midazolam 0.6 mg/kg s.c. It was compared to ketamine 20–27 mg/kg and acepromazine 1 mg/kg s.c.; ketamine 20–27 mg/kg and diazepam 2–5 mg/kg s.c.; ketamine 20–27 mg/kg and medetomidine 0.1–0.2 mg/kg s.c. It is a comprehensive study which details the clinical aspects of the various combinations including the complications they experienced. Another group (Ajadi et al., 2008) used ketamine 10–20 mg/kg, xylazine 2 mg/kg, and midazolam 0.25 mg/kg i.m. for light sedation and minor surgery.

The α-2-adrenergic agonists xylazine and dexmedetomidine are commonly included as combinations with the dissociative agents. The combination with xylazine provides short-term analgesia (5 min) but prolonged cardiodepression and heart block, which may be reversed with anticholinergics. Dexmedetomidine has less severe cardiodepression than xylazine (Flecknell, 1997). Other less commonly used agents in this class are romifidine (80 μg/kg i.m. or s.c.) and detomidine (2 μg/kg i.m. or s.c.).

The combinations with acepromazine, diazepam, and azaperone are similar in action but do not provide enough relaxation to perform intubation or to perform other than minor surgery. They all have the side effect of peripheral vasodilation but not as profoundly as the combinations with xylazine and midazolam. Atipamezole (1 mg/kg i.m., s.c., or i.v.) is a specific antagonist to the α-2-adrenoreceptors (Flecknell, 1997).

Intravenous infusions of ketamine combined with other agents can provide visceral analgesia suitable for major surgical procedures. After induction with a loading dose of the agent, the following infusions can be used: ketamine (1 mg/mL) and xylazine (1 mg/mL) and glycerol guaiacolate (guaifenesin) (5%) mixed in 5% dextrose (1 mL/kg i.v.) followed by 1 mL/kg/h of the mixture; ketamine (8–33 mg/kg/h) and midazolam (0.5–1.5 mg/kg/h); or ketamine (5 mg/kg/h) and medetomidine (10 μg/kg/h). Ketamine (5–19 mg/kg/h i.v.) and pentobarbital (6.5–20 mg/kg/h) as separate simultaneous infusions have been used for long-term 96-h shock studies and CPB (Goldmann et al., 1999; Liu et al., 2009). Following ketamine–azaperone sedation, the i.v. infusion was started at the lower dosages and gradually had to be increased as tolerance developed. However, they were able to maintain a stable anesthetic plane as monitored by heart rate and mean arterial pressure.

All these agents as i.v. infusions can provide a stable plane of anesthesia. The ketamine/xylazine/glycerol guaiacolate (guaifenesin) solution, in particular, causes minimal cardiovascular depression and can be used as a substitute for α-chloralose infusions (Thurmon et al., 1986). Its main disadvantage is the need to mix the combination in the laboratory. The infusion combinations with dexmedetomidine (Vainio et al., 1992) and midazolam are useful for cardiac catheterization protocols but do not provide good muscle relaxation. A ketamine/pentobarbital infusion has been shown to provide better survival rates than a fentanyl/pentobarbital infusion (Liu et al., 2009).

The tiletamine/zolazepam (Telazol, Ft. Dodge Labs, Ft. Dodge, IA) combination can provide 20 min of immobilization for minor surgery in the commercially available combination (2–8.8 mg/kg i.m.); however, the combination should not be used in animals with cardiovascular compromise because of cardiodepression and hypothermia (Lefkov and Muessig, 2007). The cardiodepressive

effects have been demonstrated to last for hours to days and also have been demonstrated to result in high mortality in animals with induced heart failure, especially when combined with xylazine. In a study of the effects of anesthesia on ^{18}F-FDG, a glucose analog, evaluated by positron emission tomography–computed tomography (PET-CT), Tiletamine-zolazepam decreased the accumulation in the hearts and brains when compared to other anesthetics (Lee et al., 2012). In agricultural and pet pig situations, Telazol is combined with xylazine or xylazine and ketamine (Ko et al., 1993a,b, 1997). The combination was also compared to Telazol combined with medetomidine with comparable cardiorespiratory results (Lee et al., 2010). Single-dose administration of this agent to normal animals does not cause problems with homeostasis; however, it has been associated with problems with arrhythmias in manipulative cardiac surgical protocols. The combinations have also been shown to reduce insulin levels and be a potential complication in metabolic research (Heim et al., 2002). Telazol and its combinations are useful for large research swine >75 kg because of the lower volume of injection required to achieve sedation as compared to ketamine combinations.

All of the combinations are suitable for intubation and short-term anesthesia; however, hemodynamic data have not been published to prove the usefulness of the combinations in research settings. Clinical observation of animals under anesthesia with these combinations has included peripheral cyanosis, hypothermia, and death in animals with cardiovascular compromise. The i.m. combination dosages to provide 20–30 min of immobilization are the following:

Telazol (4.4 mg/kg) and ketamine (2.2 mg/kg)
Telazol (4.4 mg/kg), ketamine (2.2 mg/kg), and xylazine (2.2 mg/kg)
Telazol (4.4 mg/kg) and xylazine (2.2 mg/kg)
Telazol (4.4 mg/kg) and dexmedetomidine (0.04 mg/kg)

Combinations useful for restraint of large swine are as follows:

Ketamine (100 mg/mL), 0.1–0.15 mL/kg + diazepam (5.0 mg/mL), 0.2–0.3 mL/kg + dexmedetomidine (1.0 mg/mL), 0.1 mL/kg + atropine (0.54 mg/mL); 2 mL/pig reversed with atipamezole (5.0 mg/mL) 1–2 mL/pig. Used for restraint of sexually mature minipigs (Goodrich, J.A., personal communication. Department of Comparative Medicine, Medical University of South Carolina, Charleston, SC.).

Tiletamine/zolazepam, 125 mg each (one vial) + dexmedetomidine, 6.5 mL of 1 mg/mL solution + ketamine, 1.25 mL of 100 mg/mL solution + butorphanol, 2.5 mL of 10 mg/mL solution mixture. Give 0.67 mL/10 kg with atropine (Ravn, K., personal communication, Novo Nordisk A/S, Gentofte, Denmark.).

Barbiturates

Barbiturate anesthesia (Riebold et al., 1995; Smith and Swindle, 2008; Smith et al., 1997; Swindle, 1983, 1991; Thurmon and Tranquilli, 1986) must be administered i.v., usually after induction with one of the dissociative anesthetics to allow the anesthetist to insert an i.v. catheter (Table 2.3). The barbiturates have become increasingly expensive and difficult to procure in recent years. The initial administration of these agents as a bolus will frequently induce apnea, which can be overcome by the stimulation of endotracheal intubation. They have a dose-related cardiopulmonary depressant activity that increases over time with repeated i.v. boluses. The cardiovascular effects can be minimized by using thiobarbiturates, which have minimal hepatic metabolism as i.v. infusions. These agents are largely excreted by the kidneys and can be flushed out of the system with i.v. fluid administration. Pentobarbital is best reserved for nonsurvival procedures and should be administered as a continuous i.v. infusion. However, a pentobarbital infusion was found to be superior to a combination of medetomidine/ketamine/fentanyl for anesthesia during ischemia produced ventricular fibrillation (Grund et al., 2003). In that study, the incidence of ventricular fibrillation and hemodynamic

TABLE 2.3
Barbiturates

Drug	Dosages	Route of Administration
Pentobarbital	20–40 mg/kg 5–40 mg/kg/h	i.v. infusion
Thiopental	6.6–25 mg/kg 3–30 mg/kg/h	i.v. infusion[a]
Thiamylal	6.6–25 mg/kg 3–30 mg/kg/h	i.v. infusion

[a] Most highly recommended.

depression was significantly less than with the balanced anesthesia combination. As discussed in preceding sections, it has also been used successfully for long-term anesthesia and imaging procedures. Dosages of barbiturates are guidelines and may be affected by other drugs and homeostatic factors. They should be given to effect with close monitoring of vital signs. The i.v. dosages of the most commonly used barbiturates are as follows:

Thiopental (6.6–30 mg/kg, 3–30 mg/kg/h)
Thiamylal (6.6–30 mg/kg, 3–30 mg/kg/h)
Pentobarbital (20–40 mg/kg, 5–40 mg/kg/h)

Opioid Infusions

Opioids can be used as i.v. infusions to provide the primary analgesia for cardiac surgery protocols; however, they have to be combined with other anesthetics, such as the inhalants, during the major manipulative procedures. In general, when isoflurane is used as an adjunct, a level of 0.5% is adequate. They can also be used as low-dose infusions during general surgery to provide balanced anesthesia and analgesia (Demestiha et al., 2010; Ehler et al., 1985; Lunn et al., 1979; Merin et al., 1982; Schumann et al., 1994; Smith and Swindle, 2008; Smith et al., 1997; Swindle et al., 1986). The concept of analgesia with these agents is discussed in the section on analgesics in this chapter.

Opioid infusions have the advantages of not decreasing myocardial contractility and coronary blood flow. They produce a dose-related bradycardia that can be reversed with anticholinergics. They can also be used as stabile i.v. infusions for taking physiological measurements for cardiovascular protocols. Hypertension occurs in the higher dose ranges over time. In that situation, the adjunctive anesthetic agents can be eliminated after all the major surgical manipulations have been performed.

The most commonly used agents for these infusion procedures in swine are fentanyl (30–100 μg/kg/h) and sufentanil (7–30 μg/kg/h), although other agents in this class should be equally effective such as alfentanil (6 μg/kg/h) or remifentanil (30–60 μg/kg/h). They may be used for induction following placement of an i.v. catheter or following restraint with midazolam or ketamine. Starting the i.v. infusion prior to administering an i.v. bolus to induce anesthesia prevents the animals from exhibiting a sudden onset of bradycardia and muscle rigidity. These dosages are guidelines, and the i.v. infusion must be given to effect with constant monitoring of cardiovascular parameters. The i.v. dosages are as follows:

Fentanyl, 0.050 mg/kg (50 μg) i.v. bolus, 0.030–0.100 mg/kg/h i.v. infusion
Sufentanil, 0.007 mg/kg (7 μg) i.v. bolus, 0.015–0.030 mg/kg/h i.v. infusion
Remifentanil, 0.5–1 μg/kg/min (0.030–0.060 mg/kg/h)
Alfentanil, 0.1 μg/kg/min (0.006 mg/kg/h)

Miscellaneous Agents

Propofol (4–20 mg/kg i.v.) is an i.v. hypnotic agent that can be used in combination with other agents to induce anesthesia (Table 2.4). It has a relatively narrow therapeutic margin in swine and can produce severe hypotension and apnea. In lower dosages, cardiac output and coronary blood flow is minimally depressed; however, there is also poor analgesia. A continuous infusion rate of 12–20 mg/kg/h can be used to provide stable general anesthesia (Foster et al., 1992; Raff and Harrison, 1989; Ramsey et al., 1993). Propofol (2.0–4.4 mg/kg/h) combined with fentanyl (0.003–0.005 mg/kg/h) and midazolam (0.4–0.7 mg/kg/h) has been described as effective for 6–7 h of anesthesia (Kaiser et al., 2003, 2006) following induction with ketamine/azaperone and an i.v. loading dose of the agents. Propofol (3.5 mg/kg/h) and fentanyl (17 μg/kg/h) have also been used to induce general anesthesia as an i.v. infusion (Bollen et al., 2007). Propofol increased ^{18}F-FDG as graded by PET-CT in the brain and heart (Lee et al., 2012).

Etomidate (4–8 mg/kg i.v.) is a hypnotic sedative that can be combined with other agents to produce analgesia. The combination of 0.6 mg/kg i.v. with a ketamine infusion of 10 mg/kg/h i.v. can be used for anesthesia (Holzchuh and Cremonesi, 1991; Worek et al., 1988). In low doses, it has minimal effects on heart rate, blood pressure, and cardiac output. It does suppress the adrenal glands for hours after administration. It is a useful agent for induction of anesthesia in animals with cardiac compromise.

The agent α-chloralose (55–86 mg/kg i.v.) has been used for nonsurvival surgery to record physiological measurements with minimal cardiovascular depression and minimal effects on the baroreceptors and chemoreceptors. It has questionable analgesic value except at the higher dose range, at which time the sparing effects on cardiovascular parameters are lost (Silverman and Muir, 1993; Thurmon and Tranquilli, 1986). It is best replaced by ketamine infusion protocols, opioid infusions, or inhalational anesthesia with isoflurane or sevoflurane.

Prolonged i.v. anesthesia may be required for protocols that do not allow the use of inhalant anesthetics. Intravenous infusion with sufentanil, thiopental, ketamine/xylazine/glycerol guaiacolate, ketamine/midazolam, ketamine/pentobarbital, or propofol/fentanyl/midazolam as described in the preceding text is acceptable for these procedures.

No particular advantage of using these agents or older agents, such as alphaxalone, alphadolone, or etorphine, over other agents in this chapter has been published.

INHALANT ANESTHETICS

Inhalant anesthetics should be the primary agents considered for general anesthesia in swine (Smith and Swindle, 2008; Smith et al., 1997). They provide a better control of the plane of anesthesia and analgesia and have a reduced recovery time over many of the injectable agents. When used in combination with intraoperative analgesics to provide balanced anesthesia, the anesthetist has the highest assurance that the animal is in an appropriate plane of anesthesia for major surgical procedures.

All these agents should be used in combination with gas scavenging and periodic monitoring of the gas anesthesia machines for leakage, because of the human health problems associated with chronic exposure to low levels of these agents. In particular, the exposure of personnel, especially pregnant women, to waste gases should be avoided with methoxyflurane, halothane, and nitrous oxide. The use of scavenging systems and absorbent filters in combination with closed or semiclosed anesthetic systems should be routine. The gas anesthetic machines should be modern in design and kept in good repair to provide the best assurance that the level of anesthesia indicated by the vaporizer is correct and to prevent personnel exposure to gases leaking from the equipment (Thurmon and Benson, 1996; Tranquilli et al., 2007).

The agents that should be considered as the primary choices for use in porcine anesthesia are isoflurane and sevoflurane. Desflurane requires specialized equipment and has a higher cost than those agents. Methoxyflurane is difficult to control because of its low potency, and the agent has

TABLE 2.4
Miscellaneous Drugs and Combinations

Drug	Dosages	Route of Administration
Alphaxolone/alphadolone	6–8 mg/kg followed by 2–3 mg/kg	i.m. i.v. infusion
α-Chloralose	55–100 mg/kg	i.v.
Atipamezole	0.24–1 mg/kg	i.v., i.m., s.c.
Atropine	0.02–0.05 mg/kg	i.m., s.c., i.v.
Azaperone	2–8 mg/kg	i.m., s.c.
Brontizolam	1–10 mg/kg	p.o.
Etorphine/acetylpromazine (Imobilon®)	0.245 mg/10 kg	i.v.
Climazolam	0.5–1 mg/kg	i.m.
Diazepam	0.5–10 mg/kg	i.m., s.c., p.o.
	0.44–2 mg/kg	i.v.
Diprenorphine	0.3 mg/kg	i.v.
Etomidate	4–8 mg/kg	i.v.
Etomidate	4–8 mg/kg	i.v., s.c., i.m.
Azaperone	2 mg/kg	
Etomidate/ketamine	6 mg/kg followed by 10 mg/kg/h	i.v. followed by i.v. infusion
Flurazepam	2 mg/kg	i.v., p.o.
Glycopyrrolate	0.004–0.01 mg/kg	i.m., s.c., i.v.
Lorazepam	0.1 mg/kg	i.v.
Midazolam	100–500 µg/kg	i.m. or i.v.,
	0.6–1.5 mg/kg/h	i.v. infusion[a]
Metomidate	4 mg/kg	i.v.
Nalbuphine	1–2 mg/kg	i.v.
Naloxone	0.5–2 mg/kg	i.v.
Pancuronium	0.02–0.15 mg/kg	i.v.
Propofol	0.83–1.66 mg/kg	i.v. followed by 14–20 mg/kg/h infusion
Propofol	2.0–4.4 mg/kg/h	i.v. infusion after loading dose
Midazolam	0.4–0.7 mg/kg/h	
Fentanyl	0.003–0.005 mg/kg/h	
Telazol® (Tiltamine:zolazepan)[b]	2–8.8 mg/kg	i.m., s.c.
Telazol	4.4 mg/kg	i.m., s.c.
Xylazine[b]	4.4 mg/kg	
Telazol	4.4 mg/kg	i.m., s.c.
Xylazine	2.2 mg/kg	
Butorphanol[b]	0.22 mg/kg	
Telazol	4.4 mg/kg	
Xylazine	2.2 mg/kg	i.m., s.c.
Azaperone[a,b]	0.88 mg/kg	
Vecuronium	1.0 mg/kg	i.v.
Xylazine	0.2 mg/kg	i.m., s.c.

[a] Most highly recommended.

[b] Telazol and combinations contraindicated in protocols involving cardiovascular or other significant physiologic compromise.

the possibility of causing nephrotoxicity in humans. Consequently, it is recommended that its use be discontinued along with older agents such as ether. Halothane sensitizes the myocardium to catecholamine-induced arrhythmias and has more severe depressant effects upon the myocardium than the more recently developed agents. These physiological effects combined with the possibility of hepatotoxicity in humans should preclude its use as an anesthetic in swine. Enflurane is associated with seizure episodes in susceptible animals and does not offer any advantages over the use of other agents (Smith and Swindle, 2008; Smith et al., 1997). The older agents are no longer available commercially in most countries and their continued use is discouraged.

Isoflurane, desflurane, and sevoflurane all have similar physiological effects in swine and are relatively safe for personnel compared to the other inhalants (Weiskopf et al., 1992). The cost differential between isoflurane and the other two agents is significant. Because of the similar physiological effects of these three agents and the significant difference in cost, isoflurane is recommended as the primary inhalant anesthetic in swine at present, with special exceptions (Smith and Swindle, 2008; Smith et al., 1997).

Sevoflurane may be an appropriate choice for high-risk cases, and it has been documented in the case of myocardial infarction projects in which sevoflurane had a significant decrease in the complications of arrhythmias and mortality as compared to isoflurane (Regueiro-Purrinos et al., 2011). Sevoflurane has also been shown to be more protective of the endothelium in ischemia reperfusion studies of the aorta as compared to propofol (Annecke et al., 2011). Induction by face mask (Figure 2.25) followed by endotracheal intubation may be performed with isoflurane or sevoflurane without nitrous oxide for protocols in which it is necessary to have a sole agent for anesthesia. The face mask should be free of leaks and the area adequately ventilated. This procedure carries minimal risk for personnel.

All the inhalant anesthetics increase cerebral blood flow and decrease coronary blood flow in a dose-dependent fashion. These effects are minimized with isoflurane, and it may increase coronary blood flow at some dosages. All produce a dose-related depression in myocardial contractility. Isoflurane, desflurane, and sevoflurane have significantly less deleterious effects on the myocardium than the other agents discussed in the preceding text (Smith and Swindle, 2008; Smith et al., 1997; Weiskopf et al., 1992). Desflurane has also been shown to impair hepatic and small intestinal O_2 capacity, but not to cause severe tissue hypoxia (Armbruster et al., 1997). Serum biochemical levels have been demonstrated to be decreased by isoflurane and sevoflurane anesthesia in minipigs (Tanaka et al., 2009). Sevoflurane decreased serum albumin, potassium, inorganic phosphorus, γ-glutamyltransferase peptidase, cholinesterase, insulin, and glucose. Isoflurane decreased total protein, albumin, triglyceride, phospholipids, sodium, potassium, calcium, alanine aminotransferase, alkaline phosphatase, insulin, and glucose. Isoflurane has been demonstrated to increase uptake of ^{18}F-FDG in the heart and brain of minipigs examined by PET-CT (Lee et al., 2012).

The percentage of the inhalant used for anesthesia may be reduced by the administration of nitrous oxide. Nitrous oxide as a sole agent does not provide visceral analgesia in swine; however, it is effective as an adjunct agent when used in a 1:1 or 2:1 combination with oxygen to deliver the inhalant anesthetic. The combination of isoflurane with nitrous oxide and oxygen (2:1) provides the least myocardial depressant effects of any of the inhalant anesthetic agents and reduces the concentration of isoflurane required by approximately 50%. However, nitrous oxide has the potential of having adverse effects on the health of personnel, and adequate scavenging of waste gases should be assured (Smith and Swindle, 2008; Smith et al., 1997).

The mean alveolar concentration (MAC) value is utilized as a measure of an inhalant anesthetic's potency and provides a guideline for the percentage of an anesthetic that should be required for general anesthesia (Table 2.5). The MAC value will vary with the age of the animal as well as other variables, such as the delivery system and protocol (Smith and Swindle, 2008; Smith et al., 1997). The MAC values (volume %) of these agents in swine are nitrous oxide, 195 (Tranquilli et al., 1985); halothane, 0.91–1.25 (Eisele et al., 1985, 1986; Tranquilli et al., 1983); isoflurane, 1.2–2.04 (Eger et al., 1988; Eisele et al., 1985; Koblin et al., 1989; Lundeen et al., 1983); sevoflurane, 2.53 (Thurmon and Benson, 1996); enflurane, 1.66 (Thurmon and Benson, 1996); and desflurane, 8.28–10 (Eger et al., 1988).

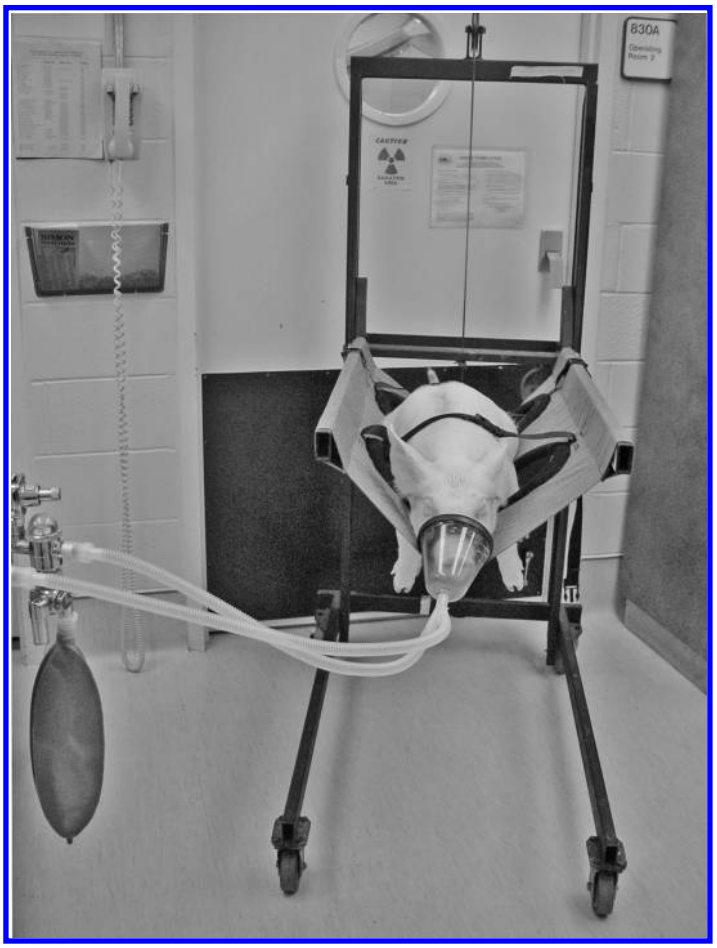

FIGURE 2.25 The pig is being induced with gas anesthesia in a humane restraint sling, using a face mask.

Xenon, a rare gas, has been used experimentally in swine to compare its effects to isoflurane (Baumert et al., 2005). It is unlikely that it will be used extensively due to its high cost.

The flow rates for anesthetics will vary with the equipment and the procedure; however, a rate of 5–10 mL/kg/min is generally adequate. Anesthetic monitoring procedures described in the preceding text should be utilized to determine if the anesthetic level and oxygenation are adequate.

TABLE 2.5
Inhalant Anesthetics

Agent	Mean Alveolar Concentration (MAC) (%)
Isoflurane	1.2–2.04
Desflurane	8.28–10.0
Halothane	0.91–1.25
Sevoflurane[a]	2.53
Enflurane	1.66
Nitrous oxide 1:1 or 2:1	195

[a] Most highly recommended agents.

CARDIOPULMONARY BYPASS

If performed as survival procedures, CPB (Figure 2.26) and extracorporeal membrane oxygenation (ECMO) (Figure 2.27) in swine are more difficult procedures to perform successfully than in most other species. Difficulties include friability of the atria, postperfusion pulmonary hypertension, cardiac arrhythmias, and edema of visceral tissues (Belanger et al., 2002; Cameron et al., 1992; Martens et al., 2004; Mohr et al., 1996; Myung et al., 2004; Pokela et al., 2002; Purohit et al., 1993; Qayumi et al., 1992; Smerup et al., 2004; Smith and Swindle, 2008; Smith et al., 1997; Swan and Meagher, 1971). The methodologies described in this section are derived mainly from the laboratory experiences described by Ehler and Swindle (Ehler et al., 1985; Smith et al., 1997; Swindle et al., 1986) with some recent modifications by Smerup et al. (2004). The use of a cooperative team approach among the perfusionist, surgeon, anesthetist, and veterinary staff is essential to the success of these protocols.

In its simplest form, blood is collected prior to entering the right side of the heart, circulated through the bypass system for oxygenation and filtering, and then returned to the arterial circulation without passing through the left side of the heart. Many variations to the surgical cannulation are performed depending upon the surgical protocol under investigation. The procedure may be performed with or without the induction of hypothermia and cardioplegia to stop electrical activity and lower myocardial metabolism. In general, the longer the procedure, the more the necessity for those procedures to protect the myocardium. CPB with aortic cross-clamp time of less than 30 min is more likely to be uneventful in swine. Time spans of greater than 45 min are likely to involve more manipulations during weaning and recovery from CPB. Indomethacin suppositories (50 mg) administered to swine the night before surgery greatly reduces the incidence of postperfusion pulmonary hypertension due to the antiprostaglandin effect (Landis et al., 2001). In a recent publication

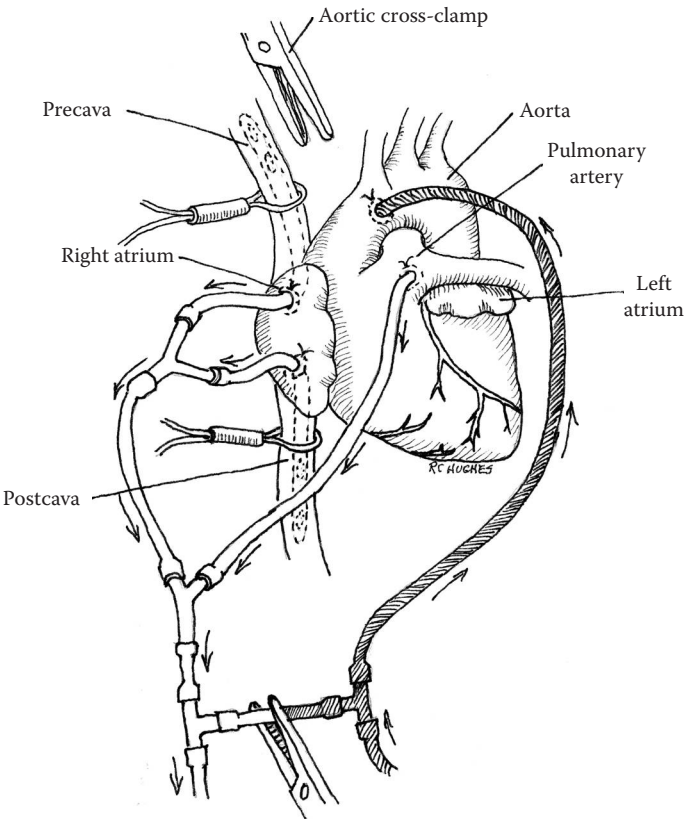

FIGURE 2.26 Catheterization circuit for cardiopulmonary bypass.

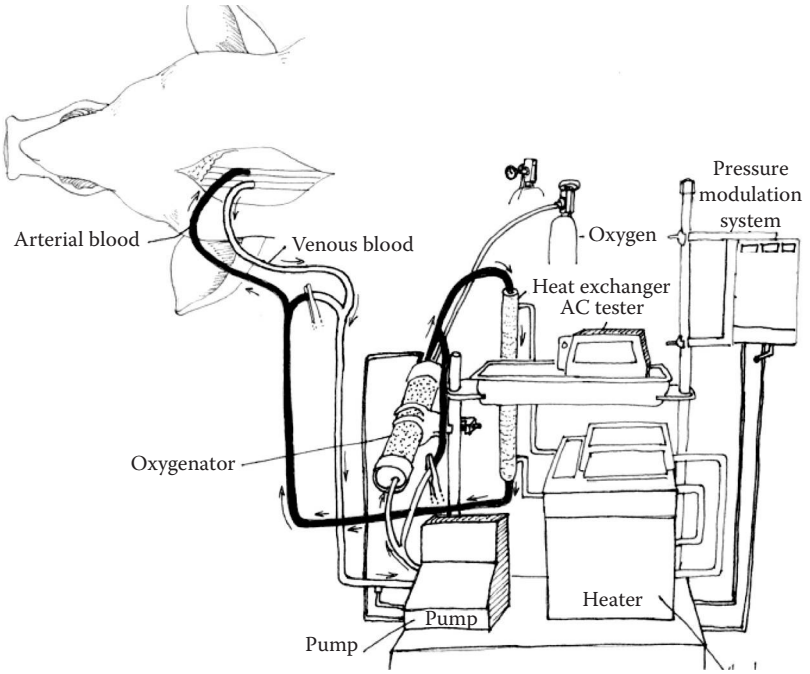

FIGURE 2.27 Extracorporeal membrane oxygenation circuit in swine. (From Purohit, D.M. et al. 1993. *J. Invest. Surg.* 6(6): 503–508. Reprinted with permission.)

(Smerup et al., 2004), methylprednisolone (500 mg i.v.) was demonstrated to also be protective against these complications if administered preoperatively.

Cardiopulmonary Bypass Equipment

Membrane oxygenators are preferred for survival procedures. Generally, the bypass equipment should be a pediatric unit with adequate monitoring devices. Cardiopulmonary monitoring, including ECG, core temperature, arterial blood gases, blood pressure, and serum electrolytes, is essential. Monitoring of activated clotting time (ACT) is essential because of the necessity of systemic heparinization of the animal during the procedure. Monitoring of the hematocrit is also essential.

Surgery

The surgical approach usually is via a median sternotomy (Chapter 9), and the heart is placed in a pericardial cradle. Femoral cutdown is performed for peripheral venous and arterial access during the procedure (Chapter 9). The animal is heparinized with 300 IU/kg. The pre- and postcava are cannulated after an atriotomy is performed in the right atrial appendage. The inserted cannulae are snared around the vessels with umbilical tape or elastic bands. This approach reduces the risk of tearing the friable right atria. Blood is returned to the arterial circulation by implanting a cannula in the aorta distal to the aortic valve and cross-clamping of the aorta between the valve and the cannula. Depending upon the procedure, insertion of a cannula to act as a pulmonary arterial vent may be required to provide drainage from the right ventricle and lungs and to manage postperfusion pulmonary hypertension (Horneffer et al., 1986). All the cannulae are held in place with purse-string sutures at their site of entrance into the cardiovascular system. After the cannulae are in place, the aorta may be cross-clamped, and cardioplegia solution containing potassium is then injected into

the aortic root. The surgical descriptions for thoracotomy and postoperative considerations discussed in Chapter 9 should be reviewed as they are applicable to CPB procedures.

Monitoring and Perfusionist Parameters

The priming solution should be fresh donor blood to maintain the hematocrit above 25. Blood groups with weak blood group antigens occur in swine (16 types, A–P); however, cross matching is not usually necessary for a single procedure. Domestic swine may be used as donors for miniature pigs. It is preferred that the last unit of blood collected during terminal collection be discarded because of the potentially high level of catecholamines (Cameron et al., 1992). Crystalloid primes have been associated with visceral edema, and colloid solutions should be used if a supplement is required for the whole blood.

Heparinization of the animal should be initiated with 300 IU/kg of heparin and the ACT maintained at 180–400 s with supplemental dosages in the 100–200 IU/kg range. An ACT of greater than 400 s may be required with complex cardiac procedures and the lower range for procedures such as ECMO. These dosages of heparin are usually required approximately every 45 min if the higher ACT range is utilized. Unless absolutely essential, protamine should not be used to reverse heparinization at the end of the procedure because of the possibility of adverse reactions, which are common in swine. If it is required, it is given in a ratio of 1.3:1 (protamine:heparin). Control of hemorrhage should be a factor of careful attention to hemostasis by the surgeon rather than drugs.

Arterial pump flow rates are dependent upon the size of the animal and the core temperature. The smaller the animal, the higher the flow rate; and the cooler the temperature, the lower the flow rate. Flow rates for swine range from 60 to 100 mL/kg/min with an average of approximately 75 mL/kg/min. The mean arterial pressure is maintained at 50–60 mmHg with a central venous pressure (CVP) of 0–5 mmHg during bypass. The systemic vascular resistance is maintained between 800 and 1400 dyn/s/cm^{-5}. The core temperature is maintained between 15°C and 37°C dependent upon the requirements of the protocol.

Neonatal piglets have been used in experimental protocols in which regional low-flow perfusion gave an improved neuorologic outcome with deep hypothermic circulatory arrest (18°C, 10 mL/kg/min) (Myung et al., 2004). CPB studies involving the injection of air versus carbon dioxide at 1 mL/kg have demonstrated improved cerebral protection with carbon dioxide (Marten et al., 2004). Swine and other animals are intrinsically more resistant to air emboli than humans. Pigs subjected to hypothermic circulatory arrest at 20°C for >75 min develop an increase in intracranial pressure >17 mmHg, which leads to brain infarction and other complications of increased intracranial pressure (Pokela et al., 2002).

Other parameters to be monitored by the perfusionist include blood gases and electrolytes. The approximate arterial blood gas parameters to be maintained include PaO_2, 100 mmHg; $PaCO_2$, 40 mmHg; pH, 7.38–7.42; base excess 0 or less. Potassium should be maintained at 4.5–5.5 mmol/L. Venous O_2 saturation is usually 65–75.

Anesthesia

Most anesthetic regimens that have been utilized in swine have been tried during CPB. Isoflurane should be the primary agent of choice unless contraindicated by the protocol or the medical status of the patient. If the animal is in cardiovascular compromise or if malignant hyperthermia is a potential risk, the high-dose opioid infusion protocols described earlier should be the primary choice. The anesthetist will need to coordinate the procedures closely with the perfusionist. Adjunct agents that may be required include the usual list of emergency drugs listed in the appendix and antiarrhythmic agents such as bretylium. The procedures for administration of these anesthetics as described in the preceding text should be used.

Weaning from Cardiopulmonary Bypass

Most of the problems encountered in CPB in swine will occur within the first 2 h following termination of the procedure. Weaning from the procedure should be performed slowly and with careful monitoring of the parameters described in the preceding text. Measurement of CVP and pulmonary artery (PA) pressure should be performed with a Swan-Ganz catheter inserted from the femoral vein. Blood gas monitoring and ventilation adjustments to treat acidosis should be performed.

The right side of the heart is refilled by gradually occluding the venous return from the cannulae after releasing the snares from the caval vessels. The arterial cross-clamp is removed, and arterial perfusion is gradually stopped. Monitoring of CVP to return to values of 8–15 mmHg is performed. Mean arterial pressure is gradually returned to 90–110 mmHg. Donor whole blood is preferred to reinstitute adequate volume, but colloidal solutions may be administered if necessary. Rewarming should be gradual if hypothermia was used in the procedure.

Intravenous nitroglycerine and inhaled nitric oxide have been demonstrated to be useful in reducing central venous and left atrial pressures, thus attenuating pulmonary hypertension. Nitric oxide has the beneficial effect of also improving arterial oxygenation and pulmonary vasodilation without effects on systemic pressure; however, nitroglycerine reduces arterial blood pressure (Troncy et al., 1996, 1997).

Insulin-like growth factor 1 (1.2 mg/h i.v.) has been shown to significantly reduce oxygen consumption, increase cardiac output, and increase oxygen delivery with decreased oxygen extraction in neonatal piglets post hypothermic CPB. The improved oxygen transport may be beneficial to the survival of neonates following this procedure (Li et al., 2004).

Careful monitoring to return the animal to homeostasis using the parameters described in this section is essential, and demonstration of normative cardiac output should be determined prior to closing the surgical incisions. If the pig is not responding to the weaning procedures, CPB should be reinstituted and the procedure retried after 10–15 min. Pulmonary hypertension and acute right-sided heart failure or irreversible ventricular fibrillation are the two most common causes of failure during weaning procedures. Returning to CPB or PA venting should be instituted as an immediate step while controlling this condition. Pulmonary vasoconstriction or clumping of blood products may be involved and should be treated if necessary (Cameron et al., 1992).

Treatment of cardiac arrhythmias may be required, and the therapeutic protocols used are the same as for other species, with the exception of the species-specific considerations discussed in the section on arrhythmias. In our experience, prophylactic treatment with bretylium (5 mg/kg i.v.) every 30 min or amiodarone (10–12 mg/kg i.v.) followed by 0.5 mg kg/h i.v. infusion during the cardiac manipulations prevents arrhythmias in most cases (Horneffer et al., 1986; Schumann et al., 1993; Swindle et al., 1986).

Postoperative Considerations

The postoperative considerations discussed in other sections of this text and for thoracotomies in Chapter 9 are applicable to these procedures. Postoperative recovery and return to homeostasis should be gradual and require at least hourly, if not continuous, monitoring for the first 6–24 h. Close attention to thermoregulation and analgesia is as important as monitoring the cardiovascular parameters. The use of intensive care cages with ECG, pulse oximetry, and blood pressure monitoring should be utilized. A team approach to recovery should include a veterinarian with experience in postoperative care for cardiothoracic procedures. Over time, the team members will be able to develop protocols for performing the procedures in their laboratories, and most of these procedures will become routine. Most swine that develop a righting reflex and can be extubated will recover and be able to eat the following day.

REGIONAL LOW-FLOW PERFUSION AND NEURAL PROTECTION

Regional low-flow perfusion is an alternative to deep hypothermia and circulatory arrest used in infants (Myung et al., 2004). In this technique in neonatal swine, an aortic or carotid cannula is advanced cranially and low-flow perfusion of 10 mL/kg/min is maintained in an effort to provide neurologic protection during CPB. Modifications of this technique may also be useful for prevention of spinal cord ischemia during aortic cross-clamping.

Increases in intracranial pressure and brain infarction can also be complications of CPB (Pokela et al., 2002). Air emboli may also be accidentally introduced in the pump circuit resulting in death or dysfunction (Martens et al., 2004). Use of CO_2 has been shown to be protective at 1–2 mL/kg as compared to air emboli at 1 mL/kg following catheterization of the carotid artery, which may indicate its use in cardiac surgery. Experiments from cardiopulmonary resuscitation experiments have shown that a combination of vasopressin (0.4–0.8 IU/kg) combined with epinephrine (45 µg/kg) as an i.v. infusion is protective following cardiac arrest (Stadlbauer et al., 2003).

ANALGESICS

The use of analgesics should be routine in porcine surgical protocols (Tables 2.6 through 2.8). Preemptive administration of analgesics prevents the pain reflex from being stimulated and reduces the postoperative recovery time. Preemptive analgesia must be administered prior to making the skin incision in order to be effective. Combinations of local anesthetics infiltrated along the incision line with parenteral opioid or nonsteroidal anti-inflammatory drug (NSAID) analgesics and general anesthesia provide the most comprehensive preemptive prophylaxis. Postoperatively, the opioids

TABLE 2.6
Postoperative Analgesia

Drug	Dosages	Route/Frequency
Aspirin	10–20 mg/kg	p.o./qid.
Butorphanol	0.1–0.3 mg/kg	i.m., s.c./bid. or tid.
Buprenorphine[a]	0.01–0.05 mg/kg	i.m., s.c./bid. or tid.
	0.5–10 µg/kg/h	i.v. infusion
	0.01–0.02 mg/kg	s.c. (sustained release) q96 h
Carprofen[a]	2 mg/kg	s.c./sid.
	2–3 mg/kg	p.o./bid.
Fentanyl	30–50 µg/kg/h	i.v. infusion
Fentanyl transdermal patches	5 µg/kg/h (highly variable)	Topical
Flunixine[a]	1–4 mg/kg	s.c., i.m./sid. or bid.
Ketoprofen[a]	1–3 mg/kg	i.m., s.c., p.o./bid.
Ketorolac[a]	1 mg/kg	p.o., i.m., s.c./bid.
Medetomidine	2 µg/kg/h	i.v. infusion
Meloxicam[a]	0.4 mg/kg	s.c./sid.
Meperidine	10 mg/kg	s.c./tid.
Morphine epidural	0.1 mg/kg	Epidural
Oxymorphone	0.15 mg/kg	i.m., s.c./bid. or tid.
Phenylbutazone	5–20 mg/kg	p.o./bid.
Piritramide	75 µg/kg/h	i.v. infusion
Sufentanil	10–15 µg/kg/h	i.v. infusion
Tramadol	1–4 mg/kg	p.o./tid.

[a] Most highly recommended agents.

TABLE 2.7
Potential Physiological Effects of Opioid Analgesics

Anatomic Region of System	Effects (+ or –)
Pulmonary	
Respiratory function	–
Cough reflex	–
Cardiovascular	
Vasodilation	+
Peripheral vascular resistance	–
Baroreceptor reflexes	–
CO_2 reflex vasoconstriction	–
Heart rate	–
Cardiac output	–
Gastrointestinal	
Gastric motility	–
Gastric emptying time	+
Intestinal secretions	–
Anal sphincter tone	+
Intestinal contraction amplitude	+
Gastric acid production	–
Increase GI tone	+
CNS	
Depression	+
Cognitive dysfunction	+
Locomotor activity	+
Increase vagal tone	+
Miosis	+
Cerebral blood flow	–
Hepatic	
Biliary secretions	–
Bile duct pressure	+
Spasm of bile duct and sphincter	+
Pancreas	
Pancreatic secretions	–
Spasm of pancreatic duct and sphincter	+
Urogenital	
Urinary voiding reflex	–
External bladder sphincter tone	+
Spasm of ureteral smooth muscle	+
Uterine tone	–
Immunologic/Endocrine	
Natural killer (NK) cell activity	–
Immunoglobulin production	–
Phagocytic activity	–
Antidiuretic hormone (ADH) release	–
Protactin release	–
Somatotropin release	–
Luteinizing hormone	–
Histamine release	+

TABLE 2.8
Potential Physiological Effects of NSAID Analgesics

Anatomic Region of System	Effects (+ or –)
GI ulceration	+
GI hemorrhage	+
Platelet aggregation	–
Renal papillary necrosis	+
Intestinal nephritis	+
Prostaglandin function	–
Kinin function	–
Cyclo-oxygenase (COX) enzymes	–
Liver necrosis	+
Uterine contractions	–
Fetal circulation	–
Fetal abnormalities	+
Cognitive dysfunction	+
Cartilage metabolism	–
Bone fracture healing	–
Bone blood flow	–
Bone and cartilage remodeling	–
Bone ingrowth into implants	–
Soft tissue healing to bone	–
Spinal fusion healing	–

may be combined with NSAIDs in cases in which the musculoskeletal component of the surgery is extensive. If preemptive analgesic regimens are used, it may not be necessary to readminister postoperative analgesia in cases of minor surgery and only for a few days with major surgical procedures (Jenkins, 1987; Smith and Swindle, 1994, 2008; Smith et al., 1997). Most of the physiological variables and complications associated with the administration of opioids and NSAIDS are associated with long-term use and not a short-term period of administration postsurgically.

Opioids

Most of the opioid analgesics have relatively short half-lives in swine (Tables 2.6 and 2.7), which limits their usefulness in postoperative protocols (Blum, 1988; Flecknell, 1997; Swindle, 1991). Agents that have half-lives of less than 4 h include fentanyl (0.05 mg/kg i.v., s.c., or i.m.), sufentanil (0.005–0.010 mg/kg i.m., s.c., or i.v.), meperidine (2–10 mg/kg s.c. or i.m.), oxymorphone (0.15 mg/kg s.c. or i.m.), morphine (2.2 mg/kg s.c. or i.m.), piritramide (μg/kg/h i.v. infusion), and pentazocine (1.5–3.0 mg/kg s.c. or i.m.). Morphine has been reported to cause excitement and other adverse behavioral patterns in swine and has a short half-life (Risdahl et al., 1992). However, morphine epidural solution (0.1 mg/kg) administered preoperatively is effective for preemptive analgesia for abdominal procedures. Tramadol (1.0–4.0 mg/kg) is not a controlled substance and has weak mu agonistic activity which can be used for mild pain.

If these agents are used in opioid infusion protocols as described in the preceding text, then they may be continued as the postoperative analgesic as a gradually decreasing infusion. During the withdrawal phase from opioid infusions, some excitement or muscle rigidity may be encountered, which can be reversed by administration of acepromazine (0.5 mg/kg s.c. or i.m.) or diazepam (2–5 mg/kg s.c. or i.m.).

Fentanyl patches have been tried for postoperative analgesia in swine; however, titration of the dosage may be difficult, and the patches must be secured to the skin with bandages to prevent their ingestion. Experience has demonstrated that transdermal fentanyl patches may be highly variable in their efficacy in swine. Variables include breed, age, site of application, presence of moisture or heat on the patch, and type of procedure. It is possible to overdose swine with these patches, especially if they ingest them. There is also an increased potential for drug abuse, compared to administering parenteral injections, by humans because they are topical patches.

In Yucatan miniature pigs 17–22 kg, 100 μg/h patches provided therapeutic levels which peaked at 42–48 h after application. Lower dosages (25–50 μg/h patches) may be required for farm breeds, which tend to be younger at the same body weight and have thinner skin than minipigs (Harvey-Clark et al., 2000; Wilkinson et al., 2001). As a starting point, if the dosage is not known, a patch delivering approximately 5 μg/kg/h should be applied to the side of the chest. Clinical monitoring for signs of overdosage or underdosage is essential.

Fentanyl has also been associated with long-lasting hyperalgesia (pain facilitation) in other species. Mu-receptor stimulation by fentanyl produces *N*-methyl-D-aspartate (NMDA) receptor activation, which can be associated with increased sensitivity to pain (windup phenomenon). The higher the dosage of fentanyl, the more pain is facilitated. This effect can be blocked by administration of ketamine or dextromethorphan, which act as NMDA receptor antagonists. Thus, caution should be taken if fentanyl is being used as the preemptive analgesic (Celerier et al., 2000). Because of the many variables associated with the use of these patches, administration of appropriate levels of analgesia should not be assumed unless the lab has blood samples indicating an adequate level of analgesia with their particular model and application method.

Butorphanol (0.1–0.3 mg/kg s.c. or i.m. qid.) and high-dose buprenorphine (0.05–0.1 mg/kg s.c. or i.m. bid.) are long acting and have few side effects in swine. Buprenorphine has been extensively used without significant respiratory depression noted in postoperative protocols, including thoracotomy. It should be considered as one of the primary opioid analgesics used in porcine protocols (Hawk et al., 2005; Hermansen et al., 1986; Swindle, 1991).

Some confusion exists concerning the wide dosage ranges of buprenorphine that have been reported in swine (0.001–0.1 mg/kg i.m., s.c., or i.v.). In our experience, lower dosages (<0.01 mg/kg) do not provide prolonged analgesia for major surgical interventions. The lower dose range may be useful for preemptive analgesia or in combination with other agents. A recent publication indicates that 0.01–0.02 mg/kg or higher dosage q 8–10 h provides postoperative analgesia for a significant number of pigs (Rodriguez et al., 2001). Buprenorphine may also be given as a continuous i.v. infusion at a rate of 0.5–10.0 μg/kg/h. Newer formulations of sustained release buprenorphine can be administered s.c., 0.01–0.02 mg/kg every 96 h (Hanks et al., 2013).

Buprenorphine patches have been developed but have not been extensively tested in swine and may have the same problems with variables as fentanyl patches. Minipig data with 8 mg patches tested over 6 months in normal animals showed a wide range of variability between animals in blood levels but toxicity was not noted (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2010/021306Orig1s000PharmToxR.pdf).

Nonsteroidal Anti-Inflammatory Drugs

Traditional NSAIDs can be used in combination with opioids for balanced analgesia protocols involving muscular or orthopedic surgery (Table 2.8). Some of the newer agents, however, are adequate for postoperative analgesia as sole agents. NSAIDs are associated with fewer platelets, renal function changes, and gastric ulceration (Junot et al., 2008). These effects are more pronounced with the older agents such as aspirin. Most of the side effects are related to chronic administration of these agents and not the short-term usage for postoperative analgesia. For example, it is unlikely that short-term administration of NSAIDs for a few days for postoperative analgesia will have significant effects on bone healing or cartilage metabolism in swine.

Phenylbutazone (5–20 mg/kg p.o. bid.) and enteric-coated aspirin (10–20 mg/kg qid.) may be used as adjuncts. Aspirin is also used for anticoagulation in some protocols as a once daily dosage. Aspirin should be enteric coated to prevent gastric ulceration with chronic usage.

Ketoprofen (1–3 mg/kg i.v., s.c., i.m., or p.o. bid. or tid.) and ketorolac (1 mg/kg i.m., s.c., or i.v. bid.), flunixine (1–4 mg/kg s.c. or i.m., sid. or bid.), meloxicam (0.1–0.4 mg/kg s.c., p.o. sid.), and carprofen (2–4 mg/kg p.o. bid. or s.c. sid.) are newer agents that may have some opioid-receptor activity (Andersen et al., 1997; Flecknell, 2009; Fosse et al., 2008; Rauser et al., 2010; Smith and Swindle, 2008). They provide adequate analgesia to be used as sole agents. Carprofen and meloxicam in particular have efficacy as preemptive analgesics. In our experience, they are equivalent to buprenorphine if used in this manner. They have the advantages of parenteral administration q 24 h and of not being controlled substances. Carprofen may be increased to bid administration if increased analgesia is required in the immediate postoperative period. Currently, carprofen is the primary analgesic used in our laboratories, and swine readily ingest the chewable tablets. Meloxicam but not carprofen extends ACT in experiments conducted in our lab and in the experience of others (Friess et al., 2012).

Reversal Agents for Injectable Anesthetics, Sedatives, and Analgesics

There may be an indication for reversal of various agents. However, caution should be taken to avoid using reversal agents for major invasive procedures in which it might be beneficial to keep the pig sedated. Generally, reversal agents are utilized for emergencies and reversal of chemical restraint protocols (Branson and Gross, 2001; Gross, 2001; Smith and Swindle, 2008; Smith et al., 1997).

Naloxone (0.005–0.02 mg/kg i.v., i.m., or s.c.) is an opioid reversal antagonist at mu and kappa receptors. Nalbuphine (1–2 mg/kg i.v.) antagonizes the effects of opioids as a mu agonist while maintaining an analgesic effect at kappa receptors.

Diprenorphine (0.3 mg/kg s.c.) is a nonspecific opioid antagonist with activity at kappa, delta, and mu receptors. It is the agent used to reverse etorphine when it is used in dart guns in the field.

Atipamezole (0.24 mg/kg i.v. to 1.0 mg/kg i.m. or s.c.) is an α_2-receptor antagonist and is generally the preferred agent for this class of drugs. Others include yohimbine (1 mg/kg i.v.), tolazoline (1.5 mg/kg i.v.), and idazoxan (0.03 mg/kg i.v.).

Flumazenil is a competitive benzodiazepine antagonist when administered at 1 part to 13 parts of the benzodiazepine.

Local and Regional Analgesia

It is possible to use the local anesthetics to provide local, regional, and spinal analgesia. These procedures are not recommended in the research setting as the sole analgesic unless it is part of the protocol. Rather, they should be used as adjuncts to systemic analgesia. Long-acting agents, such as bupivacaine, can be used as dorsal nerve root blocks (Figure 2.28) in the paravertebral region to provide analgesia for such vertical incisions as lateral thoracotomies. They can also be used to preemptively anesthetize the incision site locally or the peritoneum during general surgery in combination with parenteral agents. Bupivacaine (2.5 mg/kg) should not be given i.v. because it has been associated with cardiac arrest. Ropivicaine (5 mg/kg) was developed as a less cardiotoxic long acting local anesthetic. Lidocaine (Xylocaine; 4.5 mg/kg) is shorter acting but is an acceptable agent for preemptive analgesia using the same techniques. Lidocaine with epinephrine is longer acting and can be administered in a dose of 7 mg/kg. As a general rule, a maximum total administration should be limited to less than 200 mg and the dosages listed above are maximal dosages. For incisional blocks all of these amide local anesthetics will be effective in a 1–2 mg/kg dosage.

The difficulties associated with performing epidural blocks in swine (Chapter 10) can be overcome with practice, and this procedure is effective as a preemptive analgesic for abdominal procedures. However, epidural morphine (0.1 mg/kg) is just as effective as local anesthetics for these

FIGURE 2.28 Dorsal nerve root block with a local anesthetic.

procedures and does not produce the side effects that can be encountered with systemic administration of the opioid. Other epidural analgesics have been reported for use in the agricultural setting and may be useful for some research protocols. They include 2% lidocaine (1 mL/9 kg), xylazine (2 mL/kg diluted in saline), xylazine (1 mg/kg 10% solution) + lidocaine (10 mL 2% solution), and medetomidine (0.5 mg/kg diluted in saline) (Ko et al., 1992, 1993a,b; Scarda, 1996; St-Jean and Anderson, 1999). Precaution to prevent ascending flow of some of the agents to the brain should be taken to prevent seizures. This is usually not a problem with small volumes, and it has not been experienced with the standard human epidural preparation of morphine. The technique is illustrated in Chapter 10.

Topical anesthetic patches, usually containing lidocaine and prilocaine, have been developed that anesthetize skin as well as mucous membranes. These patches help relieve the distress caused by repeated vascular access needle punctures (Smith et al., 1997; Thurmon and Benson, 1996). The transdermal applications contain lidocaine with or without prilocaine. The transdermal analgesics require 30–60 min to provide effective analgesia after application, and the analgesic effects may last up to 12 h, depending upon the product. Use of transdermal analgesic patches or creams or regional nerve blocks with local anesthetics may be desirable if the protocol requires repeated needle access to the port over a short period of time (Swindle et al., 2005).

COMMONLY RECOMMENDED PROTOCOLS

Some of the commonly recommended protocols that have been routinely used in particular research settings are tabulated in this section. Please refer to more complete information within the description of the surgical procedure and the discussion of the classes of anesthetic agents within this chapter.

Nonsurvival Teaching Protocols

Induction: Ketamine (33 mg/kg i.m.), acepromazine (1.1 mg/kg s.c.), atropine (0.05 mg/kg s.c.).
Maintenance: Thiopental (3–30 mg/kg/h i.v.) infusion or pentobarbital (5–40 mg/kg/h i.v.) infusion.
Comment: This protocol can be used for survival surgery if gas anesthesia is not available. Buprenorphine, meloxicam, or carprofen can be administered intraoperatively; however, analgesics will decrease the amount of barbiturate required by approximately 50%.

General Surgery (without Physiological Measurements)

Induction: Ketamine (33 mg/kg s.c.), acepromazine (1.1 mg/kg s.c.), atropine (0.05 mg/kg s.c.).
Maintenance: Isoflurane, 1.5%–2% in oxygen or 0.5%–1.5% in nitrous oxide:oxygen, 2:1.
Comment: This protocol does not allow endotracheal intubation without administration of isoflurane via face mask during induction. Buprenorphine, meloxicam, or carprofen may be administered intraoperatively and may reduce the percentage of isoflurane required.

General Surgery (with Physiological Measurements)

Induction: Isoflurane, 3%–5% in oxygen via face mask.
Maintenance: Isoflurane 0.5%–1.5% in nitrous oxide:oxygen, 2:1.
Comment: This is the simplest protocol that minimizes hemodynamic effects but provides sufficient analgesia and relaxation for major surgery. Following surgical manipulation and closure, it is possible to minimize the administration of the inhalant. Buprenorphine, meloxicam, or carprofen can be administered intraoperatively after the measurements are made.

Cardiothoracic Surgery (with Cardiovascular Manipulations)

Induction: Ketamine, 33 mg/kg s.c.; isoflurane, 3%–5% in oxygen via face mask.
Maintenance: Isoflurane, 0.5%–1.5% in nitrous oxide:oxygen, 2:1.
Adjunct agents: Bretylium (5 mg/kg i.v.) by slow injection every 30 min before and during cardiac manipulation or amiodarone i.v. infusion 0.5 mg/kg/h.
Comment: An emergency kit for cardiopulmonary emergencies and a defibrillator should be available.

Cardiothoracic Surgery (with Cardiovascular Compromise)

Induction: Start i.v. infusion with sufentanil, 0.015 mg/kg/h (15 μg). Bolus, 0.007 mg/kg (7 μg) i.v. 5 min after infusion is started. If relaxation is required to induce the i.v., then administer ketamine (11 mg/kg s.c.) first.
Maintenance: Sufentanil, 0.015–0.030 mg/kg/h (15–30 μg) i.v. infusion. Supplement with 0.5% isoflurane in oxygen if required for major surgical manipulations.
Adjunct agents: Atropine (0.02 mg/kg i.v.) may be required for bradycardia, which can be profound during the induction. Bretylium (5 mg/kg i.v.) every 30 min or amiodarone (0.5 mg/kg/h i.v.) infusion during cardiac manipulation.
Comments: This protocol is useful for CPB procedures, especially if there is a predisposition for malignant hyperthermia. It is also useful as anesthesia for endoscopic thoracic surgery or coronary artery surgery in which bradycardia is required. The protocol minimizes the anesthetic effects on coronary blood flow and myocardial contractility. It has a protective antiarrhythmic effect during cardiovascular catheterization and electrophysiology studies. Acepromazine or diazepam may be required during the withdrawal phase of the protocol to counteract muscle tremors and rigidity. Other analgesics should not be administered until the i.v. infusion is decreased to less than 0.007 mg/kg/h (7 μg). A cardiopulmonary emergency drug kit should be available along with a defibrillator.

Coronary Artery Catheterization

Induction: Ketamine (33 mg/kg s.c.) followed by mask induction with isoflurane (3%–5%).
Maintenance: Isoflurane, 0.5%–1.5% in nitrous oxide:oxygen, 2:1.

Adjunct agents: During the last meal prior to induction of anesthesia, animals are administered diltiazem (4 mg/kg) and aspirin (10 mg/kg p.o.). Bretylium (5 mg/kg i.v.) or amiodarone (0.05 mg/kg/h) and heparin (200 IU/kg) are administered i.v. prior to introducing the catheter into the coronary artery. A slow 200-μg infusion of nitroglycerin is administered at the aortic root prior to introducing the catheter.

Comment: If stents are implanted, anticoagulant therapy may have to be administered postoperatively. This includes such agents as enteric coated aspirin, clopidogrel, or reviparin. Buprenorphine, meloxicam, or carprofen may be administered preemptively.

GENERAL PHYSIOLOGICAL EFFECTS OF ANESTHETICS

The general physiological effects of common anesthetic agents are summarized in the following text for reference when designing protocols for particular research projects (Table 2.9). Combining agents between classes may cause different effects. All effects are dose dependent and may vary among breeds (Benharkate et al., 1993). The information is summarized from Heavner (1994) and Smith et al. (1997). A complete discussion, by multiple authors, of the physiological effects of anesthetics and analgesics in all laboratory animals is available in Fish et al. (2008).

Dissociative Agents

The physiological effects of these agents are bronchodilation, tachycardia, increased cardiac output, increased blood pressure, and increased circulating catecholamines.

1. Ketamine: Prolongs myocardial refractory period; peripheral and coronary vasodilation, increased PA vascular resistance, increased cerebral blood flow, poor analgesia, and little muscle relaxation; it is an NMDA antagonist, and induces hepatic enzymes.
2. Tiletamine/zolazepam (Telazol): Mild myocardial depression, respiratory depression, and persistent hypothermia; contraindicated in renal disease and cardiovascular compromise; poor analgesia.

Barbiturates

1. Thiobarbiturates: Depressed cardiopulmonary function, decreased cerebral blood flow and intracranial pressure, decreased myocardial contractility, respiratory depression, minimal effects on peripheral vascular resistance, and short acting; redistribution from brain to visceral tissues occurs rapidly.
2. Pentobarbital: Respiratory depression, decreased myocardial contractility and cardiac output, increased peripheral vascular resistance, decreased cerebral blood flow, decreased hematocrit, and decreased rate of dissociation of gamma-aminobutyric acid (GABA) from its receptor; metabolized by liver.

Sedatives/Hypnotics

1. Phenothiazines (acepromazine): Hypotension, alpha adrenergic blockage, vasodilation, decreased systemic vascular resistance, and reduced sensitivity to circulating catecholamines; cardiac output and heart rate not significantly affected.
2. Butyrophenones (azaperone, droperidol): Extrapyramidal activity due to GABA blockade, hypotension, decreased cardiac output and heart rate, and some antiarrhythmic activity.
3. Benzodiazepines (zolazepam, diazepam, and midazolam): Decreased catecholamine release, increased coronary blood flow, slight cardiovascular effects unless combined with opioids (synergistic effect with severe cardiodepression), little effect on hepatic or renal function.

TABLE 2.9
Potential Physiological Effects of Intraoperative Drugs in Swine

	Cardiac Arrhythmias	Heart Rate	Cardiac Output	Myocardial Contractility	Blood Pressure	Right Atrial Pressure	Myocardial Oxygen Consumption	Cerebral Blood Flow
Atropine	Sinus tachycardia	–	–	–	NC or –	–	–	NC or –
Phenothiazine tranquilizers	Sinus tachycardia	–	–	–	–	–	NC or –	–
Dissociative agents	Sinus tachycardia	–	–	–	–	–	–	–
Butyrophenones	Extrapyramidal activity	–	NC or –	–	–	–	NC or –	–
α-2-Agonists	Sinus bradycardia, 2°–3° AV block, sinus arrest	–	–	NC or –	– then –	–	–	–
Benzodiazepines	Sinus bradycardia	NC	NC	NC	NC	NC	–	–
Barbiturates	Bradyarrhythmias, PVC, sinus tachycardia, ventricular arrhythmias	–	–	–	–	–	NC or –	–
Etomidate, metomidate		NC or –	NC or –	NC or –	NC or –	NC or –	NC or –	–
Inhalant anesthetics	Sinus bradycardia, PVC, ventricular tachycardia, ventricular fibrillation	–	–	–	–	–	–	–
Propofol	Sinus bradycardia, ventricular arrhythmias	NC or –	–	–	–	–	NC or –	–
Opioids	Sinus bradycardia, 1°–2° AV Block	–	–	NC or –	NC or –	NC	NC	–
Pancuronium		–	–	NC	–	NC	–	–
Vercuronium		NC	NC	NC	NC	NC	NC	NC

Note: NC = no change; – means a decrease in the value.

4. α_2-Adrenergic agonists (xylazine, dexmedetomidine, and detomidine): Bradycardia, first- to third-degree heart block, decreased cardiac output, increased CVP, hypotension, decreased myocardial contractility, decreased sympathetic tone, decreased coronary blood flow, increased susceptibility to catecholamines and arrhythmias, vasoconstriction, transient analgesia, reduced GI motility, increased urine production, and increased atrial natriuretic factor (ANF).
5. Propofol: Decreases cerebral blood flow and intracranial pressure; a cardiodepressant, it causes respiratory depression, but little analgesia.
6. Etomidate: Decreases cerebral blood flow and intracranial pressure, causes minimal cardiodepression, as well as adrenocortical suppression, has little effect on renal blood flow, and may induce seizures.

Opioids

Decreased cerebral blood flow, respiratory depression, minimal cardiovascular effects, increased coronary blood flow, slight decrease in peripheral vascular resistance, depressed catecholamine response, bradycardia; some agents (morphine) may have stimulant effects; histamine release.

Nonsteroidal Anti-Inflammatory Drugs

Effects vary widely between different classes of agents and are dependent upon dosage and length of administration. These are anti-inflammatory and inhibit cyclooxygenase, lipoxygenase, kinins, and prostaglandins; cause GI irritation, antiplatelet activity, renal and hepatic dysfunction, and inhibition of bone healing.

Inhalant Anesthetics

All agents increase cerebral blood flow and cerebrospinal fluid, increase intracranial pressure, depress ventilation, depress oxygen consumption, produce hypercapnia, and induce bronchodilation.

1. Isoflurane: Decreased systemic vascular resistance, little effect on heart rate and cardiac output, coronary arterial dilatation, little circulating catecholamine sensitivity, least effect on cardiac output, circulating catecholamines, best tissue perfusion.
2. Sevoflurane: Similar to isoflurane but more rapid induction and recovery. It may be a safer choice for protocols involving severe cardiovascular compromise.
3. Desflurane: Similar physiologic effects as isoflurane and sevoflurane, requires specialized equipment.
4. Halothane: Decreased cardiac output, myocardial depression, depressed baroreceptor reflexes, peripheral vascular resistance unchanged, greatest effects on catecholamine sensitivity. It is not recommended because of human health hazard.
5. Enflurane: Associated with seizures, more cardiodepressant than halothane and isoflurane; decreases blood pressure, cardiac output, and systemic vascular resistance. It is not recommended due to being more depressive than isoflurane and sevoflurane.
6. Methoxyflurane: Decreased cardiac output, blood pressure, and systemic vascular resistance; most metabolized effects, most renal effects, and most hepatic defluorination. Should not be used because of poor efficacy and human health hazard.
7. Nitrous oxide: Unacceptable as sole agent, slight cardiodepression.

EUTHANASIA

The 2007 Report of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia (AVMA Panel on Euthanasia, 2001, https://www.avma.org/KB/Policies/Documents/

euthanasia.pdf) is generally accepted as the standard for acceptable euthanasia criteria for swine and other species. The AVMA now publishes its guidelines online and periodically updates them as new scientific information becomes available (https://www.avma.org/Pages/home.aspx). The highest recommendation is euthanasia with an i.v. overdose of pentobarbital (>150 mg/kg). Larger animals may require sedation to facilitate i.v. access. Neonatal animals can be euthanized i.p. with pentobarbital. Commercially available euthanasia solutions used for pets can also be used. Other acceptable methods are the administration of KCl (2 mmol/kg i.v.) or terminal exsanguination, both of which must be performed while the animal is under general anesthesia. Overdosage with an inhalant anesthetic as part of a terminal surgery should also be acceptable. In agricultural practice, CO_2 asphyxiation or captive bolt cerebral trauma are performed, mainly to prevent drug residues in meat products. These methods and other physical methods would generally not be used in research, and they would have to be approved on a scientific necessity basis by most institutional animal care and use committees (IACUCs).

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3 Wound Closure and Integument

M. Michael Swindle

CONTENTS

GENERAL PRINCIPLES AND SURGICAL PREPARATION

The pig is a relatively hairless animal with a fixed skin tightly attached to the subcutaneous tissues similar to that in humans. The cutaneous blood supply and sequence of events in wound healing are also similar to that in humans. However, the skin of the pig is thicker and less vascular overall than human skin. The thickness of the skin is especially pronounced in sexually mature animals on the dorsal surface of the neck and back and in some breeds such as the Yucatan. There are also differences in the accessory tissues, such as the variations in sweat glands and the presence of an intrafollicular muscle in swine (Figures 3.1 and 3.2). Pigs have sebaceous glands, which are relatively insignificant. There is a variation from humans in the number and function of the apocrine and eccrine sweat glands. Eccrine glands in pigs are limited to the snout and carpal glands, whereas in humans they are extensive and active in the sweating phenomenon. Apocrine sweat glands are more extensive in pigs but, as in humans, do not contribute to sweating or thermoregulatory functions to a high degree. Secretions on the skin may help to prevent fluid loss, but pigs in outdoors subjected to high temperatures thermoregulate by wallowing or seeking shade rather than actively sweating. There is a mental gland between the mandibles that contains tactile hairs and provides sebaceous and apocrine secretory functions (Argenzio and Monteiro-Riviere, 2001; Monteiro-Riviere, 1986, 2001). Colored histologic sections are located on the textbook DVD.

The pig has been used extensively as a model for superficial and deep wound healing, including thermal injury, and plastic surgical techniques, and it has been developed as a model of dermal toxicity, percutaneous absorption, and phototoxicity (Bolton et al., 1988; Chvapil and Chvapil, 1992; Kerrigan et al., 1986; Mertz et al., 1986; Middelkoop et al., 2004; Monteiro-Riviere, 1986, 2001; Monteiro-Riviere and Riviere, 1996, 2005; Ordman and Gillman, 1966; Pietrzak et al., 2006; Riviere et al., 1986; Sullivan et al., 2001; Wang et al., 2001).

Any standard method of aseptic preparation of the skin for humans or other species may be applied to the pig. None of these methods, such as povidone–iodine and alcohol, ensures a completely sterile environment on the skin (Mertz et al., 1984), and, consequently, skin contact with other organs and tissues should be minimized when performing surgery in body cavities. The methodology preferred in the author's laboratories is described here (Figures 3.3 through 3.6).

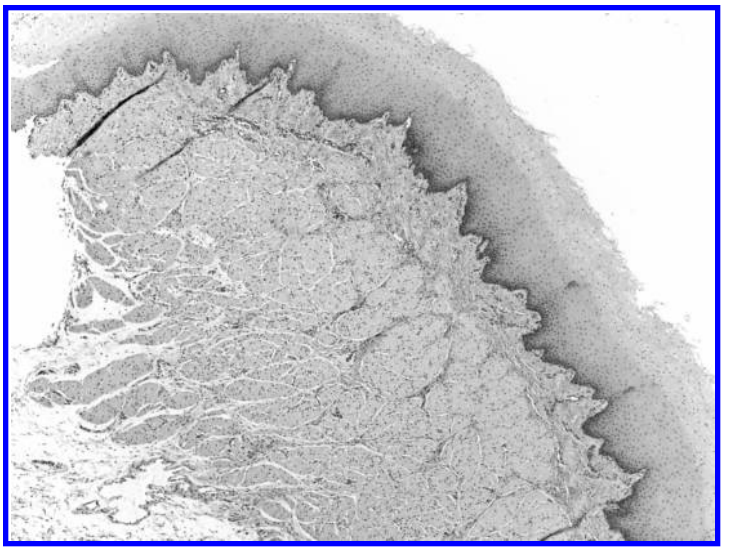

FIGURE 3.1 Histologic section of skin. H&E, ×10.

Swine are cleaned of gross contamination from the total body in a separate surgical prep room for animals. The area of surgical intervention is clipped with electric clippers and in some cases is shaved. The skin is scrubbed three times with iodine surgical scrub and rinsed with alcohol. The animal is then transported to the operating room where a sterile preparation of iodine prep solution is applied using sponge forceps and sterile sponges. In most cases, the iodine solution is removed with alcohol, and the skin dried with sterile gauze sponges. A transparent iodine-impregnated adhesive drape is applied over the dry skin. If the iodine-impregnated drape is not used, as for some minor procedures, the last solution of iodine remains on the skin. The adhesive iodine-impregnated drapes work well on swine and provide protection against contamination of tissues and organs from

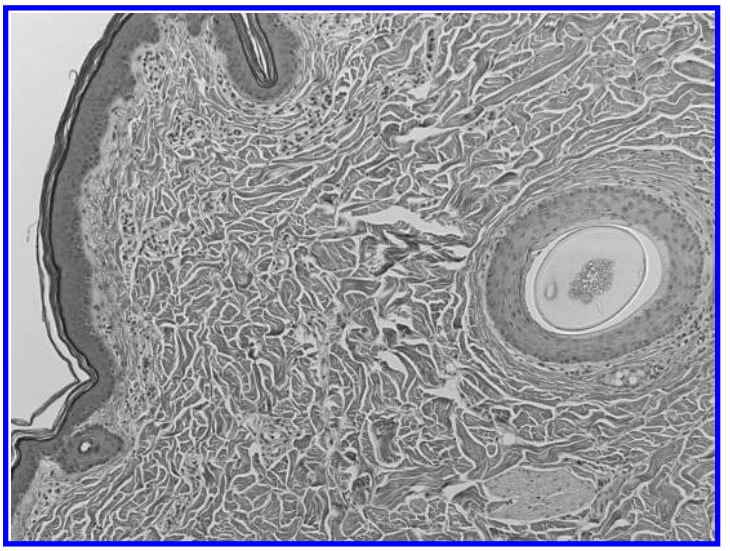

FIGURE 3.2 Histologic section of skin. H&E, ×40.

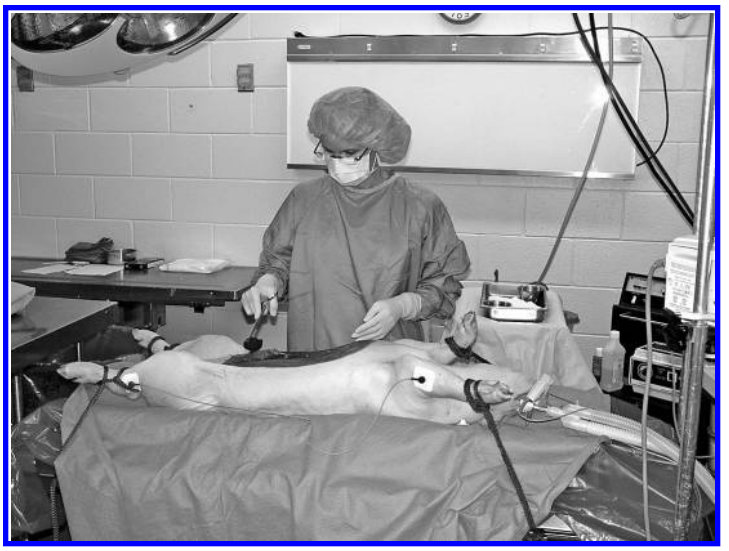

FIGURE 3.3 Sterile skin prep of a pig in the operating room.

the skin after the incision is made, because they adhere to the edges of the incision if the skin preparation has been performed as described in the preceding text (Figure 3.7).

The principles of surgery are the same for swine as they are for other species. Careful attention to hemostasis, atraumatic handling of tissues, proper use of surgical instruments, closure of dead space, and aseptic technique will minimize complications associated with surgery (Ethicon, 1999; Swindle, 1983).

SUTURE SELECTION FOR WOUND CLOSURE

Selection of the appropriate size and type of suture material is important for the prevention of postoperative complications. In general, the suture material should not cause reactions that may impede

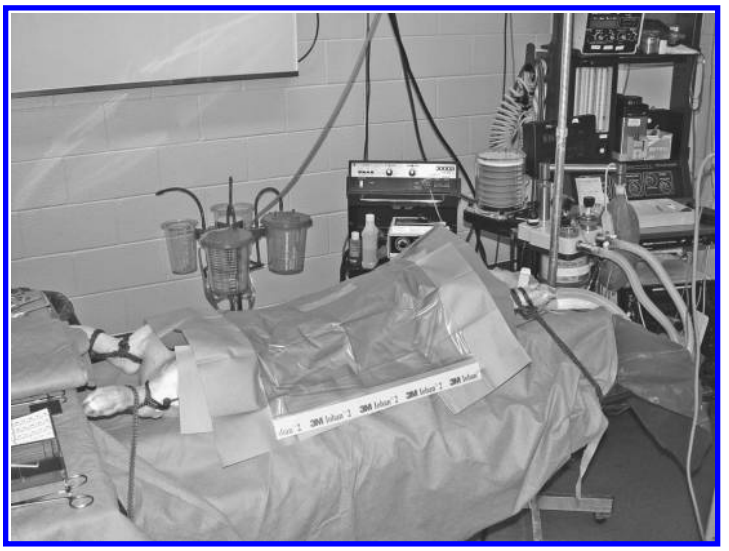

FIGURE 3.4 Application of a sterile, adhesive iodine-impregnated drape.

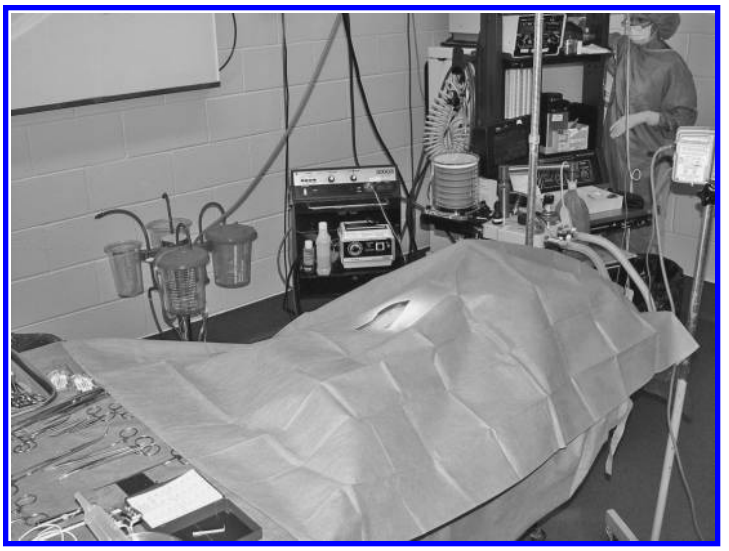

FIGURE 3.5 Application of a full-body sterile drape over the adhesive drape.

healing and should be of the smallest appropriate size to provide appropriate wound closure. If too many sutures are placed or if the suture is much larger than necessary, it overloads the region with foreign material and may cause regional ischemia or create an inappropriate foreign body reaction. These generalities depend upon the region in which the suture is implanted and the type of suture material. It is beyond the purpose of this text to provide an instruction manual for beginning techniques in surgery or for a discussion of all of the aspects of suture selection. However, a technical training book is available detailing beginning surgical techniques in swine (Swindle, 1983), and a complete discussion of the principles of suture selection and wound healing are also available (Ethicon, 1999).

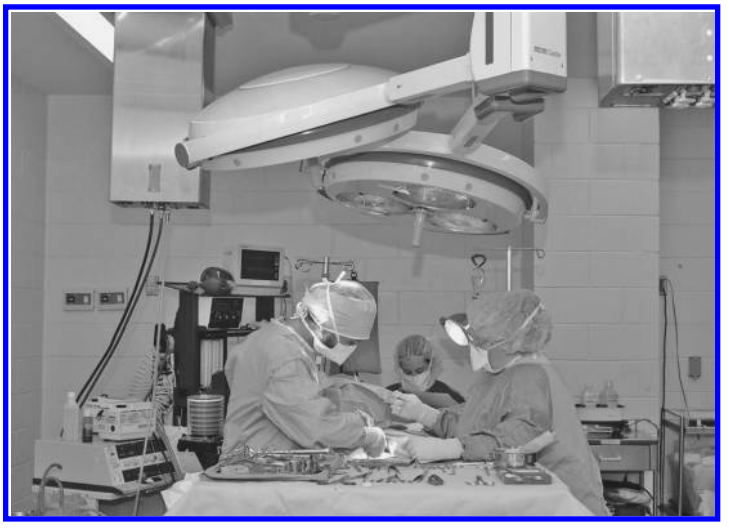

FIGURE 3.6 Surgical team in proper protective wear for sterile surgery.

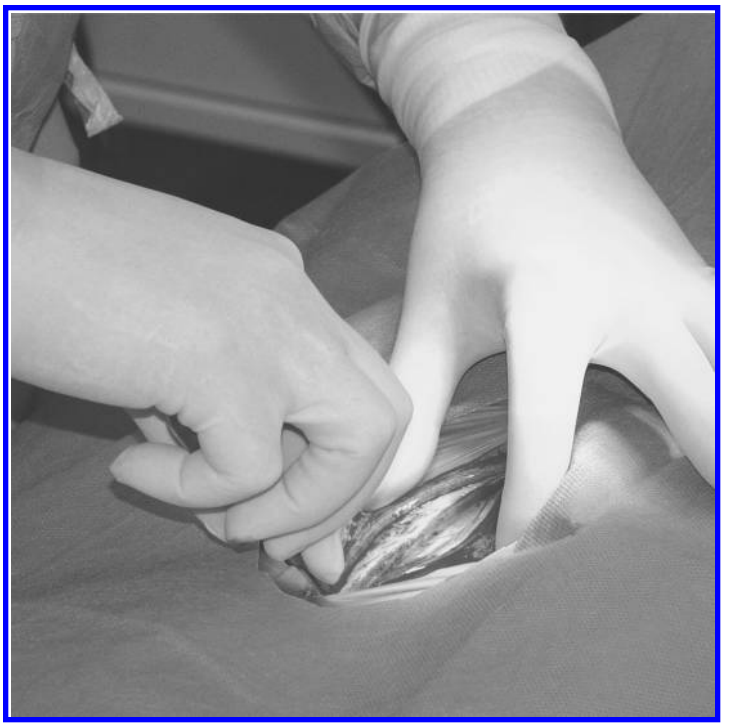

FIGURE 3.7 Making a skin and muscle incision with a scalpel through an adhesive drape.

Selection of the appropriate-sized suture material is subjective and depends upon the experience of the surgeon. Several examples based upon experience may be of value in that selection and are listed here. When closing incisions in 25-kg swine, the selection of 2/0 suture for closure of the muscle layers and 3/0 suture for closure of the subcutaneous, subcuticular, or skin layers works well. If closing the same incision in 50-kg swine, selection of 0 or 1 suture for the muscle layers, 2/0 suture for subcutaneous layers, and 3/0 suture for the subcuticular or skin layer would be appropriate.

One way of classifying suture material is whether the material is absorbable or nonabsorbable. Another classification would be whether the suture is manufactured from synthetic or natural materials. Other classifications based upon manufacturing techniques include braided or monofilament and coated or noncoated. Generally, sutures that are braided are manufactured to provide greater strength, and sutures that are coated materials are manufactured to prevent reactions and delay absorption. Selection of the type of material and the intended usage will also dictate the number of throws required in the surgical knot, with the coated or monofilament sutures usually requiring more than three throws (Ethicon, 1999).

Synthetic sutures of either absorbable or nonabsorbable material work well depending upon the surgical incision that is being closed. Silk, linen, cotton, and gut are natural materials that should not be routinely used to close surgical wounds in swine. All these materials are reactive and may lead to inappropriate bodily responses, such as seromas, which may impede wound healing. Use of these materials should be reserved for a special circumstance dictated by the protocol.

Absorbable materials that have been routinely used without complications include the following synthetics: poliglecaprone 25, polydiaxonone, polyglycolic acid, polyglactin 910, polyglyconate, poliglecaprone 25, and lactomer 9-1. Nonabsorbable materials routinely used have included nylon, polyester, polypropylene, polyamide, polytetraflurethylene, polyglecaprone 25, and stainless steel. This list is not all inclusive, and, as a general rule, the synthetic manufactured materials developed in recent years have all proved to be safe and effective.

FIGURE 3.8 Subcutaneous stapling device and specialized tissue forceps for the device.

Both absorbable and nonabsorbable surgical staples have been developed, and staple surgical techniques greatly increase the speed of wound closure. These devices are essential in endoscopic surgical techniques. However, closure of the skin of animals with staples provides some opportunities for complications that are not as likely to occur with suture materials. Staples may be caught on cages and may hold contaminants, such as hair or feces, close to the skin incision more readily than some suturing techniques. These problems can be minimized by caging and bandaging procedures; however, the surgeon should be aware of their potential occurrence prior to selecting staples for skin closure. Absorbable staples (polylactic acid and polyglycolic acid) for subcutaneous closure of wounds have been recently developed (Insorb®; Incisive Surgical, Plymouth, MN), and this type of stapling technique may be useful for larger pigs (Figures 3.8 and 3.9). In small fast-growing pigs, it

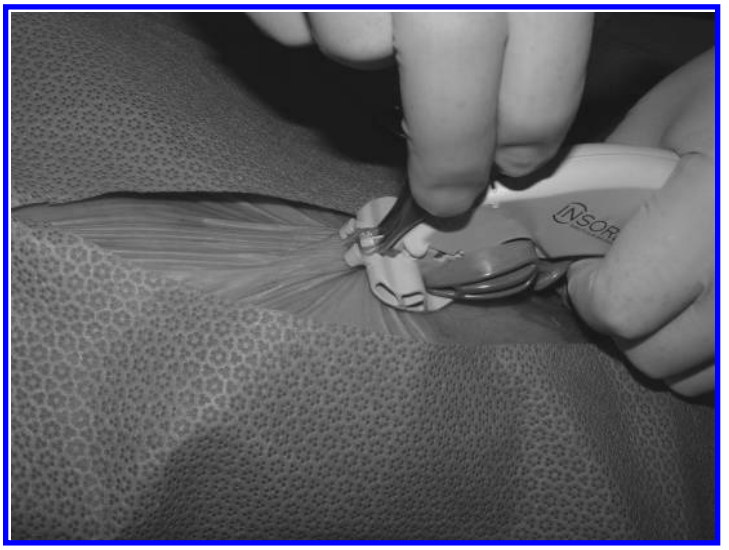

FIGURE 3.9 Using the subcutaneous stapling device in the abdomen.

is likely that the staple material will grow outward with the skin. At this time, 3-0 poliglecaprone 25 (Monocryl®; Ethicon, Sommerville, NJ), on a cutting needle, is the preferred suture material for subcuticular closures in pigs <4 months of age in our laboratories. It is a synthetic monofilament, which is absorbed in approximately 3 weeks. Synthetics with a slower absorption rate (Dexon®, Vicryl®, and PDS®; Ethicon) may grow outward with the epidermal layer onto the skin surface as the pig grows. This creates skin irritation and potential infection, even after the incision site is healed, but before the suture is completely absorbed. However, the longer absorption time may be needed for sexually mature pigs with slower healing times.

Wound Closure and Bandaging Techniques

The same suture patterns and techniques that are used for wound closure for other species are applicable to the pig. Bandaging techniques are somewhat species specific and will be described in this section with a brief review of wound closure techniques (Smith and Swindle, 1994). Specific closures for areas, such as the abdomen or thorax, and suture techniques for visceral organs are discussed in the appropriate chapters of this text.

Closure of a wound should be performed in anatomically correct layers. Either simple interrupted or continuous sutures can be used on internal layers, such as peritoneum, muscle, and subcutaneous tissues (Figures 3.10 and 3.11). Simple interrupted sutures provide more security but increase the foreign material load that is implanted and may provide uneven tension in a given layer because of variability between the individual sutures. Care must be taken when using continuous sutures to close these layers, because an improperly tied knot at either end of the suture line can lead to dehiscence of the wound. The distance between the suture insertions will depend upon the type of tissue, type of suture, and skill of the surgeon. Generally speaking, the suture placement should approximate the edges of the wound in an anatomically correct fashion without placing undue tension on the suture line and making sure that the closure does not leave dead space, which may lead to seromas or pockets of infection.

Thin muscle layers can be closed with a suture pattern that penetrates the full thickness of the muscle. Thick muscles can be closed with the external fascia only, as that is the layer that provides

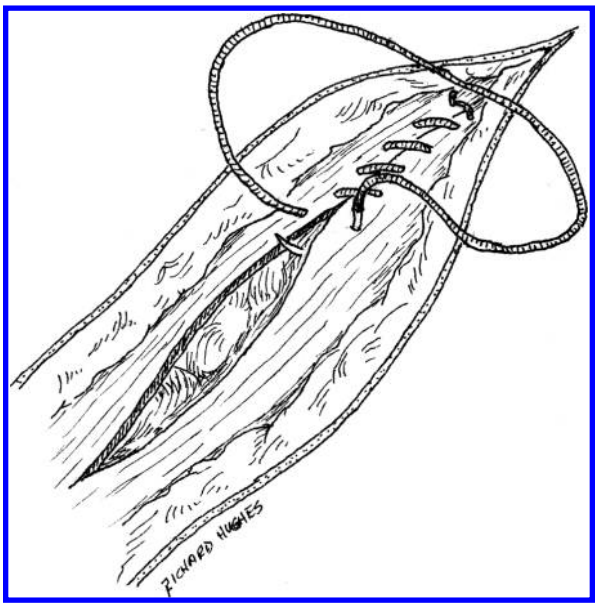

FIGURE 3.10 Closure of muscle layers for an abdominal incision.

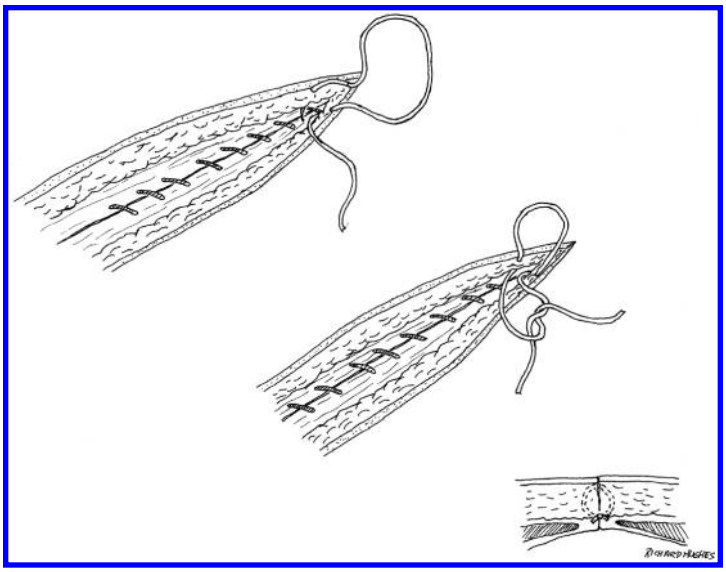

FIGURE 3.11 Simple continuous pattern in the subcutaneous tissues.

the strength for holding the sutures. Fat does not hold sutures and must be approximated with the subcutaneous layers. The subcutaneous layers are generally closed with a continuous suture pattern. In addition to closing dead space, this layer reduces the tension that must be applied to the skin sutures (Figures 3.10 and 3.11).

Problems with closure of abdominal or subcutaneous incisions may be encountered in large swine because of excess fat deposition. Use of a rubber surgical retractor (surgical fish) to hold abdominal tissues *in situ* during closure of the peritoneal and initial muscle layers is recommended. These devices can be removed through the small opening when the last few sutures are ready to be placed. Fat deposition also makes the area greasy and suture materials difficult to tie. Horizontal mattress sutures may have to be placed in the muscle layers of large animals for tension relief on the suture line.

The skin must be approximated with a minimal amount of tension, because it swells during the healing process; too tight a closure will cause irritation and secondary cutting of the skin. The ideal closure for pigskin is subcuticular suture with buried knots using 3/0 absorbable synthetic suture (Figure 3.12). If the knot is buried at both ends, it provides a cosmetic closure with minimal complications. Simple interrupted sutures (Figure 3.13) may be used to close the skin using 3/0 synthetic nonabsorbable suture. As a general rule, simple interrupted sutures should be placed 5 mm from the skin edges with approximately 5–10 mm between sutures. Horizontal mattress sutures (Figure 3.14) may be used outside the suture line as tension-relief sutures on long incision lines but should not be used as the primary skin closure pattern. Vertical mattress sutures (Figure 3.15) can be used to give a secure cosmetic closure if an eversion pattern is desired. Skin staples can be problematic for the reasons described in the preceding text. Continuous suture patterns are best not used for routine closure of skin but may be acceptable in some situations related to the research (Figure 3.16).

If the skin closure is not anatomically correct and there is an eversion or outpouching of the skin at the end of the suture line due to uneven closure, corrective measures should be taken. Cutting through the outpouching at a 45° angle and placing a few simple interrupted sutures will usually correct most situations.

The suture line should not be placed over subcutaneously implanted devices. It is best to have the suture line either caudal or cranial to the device rather than dorsal or ventral. This prevents undue tension on the suture line from the weight or pressure of the device.

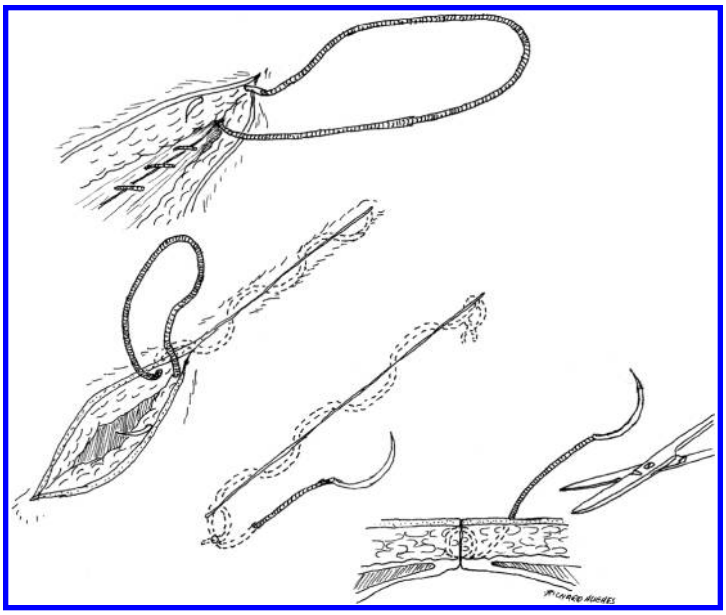

FIGURE 3.12 Subcuticular suture pattern with the knot buried at both ends.

Bandaging of the incision may be indicated in some situations, including large incisions under tension, implantation of subcutaneous devices, suture lines that are likely to be contaminated from urine or feces, and incisions that are placed in areas that the animal is likely to irritate. The use of topical spray bandages or tissue glues provides a short-term seal of the skin incision and is a good idea for most incisions. Bandages should be changed every 1–2 d, or earlier if they become contaminated with moisture, urine, or feces.

The pig should be bandaged in a circumferential fashion for incisions on the trunk and neck. Nonadhesive self-adhering bandage material can be used after placing sterile gauze pads to protect the suture line; however, these are easily removed by the animal after recovery. If they are used,

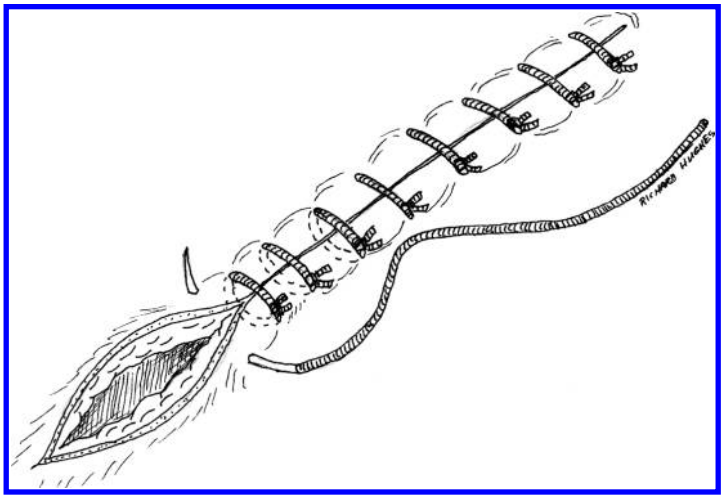

FIGURE 3.13 Simple interrupted skin suture pattern.

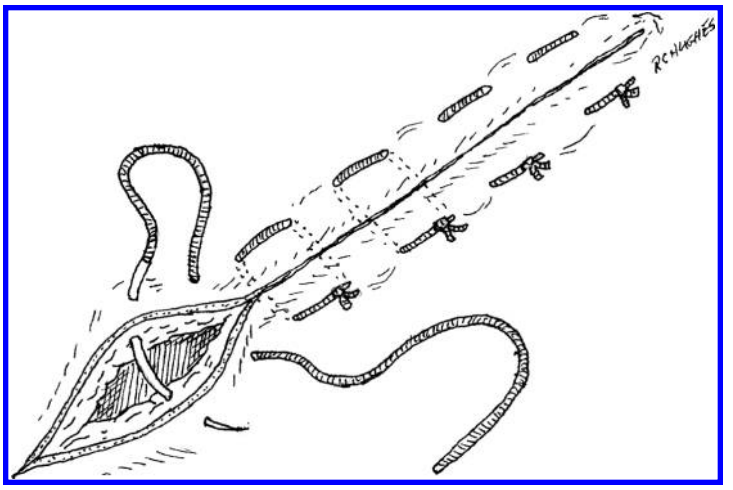

FIGURE 3.14 Horizontal mattress suture pattern in the skin.

the cranial and caudal ends of the bandage should be held in place with adhesive bandage material that includes the bare skin with the edge of the bandage material. Orthopedic stockinette can be used for a total body bandage after cutting out leg holes. This will provide a loose covering for wound-healing studies without directly applying pressure to the wound. Both the cranial and caudal ends of the stockinette should be secured with adhesive tape as described in the preceding text. Porous elastic adhesive tape in wide widths functions better than ordinary white bandage tape in swine because it provides a better contour fit and can be secured more tightly. Soft cloth surgical tape and clear self-adhering wound dressings are currently used in our laboratories for most applications (Figure 3.17).

Bandaging of the extremities and difficult areas, such as the perineum, requires some creativity. The same principles that apply to other animals apply to the pig. Use of skin adhesives may be necessary to provide additional security for the tape. Some of the plastic skin drape material used

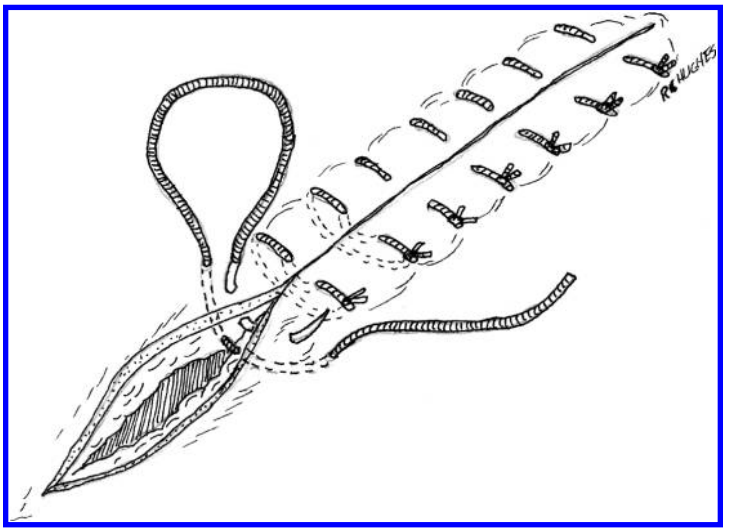

FIGURE 3.15 Vertical mattress suture pattern in the skin.

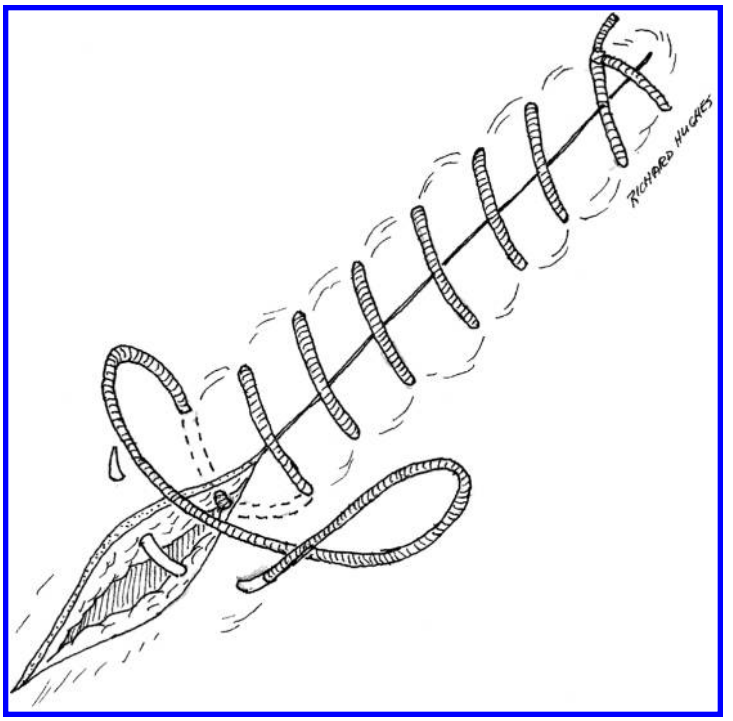

FIGURE 3.16 Continuous suture pattern in the skin.

during surgery can be used for the short term; however, this material is nonporous and will keep the wound moist and impair healing if used for long-term bandaging. Adhesive bandages that permit air transport are also useful for these areas.

Tether and harness systems can be designed to maintain chronic catheterization models (see Chapter 8, Figure 8.12). Jacket systems have also been designed for swine and are commercially

FIGURE 3.17 Soft cloth surgical tape and clear self-adhering bandage.

available. Care must be taken to have different-sized jackets available for chronic models, because small swine will tend to outgrow them in a few weeks (Davies and Henning, 1986; Smith and Swindle, 1994; Swindle et al., 1996).

SKIN FLAPS AND GRAFTS

Swine have been important models in the study of skin flaps and grafts, and a technical manuscript reviewing the various types of flaps has been published (Figure 3.18; Kerrigan et al., 1986). Swine have also been used for burn studies for both techniques and as a replacement for human skin, cutaneous pharmacology and toxicology, wound healing, and surgical techniques (Daniel et al., 1981; McGraft and Hundahl, 1982; Riviere et al., 1986; Sasaki and Pang, 1984; Shircliffe et al., 1974). The porcine skin has a cutaneous blood supply similar to humans because it is a fixed skin animal unlike most animal species (Forbes, 1969).

The classification of flaps described by Kerrigan et al. (1986) will be used here (Figures 3.19 through 3.21). Experimentally, the lengths of the flaps are longer than the described survival length to ensure a zone of necrosis. The zone of necrosis is the region generally studied for techniques and agents that enhance survival. If a zone of necrosis is not required, then the length of the flap is reduced to ensure that adequate blood supply to the flap remains intact.

Random Skin Flaps

Random skin flaps include the dorsal flank flap, the random buttock flap, and the random limb flap. The dorsal flank flap is raised 4 cm ventral to the midline. The survival length of a 4-cm-wide flap is 6.75 cm. The full thickness flap is raised and elevated to its dorsal base. The length of the flap is varied depending upon the experimental parameters. Random buttock flaps are raised over the lateral thigh and will be free of panniculus carnosus, unlike the flank flap. Survival flaps are 10 cm in width and 4.6 cm in length. The flap is incised on three sides, raised to its dorsal margin, and sutured upon itself. The length of the flap is varied as required. Random skin flaps are used on both the forelimb and hind limb and are elevated in the subcutaneous tissues as before. Survival lengths for forelimb flaps are 7.2 cm and for hind limb are 6.7 cm.

Arterial Skin Flaps

Arterial skin flaps include an arterial pedicle. The ventral flank flap is raised with the base 4 cm lateral to the nipple line and has a branch of the intercostal artery as its vascular branch. The survival length of a 4-cm-wide flap is 8.6 cm. A buttock arterial flap may be raised using the superficial circumflex iliac artery as the vascular pedicle. The 10-cm-wide flap is raised over the cranial dorsal iliac spine, with a survival length of 13.3 cm. Because the lateral femoral cutaneous nerve is included

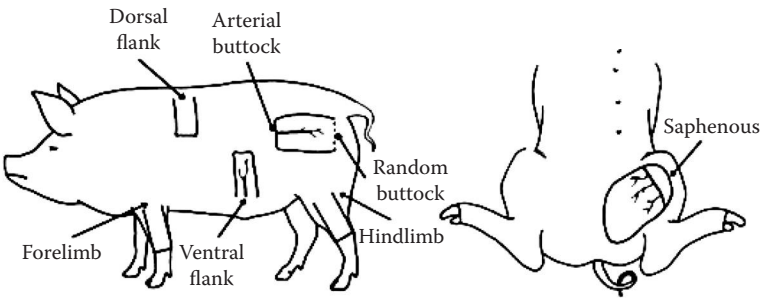

FIGURE 3.18 Location of the sites of various skin flaps and grafts. (Reprinted from Kerrigan, C.L. et al., 1986, *Lab. Anim. Sci*., 36(4): 408–412. With permission.)

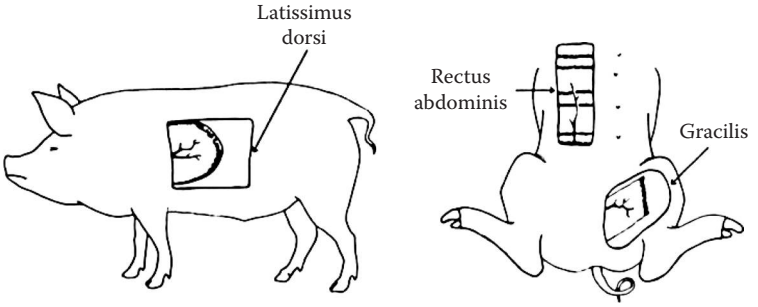

FIGURE 3.19 Location of myocutaneous flaps. (Reprinted from Kerrigan, C.L. et al., 1986, *Lab. Anim. Sci*., 36(4): 408–412. With permission.)

in this flap, it also qualifies as a neurovascular pedicle. The saphenous flap uses the saphenous artery as its vascular pedicle and is located on the medial aspect of the hind limb. The location of the flap is its main disadvantage, and, because of the vascular supply, there is not a defined survival length.

Myocutaneous Flaps

Myocutaneous flaps include the underlying muscle and a vascular supply. The latissimus dorsi flap is most commonly used, and the large thoracodorsal artery provides predictable survival. A flap of 10×16 cm starting at the caudal border of the shoulder is predictably survivable. The latissimus dorsi muscle is dissected and isolated relatively easily compared to other models. The gracilis myocutaneous flap is raised on the medial hind limb and includes the deep femoral artery as its arterial pedicle. The flap usually measures 10×20 cm and is one of the few areas where a skin flap can be raised on the contralateral side for comparison. Its main disadvantage is its location and the relative difficulty in dissection. A rectus abdominus muscle may be raised on the ventral surface of the

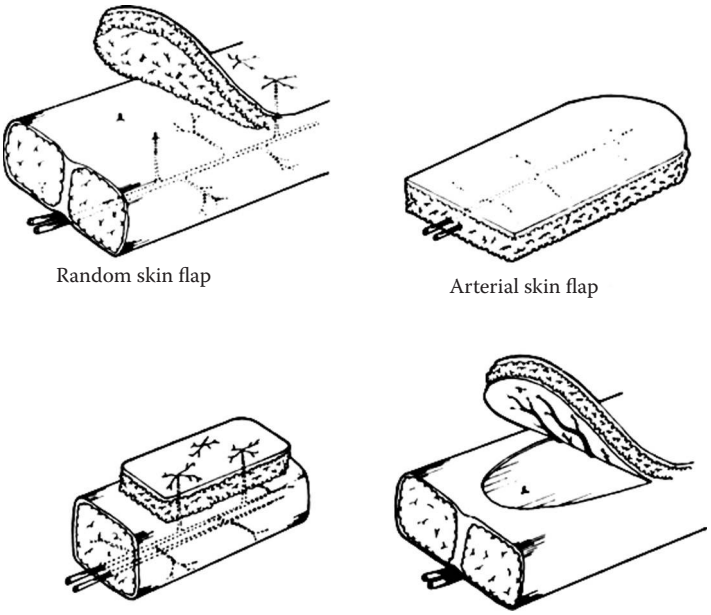

FIGURE 3.20 Location of fasciocutaneous flaps. (Reprinted from Kerrigan, C.L. et al., 1986, *Lab. Anim. Sci*., 36(4): 408–412. With permission.)

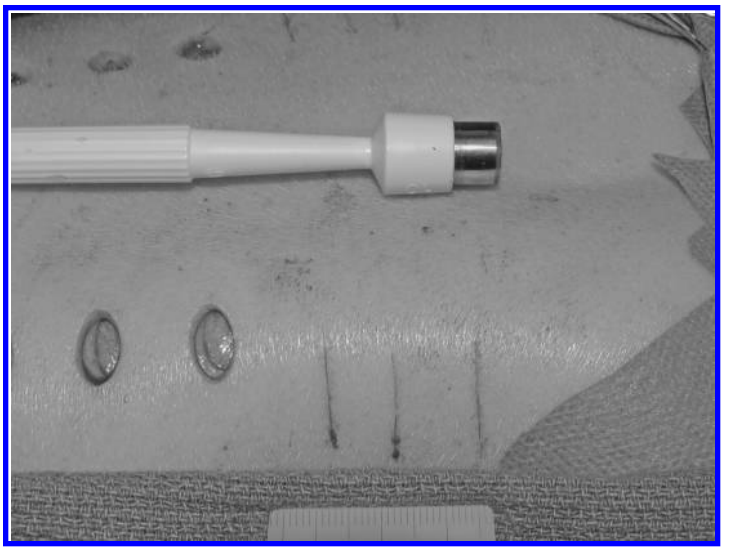

FIGURE 3.21 Full-thickness biopsy punch (1 cm) and scalpel incisional surgical wounds in the flank.

abdomen using the cranial epigastric artery. Flaps of two sizes are used: 5.5 cm and 6.5 × 18 cm. No predictable survivable flap size has been described. Besides location, there are differences in classification of the three muscles. The latissimus dorsi is a type V, the gracilis is a type II, and the rectus abdominus is a type III. Trapezius, pectoralis profundus ascendens, and biceps femoris myocutaneous flaps have found less usage and are not as well defined as these models because of the differences in conformation between swine and humans.

Fasciocutaneous flaps have been raised on the forelimb and hind limb to include the skin and deep fascia. The forelimb flap is raised 5 cm wide over the lateral condyle of the humerus at the juncture of the lateral head of the triceps and the extensor carpi radialis. The survival length of the flap is 8.2 cm. The hind limb flap is raised on the lateral aspect one-half to two-thirds of the distance between the major trochanter of the femur and the calcaneus. A 5-cm-wide flap has a survival length of 7.9 cm.

Skin flaps in swine can be problematic if multiple flaps are performed on the animal at the same time. If the suturing technique is adequate to prevent mobility of the flap, then problems of acute surgical pain can be largely avoided. If flaps are designed to have a zone of necrosis, then the animals should be monitored closely for signs of discomfort and infection. Use of stockinette bandages, as described in the preceding text, can protect the wound from contamination. The use of analgesics and antibiotics should be considered strongly unless they are contraindicated by the protocol.

WOUND-HEALING MODELS

The pig has been used for both superficial and deep wound-healing studies (Bolton et al., 1988; Chvapil and Chvapil, 1992; Mertz et al., 1986; Ordman and Gillman, 1966). Epidermal and dermal repair models have been standardized in the pig and in many other protocols, such as those involving skin flaps, described in the preceding text; wound healing is a part of the protocol. Swine have also been used for cutaneous toxicological research (Riviere et al., 1986). Differences in rates of wound healing may be noted between breeds, possibly due to age and genetic differences when comparing animal studies (Chvapil and Chvapil, 1992). Consequently, use of mature miniature pigs rather than farm breeds is a good alternative for studying more chronic wounds. Using multiple wounds on the same animal provides the ability to have the animal serve as its own control and also to have different treatments represented on the same animal. A technique of implanting

subdermal titanium wound chambers in which wound infections can be studied has been described (Steinstraesser et al., 2006). In this technique, multiple chambers can be implanted to study the effects of therapy on different organisms independently.

Pig skin is relatively thick compared to other species and is similar in blood flow to humans. In sexually mature pigs, the epidermal back thickness is approximately 52 μm with a stratum corneum thickness of 12 μm. In contrast, the skin of the abdomen is approximately 47 μm with a stratum corneum thickness of 15 μm. The blood flow in mL/min per 100 g varies between areas of the skin as well. Approximate measurements are as follows: buttocks, 3; ear, 12; humeroscapular joint, 7; thoracolumbar junction, 3; and ventral abdomen, 11 (Monteiro-Riviere and Riviere, 2005).

Epidermal wound healing is studied to evaluate pharmaceutical and bandaging interventions and their effects on epidermal regeneration. The pig has been evaluated both as a model of epidermal migration (Chvapil and Chvapil, 1992; Mertz et al., 1986) and as a model of epidermal proliferation (Winter, 1972). The method of wounding may vary slightly between studies but basically involves infliction of multiple epidermal wounds of the same size and depth bilaterally, using a keratome. A common configuration involves excision of 16–24 wounds 2.2 × 2.2 cm and 0.04 cm deep.

In contrast to the superficial wounds described in the preceding text, deep wounds heal by scarring rather than regeneration. Dermal wounds may involve the use of skin flaps, described previously, simple full-thickness incisions or excised full-thickness wounds. A size of 3 × 5 cm and 0.8–1.5 cm deep has been standardized for excision wounds in the miniature pig (Chvapil and Chvapil, 1992). Alternatively, circular full-thickness wounds with a diameter up to 2 cm can be created. Circular wounds generally show less, and more uniform, contraction, allowing for more time for wound evaluation until wound closure (Glerup, P., 2006, personal communication, SCANTOX, Ejby, Denmark). Our laboratories have used circular 1-cm biopsy punches for full-thickness excisional wounds for preclinical treatment evaluations (Figure 3.21).

In the case of both split-thickness and full-thickness wounds, the pig may experience postoperative discomfort. It is advisable to use the orthopedic stockinette bandaging technique described in the preceding text. The use of antibiotics and analgesics is strongly advised unless contraindicated by the research protocol. In our experience, epidural morphine administered preoperatively prevents most of the discomfort and shortens the period of time postoperatively required for analgesic administration when the wounds are made on the flank.

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4 Gastrointestinal Procedures

Mary Ann McCrackin and M. Michael Swindle

CONTENTS

SURGICAL ANATOMY

The applied gross (Schantz et al., 1996; Yen, 2001) and detailed vascular anatomy (Dondelinger et al., 1998) of the gastrointestinal (GI) system has been described in detail in the literature and in Chapter 1. The oral cavity is described in Chapter 10. In this section, the anatomy important to surgical procedures is discussed, and views of the intestinal viscera *in situ* are illustrated in Figures 4.1 through 4.3. Colored histologic sections of these structures are located on the textbook DVD.

The esophagus originates from the pharynx at the second cervical vertebra and passes along the left side of the trachea. The tunica muscularis of the porcine esophagus is composed of inner circular and outer longitudinal layers which are composed of striated muscle fibers in the proximal third, mixed striated and smooth muscle fibers in the middle third, and smooth muscle fibers in the caudal third of the esophagus (Banks, 1981). In humans, only the distal half of the esophageal muscularis propria has solely smooth muscle cells (DeNardi and Riddell, 1992). The mucosa is stratified squamous epithelium throughout the length. The esophageal diameter in domestic swine 10–50 kg ranges from 15 to 20 mm, and in Yucatan swine 45–50 kg, the range is 12–14 mm.

The stomach is divided into esophageal, cardiac, fundic, and pyloric regions with a diverticulum in the fundic region. The secretory functions and glandular mucosa of these gastric regions are similar to that of other mammals (Figures 4.4 through 4.6). The stomach in farm pigs after weaning is generally 5–9 g/kg body weight (BW) and GI maturity at 12 weeks in swine is approximately equal to that of a 1-year-old human (Sangild, 2001). The stomach of an adult Yucatan weighing 47 kg has a fluid volume of approximately 1300 mL. Depending upon the bulk and particle size of the meal, the stomach is generally emptied in 2–8 h. Fasting before and feeding after administration of a test substance reliably empties the stomach in 2–4 h (Casteel et al., 1998). The muscular

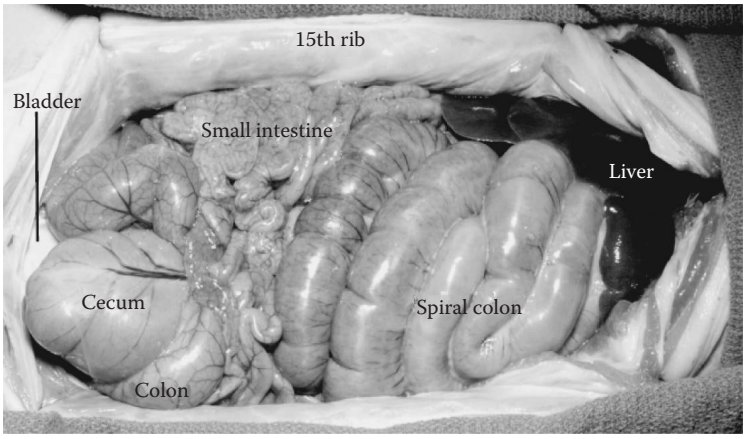

FIGURE 4.1 Ventral view of abdominal viscera.

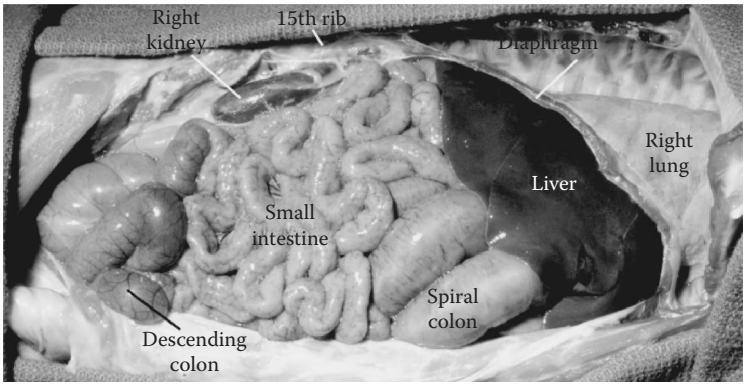

FIGURE 4.2 Right lateral view of abdominal viscera.

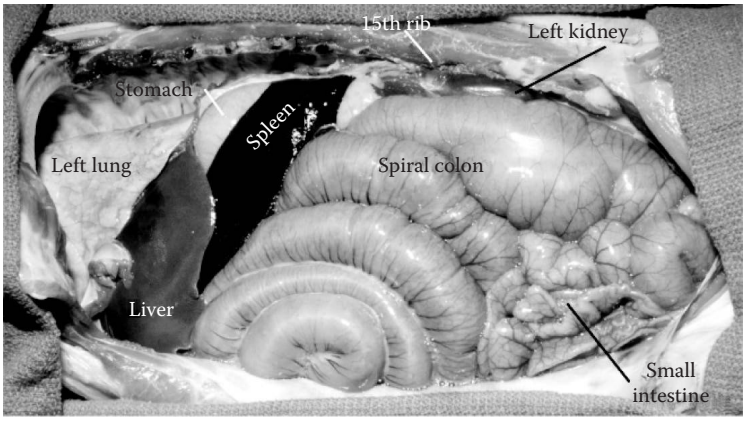

FIGURE 4.3 Left lateral view of abdominal viscera.

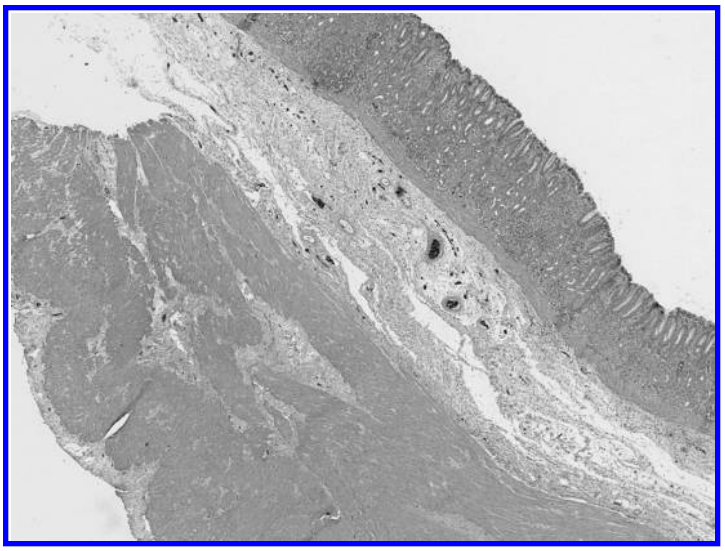

FIGURE 4.4 Histologic section of the esophageal region of the stomach. H&E, ×40.

torus pyloricus must be considered in planning gastric surgical procedures because of its size and location near the pylorus.

The duodenum generally is located in the right cranial medial aspect of the abdominal cavity in the region of the tenth through twelfth intercostal spaces. It is tightly attached and relatively fixed in its position in its cranial aspect, and the pancreas is closely adherent to it. The bile duct enters the duodenum approximately 1.5–6 cm distal to the pylorus, and the separate pancreatic duct enters the duodenum 10–20 cm from the pylorus (Schantz et al., 1996; Yen, 2001). The distances increase depending upon the size of the pig; the smaller value represents weanlings, and the larger value represents adults. The duodenal portion of the small intestine courses medially and passes through

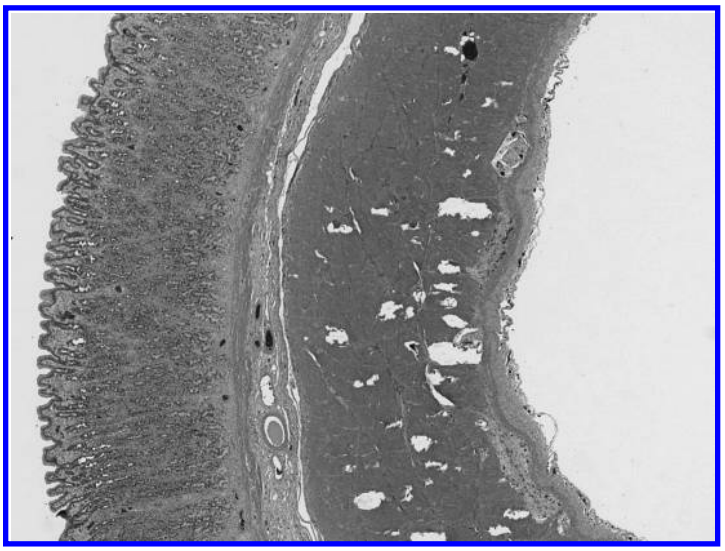

FIGURE 4.5 Histologic section of the glandular region of the stomach. H&E, ×40.

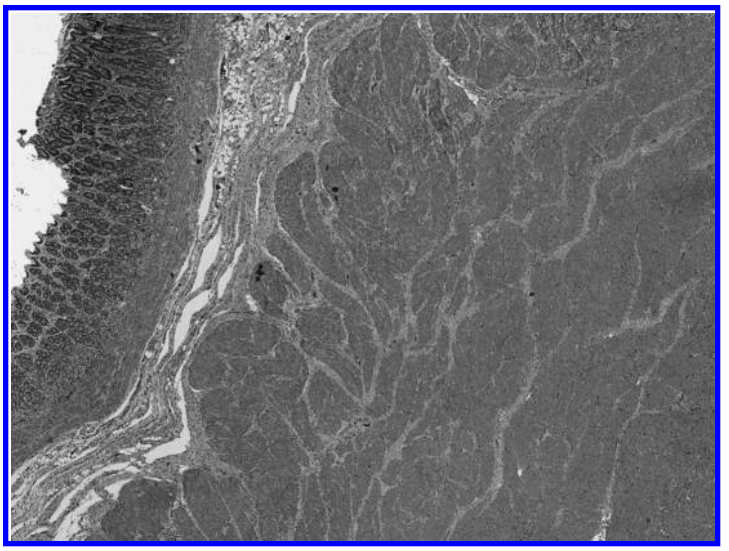

FIGURE 4.6 Histologic section of the pyloric region of the stomach. H&E, ×40.

the ligament of Treitz caudal to the stomach, after which it gradually develops into the jejunum. The linear distance from the pylorus to the ligament of Treitz in a 20-kg female domestic farm pig is about 30 cm (M.A. McCrackin, unpublished data). The duodenum is generally considered to be approximately the proximal 5% of the small intestinal mass. Small intestinal diameter in domestic swine of 25 kg is approximately 18–20 mm, and in 50 kg domestic swine, it is approximately 30 mm as compared to 50 kg Yucatan swine, in which it is approximately 20 mm.

The jejunum comprises approximately 90% of the small intestinal mass. Most of it is located in the right caudal ventral side of the abdominal cavity in tight coils. The gross differences between the jejunum and ileum are not distinct; however, the wall of the ileum is slightly thinner. The ileum is the distal 5% of the small intestine and generally originates in the middle of the abdominal cavity, in which it courses cranially to attach to the cecum in the spiral colon at the level of the left paralumbar fossa.

The mesentery of the small intestine is thin and friable with a unique fanlike pattern of mesenteric vessels with vascular arcades forming in the submucosa (Figure 4.7). Prominent lymph nodes are present in the root of the mesentery. The surgical modifications required for suturing the mesentery are discussed in the description of intestinal anastomosis in this chapter.

Generally, the small intestine comprises 20–40 times the length of the pig, approximately 4.5% of the BW (7–8 g/kg BW) and requires 2–6 h for complete transport and emptying. GI transport is dependent upon the type of diet and age of the animal. In general, the length and width of the small and large intestine of a 30- to 40-kg pig equals that of an adult human (Schantz et al., 1996). A chart of the percentage of BW of various tissues and organs of the digestive system for 12-week-old pigs is given in the appendix (Table A.31).

Histologically, the small intestine is composed of the mucosa, submucosa, muscularis, and serosa (Figures 4.8 through 4.10). Beneath the serosa, the muscularis layer is composed of an outer longitudinal layer and an inner circular layer of smooth muscle. The submucosa is located on the luminal side of the muscularis layer, separating it from the mucosa. The mucosa is composed of muscularis mucosa, lamina propria, and columnar epithelium. The muscularis mucosa has two muscular layers, with longitudinal and circular layers reversed in position compared to the muscularis layer described above. Peyer's patches (lymph nodules) are indistinct whitish oval nodules in the jejunum and ileum.

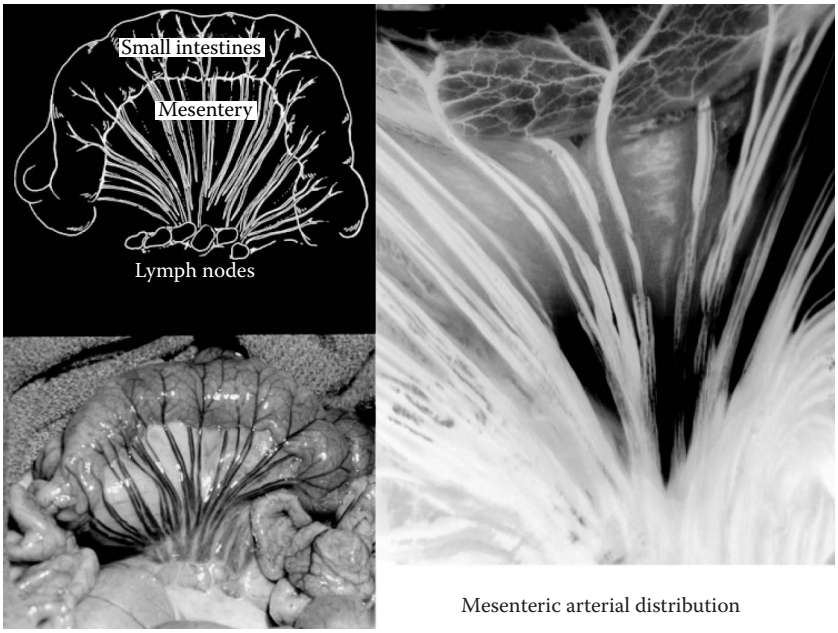

FIGURE 4.7 Small intestine showing the anatomy of the mesenteric vessels. The inset on the right is a negative image showing the arterial distribution in the intestinal wall.

The spiral colon is located in the left cranial quadrant of the abdomen and consists of the cecum, ascending, and proximal part of the transverse colon. Initially, the large intestine coils centripetally inward and then reverses after four coils to the centrifugal portion. The transverse colon exits the spiral colon cranially and then traverses caudally as the descending colon and rectum (Figures 4.11 through 4.13).

The large intestine is generally about 25% the length of the small intestine but significantly slows the transit time of ingesta, requiring 24–48 h for complete emptying. It weighs 4.5–5.5 g/kg BW.

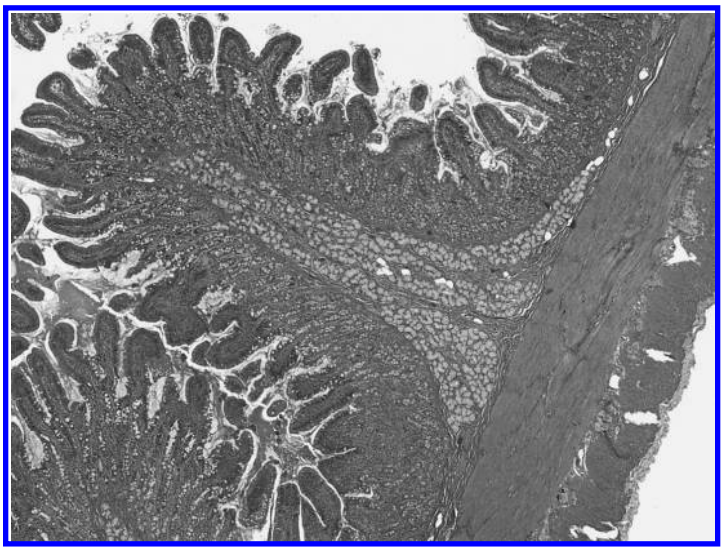

FIGURE 4.8 Histologic section of the duodenum. H&E, ×100.

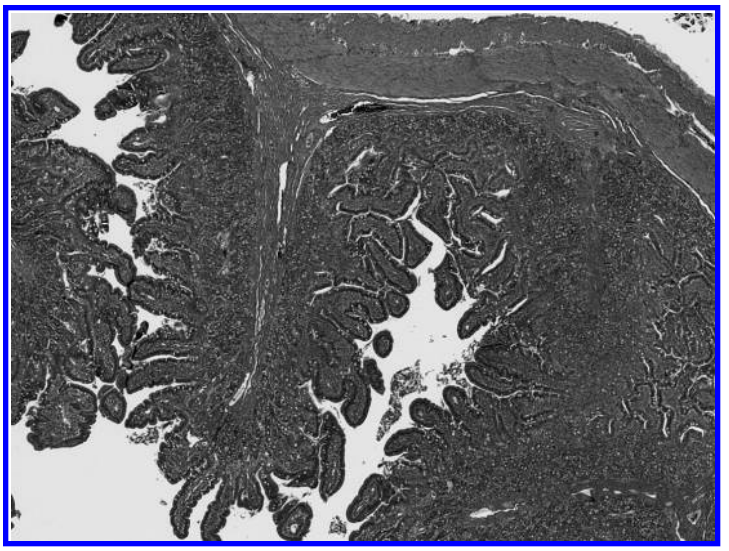

FIGURE 4.9 Histologic section of the jejunum. H&E, ×100.

The large intestinal diameter in 25-kg domestic swine is approximately 25 mm, in 50-kg domestic swine, approximately 55 mm, and in 50-kg Yucatan swine, 25 mm.

GENERAL PRINCIPLES OF ABDOMINAL SURGERY

Swine need to be fasted prior to surgery to facilitate the approach to the organs and to prevent vomiting, which rarely occurs. Unless a gastrotomy is to be performed, it is not necessary or advisable to withhold water because of their susceptibility to "salt poisoning" (Chapter 1). It is advisable to fast swine if the intestines are to be exteriorized for major abdominal surgery, because it

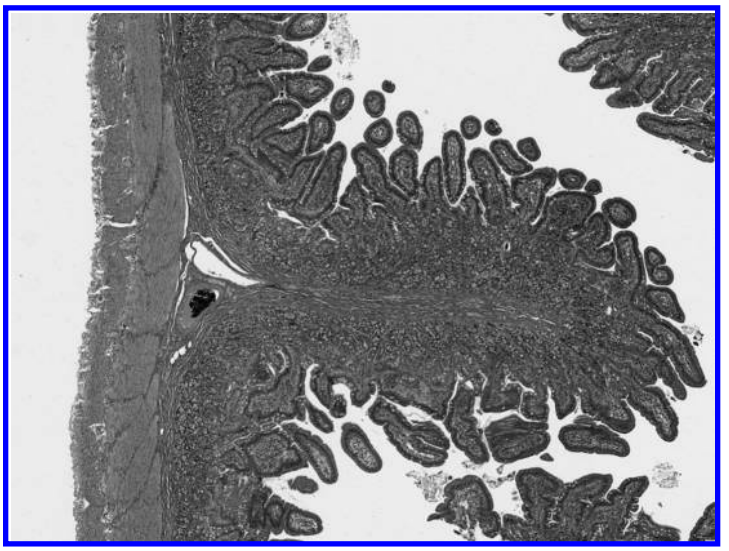

FIGURE 4.10 Histologic section of the ileum. H&E, ×100.

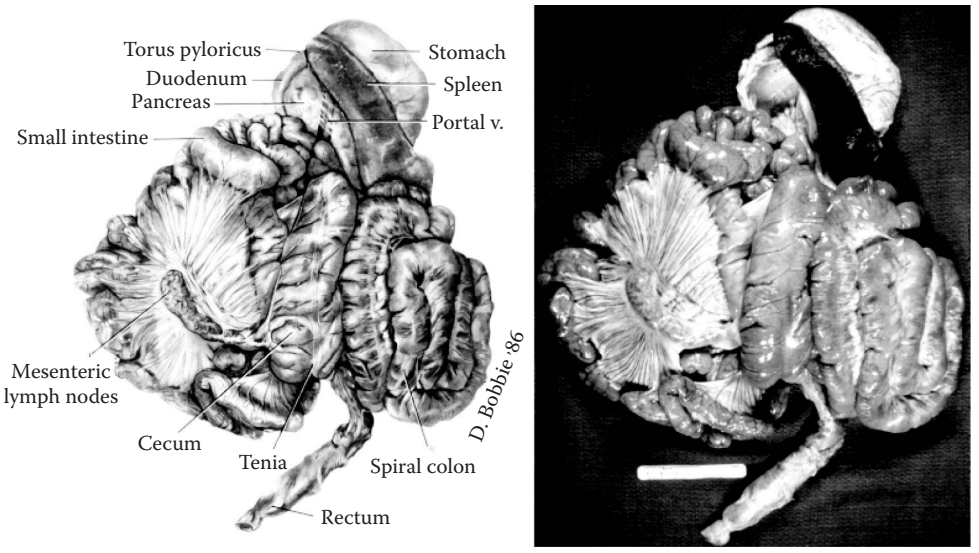

FIGURE 4.11 Gross anatomy of the spiral colon and large intestine.

decreases the edematous response. GI transit time varies with the breed, diet, and size of the pig; however, the stomach and small intestine may generally be considered to be emptied by a 12-h fast. The large intestines, including the spiral colon and cecum, generally require 48 h to empty. Use of high-volume hypertonic osmotic purgative solutions will provide a more thoroughly cleansed intestinal tract; however, these solutions must be administered by stomach tube. Approximately 1 L/25 kg is required for purgation. The pills used for human colon cleansing (Visicol™, InKine Pharmaceuticals, Inc., Blue Bell, PA) are tolerated in feed and are effective. Use of enemas and stimulant purgatives is discouraged because of the difficulty of administration, discomfort to the animal, and the associated messiness of the procedure; however, they are effective. Bedding must

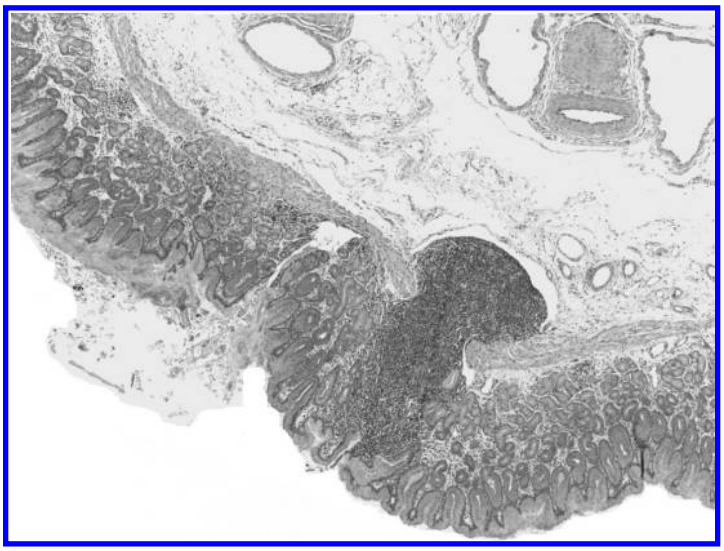

FIGURE 4.12 Histologic section of the large intestine from the spiral colon. H&E, ×40.

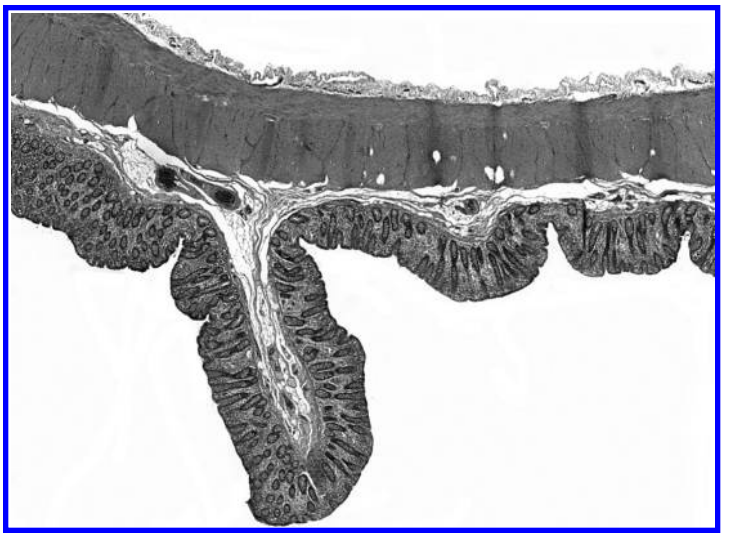

FIGURE 4.13 Histologic section of the colon. H&E, ×40.

be removed from the cages of fasted swine, because they will readily consume it, as well as any other foreign material that is available, in the absence of food. Pigs will readily consume commercially available glucose/electrolyte solutions (i.e., Gatorade®, Gatorade, Chicago, IL) or protein supplements (i.e., Ensure™, Abbott Labs, Abbott Park, IL). These can be given to pigs during the prolonged 48 h fasts necessary for some procedures without increasing residue in the GI tract. It may also be useful in postoperative care situations in which the pig cannot be given solid food for a prolonged period of time. Prophylactic antibiotics preoperatively and intraoperatively are indicated for clean-contaminated surgery that enters the intestines (Becker et al., 1992; Swindle, 1983). The pH of the stomach is generally <3.6 and is protective against microbes. Prior to surgically preparing the male pig for any type of abdominal surgery, the preputial diverticulum must be expressed as described for the urinary system in Chapter 7.

The laparotomy incision may be made in several locations depending upon the area of the small intestine to be approached. In general, the duodenum and root of the mesentery are best approached using a ventral midline incision in the cranial abdomen. The bulk of the jejunum may be approached either by a midline, flank, or paramedian incision in the mid-to-caudal aspects of the abdomen. The ileum may be approached using a midline or flank incision in the cranial portion of the abdomen.

Following laparotomy, it is advisable to use saline-moistened laparotomy pads to keep the tissue at the edges of the incision moist (Figure 4.14). Also, the use of laparotomy pads facilitates collection of the inadvertent spillage of GI contents to minimize contamination of the abdominal cavity during enterotomy. Gentle handling of the intestines with moistened sponges and atraumatic instrumentation is essential to minimize the complications associated with postoperative adhesions. The mesentery of the pig is very friable and prone to edema following prolonged manipulation. As for contaminated surgery in other species, intraoperative peritoneal lavage is controversial although recent publications in dogs (Swayne et al., 2012) and humans (Whiteside et al., 2005) were unable to find evidence-based reasons for its use. Resulting recommendations for treating septic peritonitis included aspiration of gross contamination, systemic antibiotics, and correction of the source of infection (Whiteside et al., 2005).

Closure of abdominal incisions is best accomplished in layers using synthetic absorbable sutures in either a simple interrupted or a continuous pattern. In contrast, Ceydeli et al. (2005) reported that for adult humans, optimal evidence-based closure of the abdomen employs simple continuous mass

FIGURE 4.14 Laparotomy incision with edges of incision protected with moistened gauze sponges.

closure with #1 or #2 absorbable monofilament suture and suture length to wound length ratio of 4:1. Closure of the peritoneum as an individual layer is not always possible in younger animals or in the caudal abdomen. In fact, closure of the peritoneum as a separate layer is unnecessary, as it is for humans (Ceydeli et al., 2005). Muscle fascia should be included in the sutures to bring the layers into proper apposition and to provide suture holding strength. Suturing of the skin is easily accomplished with subcuticular sutures, and this pattern is less likely to have localized wound inflammation than external suture patterns or staples (Chapter 3).

All GI procedures can be performed, at least in part, using staple surgery technologies. Also, most of the procedures can be approached using laparoscopic or endoscopic surgical techniques rather than open surgical procedures. These techniques have been shown to be equivalent for wound-healing characteristics when compared to open techniques. For simplicity, the manual suturing techniques are described in this textbook (Kopchok et al., 1993; Noel et al., 1994; Olson et al., 1995). The unique anatomic features of the GI tract are outlined in Chapter 1. Photos of the abdominal viscera are included for reference when planning celiotomies (Figures 4.1 through 4.3, 4.7, 4.11). Methods of closing intestines and other hollow viscous organs are illustrated (Figures 4.15 through 4.18).

The effects of energy-based surgical devices (ESD) on porcine peritoneum, stomach, small bowel, and colon have been studied (Phillips et al., 2008). Four devices were tested: the Harmonic ACE, a bipolar Gyrus Trisector, the Harmonic LCS-C5, and the LigaSure V. Blade temperature was inversely proportional to the amount of thermal damage created in the tissues tested. The Harmonic ACE and LCS-C5 caused the least thermal damage of the devices tested.

POSTOPERATIVE CARE

Postoperative recovery should be routine as for any other surgical procedure. However, some specific advice relevant to when the intestinal tract is entered surgically is provided in this section. Water may be provided immediately following recovery, and the animal may consume solid feed only if a catheter implantation was performed. Solid food should be withheld for the first day following surgery if an anastomosis was performed. Animals may be maintained on commercially available liquid diets such as Gatorade (glucose/electrolyte) or Ensure (protein/caloric). For a 20- to 25-kg pig, the calculated amount of diet is approximately 1 qt of the glucose/electrolyte

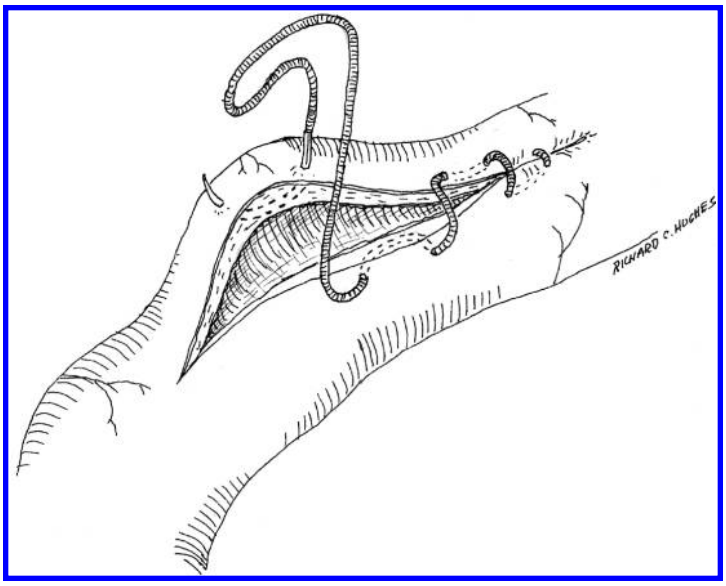

FIGURE 4.15 Closure of an intestinal incision with a Cushing pattern.

solution and two to three cans of the protein/caloric supplement provided twice a day (bid). The intention is not to fully meet daily caloric requirements for maintenance and healing but rather to provide energy and some relief from feelings of hunger. Postoperative analgesia should be provided, and the animal monitored for postoperative complications. Swine will readily eat canned pet food, chocolate syrup, pastry, or fruit, and oral medication may be hidden in these treats.

The most common complications that may occur are localized infection or peritonitis from an intestinal content leakage or intussusception. Animals should be monitored for fever and abdominal pain as well as normal postural and behavioral characteristics. Treatment should be symptomatic;

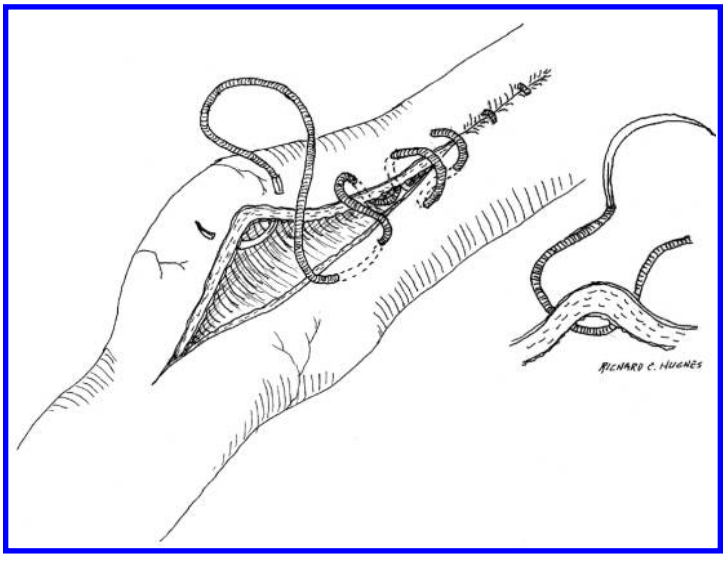

FIGURE 4.16 Closure of an intestinal incision with a Connell pattern (suture enters lumen).

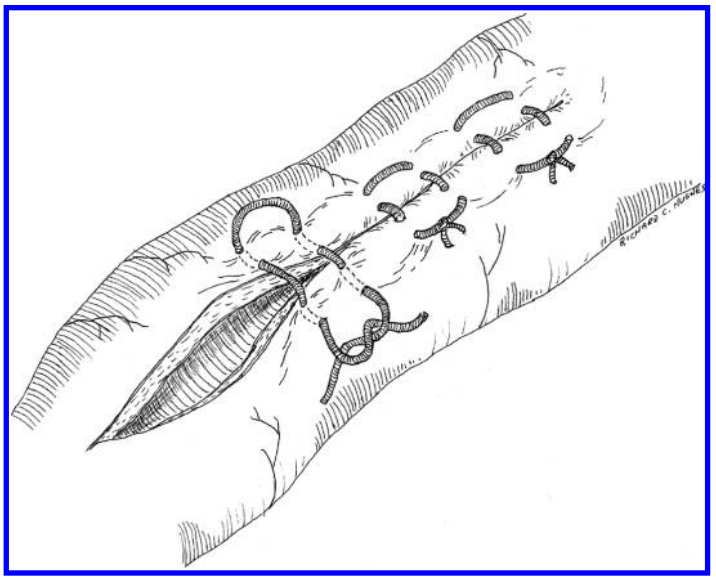

FIGURE 4.17 Closure of an intestinal incision with a Halsted pattern.

however, in a research setting animals generally are euthanized if such complications occur. Intussusception is a rare occurrence, but it may be associated with telescoping of the intestine over the silicone cuff in intestinal catheter implantations, mesenteric rents, or adhesion formation. If the animal is exhibiting fever, inappetence, and abdominal pain, radiographs should be taken to observe and diagnose the condition. Complications should be rare provided complete sterile techniques and gentle tissue handling are utilized during the procedure.

GASTROTOMY AND GASTROSTOMY TECHNIQUES

The stomach is best approached from a cranial ventral midline incision from the xiphoid cartilage to the umbilicus. Following laparotomy, the stomach may be retracted gently out of the abdomen,

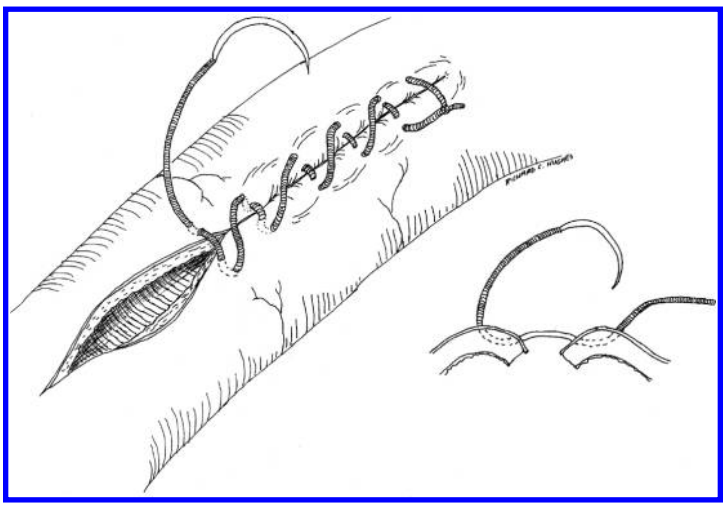

FIGURE 4.18 Closure of an intestinal incision with a Lembert pattern.

and the edges of the incision packed off with moistened laparotomy pads. The gastrotomy incision is made in the avascular plane of the greater curvature after stabilization of the plane with Babcock forceps or stay sutures. As in other species, the serosa and muscularis layers of the gastric wall are firmly attached to each other while the submucosa and mucosa are similarly intimately associated with each other. This anatomy creates a potential space between the muscularis and submucosa, so the submucosa/mucosa often falls toward the gastric lumen if the forceps or stay sutures engage only the serosa/muscularis. Care should be used to stabilize all four gastric wall layers in order to efficiently create a full thickness incision into the gastric lumen. Alternatively, the serosa/muscularis and submucosa/mucosa layers may be incised sequentially. Closure of the gastrotomy is performed with either staple surgical techniques or a two-layer inverting closure technique. The preferred suturing technique uses synthetic absorbable sutures using inverting suture patterns such as a Cushing pattern oversewn with a Lembert. The stomach should be thoroughly rinsed with sterile saline prior to replacing it in the abdomen.

Gastrostomy tubes, ports, or cannulae (Figure 4.19) may be used for serial gastric fluid sample collections or chronic administration of distasteful substances or volumes of liquids unlikely to be voluntarily consumed by swine. A variety of techniques have been described for gastrostomy placement. Gastrostomy devices may be sewn in place under direct visualization during surgical laparotomy using standard principles of abdominal surgery. Alternatively, percutaneous endoscopic gastrostomy (PEG) and percutaneous radiographic gastrostomy (PRG), with and without gastropexy in farm pigs (Maxwell et al., 2011), single port laparoscopic gastrostomy in Göttingen minipigs (Birck et al., 2014), and nonendoscopic percutaneous gastrostomy in farm pigs (Gades and Mandrell, 2001), have been described. Only the last of these reports did not describe the use of direct or radiographic visualization to immediately confirm accurate placement of the gastrostomy tube.

Gades and Mandrell (2001) reported successful tube placement in three of six pigs in which the blind technique was used with one of these resulting in unintended entrapment of the colonic mesentery. The procedure involved passing a trocar through the esophagus into the stomach, exiting the left abdominal wall to secure and retract a suture into the mouth for tying to a 26-French Foley catheter that was pulled in antegrade fashion into the stomach and through the left abdominal wall. Previous studies in dogs (Clary et al., 1996) and cats (McCrackin Stevenson et al., 2000) using blind percutaneous placement techniques demonstrated risk of suboptimal stomach orientation and mesenteric capture when tube placement was done without prior gastric insufflation. Based on the limited data available concerning blind gastrostomy placement in pigs, it may be worthwhile to

FIGURE 4.19 Cannulae for chronic fistulation of the colon (left) and the stomach (right).

consider the advice of Clary et al. (1996) to use preplacement gastric insufflation if this technique is chosen. A variation on blind placement in 18 German Landrace pigs, using a much smaller, 2.7 mm (~8 French) pleural drain as a percutaneous intragastric catheter (PIC) for administration of large volumes of ethanol, was published by Oleszczuk et al. (2009). This study used preplacement gastric insufflation and described consistent gastric fundus location for all except one catheter found in the antrum at necropsy.

Regardless of the gastrostomy placement technique used, exteriorization of gastrostomy devices is best performed on the lateral abdominal wall dorsal to the lateral border of the mammary glands or, preferably, on the dorsum of the flank. This prevents irritation of the exteriorized device when the pig is recumbent or rubs itself on the cage (Hennig et al., 1980; Pekas, 1983). In domestic farm pigs, the security of adhesion of the fibrotic stoma tract between the gastric and abdominal walls was graded subjectively as excellent at 1, 2, and 3 weeks after gastrostomy placement (Maxwell et al., 2011). Further, inclusion of gastropexy did not appear to enhance or hasten stoma tract formation or maturation. This may vary among species; cats with percutaneous gastrostomy were noted to form stoma tracts slowly and potentially inadequately for direct traction removal of tubes even after 8 weeks of healing (McCrackin Stevenson et al., 2000). Surgical placement of pharyngostomy tubes caudal to the mandible is another option.

Heidenhein pouches (Figure 4.20) may be utilized in the pig in the same manner as originally described for other species to collect gastric secretions without gastric contents (Markowitz et al., 1964). For this procedure, a sleeve of the stomach is isolated from the body along the greater curvature while maintaining the blood supply of the gastroepiploic artery. The body of the stomach and the sleeve are sutured with a double row of inverting sutures. The isolated gastric pouch may be cannulated to the outside on the lower abdomen. In performing this procedure, the branches of the vagus nerve are sacrificed. A Pavlov pouch maintains the integrity between the cranial portion of the pouch with the body of the stomach, thus preserving innervation at that end. The distal end of the pouch is fistulated to the outside.

Gastrojejunostomy techniques may also be utilized in swine (Brenner et al., 2001). The jejunum is located by finding the root of the mesentery, which is caudal to the stomach and associated with

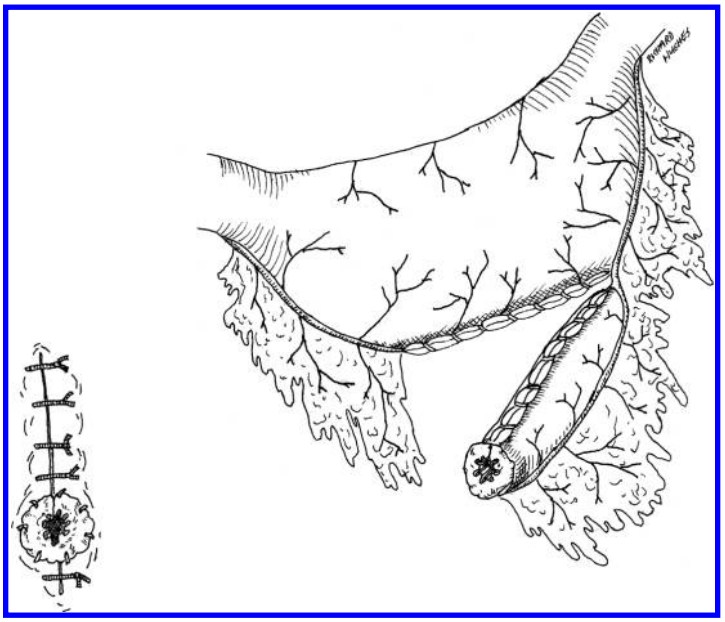

FIGURE 4.20 Creation of a Heidenhein pouch in the stomach.

the cranial mesenteric vessels. Care should be taken to ensure that there is no torsion of the mesentery and that the caudal direction of the peristalsis remains intact. A side-to-side surgical anastomosis is performed using standard techniques. Single-layer closure, using either interlocking or simple continuous sutures, is generally acceptable. Using two-layer inverting closure techniques may be necessary in large animals or in the case of existing pathology (Swindle, 1983).

A model of gastroesophageal reflux (GER) has been described (Schopf et al., 1997). This model is created by performing a longitudinal myectomy 3 cm proximal and distal to the gastroesophageal junction using a ventral midline incision from the xiphoid to the umbilicus. The approach and dissection is similar to the one described for vagotomy in this chapter. The myectomy must be performed carefully to avoid incising the mucosal layer, similar to the dissection described for pyloroplasty in this chapter. However, the myectomy incision is not closed, and the animal should be kept on a liquid diet for 3 days postoperatively.

Otherwise, the abdominal incision is closed routinely, and postoperative care is routine. This procedure results in a chronic reflux esophagitis within days if the procedure is technically successful. Duarte et al. (2013) recently described the reproducibility of gastric yield pressure (GYP) and gastric yield volume (GYV) for objectively measuring the anti-reflux barrier (ARB) in 8-week-old female Large-White pigs. It was concluded that the measurement of GYP was the more reliable of the two techniques for the testing of new chemotherapeutics targeting GER disease.

Swine have also been used for testing potential surgical techniques for preventing GER in infants (Hill and Wulkan, 2013). Eight-week-old Yucatans underwent cardiaplication with minimal dissection, and the surgical procedure increased cardia yield pressure (CYP) about five times over baseline.

PYLOROPLASTY

The pylorus may be approached either through a midline or right paramedian incision. If the paramedian incision is used, it is made along the lateral aspect of the mammary glands from the last rib to the level of the umbilicus. The pylorus is identified by palpation and must be differentiated from the muscular torus pyloricus. The pylorus is exteriorized through the incision and stabilized by an assistant with wetted gauze sponges. A linear incision is made along the longitudinal plane of the stomach and duodenum in an avascular area. Repeated gentle incisions are made until all layers of the muscularis mucosa have been incised, and the submucosa bulges from the incision. At this point, the axis of the completed incision is changed at a right angle and the incision is closed using Lembert sutures. The net effect of the procedure is to enlarge the opening of the pylorus by incising the musculature and reversing the plane of the incision (Figure 4.21). Care should be taken not to incise the submucosa or mucosa; if this occurs, however, the closure of the incision described previously will repair the defect (Swindle, 1983).

ENTEROTOMY AND INTESTINAL FISTULATION

The small intestine (Figure 4.7) can be surgically entered using standard techniques (Anderson et al., 2000; Swindle, 1983; Swindle et al., 1998a,b). The duodenum can be approached by either a cranial midline incision, a right flank incision caudal to the ribs, or a right paramedian incision lateral to the mammary glands. The ileum is best approached through the cranial midline incision as for the proximal duodenum or close to the ileocecal junction through a right flank incision caudal to the last rib. The ileocecal junction is located in the dorsum of the abdominal cavity when using the flank incision. The majority of the small intestinal mass, including most of the jejunum, is in the right middle and caudal areas of the abdomen. It may be readily approached from the midline in females; however, a right paramedian incision in the caudal quadrant of the abdomen lateral to the mammary glands or a flank incision is preferable in males. This incision can be made through relatively thin abdominal musculature with minimal vasculature requiring ligation in this region.

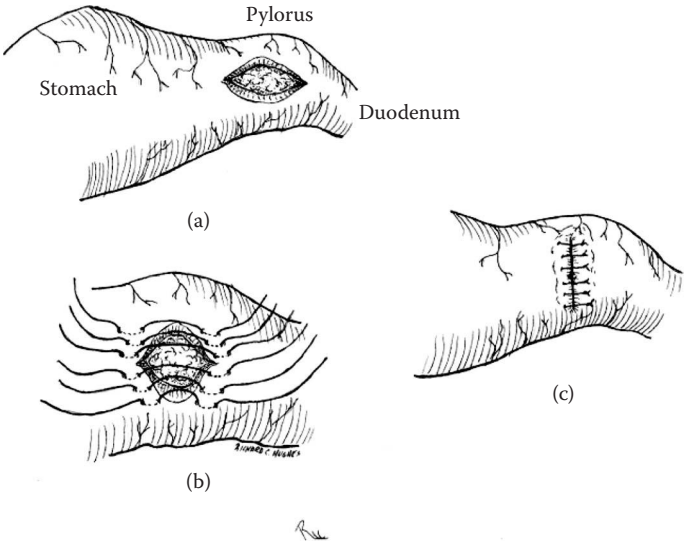

FIGURE 4.21 Closure of a pyloroplasty at right angles to the surgical incision. (a) Shows a linear incision over the pylorus to the depth of the submucosa, (b) demonstrates an interrupted Lembert suture pattern closing the pyloroplasty at right angles to the incision, and (c) illustrates the completed procedure.

Prior to enterotomy, the area of interest is brought out through the abdominal incision, and the area is packed off with wetted laparotomy sponges. A longitudinal incision is made along the antimesenteric border of the intestine in an avascular plane. If a catheter or other fistula device is to be inserted, then a stab incision may be made in the same plane with a no. 11 blade. It is not necessary to cross-clamp the intestine in most cases if the animal has been properly fasted. An assistant can preclude intestinal contents from entering the incision by pinching off the lumen at either end of the enterotomy incision. Following enterotomy, the incision may be closed using simple interrupted sutures with synthetic absorbable suture material.

For intestinal infusions, vascular access ports have been modified to function as intestinal access ports. A 7- to 9-Fr (French) silicone catheter with the end hole closed and four side slits approximately 1 cm in length has been found to be useful. The catheter has a bead preplaced 1 cm distal to a silicone flange that has been preplaced to provide an anchor for sutures to the intestinal serosa (Figures 4.22 and 4.23). The access port may either be sutured to the skin on the outside or implanted subcutaneously in the flank or over the rib cage. The site of entrance into the intestine is closed between the bead and the flange with a purse-string suture. The flange is sutured to the intestine with two to four simple interrupted sutures (Figure 4.24). The specific procedures are described in detail in the next section. If a catheter is to be placed in the peritoneal cavity for infusion, the catheter must have multiple holes to prevent occlusion by the omentum (Figure 4.25).

The small intestine may also be fistulated as a Thiry fistula or Thiry-Vella loop (see later text). When either of these procedures is performed, then the fistula is best exteriorized on the lateral portion of the right flank to minimize tension on the isolated intestinal loop. Ileocutaneous bypass has also been described, which allows complete collection of ileal contents (Anderson et al., 2000). Ports may be placed at the site of the fistulation to minimize contamination and inflammation of the exit site. T cannulation has also been used for small intestinal fistulation (Wubben et al., 2001). In this type of procedure, the tube is placed into the intestine and secured in place with a purse-string suture using nonabsorbable suture material. The barrel of the tube is exteriorized through the flank. Tubes must be designed with threads and an exteriorized cap to allow adjustment of the length of the barrel as the animal grows. The barrel should also be designed such that a plug is included to prevent ingesta from entering the tube.

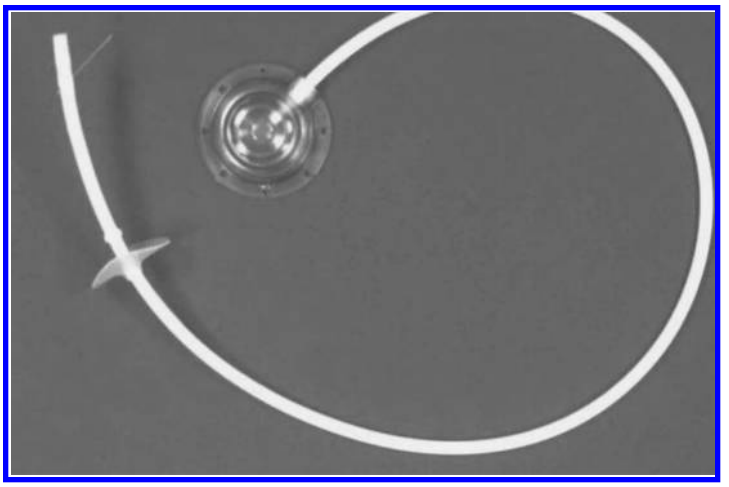

FIGURE 4.22 Intestinal access port for chronic infusion into the intestinal lumen. Note that the catheter has a bead for stability inside the lumen and a cuff for suturing to the serosa.

When fistulation is performed, the postoperative care should include local application of an antibiotic or zinc oxide ointment to prevent inflammation on the surface of the skin. Fistulas should not be accessed until the surrounding skin is thoroughly cleansed with an antibacterial soap. Depending upon the type of procedure performed, nutritional deficiencies, serum biochemical changes, or both may occur and potential corrective actions should be included in preoperative planning.

INTESTINAL INFUSION CATHETER IMPLANTATION

In pharmacological studies, it is frequently necessary to bypass the stomach and determine which area of the small intestine is responsible for absorption of the agent. This can be performed by implanting infusion catheters in various regions of the small intestine. Common sites would be the duodenum, jejunum, and ileum. Generally, duodenal catheters are implanted in the segment immediately caudal to the pancreatic duct. The proximal jejunum may be located at the root of the

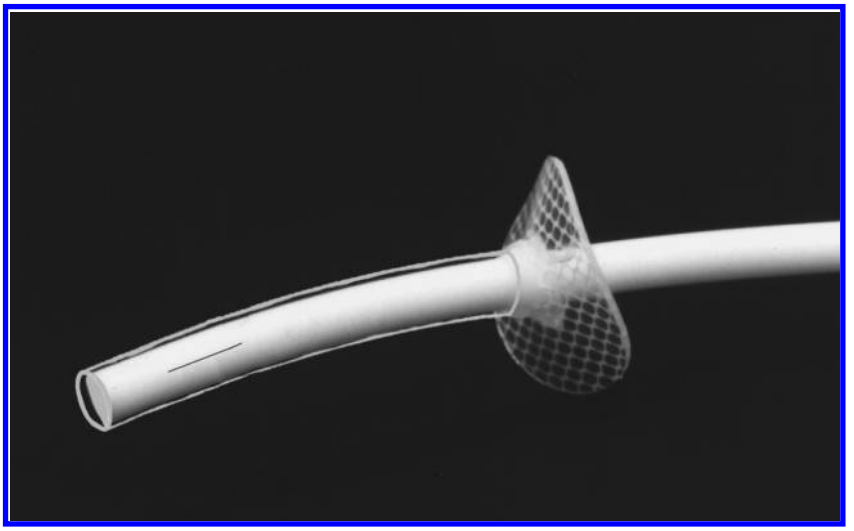

FIGURE 4.23 Tip of intestinal access port with a slit valve and closed end.

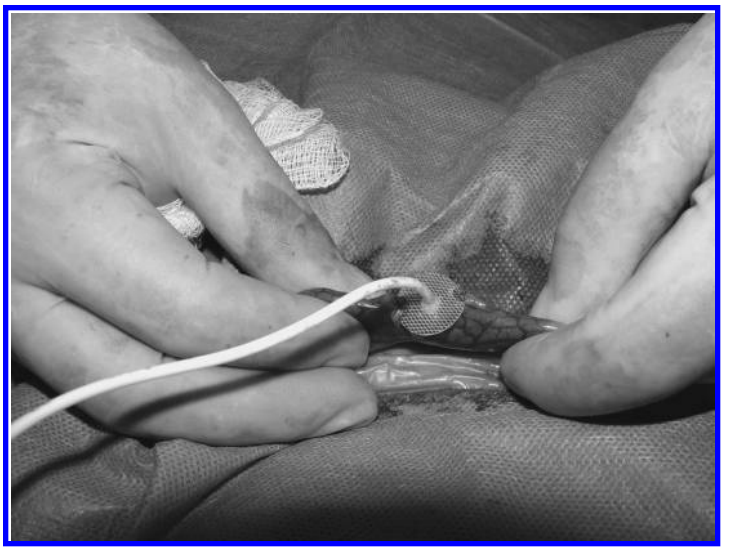

FIGURE 4.24 Intestinal access port catheter flange sutured to the serosa of the duodenum.

mesentery caudal to the stomach and midjejunum in the coils of the caudal abdomen. The ileum is best located by identifying the ileocecal junction and tracing the intestinal segment cranially.

Vascular access ports have been modified to be used as intestinal infusion ports and catheters (Swindle et al., 1998b, 2005). The port itself is of the same design but 7- to 9-Fr silicone catheters are modified for intestinal access. The tip of the catheter cannot be left open because it will rapidly become blocked with intestinal contents. The tip of the catheter is either constructed with a burp valve or four slits cut in the intraintestinal portion of the tip of the catheter. A suture retention bead is glued in place at the site of intraluminal placement and a silicone cuff is preplaced approximately 1 cm above the bead. Thus, there is an intraluminal segment with slits or a burp valve, a suture retention bead within the intestine at the site of enterotomy, and a silicone cuff for placing retention sutures on the serosa (Figure 4.22 through 4.25).

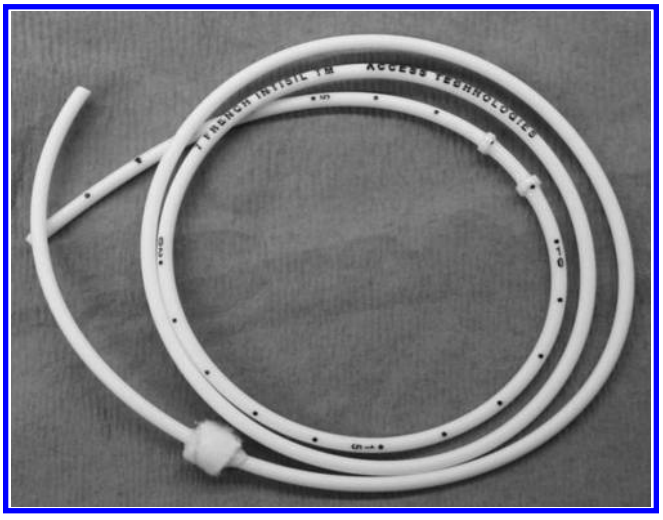

FIGURE 4.25 Intraperitoneal infusion catheter with multiple holes.

All the small intestinal sites can be accessed using a cranial to midabdominal midline incision. The procedures for isolating and packing the intestines are identical to that described earlier for anastomosis. A stab incision is made in the antimesenteric border of the intestine using a no. 11 blade, taking care to avoid direct penetration of a blood vessel. The catheter tip is inserted into the intestinal lumen in the direction of peristalsis. A purse-string suture using 3-0 PDS is placed in the serosa between the suture bead, which is in the lumen of the intestine, and the silicone cuff. The silicone cuff is tacked in place with 2–4 serosal sutures using 3-0 Ethibond or other soft nonabsorbable suture material (Figure 4.24).

An elliptical pocket for the port body is made on the lateral abdominal wall. The end of the catheter is tunneled through the wall of the abdomen into the port pocket. The end of the catheter is attached to the port, and the port and catheter are flushed copiously with sterile saline. The surgeon should be able to visualize the unobstructed passage of the fluid into the intestinal lumen without any leakage around the surgical site. The access port is sutured subcutaneously in place in the usual manner.

This procedure is repeated for every intestinal access catheter that is placed. The catheter length should be sufficient to allow for growth of the animal during the project period without putting undue tension on the intestine. Leaving too much extra catheter length in the abdomen may lead to torsion of the intestines. This technique is only useful for infusion of substances into the intestine. It is not possible to withdraw intestinal contents through the catheter. Catheters are maintained by keeping them filled with sterile saline. Access to the port site for injections should be performed under a sterile technique with skin preparation, sterile gloves, and sterile supplies.

INTESTINAL ANASTOMOSIS

The intestine may be approached surgically through any of the incisions described earlier for enterotomies (Swindle, 1983). The small intestine may be anastomosed by either end-to-end, end-to-side, or side-to-side techniques. The main variation between the pig and other species relates to the formation of the vascular arcades of the mesenteric vessels in the subserosa of the intestine rather than in the mesentery. The peculiar fanlike arrangement of the mesenteric vessels also necessitates a change in techniques to suture the mesentery (Schantz et al., 1996).

When performing an intestinal anastomosis, the vascular supply to the intestine that is to be removed is ligated at its base close to the root of the mesentery. The line of demarcation of the blood supply in the intestine should be closely observed to ensure that all infarcted nonviable intestine is removed. The intestine should be cross-clamped with intestinal forceps along the line of demarcation to ensure that the line of incision will be at the junction of viable and infarcted intestine. This line will be at an oblique angle to the long axis of the intestine. The angle will approximate the angle of the mesenteric arterial supply to the region. The section of intestine that has been ligated and its associated mesentery are excised after placing a second set of intestinal forceps at either end of the viable intestine to prevent contamination of the surgical site with reflux of intestinal contents. This pair of forceps should be applied atraumatically with the minimal amount of pressure required to prevent the movement of intestinal contents into the area of the incision. The intestinal forceps should be either linen or rubber shod to prevent cutting of the tissue by the metal forceps. Alternatively, an assistant may use digital pressure to pinch off the lumens of the viable intestine at either end of the incision. These proximal and distal clamps should be placed so that the excised ends of the remaining intestine are long enough for the edges to be sutured.

The intestinal anastomosis may be closed with simple interrupted sutures using synthetic absorbable sutures. This suture pattern enters the lumen and is pulled tightly enough when tying the knots to cut through the mucosa and become embedded in the intestinal wall. This is applicable to most small swine; however, in larger animals, either a continuous pattern or a two-layer closure of continuous sutures oversewn with inverting sutures may be required. Single-layer closure is

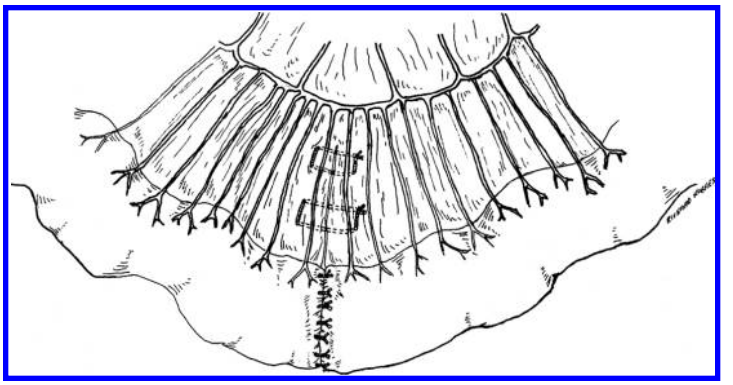

FIGURE 4.26 Closure of the mesentery with horizontal mattress sutures and an intestinal anastomosis closed with simple interrupted sutures.

usually indicated except in the case of larger animals or potential contamination that may impair healing. Regardless of which suturing technique is chosen, the sutures must be meticulously placed to provide for correct anatomic alignment and to provide a suture line without leakage. As a general rule, the sutures for this anastomosis should be approximately 5 mm from the edges of the incision and 5–10 mm between sutures. The remaining clamps should be removed and the incision line checked for leakage. In some cases, the intestinal lumen along the lines of excision may not be the same size, and it might require additional trimming of the tissue edges. Any leaks may be closed by the addition of simple interrupted or inverted sutures. Alternatively, the anastomosis may be performed using standard staple surgical devices. Suture-free methods for closure of small bowel anastomoses are under development and have been tested in pigs with initial success (Stumpf et al., 2009).

The mesenteric incision will need to be sutured to prevent bowel torsion and stricture. In most cases, the edges of mesentery will retract to the adjacent mesenteric vessels and the mesenteric tissue adjacent to the incision will become edematous. If the edges of the mesentery are readily identifiable, then the incision may be closed with simple interrupted or continuous sutures using absorbable synthetic suture material. However, this will not be the case in most animals. In those animals in which the edges of the mesentery are not readily identifiable, a series of horizontal mattress sutures are placed on the proximal and distal sides of the first viable set of mesenteric vessels at either end of the excised intestines (Figure 4.26). This pattern will close the mesenteric rent without occluding the blood supply to the remaining intestine.

The anastomosed intestine should be rinsed copiously with isotonic saline solution, with or without antibiotics, prior to replacing it in the abdomen. If contamination is suspected, then rinsing of the abdomen with antibiotic-containing saline solution may be indicated.

Intestinal resection and anastomosis have been described for neonatal gnotobiotic piglets with the surgical procedure performed inside an incubator attached to a sterile isolator (Mateo et al., 2011). The goal of this study was to remove 90% or more of the ileal Peyer patches (~60 cm) through a flank approach and to maintain the piglets gnotobiotically for about 5 weeks. Favorable outcomes were obtained and proof of principle established that the procedure was feasible.

INTESTINAL DIVERSION OR INTESTINAL BYPASS

Various techniques are used to perform small intestinal diversion or bypass in the experimental setting (Assimos et al., 1986; Fleming and Arce, 1986; Hand et al., 1981; Markowitz et al., 1964; Swindle et al., 1998a,b; Turner and Mellwrath, 1982; Wubben et al., 2001). These would include such techniques as the Thiry fistula, Thiry-Vella fistula, T-tube cannulation, and jejunoileal bypass.

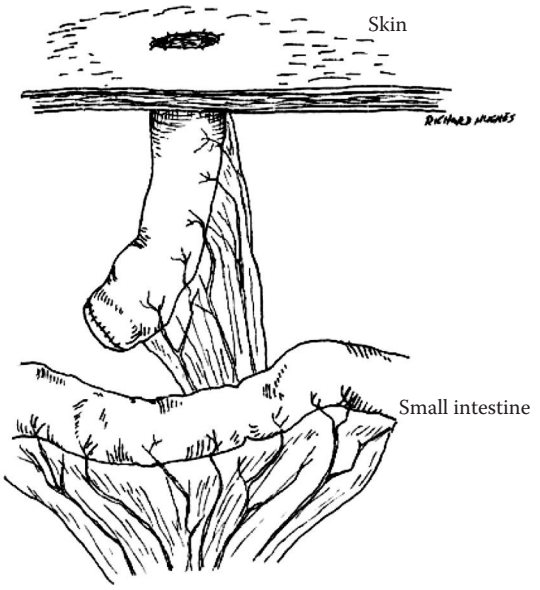

FIGURE 4.27 Thiry fistula.

The techniques for suturing the intestine are the same as those discussed for intestinal anastomosis. In this section, a review of some of the types of procedures that are possible in swine is provided. Examples of the specific porcine anatomic features that must be considered are provided.

The Thiry fistula (Figure 4.27) is performed by isolating a segment of small intestine with the vascular and nerve supply intact, closing one end by oversewing the stump, and exteriorizing the open end. The intestine from which the segment is isolated is closed using an end-to-end anastomosis. Peristalsis may either be directed into the abdomen or out of the fistulated abdominal wall depending upon the goals of the experiment. The Thiry-Vella loop (Figure 4.28) is isolated in the same manner as the Thiry fistula, except that both ends of the intestine are exteriorized. When the

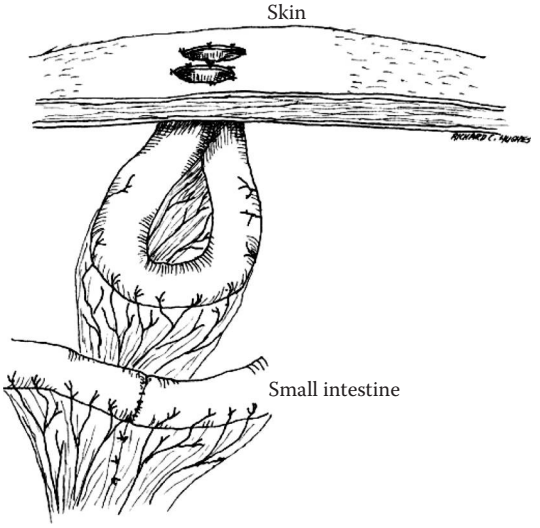

FIGURE 4.28 Thiry-Vella fistula.

intestinal segment is selected, it should be a section that will not have tension on the mesenteric attachment after isolation. The isolated segment should be flushed copiously with warm saline to minimize contamination of the abdomen and the exteriorization incision. Prophylactic antibiotics are indicated with this procedure (Markowitz et al., 1964).

The most appropriate place to exteriorize the intestine is on the right flank in a dependent portion. The thick musculature of the dorsal and lateral flank in larger swine will cause intestinal ischemia in the segment passing through the muscle. Toward the ventral portion of the abdominal wall, the muscle becomes much thinner. This problem is negated if the intestine is isolated in this area. The skin and muscle are incised, and the open end of the intestine is passed through the incision, care being taken to avoid spilling intestinal contents into the musculature. This can be facilitated by clamping the open end with atraumatic intestinal forceps. The end of the intestinal loop is sutured to the subcuticular layer of the skin using simple interrupted sutures with a monofilament nonabsorbable suture. Instead of exteriorizing the end of the intestine, it may be cannulated and left in the abdomen with only the prosthetic port exteriorized.

A model of jejunoileal bypass has been developed in swine to study nutritional complications associated with the procedure (Assimos et al., 1986). The intestine is divided caudal to the duodenum. The junction of the duodenum and jejunum is indistinct; however, it is generally considered to be caudal to the loop of the duodenum that passes cranially and then laterally from the caudal end of the body of the pancreas. The cranial end of the jejunum is oversewn and sutured to the cranial abdominal wall to prevent intussusception. The ileum is transected close to the ileocecal junction. The end of the duodenum is sutured in an end-to-end fashion to the distal end of the transected ileum. The transected proximal end of the ileum is sutured in an end-to-side technique to the ventrolateral portion of the spiral colon. This results in an almost complete bypass of the jejunum and ileum. Postoperatively, swine continue to maintain their weight with few GI complications.

Potential methods of bypassing and fistulating the intestine are almost limitless. These more traditional procedures were chosen to illustrate specific surgical considerations in swine.

COLONIC ANASTOMOSIS AND FISTULATION

The procedures described for anastomosis and fistulation of the small intestine may be applied to the large intestine, after a consideration of the unique anatomy in the pig, especially the spiral colon (Eubanks et al., 2006; Harvey et al., 2001; Swindle et al., 1998a). The spiral colon, which contains the cecum and ascending colon, is approached through a cranial midline incision and, in larger animals, may have to be extended caudal to the umbilicus. The short transverse colon can be approached through the same incision. The descending colon may either be approached through a caudal midline incision in females or a left paramedian incision in males similar to the approach described earlier for the jejunum. A schematic diagram of the anatomy and direction of peristalsis is depicted in Figure 4.29.

Anastomosis of the descending colon may be performed in a similar manner as described for the small intestine; however, it is necessary to close the colon with two layers of sutures, preferably synthetic absorbables. The internal suture pattern should be continuous or simply interrupted. The outer layer should be closed with simple inverting Lembert sutures. It is essential to prevent contamination of the abdomen with colonic contents by careful packing of the colon prior to incising it and copious flushing of the finished incision.

An attempt was made by Hoeppner et al. (2009) to develop a model of colonic leakage and ischemia in pigs as a model for humans. Anastomotic gaps in the descending colon of 18 mm and the addition of localized ischemia failed to produce a reliable model of peritonitis or intra-abdominal abscess. Areas of leakage were covered quickly by fibrin and ultimately by distinct adhesions, unlike the human condition.

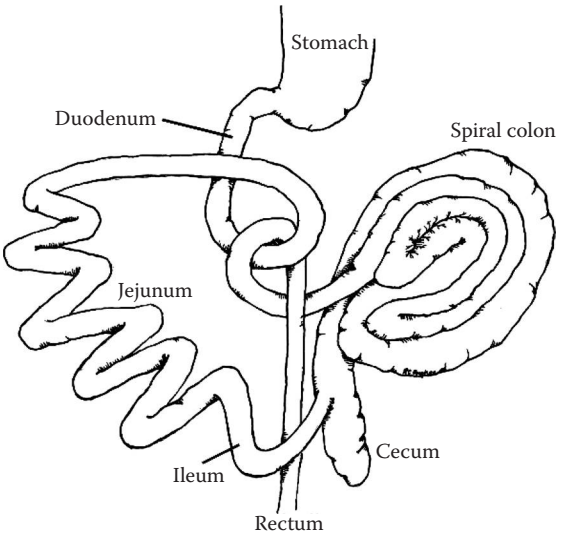

FIGURE 4.29 Schematic of the GI tract illustrating the direction of peristalsis and the orientation of the spiral colon.

The spiral colon is not amenable to anastomosis using standard techniques because of its unique anatomy (Figures 4.11 and 4.29). In the research setting, anastomosis is not likely to be attempted. However, the use of fistulas for the study of colonic contents, transport mechanisms, or infusion of pharmacological agents may be indicated. In this case, the area of the colon to be fistulated may be readily identified by its gross anatomic characteristics. The spiral colon lies in the left upper quadrant of the abdomen and contains the cecum and the majority of the ascending colon in an outer centripetal coil and an inner centrifugal coil. The cecum is located in the caudal aspect and is joined by the ileum at the base of the spiral colon dorsally adjacent to the left kidney and pancreas. A vermiform appendix is not present. The outer coil continues ventrally in a clockwise pattern (from the dorsal perspective) to form the apex. The outer coil contains two tenia. The inner centrifugal coil does not contain tenia and progresses dorsally until it exits cranially at the base of the spiral colon. The transverse colon is short and quickly turns caudally to form the descending colon. A true sigmoid flexure, analogous to humans, is not present prior to its transformation into the rectum in the pelvic cavity (Schantz et al., 1996).

Fistulas and ports may also be created in the same manner as for the small intestine (Figure 4.19). For the cecum and large intestine, however, they need to be exteriorized on the left flank caudal to the last rib (Eubanks et al., 2006; Harvey et al., 2001; Swindle et al., 1998a). If functional ostomies, such as colostomies, are performed then they are best exteriorized on the thin-walled dependent portion of the abdomen to minimize skin contact with the excreta. Spiral colon bypass has been described to treat clinical stricture in a Vietnamese potbellied pig (Gallardo et al., 2003). In this case a fecal impaction was relieved by side-to-side anastomosis of the proximal centripetal loop to the distal straight centrifugal loop of the spiral colon. This type of procedure might be employed in a research setting for partial colonic bypass procedures.

TOTAL COLECTOMY

A total colectomy may be performed in the pig. In the experimental setting this would usually be performed to simulate the conditions of colectomy following necrotizing enterocolitis or trauma. The bowel prep should include hypertonic purgatives and antibiotics preoperatively. The ileum

should be transected at the ileocecal junction and the spiral colon retracted caudally to expose the branches of the cranial mesenteric artery that provide blood supply to the structure. The dissection is continued caudally while ligating the arterial and venous branches supplying the mesocolon. The arterial branches are major subdivisions of the cranial and caudal mesenteric arteries, but the mesenteric veins have to be ligated separately. The transected ileum can be anastomosed, using an end-to-side or side-to-side anastomosis technique, to the rectum. Prior to transection of the large bowel, it should be ascertained that the ileum can be stretched to reach the area of transection.

The major postoperative complication will be diarrhea and nutritional deficiencies related to the shortened bowel. This surgery creates a similar condition as that which occurs with short-bowel syndrome in humans (Dudgeon et al., 1988). Spiral colon bypass with a side-to-side anastomosis of the proximal centripetal loop to the distal centrifugal loop has been described to clinically treat stricture in the spiral colon in geriatric potbellied pigs (Gallardo et al., 2003).

RECTAL PROLAPSE

Rectal prolapse is a clinical condition that may result secondarily to any condition that results in rectal straining such as diarrhea. It can also be produced experimentally by surgically prolapsing the rectum through the anus from a laparotomy.

Primary treatment should be targeted at reducing the swelling due to vascular congestion and edema, prior to replacement of the prolapsed segment into the pelvic cavity. If necrosis has already occurred, then the prolapsed segment must be amputated and a colonic resection performed. If the prolapsed segment is healthy, then swelling can be reduced by rinsing with hypertonic solutions such as 50% glucose. The segment should be lubricated with a water-soluble lubricant and replaced by gentle digital manipulation. A purse-string suture can be placed around the rectum temporarily to help prevent recurrence while the swelling is reduced and the condition is stabilized. A variety of prostheses have been developed to place into the rectum during this phase.

If the rectum must be amputated, then it is best accomplished with surgical staples. However, the prolapsed section of the rectum may be surgically amputated to the level of normal tissue and manually sutured, using an interlocking continuous suture with synthetic absorbable sutures. In young animals, prostheses may be placed in the rectum and rubber bands applied over the prostheses to cause necrosis of the prolapsed section of the rectum. This technique is employed in agricultural situations in the field and generally should not be used in laboratory settings in which there is availability of general anesthesia and surgical technologies (Kjar, 1976; St-Jean and Anderson, 1999; Turner and Mellwrath, 1982). Recurrence is likely if the primary cause of the prolapse is not treated.

INTESTINAL AND MULTIVISCERAL TRANSPLANTATION

Heterotopic and orthotopic transplantation of the small bowel has been described in swine and thoroughly reviewed (Weih et al., 2011). They have also been used as a model of multivisceral transplantation. Both models are performed from a ventral midline incision that may extend the complete length of the abdomen in a complex model, such as multivisceral *en bloc* transplantation (Alessiani et al., 1998; Podesta et al., 1994; Pritchard and Kirkman, 1988; Pritchard et al., 1986; Ricour et al., 1983; Tsai et al., 2010).

An isolated loop of small intestine from the jejunum to the ileum is isolated and divided between intestinal clamps as described earlier for an intestinal anastomosis. The mesentery is divided, and the segment of the cranial mesenteric artery and vein supplying the graft are identified. In this region, the vessels will have the largest diameter. The donor is heparinized, and the segment of the artery and vein are divided. The graft is flushed with chilled heparinized crystalloid solution, and the donor is euthanized.

Using a ventral midline incision, the recipient's infrarenal aorta and vena cava are isolated. The graft is placed transversely into the abdomen, and the cranial mesenteric artery and vein are sutured

in turn in an end-to-side manner to the aorta and vena cava using Satinsky clamps. The transplanted graft is placed carefully to avoid torsion of the stump. The ends of the graft are isolated as stomas in the ventral abdomen as described earlier for Thiry-Vella loops (Figure 4.30).

For orthotopic transplant, the terminal ileum is used, allowing the surgeon to use the most accessible vessels supplying the graft. The segment of bowel is isolated as described earlier, except that only a branch of the mesenteric vessels supplying the segment is divided. Consequently, this may be done as a survival procedure for the donor, because the rest of the bowel is not devascularized as described for the heterotopic transplant. A reciprocal transplant may be performed with the recipient of this graft as well, if the experimental design allows it. The graft is sutured in an end-to-end pattern to the recipient's mesenteric vessels after a similar segment of small bowel has been extricated.

Multivisceral transplantation of the liver, stomach, duodenum, pancreas, small intestine, and a portion of the large intestine has been developed in swine (Figure 4.31). The donor is prepared in a standard fashion for a ventral midline incision from the xiphoid process to the pubis. The aorta and vena cava are isolated cranial to the liver and extending caudally to the kidneys, ensuring that the celiac, cranial mesenteric, and caudal mesenteric vessels are retained intact, but ligation of the lumbar branches is necessary. The esophagus is clamped and divided at its junction with the stomach, and the descending colon is divided distal to the major blood supply of the caudal mesenteric artery. The vessels supplying the kidneys, adrenals, and mesentery are sacrificed so that only the three major vessels listed earlier remain. The ligaments of the liver are divided so that all the *en bloc* viscera are mobilized.

Cannulae are placed in the proximal and terminal aorta, and cold perfusion is initiated after heparinization. The perfusate is removed using suction in the right atrium. After perfusion is under way, the remaining attachments are divided, and the block of viscera is transferred to a bowl of cold perfusate and preservation solution. The proximal end of the aorta is oversewn.

The recipient is prepared in a similar manner to the donor. A venovenous bypass is prepared between the external jugular vein and the femoral vein. The colon and stomach are transected leaving a portion of the antrum of the stomach intact. The abdominal aorta is preserved in its entirety, including the kidneys. The vena cava remains intact, except for the portion that is

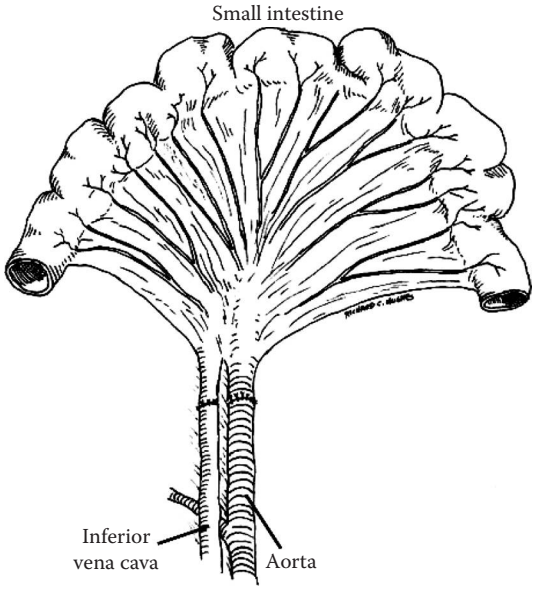

FIGURE 4.30 Segmental intestinal transplant with fistulated ends.

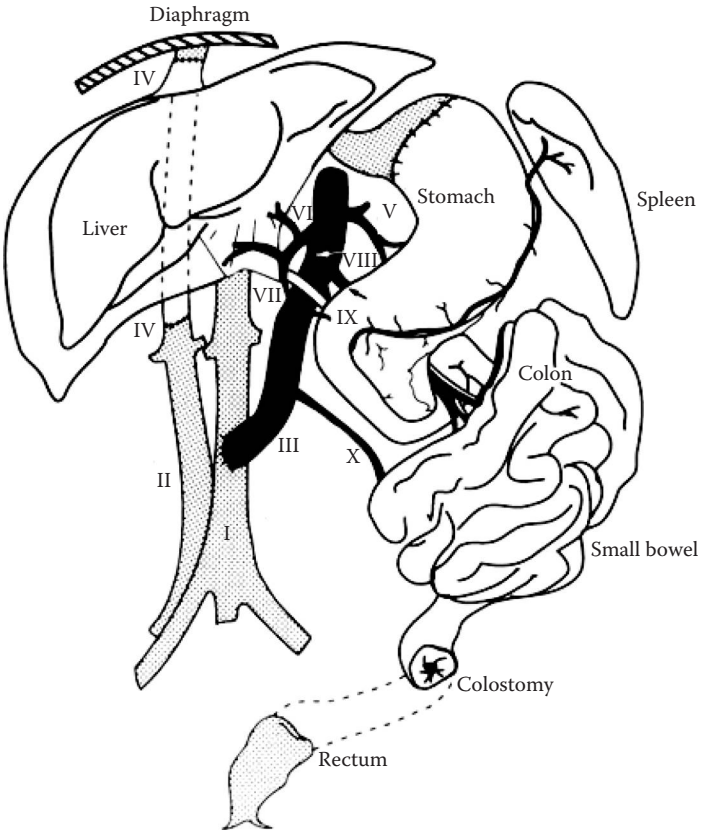

FIGURE 4.31 Diagram of multivisceral transplantation. (Reprinted from Podesta, L. et al. 1994. *Handbook of Animal Models in Transplantation Research,* Boca Raton, FL: CRC Press, pp. 231–242. With permission.)

intrahepatic. The vessels are transected starting with the liver, and venovenous bypass is initiated at the time that the celiac trunk and the cranial mesenteric artery are divided. This is the last step prior to removal of the organs.

The vessels of the donor organs are anastomosed into the recipient in the following order: suprahepatic vena cava, infrahepatic vena cava, and distal donor aorta to recipient infrarenal aorta in an end-to-side pattern. At this time, the aortic clamp is removed and the reperfusion process, including removal of the venovenous bypass, is initiated. The intestinal tract continuity is reestablished in a standard fashion, by sewing the donor stomach to the recipient stomach cuff in an end-to-side pattern, and reanastomosing the colon in an end-to-end pattern.

Intraoperative monitoring and homeostasis control is a substantial task with this type of surgery, and hemodynamic control is of prime importance. Postoperative monitoring and reinitiation of oral nutrition is also a substantive issue. The list of complications to be considered in addition to the issues of graft vs. host disease and rejection include hemorrhage, ascites, thrombosis, infection, and malabsorption. This type of study should include a multidisciplinary team approach to these issues.

VAGOTOMY

The vagus nerve may be transected intra-abdominally as the branches of the nerve traverse along the esophagus cranial to the esophageal sphincter at the cardia of the stomach (Figure 4.32). Using a midline approach, the stomach can be retracted caudally and the liver, cranially to expose the caudal esophagus. The fascia over the esophagus is opened with scissors, and the branches of the vagus

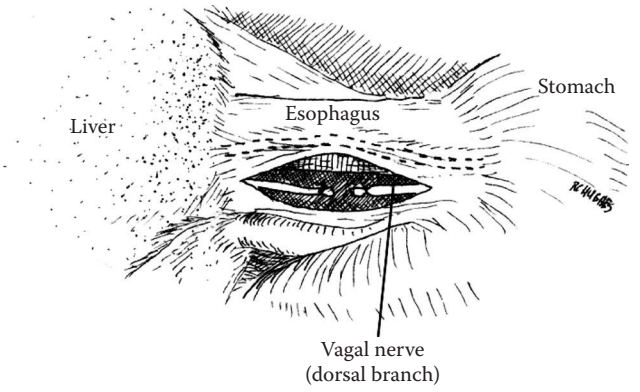

FIGURE 4.32 Technique of truncal vagotomy.

nerve can be observed along the ventral border on either side of the esophagus. The nerves can be transected with scissors and the fascia resutured. A section of the nerve can be removed to ensure that the transection remains permanent (Josephs et al., 1992; Swindle, 1983).

Selective vagotomy can be performed by tracing branches of the nerve, as they are associated with the blood vessels supplying the lesser curvature of the stomach. In a similar fashion to the truncal vagotomy described earlier, each nerve branch is isolated and severed. Manipulation of the vagus nerve has been associated with bradycardia and cardiac arrest in some cases. It is best to keep the animal atropinized during manipulation of the main branches of this nerve (Josephs et al., 1992).

INTRAPERITONEAL SEPSIS, SEPTIC SHOCK, AND HEMORRHAGIC SHOCK

Intraperitoneal sepsis leading to septic shock may be induced in swine by a variety of methods. Surgical incisions in various portions of the intestine leading to intestinal content leakage as well as infusion of lipopolysaccharides (LPSs) are standard methodologies in many species (Bathe et al., 1998; Greif and Forse, 1998; Hoban et al., 1992; Horstmann et al., 2002; Krones et al., 2005; Strate et al., 2003). Depending upon the clinical syndrome being investigated, perforations of the stomach, small intestine, and cecum will lead to development of a syndrome similar to the human situation. These procedures may be performed laparoscopically or endoscopically to avoid the complications of open abdominal incisional sepsis. Ischemia of the small bowel may also be induced by ligation of mesenteric vessels, or creation of pericardial tamponade by infusion of fluid into the pericardial sac, which leads to the development of decreased splanchnic blood flow. Reversible intestinal ischemia may be accomplished by reversing the pericardial tamponade (cardiogenic shock) or by using clamps rather than ligatures to decrease mesenteric blood supply to the intestine (Bailey et al., 1986). LPS infusions at a rate of 1 μg/kg over a 30- to 60-min time frame will also induce the symptoms of septic shock. Alternatively, *Escherichia coli* strain B7 may be infused into the peritoneal cavity at a rate of 2×10^{10} cfu/kg to produce sepsis nonsurgically.

The porcine model has the advantage of being a large animal model with a cardiovascular systemic response similar to that of humans. Swine initially develop a hypodynamic state which progresses to a hyperdynamic state within 24 h, with increased cardiac output, pulmonary hypertension, and decreased systemic vascular resistance. Multiple systemic organ failure occurs with chronic models (Hoban et al., 1992).

Hemorrhagic shock models in swine have been reviewed (Hannon, 1992). In general, two types of models are employed: fixed pressure (Wiggers model) and fixed volume hemorrhage. Fixed volume hemorrhage is more clinically applicable and relevant to the condition that occurs with trauma patients. A predetermined amount of blood is removed via a catheter implanted in the carotid artery

(Chapter 9). For awake models the catheter implantation procedure should be performed 1–2 weeks prior to the study to allow wound healing to occur and platelet levels to return to normal. Porcine models respond to various levels of hemorrhage in a similar manner to humans.

Endotoxic and hemorrhagic shock models can be performed without anesthesia; however, precautions have to be taken to ensure that animal pain and distress are monitored and controlled. In general, infusions of opioids, such as sufentanil, will have the least effects on cardiovascular parameters during these procedures. If an anesthetized animal is used for the studies, then the selection process of the anesthetic regimen must include a determination of the agents that will have the least effects on the parameters being studied.

MEDICAL TRAINING

Swine models have been used extensively for medical and veterinary surgical training, particularly open GI surgeries described in this chapter and laparoscopy training. At the authors' institution, swine stomachs with attached proximal small intestine have been removed after non-survival research or teaching procedures and frozen for later use. Upon thawing, simulated polyps were made from lymph nodes and sutured to the gastric mucosa. The attached small intestine was ligated distally to allow inflation of the *ex vivo* stomach and intestinal segment. Endoscopic training in polyp removal is then conducted with the *ex vivo* tissue secured in a specialized tray (EndoExpert tray, DeLegge Medical). Alternatively, explants can be purchased (DeLegge Medical, http://www.deleggemedical.com/index.php).

Ex vivo porcine bowel has also been used for medical resident education in obstetrics and gynecology (Thomas et al., 2010). The natural adhesions of the porcine spiral colon allow training in lysis of adhesions, a procedure often required in female pelvic surgeries. The strikingly similar anatomy of the stomach and proximal small intestine between pigs and humans also provides excellent translation of enterotomy closures from *ex vivo* porcine training to human surgery. Statistically significant improvements in resident understanding of principles, use of instruments, and comfort with surgical procedures were demonstrated using these porcine *ex vivo* training techniques (Thomas et al., 2010).

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5 Liver and Biliary System

M. Michael Swindle

CONTENTS

SURGICAL ANATOMY AND GENERAL PRINCIPLES OF SURGERY

From a surgical standpoint, the liver and gallbladder of the pig have relatively few differences from those of humans (Figures 5.1 and 5.2). The bile duct of the pig enters the duodenum 2–5 cm from the pylorus, separately from the pancreatic duct. The sizes of the bile duct and sphincter of Oddi are variable depending upon the size and breed of the pig (Table 5.1). The diameter of the bile duct seems to vary substantially even in the same size and breed of pig; however, it readily dilates when catheterized. Normal pressure in the common bile duct is usually less than 10 cm H_2O.

The liver contains six lobes: the left lateral, the left medial, the quadrate, the right medial, the right lateral, and the caudate, which contains a caudate process partially surrounding the caudal vena cava. The gallbladder is located in a fossa formed by the left and right medial and quadrate lobes in the right upper quadrant of the abdomen. The liver decreases as a percentage of body weight (BW) with age, from 3% at birth to 1.5% at sexual maturity in farm pigs (Yen, 2001). The segmental anatomy of the liver has been studied extensively and has been found to be very similar to that of the human in terms of the vascularity and biliary tree (Court et al., 2003; Farinon et al., 1981). Colored histologic sections are contained on the textbook DVD.

Bile production is variable, depending upon the weight and breed of the pig, frequency of feeding, and the composition of the diet, and normals must be established for each laboratory. As a general guideline, bile production in farm pigs is 0.6–1.1 mL/kg/h. This is extrapolated from studies of 24 h bile flow in instrumented farm pigs that had ranges of 45–60 kg BW and 24 h collections of 30–60 mL/kg bile (Yen, 2001), as well as from measurements made by the author on anesthetized animals. Composition of bile in swine (electrolytes, bile salts, phospholipids, cholesterol, mucus, pigments) is similar to that in most mammals, except that it has little cholic acid and a bile salt:phospholipid concentration of 9:1. Bile salts are reabsorbed from the small intestine and undergo enterohepatic recirculation, which helps to meet the metabolic demand (Yen, 2001).

The size and volume of the gallbladder is also variable, but in a sexually mature pig it measures approximately 3 cm wide by 5 cm long at the maximum points and contains about 25 mL of bile in a fasted animal at surgery. The hepatic bile duct exits the liver caudally dorsal to the gallbladder. It joins the cystic and common bile ducts shortly after entering the porta hepaticus. The bile duct is located in the porta hepaticus ventral to the portal vein and hepatic artery. It may be identified as a

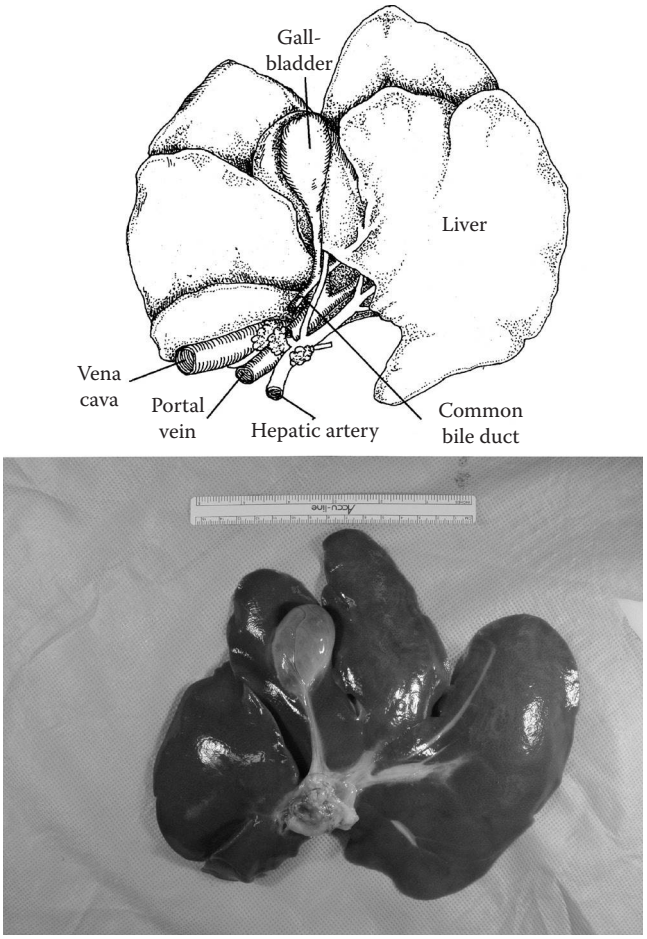

FIGURE 5.1 Visceral surface of the liver.

translucent tubular structure within the mesenteric attachments in this area, and it courses caudally to enter the duodenum as a separate duct from the pancreatic duct.

The hepatic artery and the portal vein enter the liver in close approximation to the common bile duct. Lymph nodes and fascia in the area make the dissection of these structures difficult. The liver of the pig is friable, but contains fibrous septations between lobules, which can be readily noted histologically (Figure 5.3) (Schantz et al., 1996).

The metabolic functions of the porcine liver are more similar to the human liver than even to those of most species of primates (Table 5.2). Consequently, interest in xenografic procedures has increased as immunosuppressive and transgenic technologies have improved (Institute of Medicine, 1996; Ryabinin, 1996). A porcine model of acute and chronic hepatic hemodynamics and metabolism of glucose, lactate, alanine, and glycerol was developed and validated because of its similarities to human metabolism (Drougas et al., 1996). In a comprehensive study of hepatic hemodynamics (Drougas et al., 1996) in chronically catheterized conscious farm pigs 8–16 weeks of age (20–70 kg BW), total hepatic blood flow was found to be approximately 1100 mL/min, hepatic arterial pressure was equivalent to aortic pressure, portal vein pressure was approximately 8 mmHg, and hepatic vein pressure was approximately 4 mmHg (Table 5.3). There is an inverse relationship between hepatic arterial blood flow and portal vein blood flow, in that an increase in blood flow in one circuit

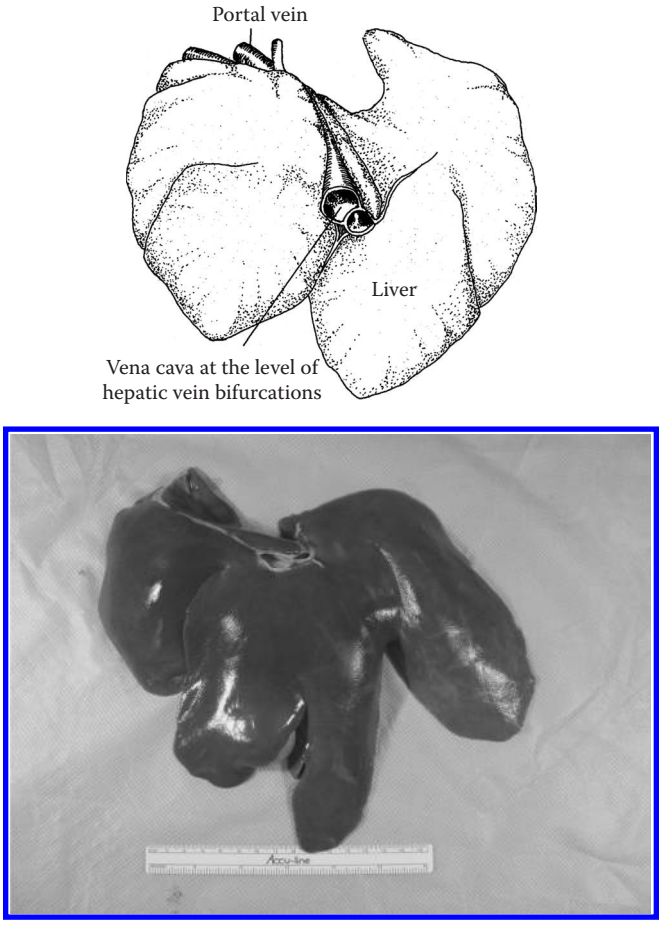

FIGURE 5.2 Diaphragmatic surface of the liver.

TABLE 5.1
Sample Sizes of Common Bile Duct Based upon Measurements

Breed	Weight (kg)	Bile Duct Diameter, ID[a] (mm)
Yucatan	29	3.0
	52	2.7–3.7
Yorkshire	16	3.0
	20	2.3
Landrace	47	2.7
	70	8–12

[a] ID = inside dimension.

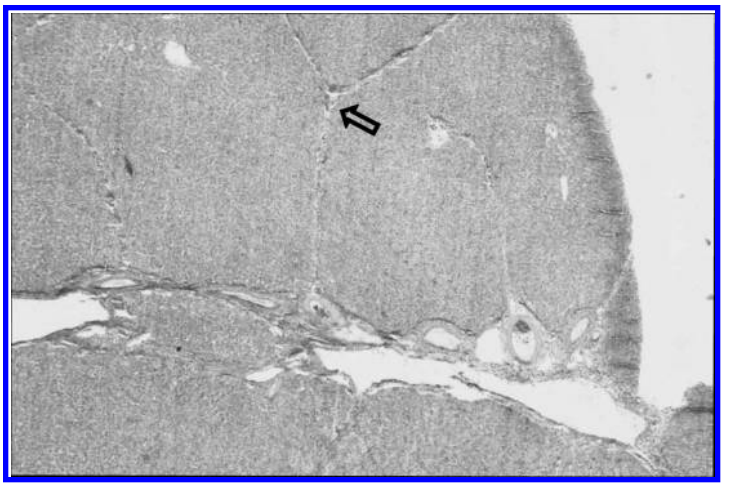

FIGURE 5.3 Histology of the liver illustrating fibrous septae (arrow). H&E, ×100.

leads to an increase in blood flow resistance in the other circuit. In general, approximately 25% of the cardiac output is included in total hepatic blood flow.

The cytochrome P450 system in swine has similar enzymatic activity to that in humans, except for the low level of CYP2C and the absence of CYP2D. Females have higher levels of some enzymes (CYP1A, CYP2E) than males (Skaanild and Friis, 1997, 1999). Bama miniature pigs have had their drug metabolizing activities compared with humans by using selective inhibitors of human CYP isozymes (Li et al., 2006). They found that the liver microsomes for metabolism of nifedipine and testosterone (CYP3A4) are similar to those in humans. Their results are summarized in Table 5.4. The oxidative biotransformation function of the liver is similar to that in humans; there is a high level of glucuronidation and acetylation, and low activity of sulfation (Skaanild and Friis, 1997). A recent review article on the porcine models in this system has been published (Puccinelli et al., 2011). More information is available in Chapter 15.

CHOLECYSTECTOMY

The gallbladder can be approached with a right paramedian celiotomy incision lateral to the mammary glands, starting cranially at the caudal border of the last rib (Figure 5.4). The length of the

TABLE 5.2
Hepatic, Gut, and Total Splanchnic Glucose Metabolic Data (Farm Pigs, 3–4 Months of Age)

		Mean ± SEM		
Variable	**Glucose**	**Lactate**	**Alanine**	**Glycerol**
Arterial value (mm/L)	4.6 ± 0.3	0.53 ± 0.07	0.30 ± 0.07	0.12 ± 0.02
Hepatic fractional extraction rate (%)	–	24 ± 4	21 ± 7	22 ± 5
Net hepatic balance (μmol/kg/min)	9.9 ± 4.0	4.2 ± 0.4	2.3 ± 1.1	0.68 ± 0.22
Net gut balance (μmol/kg/min)	2.0 ± 2.5	1.1 ± 0.5	0.73 ± 0.18	0.69 ± 0.19
Net splanchnic balance (μmol/kg/min)	7.7 ± 1.9	3.1 ± 0.2	1.5 ± 1.1	1.4 ± 0.3

Source: Reprinted from Drougas, J.G. et al., 1996, *Lab. Anim. Sci.,* 46(6): 648–655. With permission.

TABLE 5.3
Hemodynamic Data (Farm Pig, 3–4 Months of Age)

Variable	Units	Intraoperative Mean	Final Mean	Steady State (d)	*P* Value
CO	L/min	4.46 ± 0.08	7.19 ± 0.15	2.4	0.002
CAP	mmHg	65 ± 3	101 ± 2	1.8	0.0001
PVP	mmHg	5.3 ± 0.9	7.8 ± 0.5	2.1	0.07
HVP	mmHg	2.0 ± 1.0	4.3 ± 0.4	1.3	0.12
PAP	mmHg	11.7 ± 1.2	21.4 ± 2.1	3.2	0.08
JVP	mmHg	2.9 ± 1.2	1.0 ± 0.3	1.8	0.10
HR	Beats/min	112 ± 4	116 ± 4	5	0.35
HABF	mL/min	262 ± 33	116 ± 24	7.5	0.04
PVBF	mL/min	631 ± 91	880 ± 130	7.5	0.09
THBF	mL/min	799 ± 133	1132 ± 187	7.5	0.19
HABF	%	35.0 ± 3.0	15.5 ± 2.7	7.5	0.001
PVBF	%	65.0 ± 3.0	84.5 ± 2.7	7.5	0.001

Source: Reprinted from Drougas, J.G. et al., 1996, *Lab. Anim. Sci.,* 46(6): 648–655. With permission.

Note: Intraoperative values measured under isoflurane anesthesia. Final mean values were from awake, chronically catheterized pigs. CO = cardiac output, CAP = carotid artery pressure, PVP = portal vein pressure, HVP = hepatic vein pressure, PAP = pulmonary artery pressure, JVP = jugular vein pressure, HR = heart rate, HABF = hepatic artery blood flow, PVBF = portal vein blood flow, THBF = total hepatic blood flow.

incision will be variable, depending upon the size of the pig. Care should be taken to avoid the cranial epigastric vessels in the same area. The area of interest can be isolated with either laparotomy sponges or self-retaining retractors. The ventral tip of the gallbladder should be grasped with gallbladder forceps and gentle finger dissection can be used to separate it from the liver. The technique involves applying gentle pressure caudally with the forceps while making gentle side-to-side movements with the tip of the finger. Experience has shown that only in larger adult swine is it necessary to use other surgical instruments either to cut or cauterize during the dissection. Rupture of the gallbladder is rare if gentle handling and atraumatic forceps are used. The cystic

TABLE 5.4
Enzyme Activities (Mean ± Standard Deviation) of Various Marker Reactions in Liver Microsomes from Chinese Bama Miniature Pigs and Humans

Substrate	Reaction	Activity[a] (Mean ± Standard Deviation) Bama Miniature Pig (*n* = 6)	Human (*n* = 3)
Nifedipine	Oxidation	1414 ± 122	1360 ± 1102
Testosterone	6β-Hydroxylation	1140 ± 42	2065 ± 1380
Phenacetin	0-Deethylation	312 ± 49[b]	743 ± 236
Coumarin	7-Hydroxylation	30.8 ± 8.4[b]	392 ± 148
Dextromethorphan	0-Demethylation	2387 ± 264[b]	76 ± 59
Chlorzoxazone	6-Hydroxylation	278 ± 45[b]	538 ± 146

Source: Reprinted from Li, J. et al., 2006, *Comp. Med.,* 56(4): 286–290. With permission.

[a] Measured in pmol of product formed/min per mg microsomal protein.

[b] $P < 0.05$ vs. value for human enzyme.

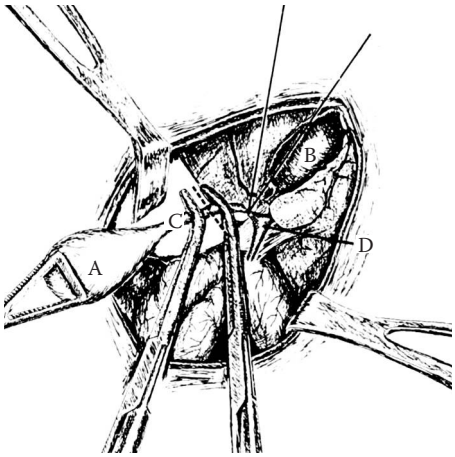

FIGURE 5.4 Cholecystectomy. A—gallbladder, B—fossa of gallbladder, C—cystic duct, D—common bile duct.

duct is cross-clamped with two pairs of right-angle forceps and cut between them following the gallbladder dissection. A double ligature with synthetic absorbable sutures is applied to the stump of the duct. Hemostasis in the gallbladder fossa is usually achieved by packing the area with gauze sponges for 3–5 min. Oxycellulose sponges may be placed in the fossa for extra security against hemorrhage. Prior to closure of the incision, any bile that has leaked into the peritoneal cavity should be removed by flushing and suction. The peritoneum, muscle layers, and skin are closed routinely (Swindle, 1983).

CANNULATION OR CONSTRICTION OF THE COMMON BILE DUCT

Reentrant cannulation of the common bile duct may be achieved with reasonable success if close attention is paid to the design of the cannula (Figures 5.5 and 5.6). Generally, this procedure is performed simultaneously with a cholecystectomy to ensure that the bile sample collected is freshly produced by the liver and not stored in the gallbladder. The size of the bile duct will vary substantially, even at similar weights within the same breed. Typically, it is approximately 3 mm (inside dimension) in a 20–30 kg pig. However, most swine greater than 15 kg will accommodate at least a 5-French (Fr) catheter, and adult pigs can be cannulated with much larger catheters (Table 5.1).

Complete ligation of the common bile duct without cholecystectomy will result in great dilatation of the gallbladder and ductal system within a few hours. The common and cystic ducts will be impressively dilated with an accompanying increase in intraductal pressure. In spite of the amount of dilatation, the onset of clinical symptoms of biliary stricture is delayed and variable between breeds and sizes of swine. As a general rule, however, swine will become symptomatic 3–4 weeks following surgery. Cholecystectomy at the time of placing the stricture will result in a more rapid onset of symptoms.

Total ligation or constriction of the bile duct should be performed close to the sphincter of Oddi at the distal end of the duct. The ligature can be made to include a premeasured rod laid along the bile duct. After the ligature is placed, the rod can be removed to achieve a stricture instead of a total blockage.

If cannulation of the duct is to be performed, then it should be a reentry cannulation, unless the procedure is to be performed acutely or the animal supplemented orally with bile salts. The general principle of reentry cannulation involves placing a small catheter proximally into the common bile duct, exteriorizing it with a port or valve on the skin to collect samples, and then returning the bile via another catheter into the duodenum. There are insignificant differences

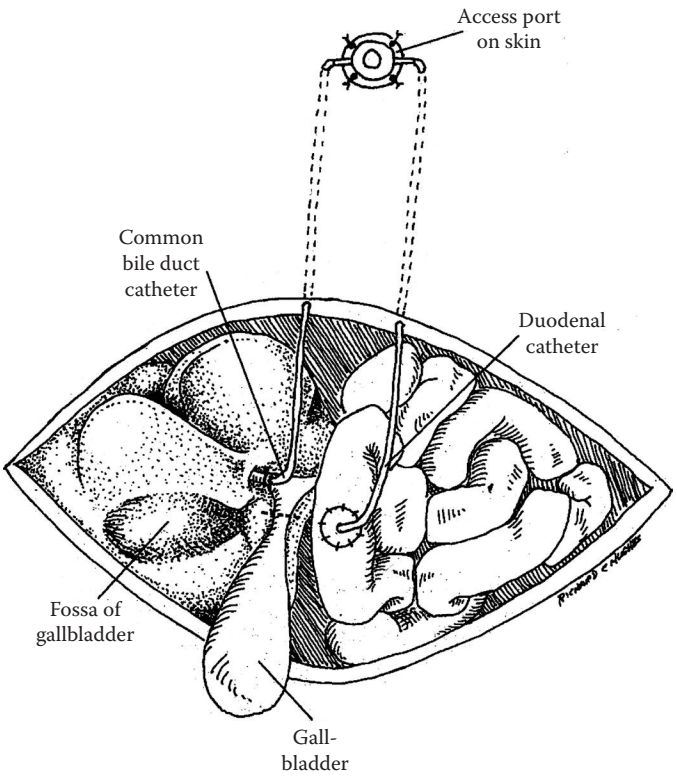

FIGURE 5.5 Reentrant cannulation of the bile duct.

between having the intestinal catheter enter through the sphincter of Oddi or another location in the proximal duodenum.

Reentry biliary catheters are commercially available or may be custom designed. Catheters within the abdomen are generally constructed of silicone for flexibility. Having a combination polyethylene catheter helps avoid kinking of the catheter. The tips of the catheter placed inside

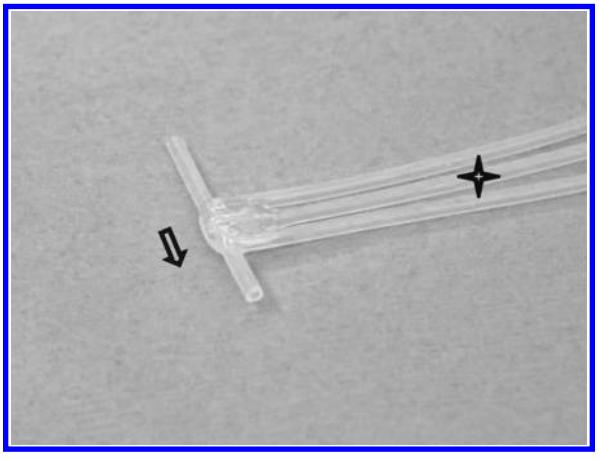

FIGURE 5.6 Reentrant cannula for bile duct and other continuous flow ducts (lymphatic, urinary). The arrow indicates the direction of normal bile flow. The star is on the catheter which inflates and deflates a balloon for occlusion of flow.

the duct are more readily situated if they are made of the sturdier polyethylene material. Having suture retention beads on the catheter helps to ensure retention within the duct. Reentry catheters typically cannulate both proximal and distal ends of the duct with a flow pathway from the hepatic side of the duct to a sampling port at the skin surface and then reflow into the duodenal side of the duct. Using this type of catheter, the flow of bile is interrupted for sampling by inflating a balloon in the catheter. Other types of catheters are inserted into the duct toward the liver with the distal end of the duct ligated. In this type of catheter, the flow of bile goes to a sampling port on the surface of the skin and then reflows into the duodenum through a catheter inserted into the duodenum. The reentrant catheter can be placed into the proximal duodenum, taking care not to disturb the pancreas or pancreatic duct. As per the description of the cannulae for an intestinal access port (Chapter 4), the catheter can be placed into the lumen of the duodenum after making a stab incision. The catheter should be open ended instead of slitlike according to the previous discussion of intestinal access ports. This modification is necessary because of the low pressure in the biliary system. In either design, the flow of bile from the liver to the duodenum is only interrupted during the sampling procedure.

A midline celiotomy is performed from the xiphoid process to the umbilicus. Self-retaining retractors and wetted laparotomy sponges are utilized to visualize the bile duct system in the right apical quadrant of the abdomen (Figures 5.7 and 5.8). The gallbladder is visualized and squeezed lightly to illuminate the common bile duct with bile. The fascial tissue is dissected to expose the common duct. A wetted sponge is placed under the duct and an elastic vessel loop is preplaced cranial and caudal to the entry site for the catheter (Figure 5.9). After retraction, the duct is nicked with iris scissors. A vascular pick is utilized to open the lumen for catheterization (Figure 5.10). The catheter is inserted into the abdomen in the right cranial paramedian area of the abdominal wall, distant to the celiotomy incision.

When utilizing the T-tube catheter with an occlusal balloon, the tube is inserted cranially and then caudally in the common bile duct. Ligatures are placed around both ends of the catheter to keep it in place.

When utilizing the bypass catheter, the catheter with retention beads is placed retrograde into the duct. Ligatures are utilized cranially and caudally after insertion. The second duodenal catheter is inserted into the cranial duodenum with the retention bead inside the lumen. The catheter is held in place within the lumen with a purse-string suture and simple interrupted sutures through the cuff and the serosa.

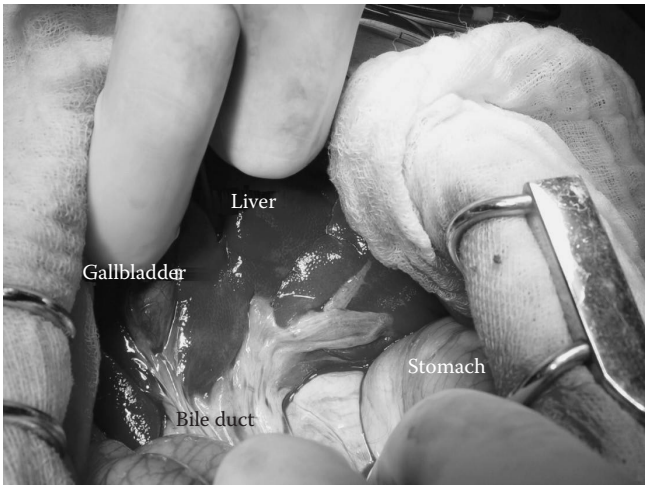

FIGURE 5.7 Surgical anatomy of the portal hiatus of the liver.

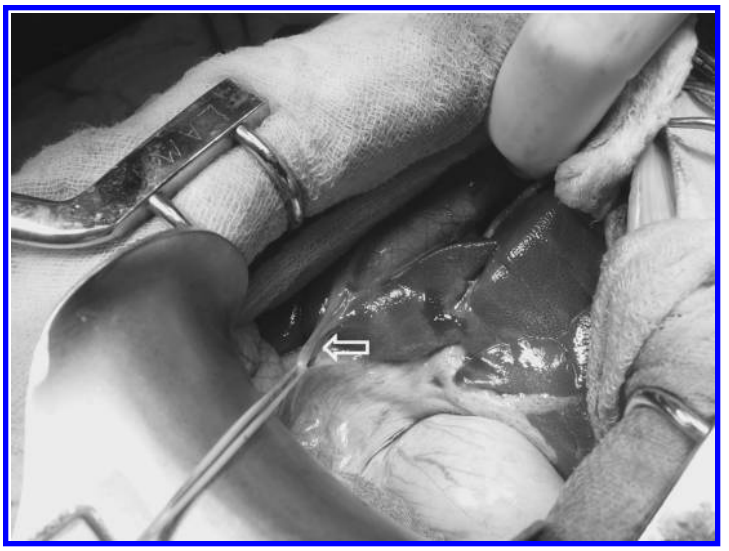

FIGURE 5.8 Surgical anatomy of the viscera and vasculature caudal to the portal hiatus.

If a cholecystectomy is to be performed, it is done at this time. The sampling port, which has been preplaced, is flushed with saline and a port pocket is made to place the device subcutaneously. For a procedure of only a few days, the sampling port may be sutured to the skin and protected with a circumferential bandage.

The abdomen is closed after sutures are placed in the peritoneum at the entrance of the two cannulae into the abdominal cavity to prevent a possible dehiscence. The midline incision is closed in a routine manner as described previously (Hand et al., 1981; Swindle, 1983; Terblanche and Van Horn-Hickman, 1978).

Patency of the catheter should be rechecked and biliary flow observed as soon as this procedure is finished. The device is flushed daily with saline if the reentry duodenal catheter is used. Flushing or filling the balloon device with hygroscopic or hypertonic solutions may result in the occlusal

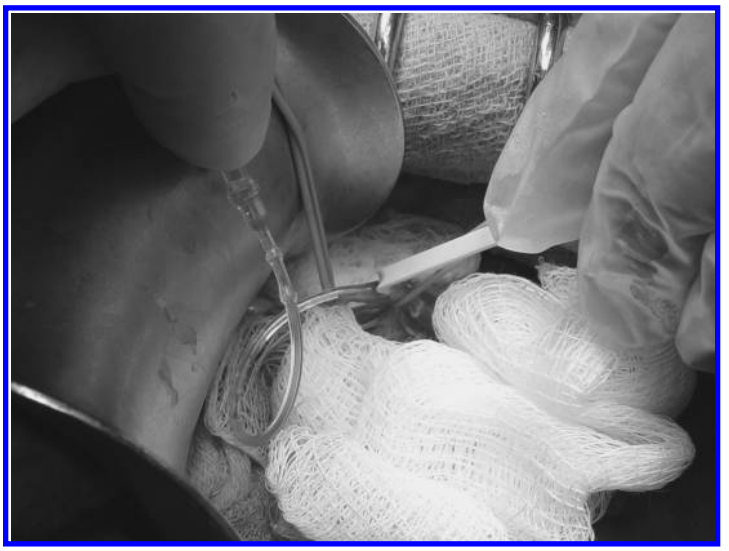

FIGURE 5.9 Isolation of the common bile duct with an elastic vessel loop.

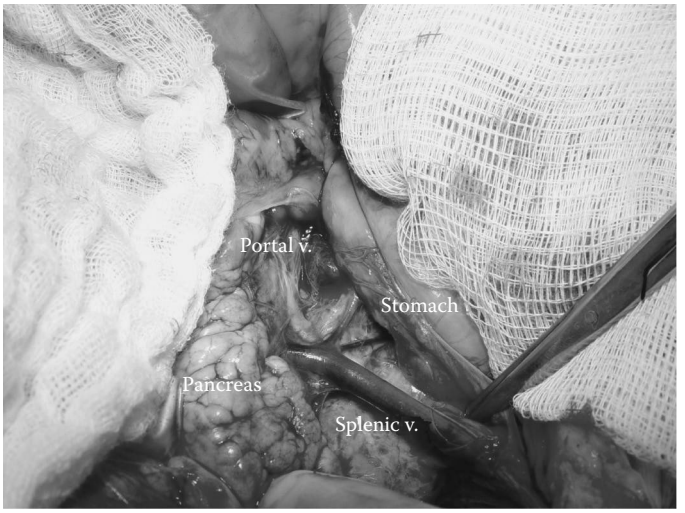

FIGURE 5.10 Use of a vascular pick to insert a catheter into the bile duct.

balloon closing spontaneously because of the infiltration of body fluids. These catheters may be maintained for months if the sampling ports are implanted subcutaneously and a meticulous aseptic technique is utilized. Ascending infections from the intestine to the biliary system and kinking of the catheter, resulting in biliary stasis and liver failure, are two potential complications of this procedure.

A porcine model of intraoperative radiation therapy combined with biliary-enteric bypass has been developed (Kaiser et al., 2005). Following resection of the gallbladder and common bile duct, pigs received 20–40 Gy of radiation. A Roux-en-Y hepaticojejunostomy is performed between the jejunum from the area of the ligament of Treitz and the residual extrahepatic biliary duct. Additional studies in pigs with biodigestive anastomosis receiving radiation therapy of the liver hilum demonstrated that 20 Gy was sufficient to provide localized necrosis in potentially neoplastic tissue (Juntermanns et al., 2014). In 20–25 kg Landrace pigs, an autologous jugular vein graft with a biodegradable endoluminal stent (6 mm in diameter) was used to repair defects in the bile duct. Animals were successfully followed for 6 months postoperatively (Heistermann et al., 2006). The same types of tissues and a stent were also used to create a neo-bile duct as an alternative to a hepaticojejunostomy (Palmes et al., 2009). As an alternative to the end-to-side biliodigestive anastomosis procedure 25–30 kg Landrace swine were used to develop a single-stitch telescopic bilioenterostomy technique (Vrochides et al., 2012). After ligation of the bile duct at the level of the duodenum it was dissected free to the cystic duct which was approximately 4 cm. A 5 cm biliary stent was inserted 3 cm into the bile duct and then into the lumen of the intestine through a small electrocautery enterotomy. A single suture was inserted through the intestinal wall and the bile duct-stent distal from the enterotomy. The wound healed without complication with slight structuring of the bile duct which became totally integrated with the intestinal wall over the 6 week period of the study.

PARTIAL HEPATECTOMY, LIVER BIOPSY, LIVER FAILURE, AND INTRAHEPATIC CANNULATION

Liver biopsies and partial lobectomies can be performed in swine using the same surgical techniques as for other species. The tip of a lobe can be removed by applying a suture ligature circumferentially around a small segment, referred to as the *guillotine technique*. Alternatively, a larger segment can be removed by cross-clamping with noncrushing intestinal clamps and placing overlapping mattress

sutures or staples in the viable segment to provide hemostasis. Oxycellulose sponges may be applied to the cut edges to provide additional hemostasis. Percutaneous needle biopsies may be performed by using trucut biopsy needles passed caudomedially through either the tenth or eleventh right intercostal spaces in the dorsal half of the lateral wall (Swindle, 1983). A nylon device (LoStRiT) using a ratchet mechanism was successfully tested in 28–33 kg pigs as a more rapid method of liver parenchymal transection (Vrochides et al., 2014). A bleeding liver model to compare hemostatic products has been described (Lewis et al., 2013). Heparinized pigs 49–56 kg had multiple circular (1 cm diameter, 3–4 mm deep) lesions created on the liver surface with a drill loaded with medium grade sandpaper. Swine, 28 kg, have been developed as a liver trauma model leading to hemorrhagic shock (Karmaniolou et al., 2013). Left lobe resection was performed to create hemorrhage leading to a mean arterial pressure of 35–40 mmHg for 40 min before experimental treatment was instituted. The liver was surgically repaired after the 40 min interval and animals were maintained under anesthesia for up to 6 h postprocedure.

Intrahepatic veins and ducts may be cannulated by modification of the procedure described earlier (Figure 5.11). After cross-clamping the left lobe of the liver, an incision is made from the edge into the parenchyma until a central vein is transected. The vein may be cannulated with an appropriate catheter. Biliary structures may also be cannulated. The incision is closed, as described previously, with suturing of oxycellulose sponges over the incision. The cannula can be externalized after surgical fixation in the abdominal cavity (Svendsen, 1997). The right lobe has been cannulated using a modified balloon catheter inserted from the external jugular vein into the right hepatic vein from the inferior vena cava (Katsimpoulas et al., 2011). The technique was developed to study whether large volumes of solution containing plasmid DNA could be injected without hepatic injury or heart failure. Two hundred milliliters of solution were successfully injected at a rate of 20 mL/s into the right lateral liver lobe without significant injury to the liver.

A model of segmental liver necrosis can be induced by hepatic artery embolization with biocompatible polyurethane (Maurer et al., 2000). Using a ventral midline abdominal incision, the hepatic artery to one lobe was dissected and cannulated. The polyurethane was infused at a rate of 1.5 mg/min. The liver hilus was checked for retrograde embolization, and recurrent arteries returning to the gallbladder, stomach, and gastrointestinal (GI) tract were ligated. The technique led to sharp hepatic necrosis without significant systemic effects due to occlusion of the arterial tree and concomitant portal vein occlusion. Ablation of hepatic parenchyma can be performed

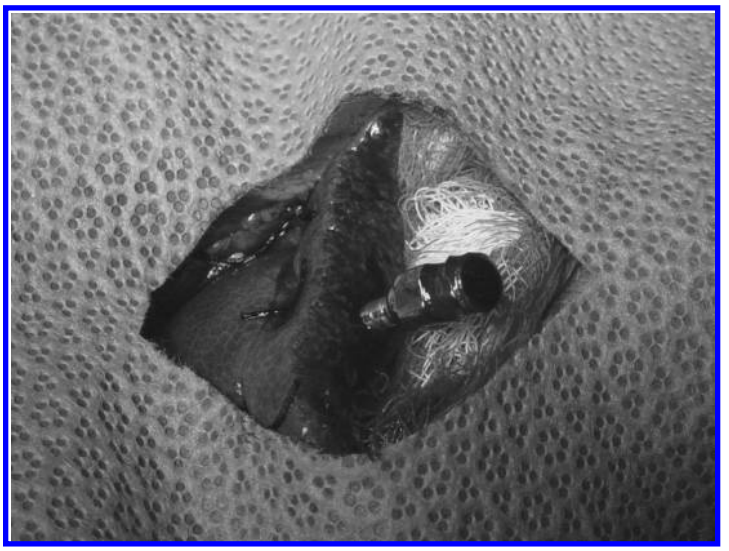

FIGURE 5.11 Cannulation of an intrahepatic vein.

with radiofrequency catheter techniques, and some animals will develop portal vein thrombosis if the procedure is performed <5 mm of the intrahepatic portal vein (Frich et al., 2006).

A model of fibrous cholangitis has been developed in pigs by oral administration of 1,4-phenylene diisothiocyanate (DITC), followed by administration of 100 mg/kg of DITC in early gestation (5th–7th week) of pregnant pigs. Administration of the drug to pigs which had not been exposed to it *in utero* did not produce the same syndrome and administration of DITC to neonates who were exposed did not have an additive effect. The histologic patterns in the pigs were similar to those found in humans (Lainakis et al., 2014).

Acute fulminent hepatic failure can be created by total hepatectomy or devascularization of the hepatic artery and vein with portocaval shunting (Fruhauf et al., 2004; Tsaroucha et al., 2013). From a midline abdominal approach, the infrahepatic vena cava and portal vein were isolated and circled by a tourniquet. Isolation and division of the hepatic artery and all accessory branches plus the common bile duct was performed. The vena cava is partially cross-clamped and an end-to-side anastomosis of the portal vein was performed to create a portocaval shunt. Animals will die in 12–21 h postprocedure with elevated serum liver enzymes, lactate, and ammonia levels. The total hepatectomy is performed in the same manner as for the donor liver in liver transplantation procedures (later text). A Y-shunting procedure between the two vena cava segments and the portal vein is performed. Landrace pigs 25–30 kg were utilized in a similar manner described by Fruhauf et al. (previous text) to study the effects of various treatment methods with albumin in acute liver failure (Tympa et al., 2011). That group performed a 70% right hepatectomy and the liver remnant was deprived of blood flow for 150 min. The models are useful for studying rescue methods using therapies such as bioartificial liver support system. Death is due to circulatory collapse and cerebral edema.

Preconditioning to prevent damage from ischemia-reperfusion in liver surgery was studied in 18–23 kg pigs (Giovanardi et al., 2009). The liver was isolated by dividing the suspensory ligaments; ischemia was induced by occlusion of the portal vein, hepatic artery and all the collaterals, and a venovenous bypass was initiated between the splenic vein and the left internal jugular vein. Ten minutes of ischemic preconditioning followed by 90 min of ischemia and 180 min of reperfusion was demonstrated to be protective by means of mitochondrial preservation and inhibition of caspase-3 activity. Another model of ischemia-reperfusion was created by occlusion of the hepatoduodenal ligament with a Satinsky clamp after the liver was mobilized (Kyriazi et al., 2011). The left lobe was resected and 30 min of ischemia was allowed for the remnant lobes followed by 6 h of reperfusion. Iron chelation was demonstrated to be protective of hepatic injury.

Complete lobectomies may be performed by modification of the techniques described earlier for larger segments. The left lateral is the most easily removed lobe. Its excision is discussed in the following section (Camprodon et al., 1977; Kahn et al., 1994; Procaccini et al., 1994).

LIVER TRANSPLANTATION

Orthotopic, heterotopic, and xenografic transplantations have all been studied in this species. Hepatic transplantation in swine was developed because of the anatomic and physiological similarities of the porcine liver to human liver, notably because of its ability to withstand total portal vein occlusion up to 10 min, resistance to hepatic venous sphincter vasospasm, and its relative resistance to immunologic rejection post-transplantation. This last characteristic may be due to the use of related individuals from linebred herds of swine, rather than other characteristics of its immune system. Allografts, autografts, and segmental and auxiliary liver grafts have all been described in detail. Multivisceral transplantation including the liver has been previously described (see Chapter 4, Figure 4.31) (Calne et al., 1967; Dent et al., 1971; Flye, 1992; Gadacz, 1988; Hickman et al., 1971; Kahn et al., 1994; Leal et al., 2013; Mizrahi et al., 1996; Oldhafer et al., 1993; Pennington and Sarr, 1988; Procaccini et al., 1994; Ryabinin, 1996; Sika et al., 1996; Terblanche and Van Horn-Hickman, 1978; Terblanche et al., 1967).

For swine, in all phases of the surgery, the mean arterial pressure should be maintained at 60–70 mmHg, and swine should be kept normothermic by adequate perfusion with maintenance fluids. Animals need to be fasted from solids for 48 h to empty the bowel. Intestines may be packed off from the hepatic region with wetted laparotomy sponges or placed in a plastic bowel bag. Anesthesia, maintenance of homeostasis, and appropriate perioperative care are as essential to the success of this procedure as performing the surgery (Eisele et al., 1986; Mizrahi et al., 1996; Stump et al., 1986).

Donor Liver

Swine are anesthetized, and a midline celiotomy incision is made from the xiphoid process to the pubis. For the donor procedure, the sternum should be split for greater exposure. The falciform, left triangular, and gastrohepatic ligaments are divided. The porta hepatis is dissected free from the peritoneum to the level of the pancreas. This will expose the portal vein and hepatic artery. The caudal vena cava is dissected free of its peritoneal attachments from the level of the adrenal glands cranially through the diaphragm. Generous lengths of these vessels should be dissected to have adequate vessel length for reanastomoses during reimplantation. Consequently, their branches are ligated. At the time of harvest, the common bile duct, hepatic artery, portal vein, and vena cava are ligated and divided. The donor liver is cooled and flushed with a crystalloid solution or a preservation solution. The perfusion is performed through the portal vein during harvesting. Harvesting of the donor liver should be timed closely to coincide with the preparation of the recipient for transplantation. Prior to implantation, the excess tissue is trimmed from the liver. Blood from the donor pig should be collected by exsanguination and used postoperatively as required in the recipient pig.

Allograft and Autograft Implantation

The recipient for whole-organ transplant is prepared in a manner similar to the preparation of the donor animal. An external jugular vein cutdown is performed to facilitate the bypass technique. A catheter is inserted into the splenic vein proximally in the heparinized recipient, and the primed catheter is placed into the external jugular vein to provide a passive bypass. If circulatory function is not adequate, then mechanical pumping may have to be provided. The same structures listed earlier for the donor organ are transected after initiation of the bypass and clamping of vessels with vascular clamps. Vascular suture in a continuous pattern is used to reanastomose the vessels in the following order: the prehepatic caudal vena cava, 90% of the infrahepatic vena cava, the portal vein, completion of the infrahepatic caudal vena cava, and the hepatic artery. After anastomosing the first two vessels, the liver is allowed to distend with blood by clamping the donor infrahepatic caudal vena cava prior to completing the anastomosis of the two segments of the vessel. Prior to tying the last knot of the anastomosis, the bypass segment is clamped, and the incomplete vascular connection is used as a vent and checked to ensure that air is not present, to remove 50–100 mL of waste blood after reperfusing the liver, and to see that the portal and vena caval circulations are restored. The hepatic artery is reanastomosed in a routine end-to-end manner; if a Carrell patch was taken during harvesting of the donor organ, it is reanastomosed to the aorta. The bile duct is the last connection and may be sutured using an intraluminal stent to guide the anastomosis (Figure 5.12).

An auxiliary liver transplant can also be performed by implanting the second liver into the right renal fossa of a recipient following excision of the kidney. The vascular anastomoses are hepatic artery or donor aorta to infrarenal aorta, hepatic vein, or infrahepatic caudal vena cava to infrarenal caudal vena cava, oversewing of the suprahepatic vena cava, portal vein to superior mesenteric vein, and common bile duct to a Roux-en-Y limb of small bowel (Kahn et al., 1994).

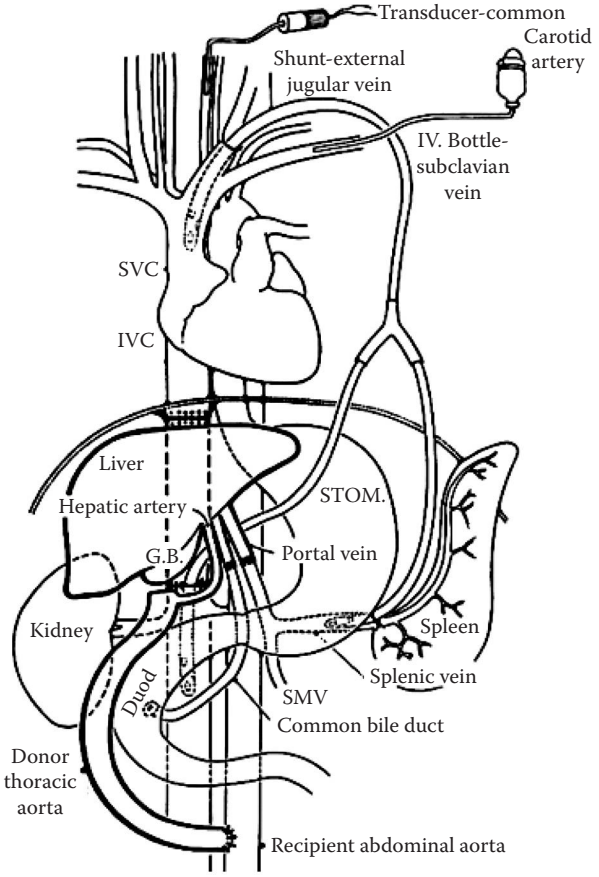

FIGURE 5.12 Orthotopic liver transplantation in the pig. During the anhepatic phase, venous blood is shunted from the portal system via the splenic vein and from the infrahepatic vena cava to the jugular vein. (Reprinted from Flye, M.W., 1992, *Swine as Models in Biomedical Research*, Ames, IA: Iowa State University Press, pp. 44–56. With permission.)

Segmental Liver Grafts

A living donor may be used to transplant the left lateral lobe of the liver into a recipient. Approximately 10 g/kg BW of liver tissue is necessary to provide normal metabolic functions. The segmental graft is harvested by incising the capsule and finger fracturing the parenchyma along the fissure of the lobe. The branches of the hepatic artery, hepatic vein, and portal vein are ligated, and the graft is treated as described earlier. The recipient liver is excised leaving the vena cava intact, and venovenous bypass is instituted as described earlier. Reimplantation is made into the site of the excised liver by anastomosing the hepatic veins, followed by the portal vein, the hepatic artery, and the bile duct (Camprodon et al., 1977; Procaccini et al., 1994). As an alternative to hepatic arterial flush in living donor transplant, 14–16 kg pigs were used to study retrograde arterial flush of the liver graft via the portal vein (Liu et al., 2009). The arterial flush only worked well when there was occlusion of the hepatic venous outflow on the back table.

Postoperatively, care must be taken to provide adequate fluid therapy intravenously and to avoid solid food for 24–48 h. Aspirin may be given as an anticoagulant, depending upon the size of the vascular anastomoses. Systemic antibiotics are indicated. Immunosuppressive agents may be given depending upon the protocol. Pigs with orthotopic transplants have been studied for the efficacy

of tissue impedance changes to detect acute liver graft rejection as compared to biochemical measurements (Harms et al., 2001).

HEPATIC XENOPERFUSION

With minor success in the past, porcine liver has been utilized clinically as a bridge to transplantation in an effort to prevent fatal levels of ammonia and other toxic metabolites from accumulating in the patient (Norman et al., 1966). Devices containing porcine hepatocytes with filtration systems to prevent immunologic reactions to the cells have also been investigated. A renewed interest in the

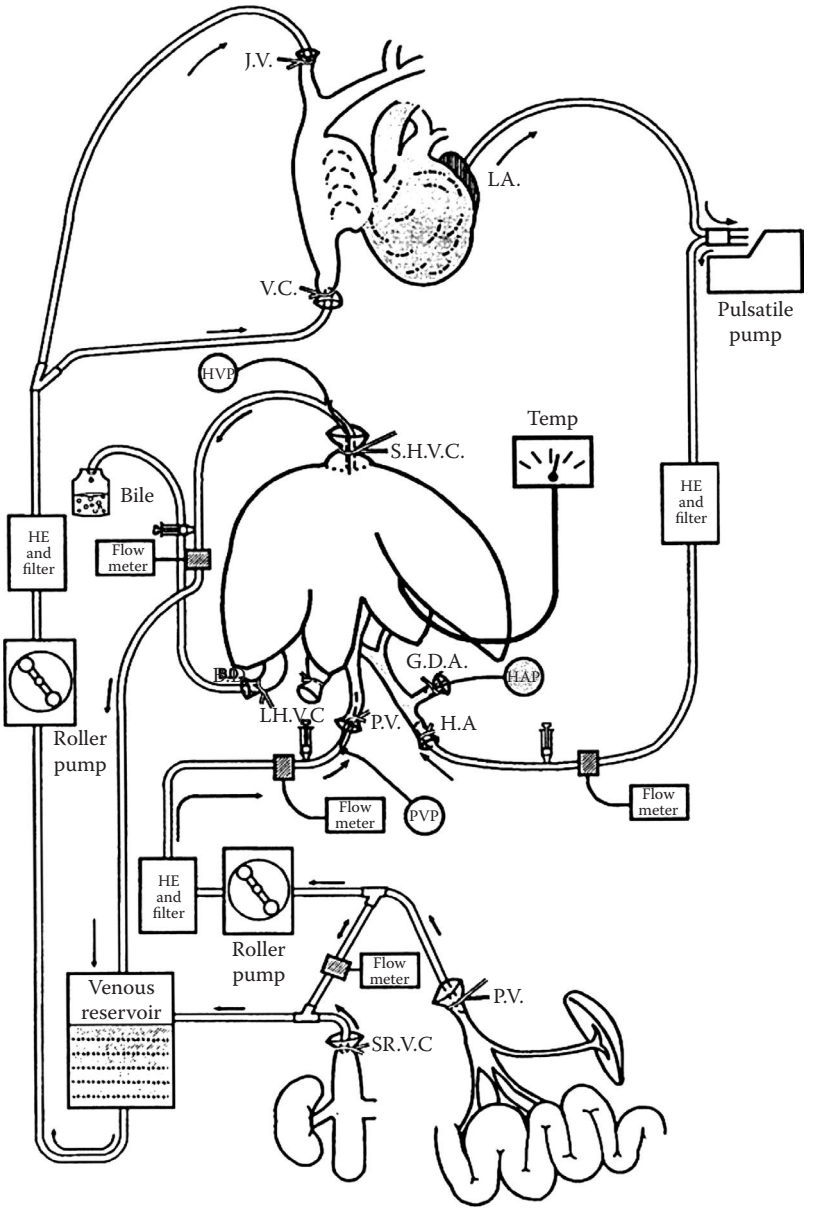

FIGURE 5.13 Isolated *in situ* liver perfusion system. (Reprinted from Travis, D.L., Paulsen, A.W., and Genyk, Y., 1996, *J. Invest. Surg.,* 9(2): 131–147. With permission.)

procedure has developed because of the possibility that transgenic manipulation of the donor swine will result in an increased success rate (Adham et al., 1996; Argibay et al., 1996; Collins et al., 1994; Mizrahi et al., 1996; Mora et al., 2002; Palmes et al., 2000; Pohlein et al., 1996; Terajima et al., 1996, 1997; Travis et al., 1996).

The liver may be harvested following the procedure for donor liver harvesting, discussed earlier in the section on liver transplantation. Female swine, 20–30 kg in BW, are preferred for the procedure because of the size of the liver and vessels. If the system is to be used for clinical xenoperfusion, then the infectious disease precautions in Chapter 14 should be followed. Modifications to the dissection technique that may be applicable have been described by Mizrahi et al. (1996) and Travis et al. (1996). Dual cannulation of the portal vein and hepatic artery has been shown to be superior to vessel perfusion of the portal vein only (Mora et al., 2002).

The modifications of Mizrahi et al. (1996) include approaching the portal vein through the splenic vein for cannulation and not occluding the inflow or outflow vessels of the liver during cold perfusion. The technique also involves placing the GI viscera in a sterile plastic bag to facilitate manipulation during the dissection.

Travis et al. (1996) also recommended adrenalectomy to prevent catecholamine production in the development of an isolated perfusion model for the study of pharmacological agents. Their system provides an *in situ* functioning liver (Figure 5.13).

When the system is to be used clinically for xenoperfusion, the isolated liver is kept in a sterile pan of iced saline and perfused through an extracorporeal system. The system involves shunting the human blood through the portal vein and suprahepatic vena cava to provide inflow and outflow lines. The hepatic artery is used for infusion in addition to the portal vein. Separate infusions with oxygenated blood are required. Using a two-pump system, the blood is circulated from the femoral vein of the patient through the liver and back into the venous system of the patient. The liver must be flushed with saline to exclude all porcine hematogenous products prior to connecting to the circuit. Hepatic hemodynamics in the isolated liver must be monitored during this procedure. Flow rates of approximately 1 mL/g of liver per minute have been recommended for the portal vein and approximately 25% of that rate for the hepatic artery (Terajima et al., 1997).

Liver function is monitored by measuring bile production and oxygen consumption, and by clinical observation of the organ for changes in color and consistency. Livers will develop progressive hypoalbuminemia and hypoproteinemia. These techniques are still experimental and will undoubtedly undergo further modification.

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6 Pancreas and Spleen

M. Michael Swindle

CONTENTS

SURGICAL ANATOMY AND GENERAL PRINCIPLES OF SURGERY

The pancreas of the pig is extensive, and the tail follows the lesser curvature of the stomach from the spleen and left kidney to a position along the proximal duodenum (Figures 6.1 through 6.5). The majority of the pancreas is retroperitoneal. The head of the pancreas gives rise to the body, tail, and an uncinate process. The pancreas encircles the portal and superior mesenteric veins and extends dorsally to the region of the left kidney. The pancreatic duct is composed of two separate ducts draining the tail and body. They anastomose to form the common pancreatic duct (accessory duct) immediately prior to the pancreatic sphincter. The pancreatic duct enters the duodenum caudal to and separate from the bile duct in the proximal duodenum approximately 20–25 cm distal to the pylorus (Figures 6.2 through 6.5). Surgically, it may be readily identified as a firm, whitish structure along the caudal third of the portion of the pancreas that is associated with the duodenum. There is usually a single pancreatic artery supplying the tail of the pancreas as a branch of the splenic or common hepatic (gastrohepatic) artery. The pancreatoduodenal artery courses between the duodenum and the pancreas along its joint border and supplies both from a series of small branches (Figure 6.4). Venous drainage is through the splenic vein. Some variations in vascular and ductal anatomy may be encountered between breeds (Ferrer et al., 2008; Truty and Smoot, 2008). The pig pancreas has a high level of cholinergic control as in the human. Both exocrine and endocrine functions of the pancreas are negatively affected by anesthetics (Laber-Laird et al., 1992; Rådberg et al., 1999). The pancreas accounts for approximately 0.1%–0.29% of the body weight (BW) and increases in size with age and consumption of solid food.

Histologically (Figure 6.6), the pancreatic islet cells are relatively indistinct, but functionally similar to those in humans (Elowsson and Carlsten, 1997; Hand et al., 1981; Kim et al., 2009; Koyama et al., 1986; Niebergall-Roth et al., 1997; Schantz et al., 1996; Stump et al., 1988; Yen, 2001).

The spleen is a pedunculated organ that is elongated in shape and located in close apposition to the greater curvature of the stomach in the left upper quadrant of the abdomen (Figures 6.7 and 6.8). It extends from the left kidney ventrally to the midline. There are three main vascular supplies to the organ. These are located in the splenic ligament and are the left gastroepiploic, the splenic, and the short gastric arteries and veins. The vascular supply enters the organ from the head to one half the distance to the tail of the spleen (Getty, 1975; Swindle, 1983). Colored histologic sections of these organs are on the textbook DVD.

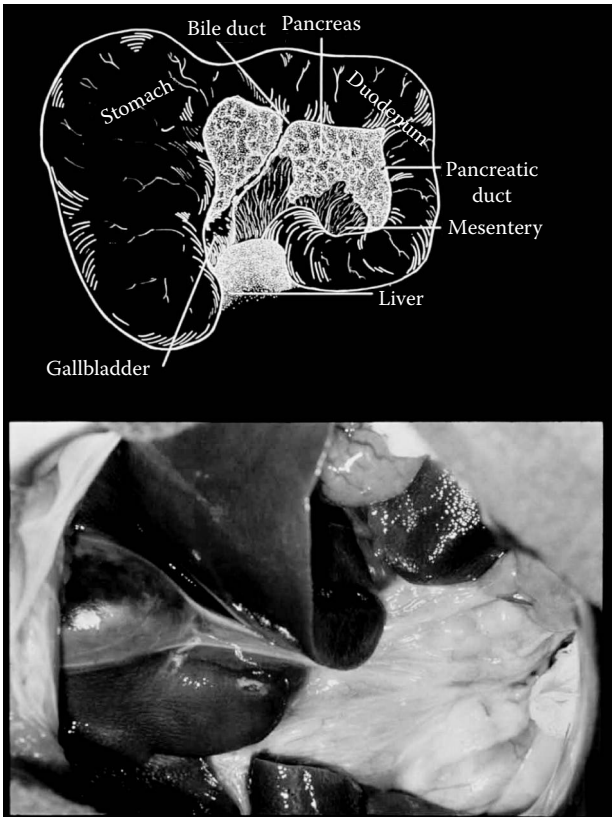

FIGURE 6.1 Relationship of the common bile duct and the pancreatic duct.

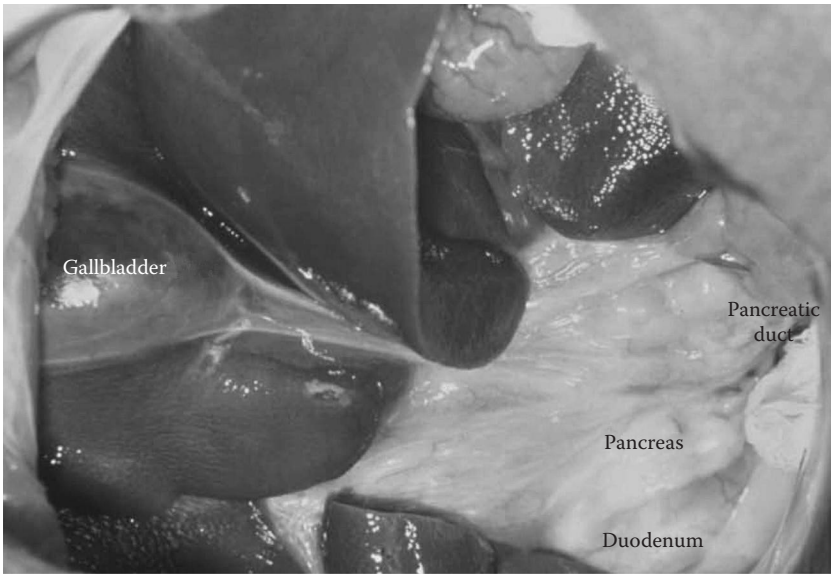

FIGURE 6.2 Surgical view of the gallbladder, pancreas, and portal hiatus.

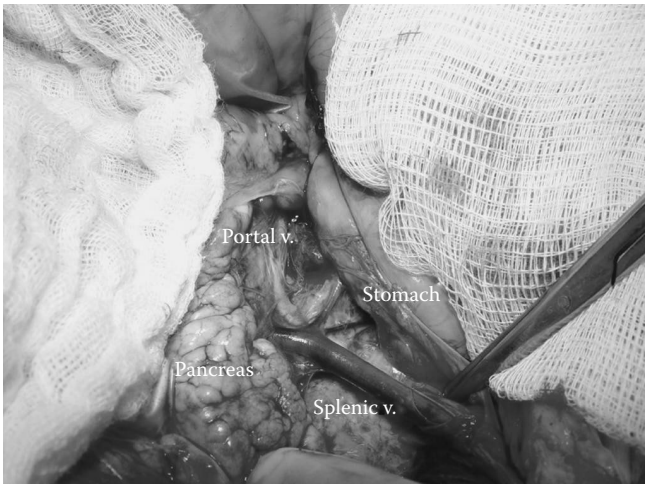

FIGURE 6.3 Relationship of the pancreas to the splenic and portal veins.

PANCREATECTOMY, ISLET CELL ABLATION, AND DIABETES

A total pancreatectomy can be performed without compromise or removal of the duodenum (Chaib et al., 2011; Stump et al., 1988). With the pig in dorsal recumbency, a midline incision is made from the xiphoid cartilage to at least the umbilicus. In larger animals, the incision may have to be extended caudally. Balfour retractors and laparotomy sponges are utilized to pack off the spiral colon and small intestinal mass to see the tail of the pancreas. In larger animals, solid food should be withheld for 48 h to empty the spiral colon.

Using gentle dissection, the retroperitoneal portion of the pancreatic tail is dissected free, and using gentle retraction, the dissection is continued until the pancreatic artery is encountered. After ligation and transection of the artery, the pancreas is dissected to the level of the pylorus. At this point, the dissection turns caudally, and the branches of the pancreatoduodenal artery supplying the

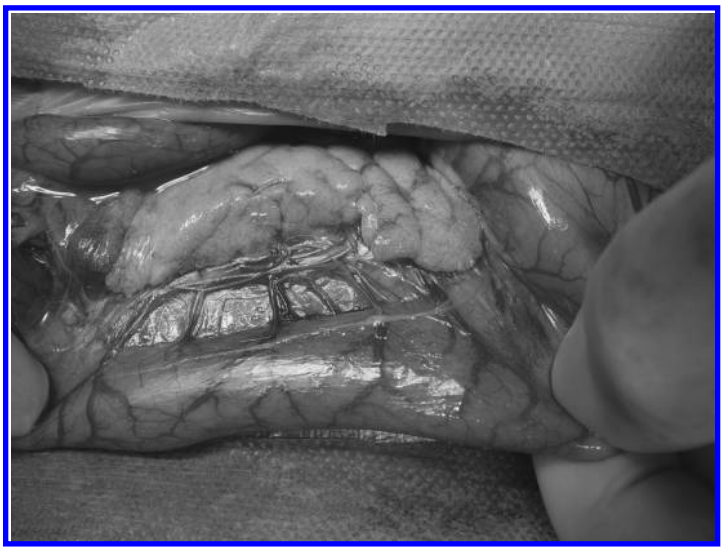

FIGURE 6.4 The pancreas and duodenum showing the pancreatoduodenal arterial supply.

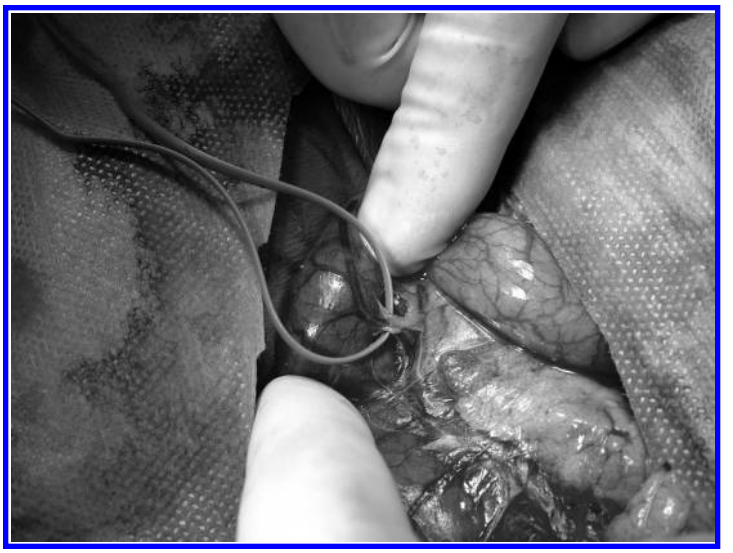

FIGURE 6.5 Pancreatic duct isolated with an elastic vessel loop.

pancreas are ligated as they are encountered. This dissection is more easily performed in the pig than in many other species because of the relatively loose connection between this artery and the pancreatic body. When the pancreatic duct is encountered, it is also ligated.

At this point, the dissection becomes more difficult. The major portion of the pancreatic body is deep and surrounds the portal vein and cranial mesenteric vessels. To perform this dissection, an assistant is required to provide additional retraction with handheld ribbon retractors. It is best to continue the dissection dorsally from the duodenum. The pancreas has to be split in order to dissect it from the portal vein. Care should be taken to minimize spillage of pancreatic enzymes, and the area should be flushed with saline following removal of the pancreas. The abdomen is closed in a routine manner.

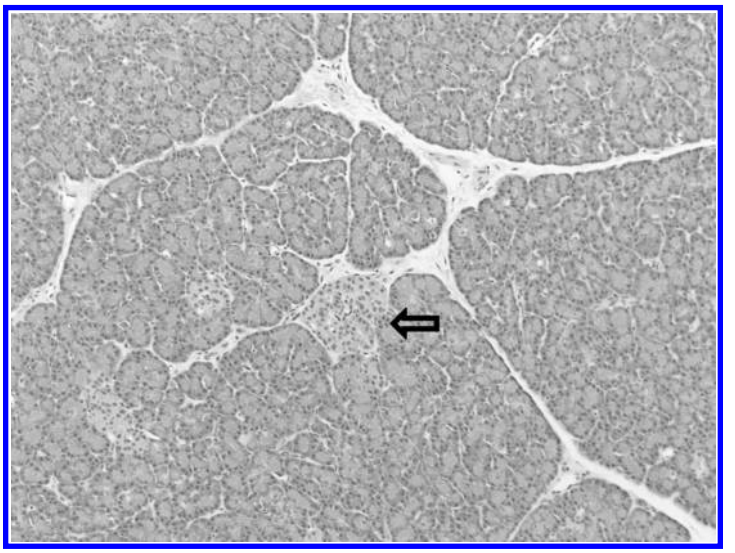

FIGURE 6.6 Islets of Langerhans (arrow) in the pancreas. H&E, ×100.

FIGURE 6.7 Midline abdominal incision showing the spleen in its normal position.

A chemical ablation of the islet cells may be performed by injecting alloxan (100–200 mg/kg intravenous (IV)) or streptozotocin (140–150 mg/kg) as an IV infusion over 5 min (Dixon et al., 1999; Larsen et al., 2003; Mullen et al., 1992). A 24-h fast is required before either treatment, and the drugs are dissolved in saline, which might have to be acidified with citrate to properly dissolve the agent. There is a difference in reaction between breeds of swine and between age groups within the same breed with either chemical agent. Toxic reactions resulting in renal or hepatic failure are possibilities. It is best to start with a low dose and repeat in a week if signs of beta cell regeneration occur. With either agent it is best to provide IV access and fluid loading during and following drug administration. Beta cell lysis may initially result in high levels of insulin release leading to fatal hypoglycemia. Hyperglycemia will occur if the chemical ablation was successful, and the pigs need to be maintained on insulin.

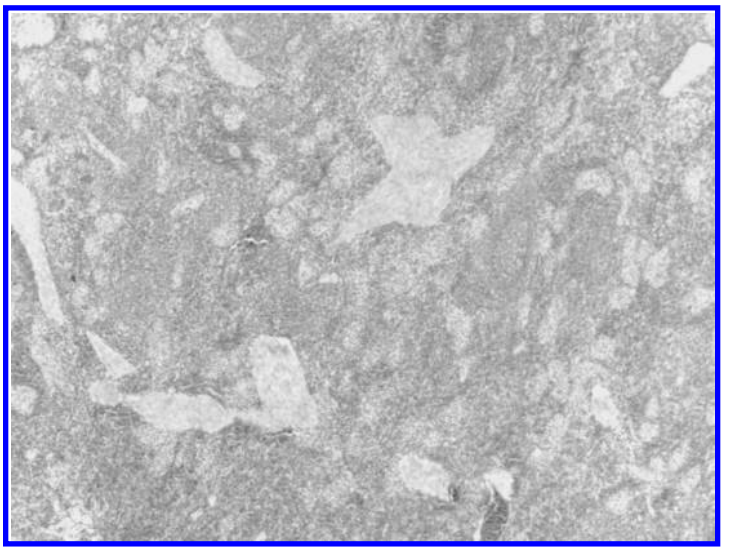

FIGURE 6.8 Histologic section of the spleen. H&E, ×100.

Diet and insulin control of glucose levels is difficult in swine. An amount of feed equivalent to 4% of the BW of the pig is provided as a starter ration. If pancreatectomy is complete, then oral pancreatic exocrine enzymes should be added to the food. Approximately 7 g of enzyme (Viokase-V®, Ft. Dodge Labs, Ft. Dodge, IA) is sufficient to digest 712 g of protein, 500 g of fat, and 1067 g of carbohydrate (Stump et al., 1988). In this species, as a general rule, elevations in blood glucose and glucosuria are moderate, and ketonuria and acidosis are not encountered as a major problem. Swine die within 10 days if not treated with insulin.

The goal of an experimental study requiring pancreatectomy is likely to be either treatment with various insulin protocols or islet cell transplantation. A detailed discussion of the monitoring and treatment of hyperglycemia is available (Mullen et al., 1992; Stump et al., 1988). Starting dosages for various types of insulin in swine 15–20 kg are as follows: regular insulin, 4–5 U (4-h duration); Lente insulin, 10 U (12-h duration); Ultralente, 20 U (24-h duration). The regular and Lente insulin injections are given together in the morning and the Ultralente in the afternoon. Another method is to calculate approximately 1.6 total units of insulin/kg for maintenance. Blood glucose levels should be maintained at approximately 200–300 mg/dL depending upon the goal of the study. Normals for insulin levels and protocols for conducting both oral and IV glucose tolerance tests have been published (Laber-Laird et al., 1992; Mullen et al., 1992; Stump et al., 1988). There are variations among breeds and ages within the breed; consequently, it is appropriate to establish normals for each laboratory experiment (Larsen et al., 2001). As a generality, fasting swine have insulin levels of 5–10 μU/mL which will increase two to three times that amount following a meal. Blood glucose levels can be determined twice daily with microcapillary tube samples from the auricular vein. Swine can be trained to accept this blood sampling procedure with gentle handling and a noncomplicating food treat (carrots, dog biscuits).

Generally, swine may be monitored for weight loss and glucose for several days, prior to initiating insulin replacement. When they are treated with insulin, the total amount may be reduced after the dosage has stabilized for 3–4 weeks. Hypoglycemia can be treated by glucose infusion. As a guideline for glucose administration, IV glucose tolerance tests (IVGTT) require 0.5 g/kg of a 50% glucose solution, and oral GTT require 1.75–2 g/kg of the same glucose solution (Dixon et al., 1999; Laber-Laird et al., 1992; Mullen et al., 1992; Sasaki et al., 1984; Stump et al., 1988).

When monitoring glucose and insulin levels, the effects of anesthesia and sedation must be considered (Heim et al., 2002; Laber-Laird et al., 1992; Rådberg et al., 1999; Vore et al., 2001). Induction of anesthesia and many sedatives may cause hyperglycemia and depressed insulin levels in most species. Included in these agents are inhalants (isoflurane, enflurane, nitrous oxide), benzodiazepines (midazolam), barbiturates, ketamine, and xylazine. Depressive effects may resolve with time; however, it is best to use chronically catheterized blood vessels for large samples, or microcapillary tubes filled after lancet pricks on the ear, as described earlier.

Atherosclerotic complications of diabetes are a possibility in this species, because they develop both spontaneous and experimental atherosclerosis. Currently, much of the development of porcine models of diabetes, complications of diabetes, and metabolic syndrome is being performed by Michael Sturek and his group, who have developed a line of wild-caught Ossabaw swine with a predisposition to the metabolic syndrome of obesity and diabetes (Dyson et al., 2006). Females of this breed have been developed as a model of the metabolic syndrome by feeding the thrifty genotype a diet with 45% kcal fat and 2% cholesterol. These animals develop obesity, insulin resistance, hypertension, and neointimal hyperplasia of the coronary arteries, and show increases in total cholesterol, triglycerides, and LDL/HDL. Other studies with the Ossabaw minipig have demonstrated their usefulness as an obesity model (Faris et al., 2012; Newell-Fugate et al., 2014). A model of metabolic syndrome has also been created in Göttingen minipigs in which females were more insulin resistant and had a higher beta cell function (Christoffersen et al., 2007).

Sturek has demonstrated that alloxan-induced diabetic dyslipidemia in Yucatan pigs fed an atherosclerotic diet leads to the development of some of the early signs of diabetic retinopathy, renal capillary basement membrane thickening, coronary artery atheroma, arterial fatty streaks, and arterial

intimal thickening (Boullion et al., 2003; Hainsworth et al., 2002; Otis et al., 2003). Similar types of studies have been conducted in the smaller Göttingen minipig (Johansen et al., 2001; Larsen et al., 2001), which developed some of the metabolic characteristics of obese humans on a high-fat and high-energy diet. Serum leptin levels and widest-girth circumference have been described as means to noninvasively estimate body fat percentage in Yucatan swine. Normal leptin levels are approximately 2 ng/mL, and the normal value of the widest-girth circumference is approximately 92 cm in 60-kg male swine (Witczak et al., 2005). Steptozotocin-induced diabetes in domestic swine on an atherosclerotic diet also developed accelerated atherosclerotic lesions (Gerrity et al., 2001). In the Chinese Guizhou minipig, it has been demonstrated that feeding a high fat/high sucrose diet without the addition of cholesterol for 6 months produced mild diabetes and atherosclerotic lesions along with obesity (Shoumin et al., 2004).

Pigs with hyperglycemia have also been used to test and evaluate the use of subcutaneous and intraperitoneal glucose sensors for continuous glucose measurements (Burnett et al., 2014; Kvist et al., 2006).

Analgesics and IV infusions of fluids and nutritional solutions should be provided for 1–3 days postoperatively (Laber-Laird et al., 1992; Mullen et al., 1992; Sasaki et al., 1984; Stump et al., 1988).

SEGMENTAL PANCREATECTOMY, PANCREATIC TRANSPLANT, AND ISLET CELL ISOLATION

Most of the swine used in pancreatic transplantation are used for islet cell transplantation techniques after pancreatectomy, digestion of the organ for purification, and isolation of the beta cells (Ferrer et al., 2008; Nielsen et al., 2002; Qiao et al., 2010). Much of the research in this area is directed toward preservation of the function of the cells and methods of delivery as xenografic transplants to humans. Fetal pancreases are frequently harvested and used in these procedures. Swine have also been employed as a model in segmental pancreatic transplant using the tail of the organ (Koyama et al., 1986; Mullen et al., 1992; Pennington and Sarr, 1988; Sasaki et al., 1984). Partial pancreatectomy has been demonstrated to result in pancreatic regeneration with hypertrophy and hyperplasia developing within 30 days (Morisset et al., 2000, 2001). Swine have also been used in a study to compare various electrosurgical and stapling devices for transection (Ikeda et al., 2013). Cryosurgical and radiofrequency devices for regional ablation for potential treatment of pancreatic cancer have also been studied in swine (Chen et al., 2010; Fegrachi et al., 2013).

The same midline incision described for the total pancreatectomy (see earlier text) is used for this procedure. Starting at the tail and extending to the distal body near the duodenum, the organ is mobilized using gentle dissection. The tail is the ventral strip of the pancreas. It contains the main portion of the pancreatic duct and has a single arterial blood supply. It is this section of the pancreas that is harvested as a segmental donor organ (Figure 6.9). The short gastric and left gastric vessels are ligated, and the origin of the pancreatic blood supply is identified. It may branch off either the common hepatic artery or the splenic artery. The common hepatic artery and splenic artery are transected distal from the branch supplying the tail of the pancreas leave the celiac artery intact and in order to have ample distance to avoid damaging the pancreatic artery. The pancreatic vein is transected as it enters the splenic vein, and the tail of the organ is transected from the distal body of the pancreas at the level of the cranial mesenteric vein, which represents its narrowest section. The celiac artery is cannulated and transected, and the organ is perfused with cold perfusate and heparin.

The recipient animal has a left nephrectomy performed from a ventral midline celiotomy as described previously. End-to-end anastomosis is performed with the donor splenic vein to the renal vein, and the donor celiac artery to the renal artery. A Roux-en-Y loop is isolated in the recipient jejunum by transecting a section of the jejunum and performing an end-to-side anastomosis of the proximal segment to the distal segment (Figure 6.10). A 40-cm section of the loop is isolated and flushed free of intestinal contents. An invaginated section of the pancreas is anastomosed into the

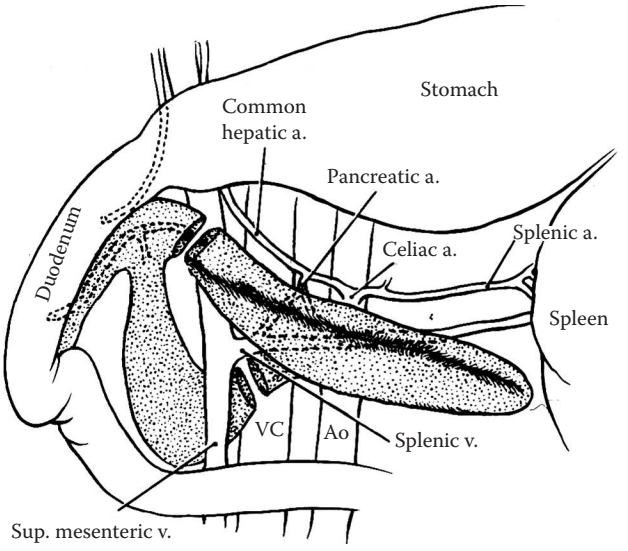

FIGURE 6.9 Harvesting the pancreas for a segmental pancreatic transplant with Roux-en-Y intestinal loop. (From Tumbleson, M.E. 1985. *Swine in Biomedical Research*, Vol. 1. New York: Plenum Press, pp. 385–389. With permission.)

proximal end of the loop to provide exocrine duct entrance into the gastrointestinal tract without contamination by intestinal contents of the anastomotic site. The abdominal incision is closed in a routine manner.

Postoperative care should include systemic antibiotics, immunosuppressive therapy, and analgesics. Animals receive fluid and nutrition by a continuous IV infusion for 3 days prior to starting solid food.

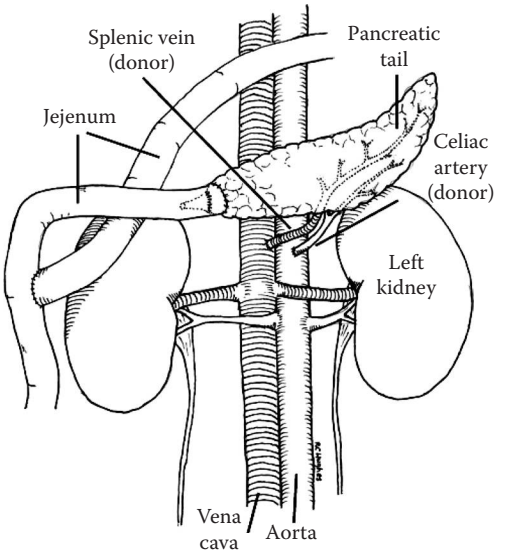

FIGURE 6.10 Ligation of the vessels of the splenic hilus for splenectomy.

Pancreatectomy is also performed to isolate islet cells for transplantation (Lundberg et al., 2014; Marigliano et al., 2011). Swine islet cells that retain physiological function are difficult to isolate in large numbers. The general methodology involves rapid removal of the pancreas following exsanguinations under anesthesia. The pancreas is washed, cooled on ice, and dissected free from adventitious tissue; the pancreatic artery or pancreatic duct is isolated for cannulation; and collagenase enzymatic solutions are infused. Islet cell yields increase with age, and more are located in the body and caudal portions of the pancreas. The pancreas undergoes digestion and isolation. Porcine islets are fragile because of a thin peri-insular connective tissue capsule. There are also differences in the amount and size of the islets and the collagen capsules between breeds of pigs. With a good technique, approximately 3000 islet equivalent number (IEQ)/g of islet cells can be isolated. A recent review of the techniques and refinement of the procedure using Liberase has been published (Kim et al., 2004; Nielsen et al., 2002).

PANCREATIC DUCT ABLATION, CANNULATION, AND PANCREATITIS

It is more difficult to induce acute and chronic pancreatitis in swine than in other species. The pancreatic duct is readily identifiable at its entrance into the duodenum from the distal portion of the body. It is palpable as a firm tubular structure with a grayish white appearance. Its entrance into the duodenum is distal from that of the bile duct (Engelhardt et al., 1982; Sarr, 1988; Thorpe and Frey, 1971).

Acute pancreatitis has been produced by the injection, under pressure, of bile incubated with active trypsin. The lesions are much milder in the pig than in other species (Caronna et al., 2003; Sarr, 1988; Thorpe and Frey, 1971; Trepte et al., 2013). Modification of the procedure by infusion of the pancreatic duct with taurocholic acid results in severe acute pancreatitis, which results in significantly impaired pancreatic oxygenation (Kinnala et al., 2001). Saline infusion in the same model results in a much milder form of pancreatitis with increased pancreatic oxygenation. Signs of pancreatitis are macroscopically detectable within minutes of performing the perfusions.

A model of chronic pancreatitis can be produced by ligation of the duct and creation of ischemia by ligating the branches of the pancreatoduodenal artery supplying the body of the pancreas. The blood supply to the tail is left intact. Lesions of chronic inflammation and fibrosis appear within weeks of the ductal ablation. Pseudocysts may also be present. There is exocrine deficiency with weight loss and gastrointestinal signs, but no signs of diabetes (Pitkaranta et al., 1989).

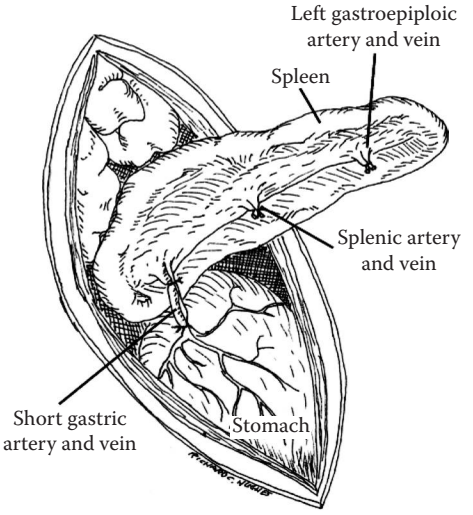

FIGURE 6.11 Splenectomy.

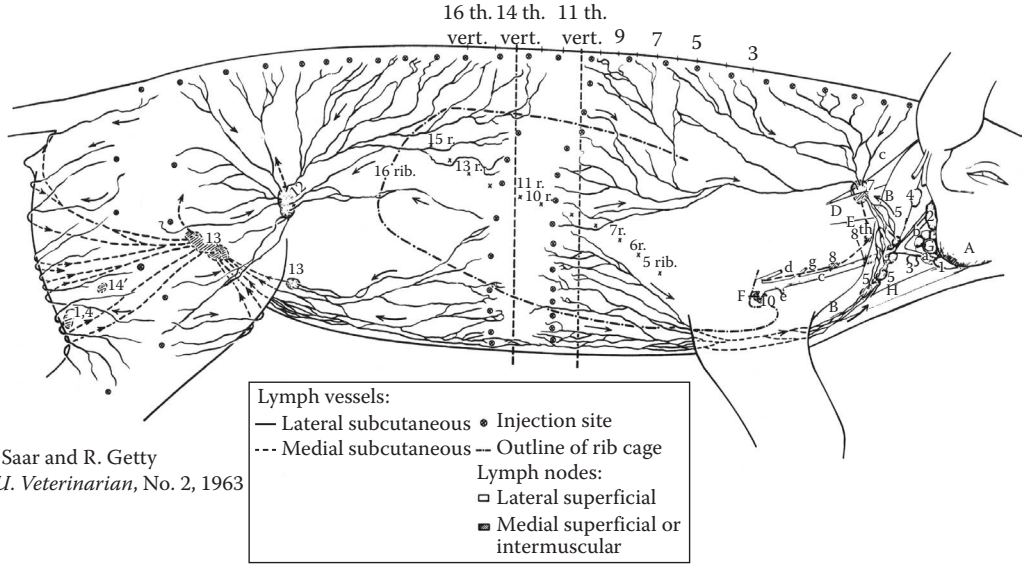

FIGURE 6.12 Superficial lymphatic system. (Reprinted from Sarr, L., Getty, R. 1963. *Iowa State University Veterinarian*. 26(2): 9. With permission.)

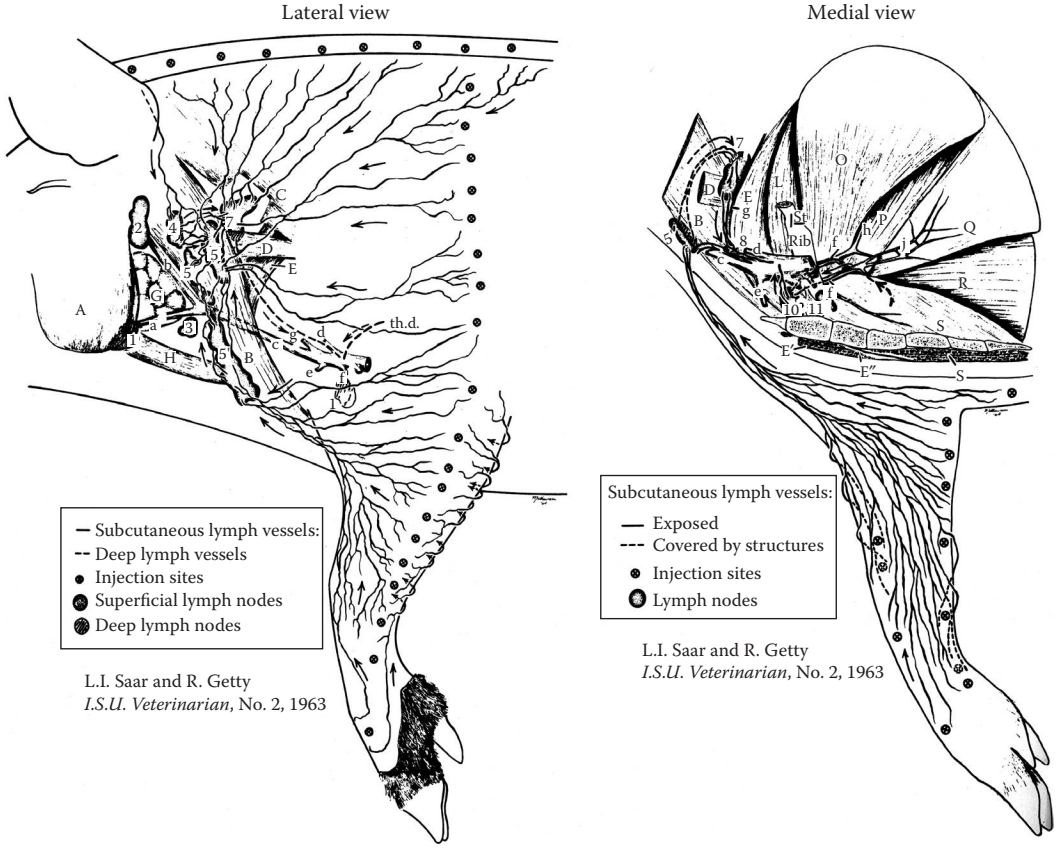

FIGURE 6.13 Lateral and medial views of the lymphatics of the foreleg. (Reprinted from Sarr, L., Getty, R. 1963. *Iowa State University Veterinarian*. 26(2): 9. With permission.)

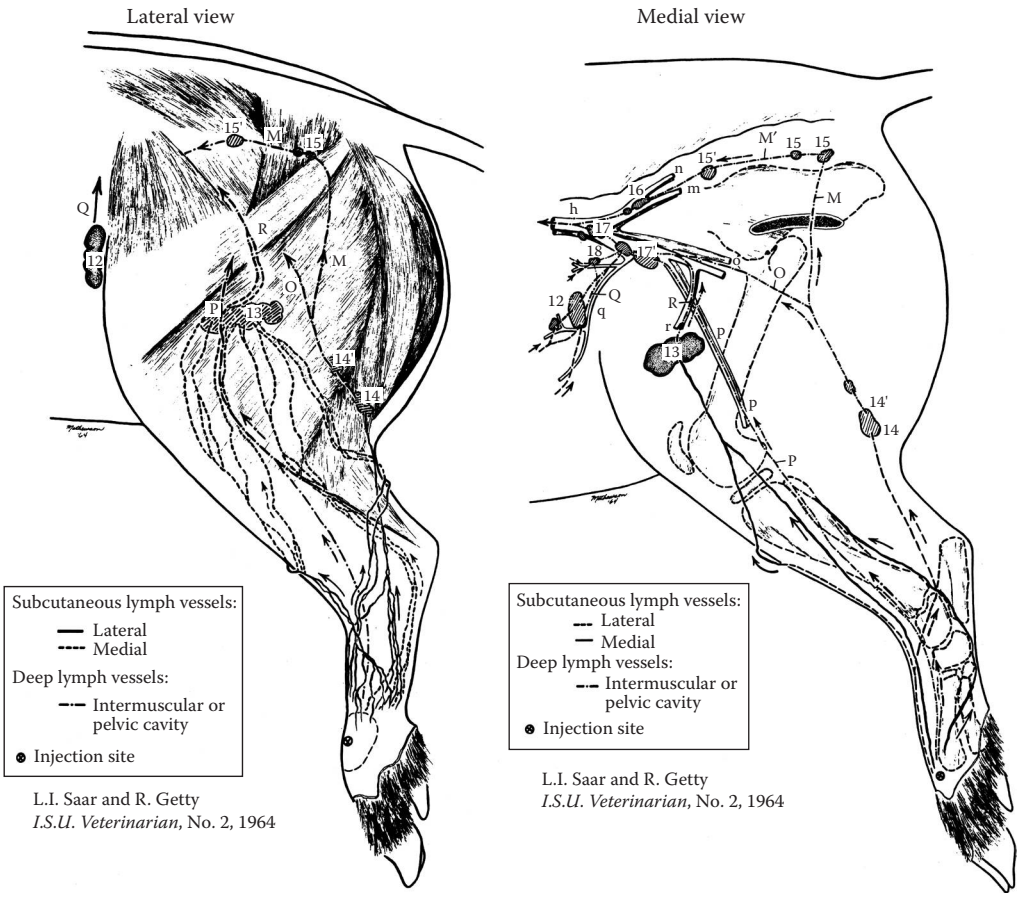

FIGURE 6.14 Lateral and medial views of the lymphatics of the hind limb. (Reprinted from Sarr, L., Getty, R. *Iowa State University Veterinarian*. 26(2): 9. With permission.)

The pancreatic duct can be cannulated for short-term collection of pancreatic enzymes; however, animals will die of electrolyte imbalances and inability to digest nutrients in approximately a week. These problems can be alleviated by using re-entry cannulation of the duodenum after exteriorizing the catheter on the abdominal wall to partially return pancreatic secretions. The catheter is passed into the main pancreatic duct and ligated in place. The catheter is exited through the ventral abdominal wall to the collection device and then reentered into the abdomen and the proximal duodenum. Suture retention beads should be used inside the pancreatic duct and the duodenum to avoid dislodgement. Alternatively, side entrance catheters can be used in the accessory duct or T-tubes in the main duct if only partial collection of the pancreatic secretions is desired (Niebergall-Roth et al., 1997; Swindle et al., 1998).

SPLENECTOMY, SPLENIC VASCULAR CATHETERIZATION, AND SPLENIC TRANSPLANTATION

The spleen is approached using a paracostal incision with the pig in dorsal recumbency. The incision starts from the lateral margin of the mammary glands at the halfway point between the first and second nipple on the left side. The incision parallels the caudal margin of the caudal edge of the rib line and extends caudolaterally to approximately the level of the third nipple. The splenectomy

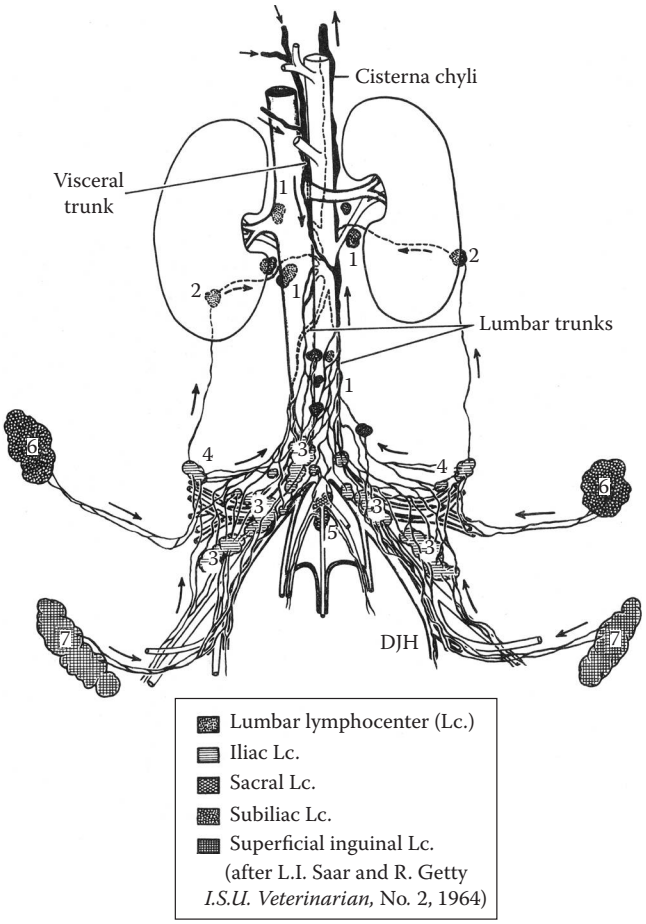

FIGURE 6.15 Intra-abdominal lymphatic system. (Reprinted from Sarr, L., Getty, R. *Iowa State University Veterinarian*. 26(2): 9. With permission.)

may also be performed from a midline incision; however, dissection of the short gastric vessels is more difficult (Swindle, 1983).

After celiotomy, the tail of the spleen is retracted out of the abdomen. The vessels supplying the splenic hilus are clamped, transected, and ligated in the following order: left gastroepiploic, splenic, and short gastric artery and veins. The arteries and veins may be ligated together. The short gastric vessels are deep in the abdomen and in close proximity to the stomach. They generally are ligated *in situ* and then transected. While dissecting this vessel, the surgeon should take care to avoid damage to the underlying pancreas (Figure 6.11).

The splenic vessels may also be used to catheterize the portal system with or without splenectomy (Figure 6.3). The surgical exposure for this procedure is easier than the exposure described for the portal vein catheterization in Chapter 9; however, the location of the tip of the catheter is not as readily discernable, and catheters made of nonflexible materials may penetrate into the abdomen at the entrance of the vessel into the portal vein. Sacrifice of a single vessel or pair of vessels in the spleen for this procedure does not cause a problem because of the extensive collateral circulation.

Splenic transplantation has been performed to study the immunologic effects in miniature swine as defined by major histocompatibility complex (MHC) (Dor et al., 2004; Gollackner et al., 2003). The technique involved excision of the spleen with its aortic and portal venous vascular pedicles

which also necessitates dissection of the pancreas. Using a Carrel patch technique, the aorta and portal vein stumps were anastomosed to the abdominal aorta and caudal vena cava of a splenectomized recipient. Following transplantation, the spleen is positioned in the flank superficial to the bowel. Hematopoietic cell chimerism was detectable and rejection could be prevented by immunosuppression with cyclosporine (10–30 mg/kg, trough level 400–800 ng/mL) or thymic irradiation (700 cGy) and/or total body irradiation (100 cGy). Post-transplant lymphoproliferative disease is a possible complication.

The spleen is friable and prone to bleeding if injured during surgery or following trauma. Biopsies may be performed if the wound is small enough to be closed with fibrin glue (Hui et al., 2013). The anatomy of the lymphatic system and its drainage is depicted in Figures 6.12 through 6.15.

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7 Urinary System and Adrenal Glands

M. Michael Swindle

CONTENTS

GENERAL PRINCIPLES OF SURGERY AND SURGICAL ANATOMY

The urinary system of the pig is anatomically similar to that of most species. The kidney of a 70 kg domestic pig is approximately the size of that of an adult human. In newborn pigs, the development of nephrons continues for approximately 3 weeks in contrast to the fully developed nephron system in humans at birth. The kidneys of a 25 kg Hanford miniature pig weigh 120 g and measure $11 \times 6 \times 3$ cm which is similar to the human (Schwalb et al., 1989). In these minipigs, the ureteral length is 22–26 cm with a diameter that accommodates a 4–6 French (Fr) catheter and a bladder capacity of approximately 150 mL. The left kidney is more cranial than the right kidney and its cranial pole is located at approximately the 13th rib (L1–L4). The renal artery and vein divide into two branches close to the renal hilus. The blood supply is divided into cranial and caudal segments rather than into longitudinal halves as in other species. This means that the avascular plane of the kidney is transverse rather than longitudinal (Figures 7.1 through 7.3). Renal blood flow is reported as 365 mL/min and medullary blood flow as 2.5–2.6 mL/min/g (Lüdemann et al., 2009). There are some differences in anatomy and function between the pig and human (Tables 7.1 through 7.4), even though the internal renal anatomy is very similar (Figure 7.4). The multirenculate, multipapillate kidney of the pig contains a greater proportion of juxtamedullary glomeruli, the loops of Henle are relatively longer, and creatinine is absorbed from the proximal tubule (Figures 7.4 through 7.6). Maximum urine concentration in the pig is 1080 mOsm/kg, which compares favorably to that of the human, which is 1160 mOsm/kg. The calyx contracts approximately 15 times/min and the renal pelvis 3–6 times/min in both the pig and human (Assimos et al., 1986; Deding et al., 2006; Friis, 1998; Giraud et al., 2011; Sampaio et al., 1998; Schwalb et al., 1989; Swindle and Olson, 1988; Dalmose et al., 2000; Terris, 1986). Danish Landrace pigs 9–13 weeks of age (12–18 kg) were extensively studied for normal urine production (Deding et al., 2006). Urine production was approximately 15 mL/kg/h during the daytime and approximately 8 mL/kg/h at night. Daytime voidings peaked around noon and were approximately 15 during the day and 3 during the night with a mean of 18.7 voidings. The mean total volume was 2845 ± 900 mL. Average urine flow was approximately 12 mL/s and residual urine was variable between 0 and 136 m. Their average fluid intake was 4151 ± 1313 mL.

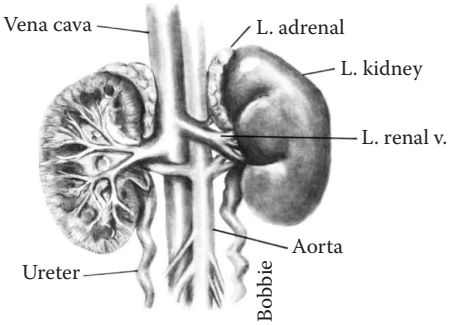

FIGURE 7.1 Anatomy of the kidneys and adrenal glands with the internal renal anatomy of the right kidney demonstrated.

The ureters extend caudoventrally to the dorsolateral aspect of the neck of the urinary bladder (Figures 7.7 and 7.8). The urinary bladder is large and thin walled but typical in morphology. It receives its innervation from S2 to S4. The urethra courses along the pelvic floor into the penis. The tip of the penis is located on the ventral abdominal wall in a preputial diverticulum. The external opening of the preputial diverticulum is located immediately caudal to the umbilicus. The contents of this structure must always be expressed prior to performing abdominal surgery in male swine. The desquamated cells and urine in the preputial diverticulum are foul smelling and contaminated with bacteria. Gloves should be worn during the expression of this material (Hodson, 1986; Russell et al., 1981; Swindle, 1983; Swindle and Olson, 1988; Swindle et al., 2000; Terris, 1986). Colored histologic sections are contained on the textbook DVD.

Catheterization of the urinary bladder through the penis is difficult, even impossible, depending upon the size of the animal and the breed, because of the male anatomy. Consequently, the urinary bladder can be emptied by manual expression in small animals or via needle aspiration. Needle aspiration should be performed very carefully because the bladder is relatively thin walled and

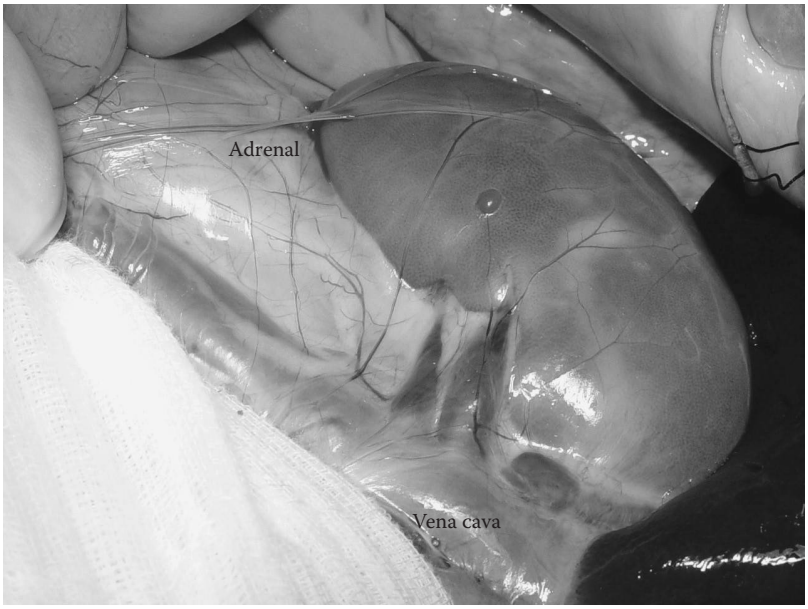

FIGURE 7.2 Surgical anatomy of the right kidney and adrenal gland.

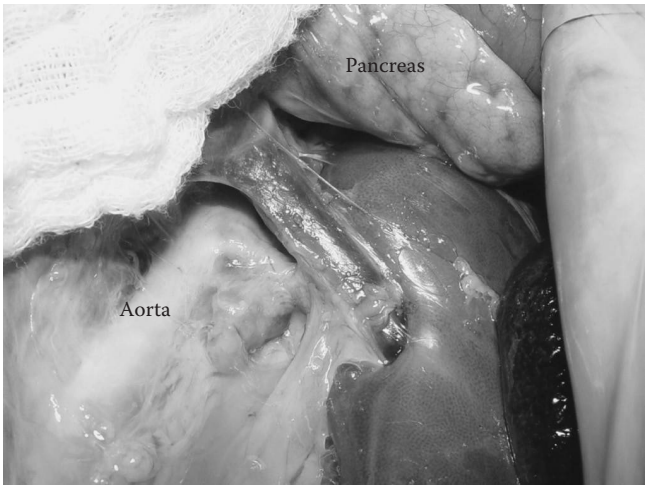

FIGURE 7.3 Surgical anatomy of the left kidney.

TABLE 7.1
Renal Function in Swine

	mL × min⁻¹ × 1.73 m²						mg × min⁻¹ × 1.73 m²					
	GFR			C_{PAH}			Tm_{PAH}			$Tm_{Glucose}$		
	Pig	Minipig	Man	Pig	Minipig	Man	Pig	Minipig	Man	Pig	Minipig	Man
Premature	–	–	16	–	–	39	–	–	14	–	–	93
Newborn	49	–	40	176	–	–	16	–	–	164	–	–
Young[a]	133	88	–	381	497	–	89	88	–	360	–	–
Adult	141	–	125	433	–	638	166	–	78	466	–	339

Source: Reprinted from Friis, C., 1998. *Scand. J. of Lab. Anim. Sci*., 25(Suppl. 1): 57. With permission.

Note: GFR = glomerular filtration rate; C_{PAH} = renal plasma flow measure as clearance of para-aminohippurate; Tm_{PAH} = transport capacity of organic anions; $Tm_{Glucose}$ = capacity to reabsorb glucose from the urine.

[a] Minipigs: 4 months, domestic pigs: 2 months.

TABLE 7.2
Urinalysis Results for Göttingen Minipigs

		Male			Female		
		N	Mean	SD	*N*	Mean	SD
pH		190	7.95	±0.64	192	8.11	±1.04
Osmolality	mmol/kg	106	543.491	±252.508	108	636.094	±241.578
Volume	mL/day	225	302	±221.5	228	290	±152.6

Source: Courtesy of Ellegaard Göttingen Minipig ApS, Dalmose, Denmark.

Note: Samples from 219 males 3.5 ± 0.6 months of age and 220 females 3.5 ± 0.7 months of age. *N* = number of pigs, SD = standard deviation.

TABLE 7.3
Urinalysis Results for Juvenile and Young Adult Hanford Miniature Swine

		Male				Female			
		Mean	SD	Min	Max	Mean	SD	Min	Max
pH		7.3	1.0	6.0	9.0	6.7	0.6	5.0	8.0
Specific gravity		1.0	0.0	1.0	1.0	1.0	0.0	1.0	1.0
Volume	mL/h	26.8	12.3	4.3	61.7	32.7	15.8	9.0	70.8

Source: Courtesy of Sinclair Research Center, Auxvasse, MO.
Note: $N = 28$ per gender group, age = ~ 4–8 months, SD = standard deviation.

TABLE 7.4
Urinalysis Results for Juvenile Sinclair Miniature Swine

		Male				Female			
		Mean	SD	Min	Max	Mean	SD	Min	Max
pH		6.9	1.0	5.0	9.0	6.7	0.8	5.0	9.0
Specific gravity		1.2	1.3	1.0	10.1	1.0	0.0	1.0	1.0
Urobilinogen	EU/dL	0	0.1	0	1	0	0.0	0	0
Volume	mL/h	14.6	9.9	4.3	58.8	13.0	7.3	3.4	30.4

Source: Courtesy of Sinclair Research Center, Auxvasse, MO.
Note: $N = 51$, age = ~3–4 months.

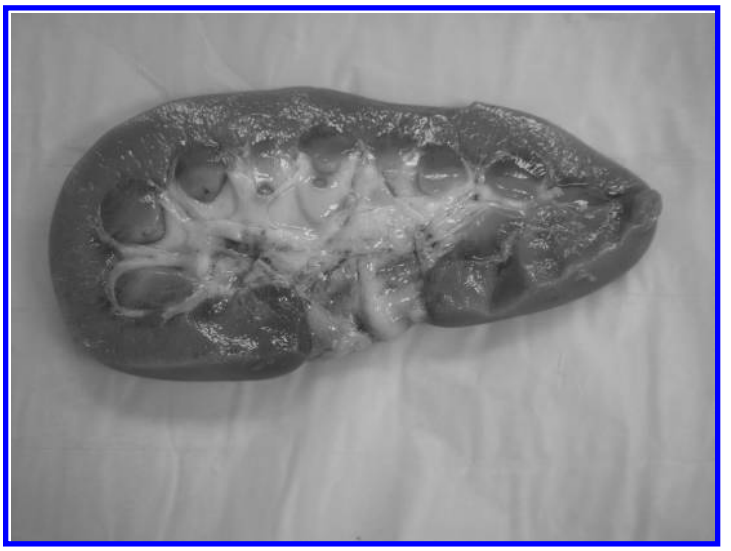

FIGURE 7.4 Longitudinal cross section of the kidney showing the multirenculate, multipapillate system.

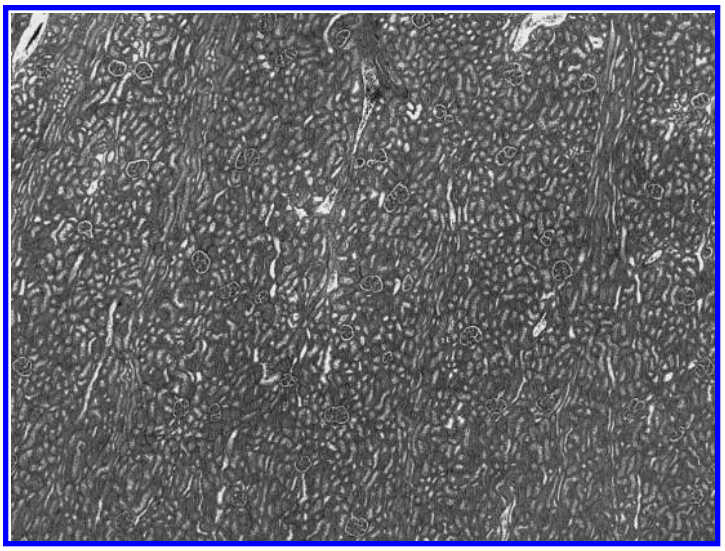

FIGURE 7.5 Histology of the renal glomerulus. H&E, ×100.

tears easily. The male urethra may be entered percutaneously with an intracath needle as it courses ventrally over the pubis in the perineum. It may be palpated on the midline. The pelvic urethra of a 25–30 kg pig is approximately the size of that of a human. This catheterization procedure can be readily performed with practice. The female urethra may be catheterized conventionally and may be entered on the floor of the vagina approximately one-quarter to one-third of the distance to the cervix. Pigs produce 5–40 mL/kg urine per day depending upon the age and water consumption (Swindle, 1983).

In studies of fetal and newborn pigs, development of storage and bladder function develops between the mid-second and early third trimesters of pregnancy. Similar to human infants, they have a dyscoordinated voiding with staccato flow. Newborns void approximately 3.3 times per hour and fetuses 5.85 times per hour (Olsen et al., 2001, 2004; Peters, 2001).

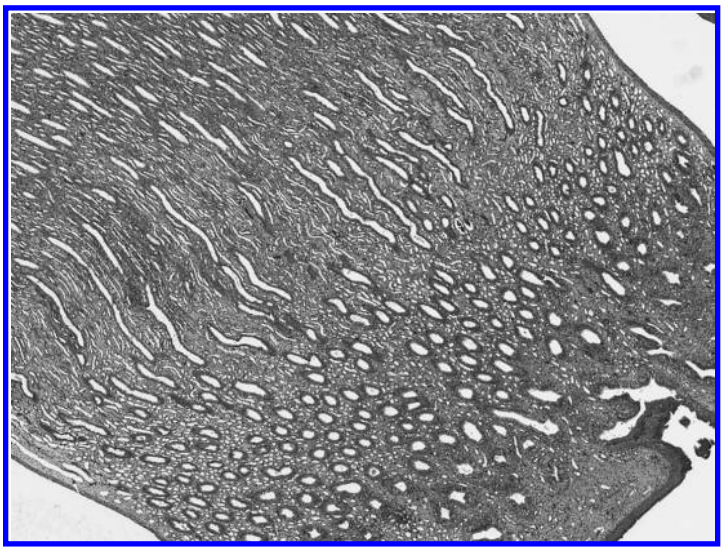

FIGURE 7.6 Histology of the renal pelvis. H&E, ×40.

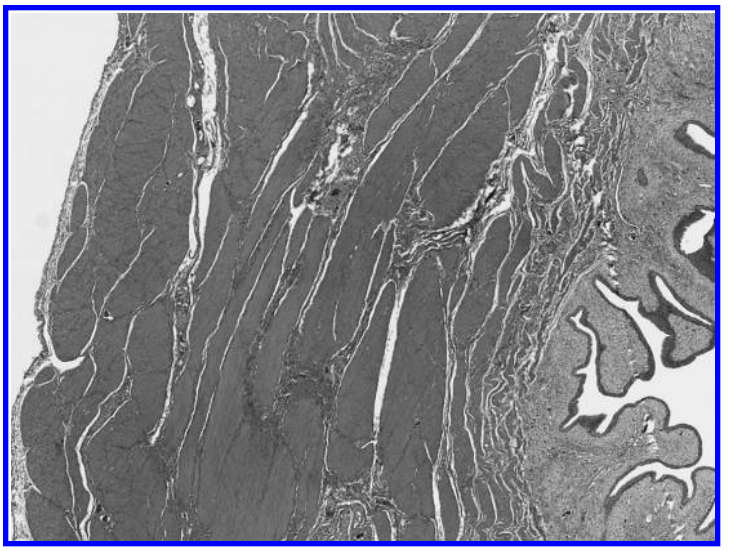

FIGURE 7.7 Histology of the urinary bladder. H&E, ×40.

The adrenal glands are associated with the medial surface toward the cranial pole of each kidney. The right adrenal gland is tightly adhered to the caudal vena cava. The arterial supply of the glands is from either the aorta or branches of the lumbar arteries, and the venous drainage is into either the vena cava or the renal veins (Figures 7.1 through 7.3) (Swindle and Smith, 1998; Venzke, 1975).

NEPHRECTOMY

Nephrectomy can be performed either through a ventral midline approach or a retroperitoneal approach through the flank (Figures 7.9 and 7.10). The flank approach is the approach of choice if only one kidney is involved in the surgical procedure (Swindle, 1983; Webster et al., 1992).

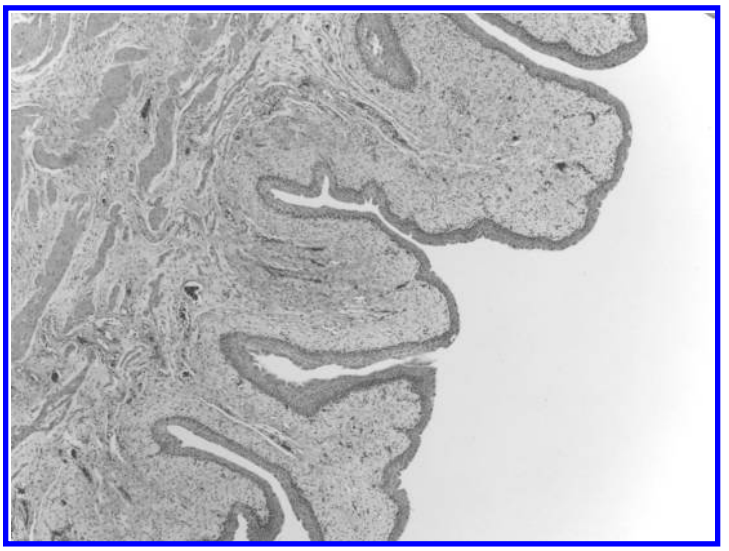

FIGURE 7.8 Histology of the bladder mucosal epithelium. H&E, ×100.

FIGURE 7.9 Flank incision to expose the kidney. The surgeon's finger is in the incision and the pig is in left lateral recumbency with the head to the right.

The flank approach is performed with the pig in ventral or lateral recumbency. A curved linear incision is made caudal to the last rib following its contour approximately one-quarter to one-third of the distance ventrally, from the lateral aspect of the vertebral wings to the midline. The muscle layers are either cut or bluntly divided and retracted until the peritoneum is exposed. Using manual manipulation, the peritoneum is retracted ventrally after peeling it from the dorsal aspect of the abdominal musculature and the kidney. When performed properly, the kidney is exposed without entering the abdominal cavity, thus avoiding interference with the abdominal viscera.

If bilateral renal procedures are to be performed or other abdominal procedures are involved in the protocol, then a ventral midline incision is the preferred approach. The spiral colon will interfere with observation of the kidneys, especially the left one, if it has not been emptied preoperatively. This may be performed either by a 48-h fast from solid foods or by administering hypertonic saline

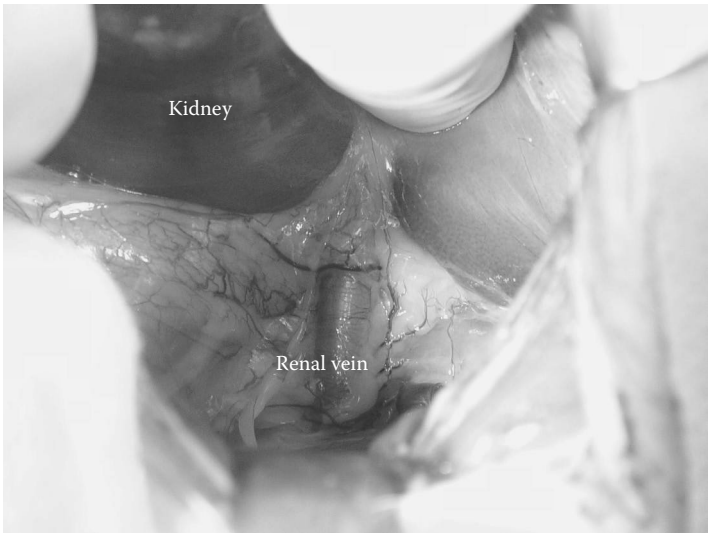

FIGURE 7.10 Close-up of the retroperitoneal approach to the kidney. The kidney is displaced dorsally to show the blood vessels.

cathartics, as previously described. The ventral midline incision is initiated at the xiphoid process and extends caudally beyond the umbilicus. Balfour retractors and laparotomy sponges are required to retract the viscera for surgical exposure of the kidneys. The intestinal mass should not be exteriorized to enhance exposure because of the complications of edema and ischemia, to which the pig is highly susceptible.

The renal artery is bluntly dissected and ligated first. A branch of the suprarenal artery supplying the adrenals will have to be ligated in some cases, especially on the left kidney. This is followed by isolation and ligation of the renal vein and then the ureter. From the flank approach, it is helpful to make a sling of surgical gauze around the cranial and caudal poles of the kidney. This aids in the manipulation of the organ to enhance retraction and exposure. When dissecting in the midline region, the surgeon should take care to avoid damaging the lymphatics. They can usually be seen and should be ligated, if damaged, to avoid chyloperitoneum.

Following removal of the kidney, the incision is closed in a routine manner. When closing a flank incision, it is not necessary to suture the retracted peritoneum back in place. The muscle layers are closed in anatomically correct layers by suturing the fascia. The skin and subcutaneous tissues are closed in a routine fashion.

PARTIAL NEPHRECTOMY AND INTRARENAL SURGERY

The branches of the renal artery supplying the kidney divide the blood supply transversely rather than longitudinally. The avascular plane of the kidney may be readily demonstrated by temporarily occluding the blood supply of one of the branches of the renal artery in the hilus of the kidney (Giraud et al., 2011; Russell et al., 1981; Swindle and Olson, 1988).

If a surgical approach to the renal pelvis is indicated, then this avascular plane forms the line of incision into the kidney parenchyma. The capsule of the kidney is incised with a scalpel, and the incision may be continued either with a scalpel or bluntly with a surgical spatula. The kidney is surgically repaired with mattress sutures of synthetic absorbable or nonabsorbable sutures, which occlude the edges of the incision and provide hemostasis. In heminephrectomy, this technique of closure will occlude most of the blood supply, but it may be necessary to provide additional hemostasis with oxycellulose sponges (Russell et al., 1981; Swindle and Olson, 1988).

The blood supply to the kidney may be reduced by surgical imbrication, inflatable circumferential cuffs (Figure 7.11), or Goldblatt clamp techniques that occlude the renal blood flow. This procedure is performed usually to produce a model of renal hypertension. To produce the model

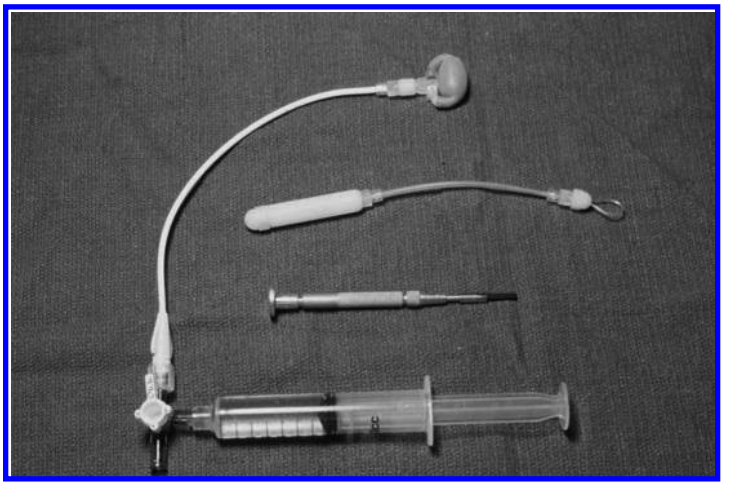

FIGURE 7.11 Inflatable cuffs to occlude the renal artery.

surgically, it is necessary to remove one kidney and significantly reduce the blood supply to the remaining kidney. The exception to this is the two-kidney deoxycorticosterone acetate (DOCA) salt model in Yucatan miniature swine (O'Hagan and Zambraski, 1986; Swindle and Olson, 1988). Renal models including ischemia/reperfusion techniques have been recently reviewed along with the physiology of the porcine kidney (Giraud et al., 2011).

To produce the model of hypertension, the left kidney is surgically removed as described previously. The right kidney, which has a longer artery than the left, is the kidney to which the blood supply is reduced. In our laboratories, a reduction of approximately 75% of the renal blood flow will produce chronic hypertension. An acute hypertensive episode may be initiated by challenge with 0.9% NaCl at a rate of 20 mL/kg iv as a bolus and maintenance infusion. An increase of 20% above baseline arterial pressure is considered significant.

Radiofrequency ablation has been used laparoscopically and percutaneously to study sequella associated with using the technique for ablation of renal tumors or other lesions (Gill et al., 2000; Wagner et al., 2005). The studies in swine have proved applicable to the postprocedural seqellae in humans. The renal tissue is dessicated, becomes necrotic, and undergoes autoamputation and resorption. A model of acute pyelonephritis was developed 19 kg Danish Landrace cross pigs by injection of 3.25 mL of *Escherichia coli* (10^6 CFU/mL) into the renal pelvis through a catheter implanted in the ureter followed by 20 min of occlusion of the catheter. The model was comparable to the situation in humans and deemed to be useful in pathogenesis studies (Isling et al., 2011). Domestic breeds have been reported as commonly having spontaneous urinary tract infections (Martineau and Almond, 2008).

RENAL TRANSPLANT

The pig has been used in renal transplantation research to study organ preservation, rejection phenomena, and surgical procedures including allographic, xenografic, heterotopic, and orthotopic techniques. In addition to the anatomic characteristics of the kidneys described previously, it is important to use anesthetic and perioperative techniques that maintain adequate blood pressure to ensure tissue perfusion (>50 torr) and avoid vasospasm (Howard et al., 1994; Kirkman et al., 1979; Pennington, 1992; Sachs, 1992; Williams, 1988).

Selection of the donor and recipient breeds will be determined by the longevity of the experiment and the type of experiment being performed. For example, if the recipient is to be another pig and the experiment is meant to be longer than 3 weeks in length, then one of the miniature breeds should be considered because of the difficulty in maintaining large farm pigs postsurgically. Differences will be noted in the histocompatibility complex among breeds as well. Many sources of farm and miniature pigs will have closely related individuals in the herd even if they are not littermates.

The surgical approach to the kidneys and the general perioperative procedures, such as fasting, are the same as the ones noted for nephrectomy. The midline incision is preferred because of the increased surgical exposure. The flank approach may be preferred if the donor animal is expected to survive the experiment, especially if a future reimplantation procedure is anticipated using the midline approach.

In a nonsurvival donor procedure to harvest the kidneys, the aorta and vena cava are isolated cranial and caudal to the renal vessels, and the kidneys are harvested *en bloc*. This procedure will involve ligation of the dorsal lumbar branches of the aorta and careful dissection to avoid blood loss. The aorta and vena cava are ligated proximally and distally after heparinization of the animal. A sufficient length of ureter to ensure successful reimplantation without stretching should be simultaneously harvested.

In a survival procedure, a decision has to be made concerning which kidney is to be harvested. For the left kidney, the renal artery will be shorter and the renal vein longer. For the right kidney, the opposite is true, and the adrenal gland is closely associated with the junction of the renal vein and vena cava. If using a flank approach, the left kidney is generally the kidney of choice for removal.

When a single kidney is harvested, it is best to use a Satinsky clamp to isolate the renal vessels and harvest them using a Carrel patch technique. Reimplantation of the renal vessels is greatly enhanced when such a patch is available. It also minimizes the manipulation of the renal artery, which is very prone to vasospasm. Care should be taken to avoid damaging the lymphatics in a survival procedure, because the accumulation of lymph in the abdominal cavity will result in a postoperative complication. They may either be ligated or cauterized. The donor kidney should be perfused with a cold preservation solution and kept in a chilled preservation solution or isotonic iced slurry until reimplantation.

The midline approach is preferred for reimplantation of the kidneys. A prolonged fast (24–48 h) ensures that the colon will be emptied, which aids both the surgical exposure and the prevention of edema postsurgically, following manipulation of the intestinal mass. The kidney is usually implanted into the distal aorta and vena cava or the iliac vessels to minimize the length of ureter that has to be reimplanted (Figure 7.12) and thus minimize the chances of ischemic necrosis of the structure. After heparinizing the recipient, a Satinsky clamp is applied to the artery and a longitudinal incision made. The cranial and caudal ends of the Carrel patch are sutured with 6/0 nonabsorbable cardiovascular suture, and the patch is anastomosed with a simple continuous pattern. The same procedure is performed for the renal vein, and blood reperfusion is allowed by removing first the venous and then the arterial clamps. The kidney is observed to return to a normal color following the resumption of blood flow through the vessels.

The ureter is trimmed to reach the dorsal surface of the bladder without stretching, and the end is spatulated. By opening the tip of the urethra longitudinally, the lumen of the anastomosed ureter is increased in size. A silicone tube or stent may be passed into the ureter to ensure that the lumen is not sutured closed. Simple interrupted sutures of 6/0 nonabsorbable cardiovascular suture are preplaced and the luminal tube removed prior to closure. A direct technique of uretero-neocystostomy has been described (Zonata et al., 2005). In this technique, an incision is made in both the ventral and dorsal portions of the bladder, and the ureter is threaded into the lumen through the small dorsal incision. The edges of the ureter are spatulated and sutured over the top of the mucosa.

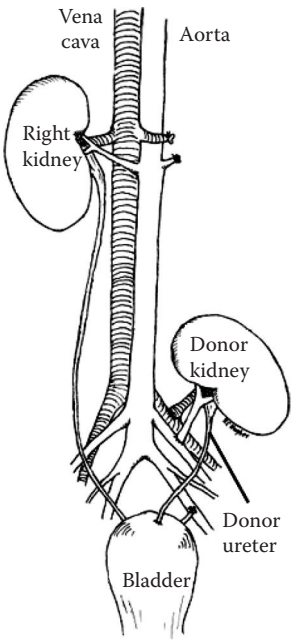

FIGURE 7.12 Implantation of a transplanted kidney (donor) into the iliac vessels.

Postimplantation, the animal should be flushed with iv fluid at a rate of 10–20 mL/kg/h and an infusion of 50 mL of 50% mannitol or glucose. An iv injection of methylene blue may be used to check for leaks in the ureteral anastomosis. Closure of the surgical incision is routine, and the postoperative recovery procedures should be aimed toward maintenance of adequate fluid levels and normal body temperature. Use of postoperative analgesics, antibiotics, and other therapeutic agents depends upon the experimental protocol. Urine output is particularly important in these studies postoperatively, and monitoring serum creatinine and blood urea nitrogen are standard techniques. Creatinine levels >6 mg/dL in association with clinical signs generally are prognostic for irreversible renal failure. Typically, these protocols are designed to test experimental renal preservation solutions or immunosuppressive regimens. One current recommendation is the use of tacrolimus 0.5 mg/kg bid and mycophenolate mofetil 250 mg bid (Zonata et al., 2005). Postoperative complications in addition to renal failure are mainly associated with stricture of the vascular or ureteral anastomosis.

CYSTOTOMY AND URETERAL DIVERSION

The bladder of the pig is thin walled and difficult to catheterize (see earlier text). Consequently, it may be desirable to suture a catheter in place during abdominal surgery in some experimental models. Atraumatic catheters have been designed for the bladder (Figure 7.13). In the pet pig population, urolithiasis is also a problem, and cystotomy may be part of the indicated treatment.

The surgical approach is via a ventral midline incision in females and a paramedian incision lateral to the penis in the male. The bladder is usually drained carefully using a needle and syringe in the male because of the difficulty in catheterization. The surgical approach may be made in any avascular portion of the bladder after packing it away from the viscera with wetted gauze sponges.

Implantation of a Foley catheter may be used for urine collection after it is sutured in place with a purse-string suture in the perineum. If a cystotomy is performed to remove uroliths, the area is copiously flushed with saline prior to suturing the incision closed. The bladder is too thin walled for a double suture layer closure except in larger animals. Any type of suture pattern that achieves a waterproof seal is appropriate. These would include a two-layer pattern of Cushing oversewn with Lembert sutures, simple interrupted, or continuous patterns using synthetic nonabsorbable sutures. The abdominal incision may be closed using a standard technique (Swindle, 1983).

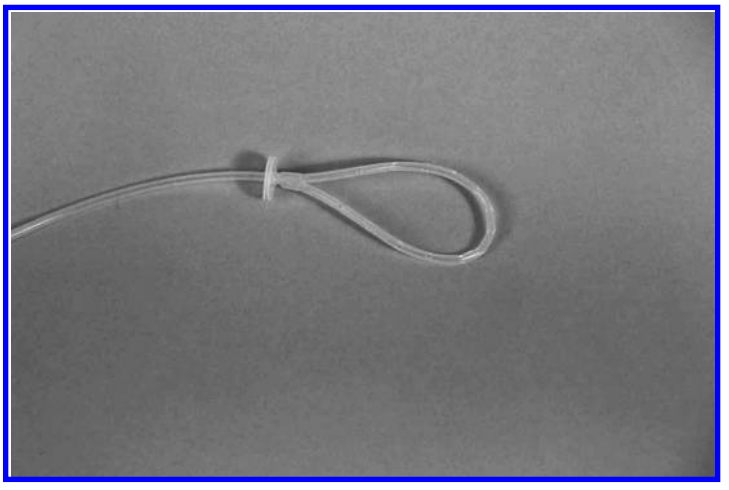

FIGURE 7.13 Urinary bladder catheter which is circular with multiple holes to prevent trauma to the mucosa.

Experimentally a porcine model has been utilized to study augmentation of the bladder (Clementson et al., 1999), stimulation to treat detrusor dysfunction (Braun et al., 2003), overactive bladder issues (Parsons and Drake, 2011) and localized anticancer treatments (Hendricksen et al., 2006).

URINARY TRACT OBSTRUCTION, REFLUX NEPHROPATHY, AND HYDRONEPHROSIS

Swine are the preferred model for the study of lower and upper urinary tract obstruction and the resulting complications because of the similarities of their internal renal anatomy to that of humans. Models have been created in various ages of swine to study the different effects of maturity. Swine less than 1 month of age are similar to neonatal humans in development of the urinary tract and swine 5–6 months of age are similar to adults. Most of the pediatric models have been studied in 8- to 12-week-old pigs. Swine can have a spontaneous occurrence of both vesicoureteral reflux and hydronephrosis and should be screened prior to surgery (Constantinou et al., 1986; Desai et al., 2005; Djurhuus et al., 1976; Jørgensen and Djurhuus, 1986; Jørgensen et al., 1983, 1984a,b; Melick et al., 1961).

A model of intrarenal reflux may be produced by surgically reimplanting the ureters. This is performed using the same ventral midline incision described for cystotomy. The intravesical ureteral roof is excised along with a small wedge of ipsilateral trigonal muscle. The ureteral roof is reimplanted at an angle to straighten the juxtavesicular portion of the ureter after performing a ventral cystotomy. The relaxation and changed angle of the ureteral orifice reliably produces vesicoureteral reflux. This is an improvement over a previous model of cutting the lower 3–4 mm of the wall of the ureteric orifice following a cystotomy. Intrarenal reflux will not occur with this model unless there is a pressure increase secondary to lower urinary tract obstruction. This is performed by partially obstructing the ureter at the neck of the bladder with a wire or plastic ring. The amount of constriction is variable, depending upon the age and breed of the pig; however, reports range from 3 to 6 mm diameters for the rings. Reflux starts to occur at a bladder pressure of approximately 10 cm H_2O in operated animals, compared to a pressure of greater than 20 cm H_2O in nonoperated animals. Infection may be studied as a complication in either of these models. The progression of the syndrome depends upon the length of time of the extravesicular obstruction as well as the degree of constriction. This procedure may lead to renal failure (Jørgensen and Djurhuus, 1986; Jørgensen et al., 1984a,b). Ureteral damage has been repaired with tubularized porcine small intestine submucosa (Duchene et al., 2004; Liatsikos et al., 2001; O'Connor et al., 2002; Smith et al., 2002) and biodegradable materials (Shalhav et al., 1999). The porcine submucosa induces ureteral ingrowth which remains functional with the characteristics of the normal ureter (Smith et al., 2002). Failures of these grafts are usually associated with inflammation and stricture.

Hydronephrosis and hydroureteronephrosis may also be produced surgically. The left ureter is primarily studied because of the increased incidence of left-sided involvement clinically in humans. The surgical approach is retroperitoneal through the flank, as described for nephrectomy (see earlier text). The ureter is identified as it progresses caudomedially to the caudal pole of the kidney in the retroperitoneal space. Complete obstruction is performed by ligating the ureter. This will lead to a rapid dilation of the kidney within hours, after which the kidney then shrinks and develops a progressive nephropathy over months (Constantinou et al., 1986; Djurhuus et al., 1976).

Partial obstruction results in more progressive changes over a period of 3–4 months. Partial obstruction may be produced by partial occlusion with cuffs or sutures tied over a premeasured rod or catheter. However, a model of progressive chronic hydronephrosis can be consistently produced by implanting a 1–2 cm length of the ureter into the psoas muscle caudal to the kidney. For this model, the fascia of the psoas muscle is incised, and the muscle fibers are split in a curved fashion medially toward the midline in the body of the muscle. The ureter is gently placed into the psoas muscle without torsion or kinking (Figure 7.14). The sheath and ventral edge of the muscle are

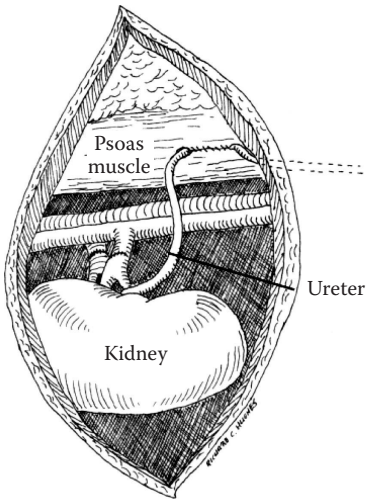

FIGURE 7.14 Suturing the urethra into the psoas muscle from a flank approach.

loosely approximated with simple interrupted sutures. This will lead to a progressive syndrome of chronic hydronephrosis secondary to obstruction within weeks. Percutaneous endopyeloplasty has been studied in a porcine model of partial obstruction created by laparoscopic ligation over a 5-Fr catheter (Desai et al., 2005). Imaging of control and obstructed pigs are illustrated in studies by Thomas Dissing of Aarhus University Hospital (Figures 7.15 through 7.18). The abdominal incision is closed in a routine manner.

PERINEAL URETHROSTOMY AND URINARY DIVERSION

In cases of urolithiasis or trauma in males, it may be necessary to permanently divert the urinary outlet to the perineum or ventral midline. In experimental models, it may be necessary to perform these procedures to facilitate chronic urine collection (Noordhuizen-Stassen and Wensing, 1983; Swindle, 1983; Tscholl, 1978).

The urethra may be palpated by using a rolling movement with the fingers on the brim of the pubis in the perineal region. If percutaneous catheterization (see earlier text) is not achieved or desired, then a perineal urethrostomy may be performed. A dorsal to ventral midline incision approximately 1–2 cm in length is made through the skin and subcutaneous tissue in the dependent portion of the perineum dorsal to the scrotum. The urethra and base of the penis can be identified between the crura and ischiocavernosus muscles and isolated in the subcutaneous tissue. The penis and urethra are ligated ventrally, and, using iris scissors, the urethra is split dorsally for a distance of at least 1 cm. Care should be taken to avoid trauma to this tissue by using atraumatic instruments, such as Debakey forceps, when handling the urethra. Complete hemostasis in the subcutaneous tissue is essential for the success of this procedure. After splitting the urethra, it is sutured to the skin using a simple interrupted pattern with synthetic absorbable or monofilament nonabsorbable sutures. This will result in a dorsal oval-shaped entrance into the urethra with a ventral rectangular-shaped apron. The skin is closed with simple interrupted sutures (Figure 7.19).

If urine collection is the goal of this surgery, pediatric adhesive urine collection bags may be attached to the skin over the incision site. The skin must be prepped with alcohol to achieve adhesion with these bags, and stay sutures may be required. The main complication encountered postoperatively will be urine scalding, especially if the pig is uncastrated and the scrotum is prominent. This complication requires the use of topical anti-inflammatory and/or antibiotic ointments on a chronic basis if it occurs. Simultaneous castration may help relieve this complication by making the

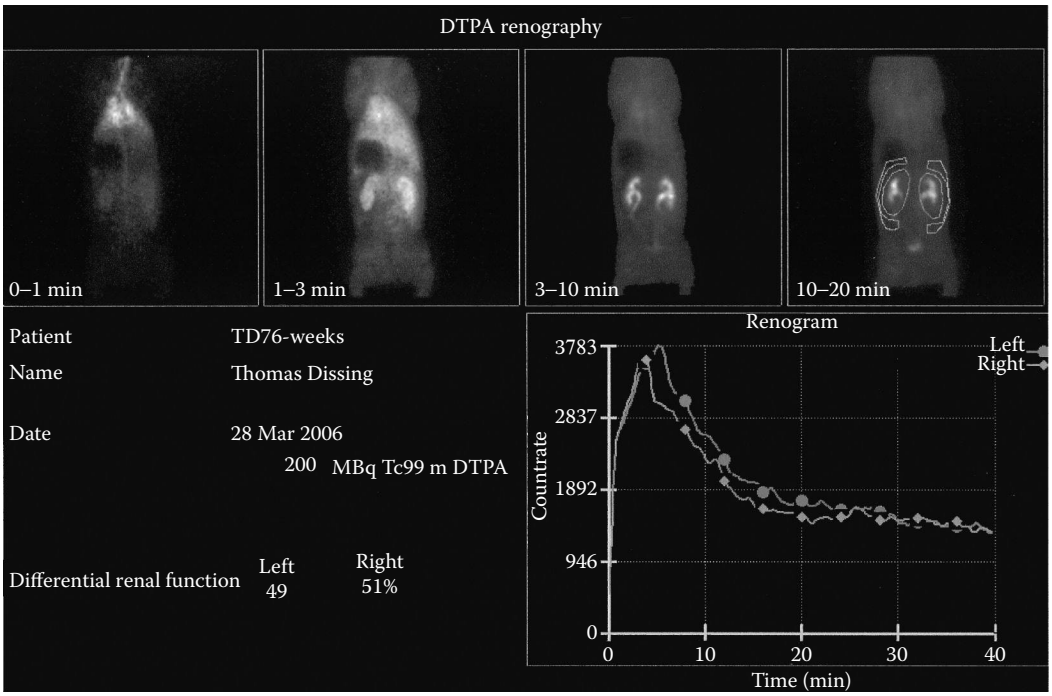

FIGURE 7.15 ^{99m}Tc-DTPA furosemide renography of a 4-week-old sham operated pig with normal kidneys. ^{99m}Tc-DTPA demonstrates glomerular function of the kidneys and urine transportation. The gamma rays emitted from the compound allow the gamma camera to obtain subsequent images displaying its distribution in the body. The resulting dynamic image recording is analyzed with regard to the kidneys filtration of ^{99m}Tc-DTPA and the clearance of the urine from the kidney. By administering furosemide diuresis is enhanced and the patency of the collecting system is demonstrated. The renogram shows the two kidneys both draining well and evenly. The differential renal function shows even function between the two kidneys. (Courtesy of Thomas Dissing, MD, Institute of Clinical Medicine, Aarhus University Hospital, Denmark.)

scrotum less prominent. Another complication to be considered is contamination with feces in this area. Alternatively, a Foley catheter may be placed into the incised urethra and directed caudally into the plastic adhesive pouch. In this case, the incision is closed with a purse-string suture.

If long-term maintenance of the animal is indicated, then it may be preferable to perform urinary diversion by performing an urethrostomy with the urethra directed ventrally cranial to the scrotum on the ventral midline.

PREPUTIAL DIVERTICULUM ABLATION

The preputial diverticulum may be surgically ablated to reduce the odor of male pigs and to relieve infection of the structure. The structure should be manually expressed and flushed with saline and dilute Betadine solution prior to surgery. There are two lateral epithelial-lined pouches within the structure. These are exteriorized one at a time by inserting Allis or Babcock forceps into the preputial opening and directing them laterally. After grasping the lining of the structure with the forceps, the lining is steadily pulled out of the opening. This process is then repeated on the opposite side. Any visible blood vessels are cauterized or ligated, and the structure is excised. The resected pockets are packed with umbilical tape or gauze for 5–10 min to provide hemostasis. After the gauze is removed, the structure is examined for hemorrhage and repacked if necessary.

The surgery may also be performed by an open technique, in which the diverticular pouches are packed with umbilical tape or gauze to identify them, and a skin incision is made over each

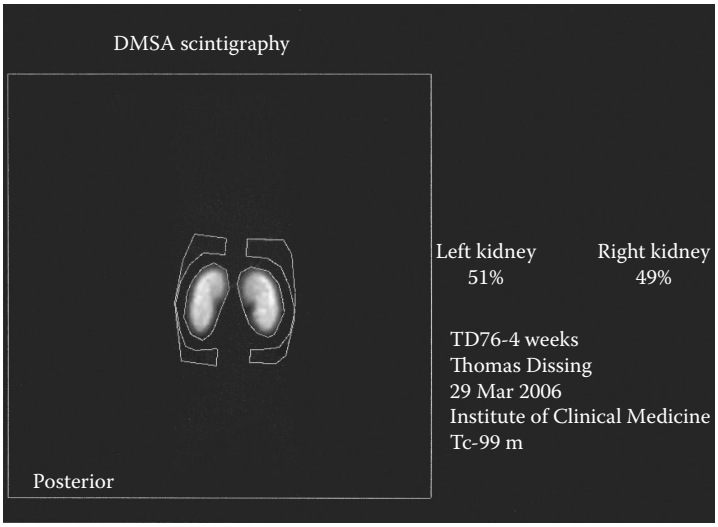

FIGURE 7.16 ^{99m}Tc-DMSA scintigraphy of the same pig (Figure 7.15) the following day. ^{99m}Tc-DMSA demonstrates renal tubular function. The compound is taken up by proximal tubular cells. The amount of uptake is in close relation to the amount of functioning renal mass. The following day after its administration the scintigraphy is performed. The image produced displays ^{99m}Tc-DMSA distributed in the body with the vast majority taken up by the kidneys. The functional distribution in this examination confirms even function of the two kidneys. (Courtesy of Thomas Dissing, MD, Institute of Clinical Medicine, Aarhus University Hospital, Denmark.)

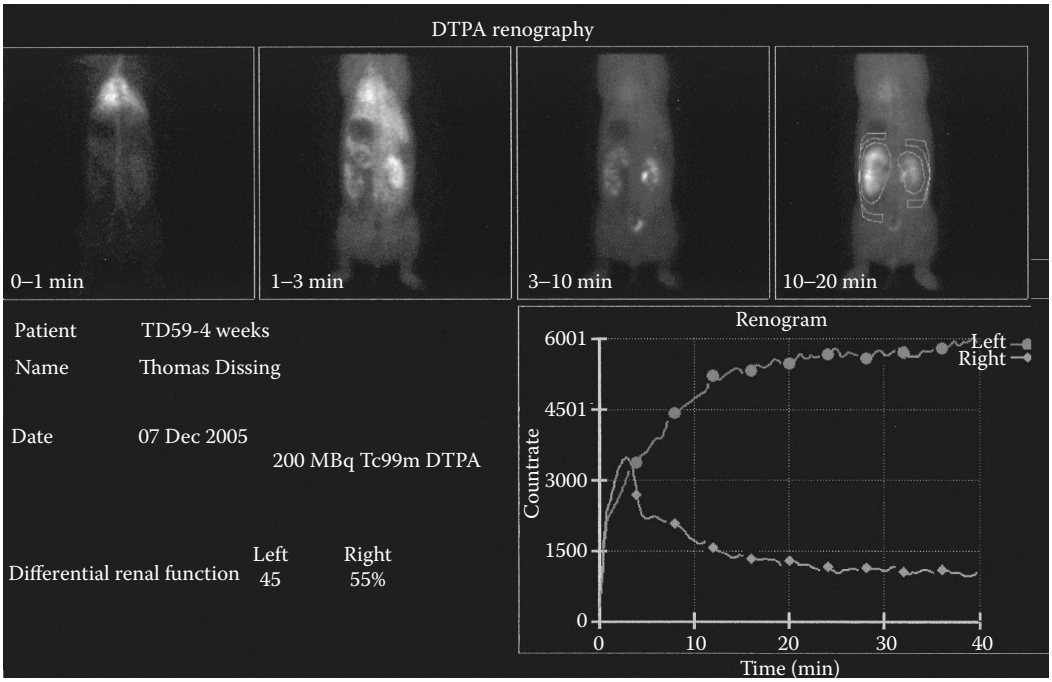

FIGURE 7.17 ^{99m}Tc-DTPA furosemide renography of a 4-week-old pig that was subjected to ureteric obstruction at the age of 2 days. The renogram shows severe hydronephrosis and impaired drainage of the left kidney. The differential renal function of the left kidney is slightly decreased, which could be interpreted as the onset of progressive kidney damage. (Courtesy of Thomas Dissing, MD, Institute of Clinical Medicine, Aarhus University Hospital, Denmark.)

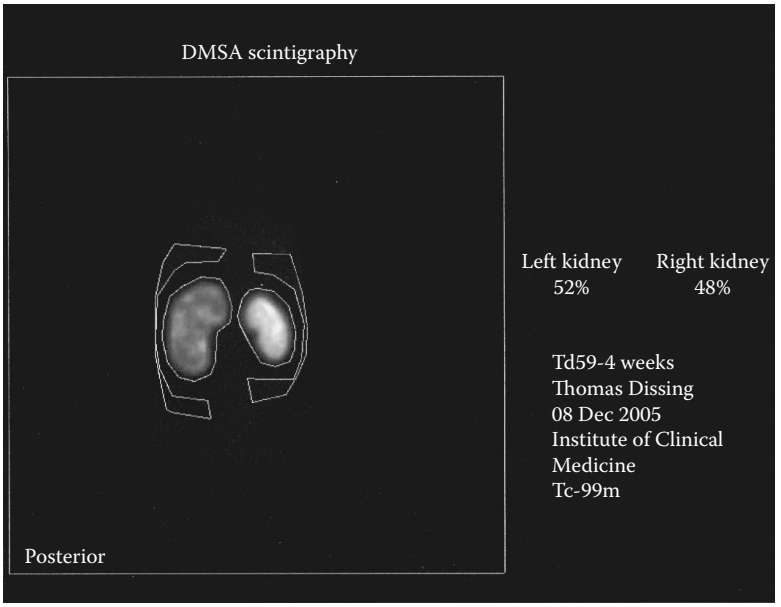

FIGURE 7.18 ^{99m}Tc-DMSA scintigraphy of the 4-week-old pig (Figure 7.17). The left kidney has a slightly higher differential renal function. The suspicion of kidney damage raised by the renography is therefore not confirmed by the scintigraphy. Hence, the two investigations together do not demonstrate abnormal function of the obstructed kidney. (Courtesy of Thomas Dissing, MD, Institute of Clinical Medicine, Aarhus University Hospital, Denmark.)

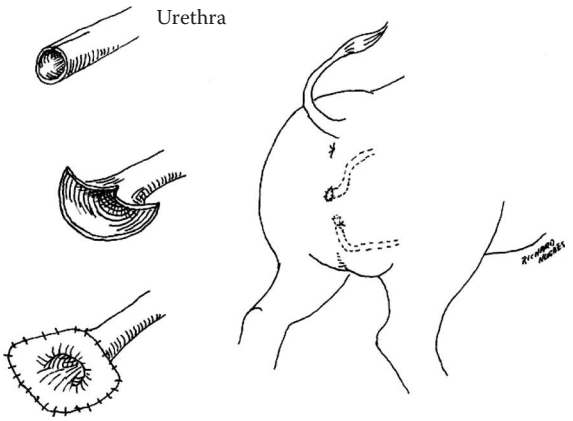

FIGURE 7.19 Perineal urethrostomy.

structure. The pouches are then dissected free from the subcutaneous tissue. The surgical wound is closed with subcuticular sutures (Bollwahn, 1992; Dutton et al., 1997; Kross et al., 1982; Lawhorn et al., 1994; St-Jean and Anderson, 1999).

ADRENALECTOMY

The surgical approaches to the adrenal gland are the same as for midline, flank, and retroperitoneal approaches to the kidney, described previously for nephrectomy. The surgical anatomy of the adrenal glands is depicted in Chapter 1 (Figure 1.41) and Figure 7.1. Indications for adrenalectomy

in swine will most likely involve the creation of a model of adrenal insufficiency and thus will probably be bilateral. If a unilateral adrenalectomy is indicated, the left adrenal gland is more readily removed (Dougherty, 1981).

Regardless of the surgical approach, the adrenal glands are readily mobilized by blunt dissection. In older animals, they will probably be covered with perirenal fat. After identification of the arterial branches, the branches are ligated and transected. Variations in the blood supply to both glands may be encountered, and care should be taken not to damage the renal vessels during the dissection. Careful dissection is required to separate the right adrenal gland from the wall of the caudal vena cava and to ligate the venous drainage. Removal of this gland may require repair of the vessel wall. After the adrenalectomy is performed, the abdominal incision is closed in a routine fashion.

Intraoperative and postoperative care is directed toward controlling the effects of glucocorticoid and mineralocorticoid insufficiency. In a bilateral procedure, these complications can rapidly lead to death from electrolyte imbalances. Unilateral procedures may induce adrenocortical insufficiency, which may require supplementation until the remaining adrenal gland hypertrophies and restores function. Monitoring of serum electrolytes, especially potassium, and glucose is essential. Fluid therapy and glucocorticoid administration, with such agents as prednisolone, will be necessary to restore imbalances. With a bilateral adrenalectomy, the exogenous maintenance therapy is permanent. Otherwise, postoperative care is routine for celiotomies and laparotomies.

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8 The Reproductive System

M. Michael Swindle

CONTENTS

GENERAL PRINCIPLES OF SURGERY

The reproductive tract of the female is typical of that of a bicornuate species that produces litters. The ovaries are located caudal to the kidneys and are only loosely attached by a thin ovarian ligament and suspended within the broad ligament of the uterus (mesometrium). The ovarian vessels are the last branches of the aorta and vena cava prior to the iliac bifurcation at approximately L5–L6. The fallopian tubes are long and tortuous and typically form coils in the caudal abdominal cavity (Chapter 1, Figure 1.43; Figures 8.1 and 8.2). The fallopian tubes of an 80- to 100-kg pig approximate the diameter of those of an adult human (Rock et al., 1979). The uterine horns are long and curve cranially from the ovaries and then reverse direction to form the short body of the uterus at approximately the same region of the ovaries in the midsagittal plane. The cervix is thick, elongated, and has a curved cervical canal. The vagina extends caudally in the pelvic cavity and contains the urethral orifice on the ventral floor at approximately the level of the caudal edge of the pubic bone and has similar morphologic characteristics to humans (D'Cruz et al., 2005). The pig has a diffuse epitheliochorial placentation with drug transport and metabolic mechanisms similar to humans. This type of placenta does not invade the endometrium. The placental membranes include the yolk sac, amnion, allantois, and chorion, and the latter two membranes fuse at an early stage to form a chorioallantoic type of placenta. The chorioallentois is responsible for transplacental transport of nutrients from the sow (Bazer et al., 2001; Swindle and Bobbie, 1987; Swindle et al., 1996; Wiest et al., 1996). Colored histologic sections are contained on the textbook DVD.

Paired mammary glands are located on the ventral surface of the abdomen and may extend to the caudal thorax and caudal inguinal regions. The number of glands is highly variable with an average of six to seven pairs. Generally, breeds that produce large litters have a greater number. The vasculature is located along the lateral edges of the row of glands. The pectoral and cranial abdominal glands receive their blood supply from branches of the internal thoracic artery, which continues along the glands on the abdomen as the cranial superficial epigastric artery. These mammary glands drain into the cranial superficial epigastric vein, which continues cranially as the internal thoracic vein. The caudal glands (two to three pairs) receive their blood supply from the external pudendal arteries, and drainage is from the pudendal veins into the iliac system (Bazer et al., 2001).

FIGURE 8.1 Anatomy of the female reproductive tract. The fallopian tube is fully extended on one horn.

The male reproductive system is typical of domestic animals in location; however, there are several variations in anatomy that are important surgically (see Chapter 1, Figure 1.42). The scrotum and testicles are located in the perineal region and may be of considerable size in adult males. The spermatic cord passes through each inguinal ring in the caudal abdomen. The spermatic vessels branch off the aorta and vena cava just cranial to the iliac bifurcation. Each ductus deferens enters the urethra independently on the dorsal surface at the neck of the bladder. The accessory sex glands are similar to those of humans except for the prominence of the various structures. The paired vesicular glands are large and located on either side along the neck of the bladder. The prostate is small and located on the dorsal surface of the urethra at the entrance of the ductus deferens. Paired bulbourethral glands extend along the dorsolateral surface of the urethra starting at the caudal brim of the pubis; these may extend along the entire length of the pubis in intact adults. The crus of the penis and the ischiocavernosus muscles form at the caudal surface of the pubis with the base of the penis. The penis extends ventrally and cranially forming a fibromuscular sigmoid flexure as it curves from the perineum to the ventral surface of the abdomen. The penis extends almost to the umbilicus before terminating in the preputial diverticulum in a corkscrew shape. The preputial diverticulum is described additionally with the urinary system; however, it is important to restate that it must be cleaned before surgically preparing the abdomen and that gloves should be worn to avoid contamination with the foul-smelling fluid contents. The tip of the penis is almost impossible to exteriorize without trauma (Swindle and Bobbie, 1987; Swindle et al., 1988).

CASTRATION (ORCHIECTOMY) AND VASECTOMY

Swine may be castrated either by scrotal, prescrotal, or ventral midline approaches. The most common approach in small swine is prescrotal, whereas the scrotal approach predominates in adult males. For either the prescrotal or the ventral midline approach, the testicle is manually manipulated cranially, and either a paramedian or midline incision is made over the testicle (Becker, 1992; Mayo and Becker 1982; McGlone and Hellman, 1988; Ostevik et al., 2012; St-Jean and Anderson, 1999; Swindle, 1983).

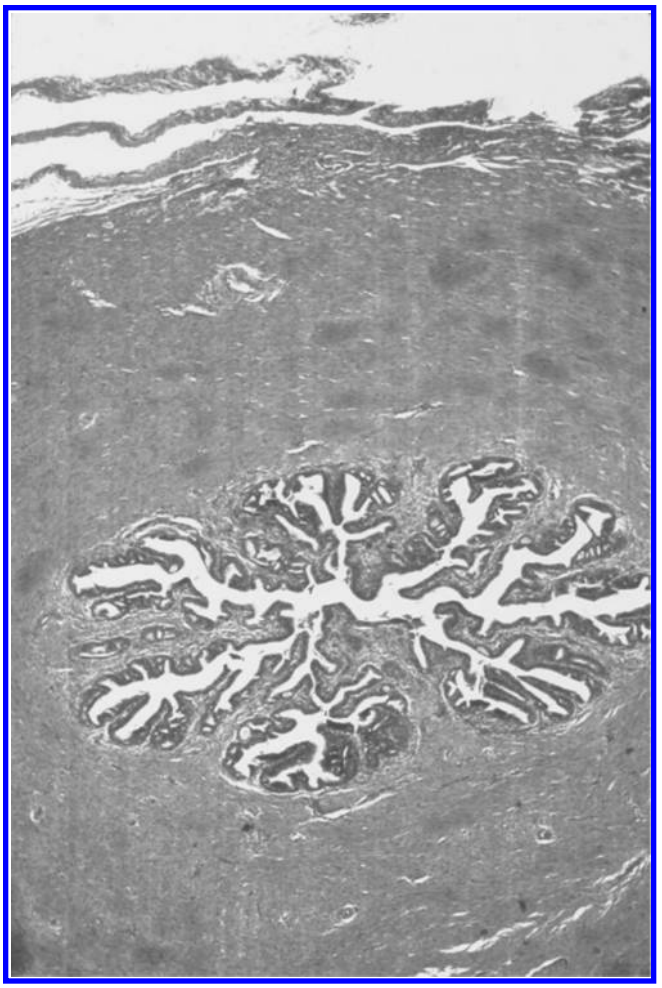

FIGURE 8.2 Histology of the fallopian tube. H&E, ×40.

The skin and subcutaneous tissue are incised in the initial incision. The tunica vaginalis is incised without incising the testicular tissue. The testicle is exteriorized, and the spermatic cord is dissected away from the mesorchium. Clamps are placed across the scrotal ligament, and the spermatic cord and the testis are removed. The spermatic cord and the scrotal ligament are ligated with synthetic absorbable suture material. The incision is closed with continuous suture patterns in the tunica vaginalis and subcutaneous tissues. The skin is closed in a subcuticular pattern. It is unnecessary and undesirable to leave the castration incision open and draining as is done in the agricultural setting. If adequate hemostasis is achieved, seromas or hematomas will not be a problem.

If a vasectomy is to be performed, the spermatic cord is identified as it passes from the scrotum to the inguinal canal at approximately a 45° angle to the midline from the scrotum to the brim of the pubis. At approximately one-half of the distance between the scrotum and the inguinal canal, the spermatic cord can be palpated as a firm tubular structure. A skin incision is made over the spermatic cord, and the tunic is incised. The vas deferens can be identified as a firm whitish structure approximately 2–3 mm in diameter. The vas deferens is isolated, doubly ligated, and a segment removed. The tunic is closed with simple interrupted sutures, and the skin and subcutaneous tissues are closed in a routine manner (Figure 8.3).

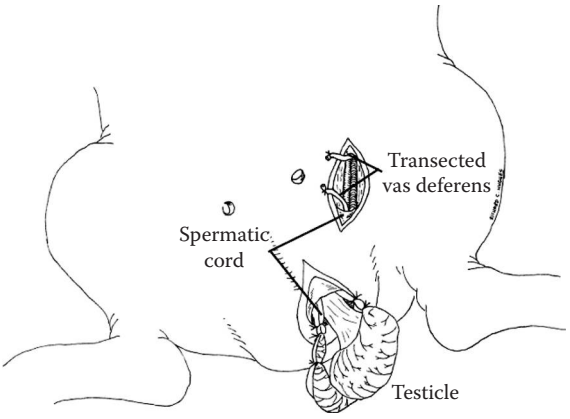

FIGURE 8.3 Castration and vasectomy surgical sites.

OVARIOHYSTERECTOMY, HYSTERECTOMY, OVARIECTOMY, OR TUBAL LIGATION

The female reproductive tract is usually approached through a ventral midline incision except in the case of a cesarean section (see the following text). The incision extends from the brim of the pubis to approximately two-thirds of the distance cranially to the umbilicus. Upon entering the abdomen, the fallopian tubes are likely to be the initial structures encountered in the nongravid system. They may be used to trace the origin of the ovaries and the horns of the uterus (Christenson et al., 1987; St-Jean and Anderson, 1999; Swindle, 1983).

The ovarian vessels are clamped, incised, and ligated proximally and distally. If only an ovariectomy is to be performed, then the fallopian tubes are divided surgically in the same manner, and the ovaries are removed. A tubal ligation may be performed by doubly ligating and dividing the structures or by placing occlusal devices on the structure for reversible sterilization (Rock et al., 1979).

If an ovariohysterectomy is to be performed, then it is necessary to dissect the horns of the uterus from the broad ligament of the uterus (Figure 8.4). This is performed by bluntly dissecting along the uterine vessels following the curvature of the uterine horns. The middle uterine vessels will have to be divided and ligated separately. The dissection is continued bilaterally to the level of the cervix.

At this point, the ovaries and uterine horns are retracted caudally, and the cervix is exteriorized. The blood vessels on the lateral sides of the vagina are individually ligated in place. The vagina proximal to the cervix is cross-clamped and divided. The vaginal stump is sutured with transfixing ligatures in the case of nulliparous animals. In sows, the stump may have to be divided using an inverting technique such as a Cushing oversewn with a Lembert pattern. In the case of a hysterectomy, the instructions for the ovariohysterectomy are followed except that the ovaries are left intact, and the initial surgical transection starts with the fallopian tubes (Figure 8.5).

FIGURE 8.4 Exposure of the uterus and ovary.

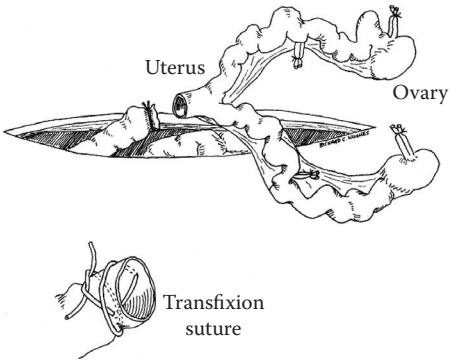

FIGURE 8.5 Ovariohysterectomy.

The middle uterine artery may be surgically constricted to reduce blood flow to the fetuses dependent upon the blood supply to the uterus. If performed in the second trimester of pregnancy, it retards fetal and placental growth and development. It also reduces maternal estrogen blood levels (Molina et al., 1985).

Unlike many species of domestic animals, such as the dog and cat, swine rarely develop pyometritis, and the indications for these procedures are rarely clinical. Consequently, problems associated with removal of the gonads and retention of the uterus are rare. Rather, these procedures tend to be either research techniques or surgical sterilization procedures for the pet pig. In the case of pet pigs, the ovaries should be removed in order to avoid the behavioral problems associated with estrus.

The pig has also been used as a preclinical model for the study of surgically implanted gynecologic meshes prior to their use in humans (Boulanger et al., 2006).

CESAREAN SECTION (C-SECTION)

A c-section may be performed using a midline, paramedian, or flank surgical approach (St-Jean and Anderson, 1999; Swindle et al., 1996). The midline and paramedian incisions are made as described previously for ovariohysterectomy. If the c-section is to be performed as a survival procedure with the pigs being allowed to nurse the sow, then it is best to avoid the midline approach. The midline will be constantly irritated by the pigs, and infection and refusal to nurse will be encountered as complications.

The paramedian incision is made along the dorsolateral margin of the mammary glands with the pig in lateral recumbency. The incision is made from the cranial margin of the retracted rear leg (approximately the brim of the pubis) to approximately the level of the umbilicus. In the pregnant sow, the muscle in this region will be thin and relatively avascular when cut; however, several branches of the caudal abdominal arteries (mainly external pudendal arterial branches) will have to be ligated or cauterized.

The flank approach is the most commonly employed approach for a c-section when the pigs are going to be allowed to nurse. A vertical incision is made from the level of the wings of the lumbar vertebrae ventrally to a point halfway to the mammary glands. The incision is located approximately one-half of the distance between the cranial surface of the thigh to the last rib. After making the skin incision, the muscles may either be bluntly dissected or transected in layers until the abdominal cavity is exposed.

Depending upon the number of fetuses and the experimental purpose of the surgery, multiple incisions will probably have to be made along the uterine horn, rather than making a single incision in the body of the uterus and removing all the fetuses through it. If the uterus and fetuses are large, it may be possible to remove all the fetuses and membranes through an incision in the uterine horn.

If fetuses have been manipulated experimentally, however, then an incision either over each fetus or between two fetuses may be required.

When the decision has been made to incise the uterus, the surgical site is packed off with warm saline-wetted laparotomy sponges. The uterotomy incision may be made using a scalpel; however, better hemostasis is achieved using absorbable GIA-60 3.8-mm staples (Figure 8.6). The device is inserted and fired after making a stab incision into the amniotic cavity. The fetus and membranes are extracted and handed to an assistant, who cleans and resuscitates the piglet after ligation of the umbilical vessels. The uterus will contract relatively rapidly following removal of a fetus, which hampers the effort to remove multiple fetuses from the same incision. Hemorrhage from removal of the fetal membranes is usually minimal, provided the c-section is performed at full-term gestation.

If a fetus is being removed before full-term gestation, then it is necessary to ligate and transect the umbilical vessels first. After this procedure, the membranes are bluntly separated from the uterine wall and extirpated. Gauze sponges may have to be used to provide hemostasis of the uterine wall following this procedure.

The uterotomy may be closed with a two-layer suture pattern of Cushing or Connell sutures oversewn with a Lembert suture pattern. It may also be closed with a staple surgical device, such as a TA-90 3.8 mm with absorbable staples, if staples were used to perform the uterotomy. In this case, the initial lines of staples are lifted together using Babcock forceps, and the TA device is closed distal to the suture line (Figure 8.7). After firing the device, a scalpel is used to trim the uterus between the device and the GIA staples. The line of TA staples is oversewn with a continuous Lembert pattern using absorbable sutures. The celiotomy incision is closed in a routine manner.

Fertility may be impaired following a c-section and this must be considered when making a clinical decision about performing the surgery as a survival procedure in the sow. Oxytocin may be given postoperatively if there is reason to believe that intrauterine hemorrhage may occur or that membranes remain in the uterus. The pathophysiology of perinatal asphyxia in perinatal pigs has been reviewed and compared to the syndrome in humans (Alonso-Spilsbury et al., 2005). Successive uterine contractions, placental insufficiency, pressure or torsion of the umbilical cord, and meconium aspiration are significant factors in mortality of pigs both intrauterine and during parturition. These factors may be important issues to prevent during procedures such as fetal surgery (described below).

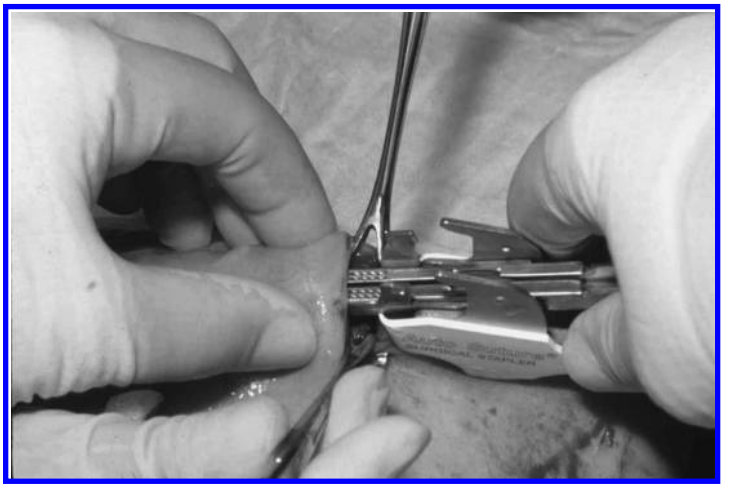

FIGURE 8.6 Uterotomy using a staple device. (Reprinted from Swindle, M.M. et al., 1996. *Lab. Anim. Sci.,* 46(1): 90–95. With permission.)

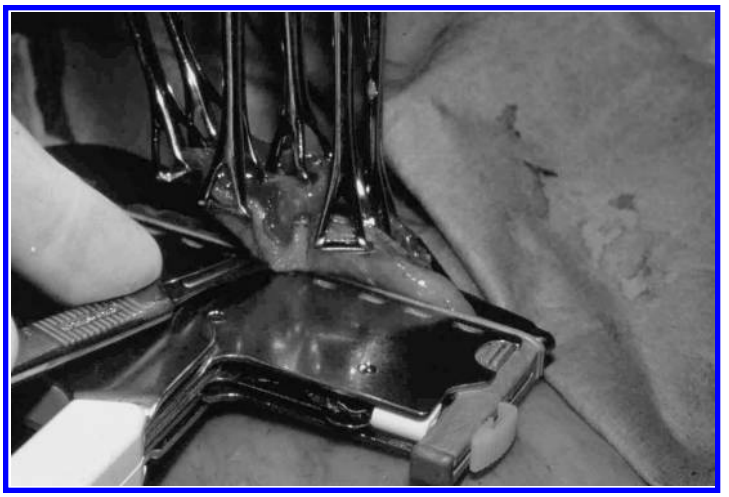

FIGURE 8.7 Closure of the uterus with staples. (Reprinted from Swindle, M.M. et al., 1996. *Lab. Anim. Sci.,* 46(1): 90–95. With permission.)

FETAL SURGERY AND FETAL CATHETERIZATION

The use of the pig as a fetal surgical model, described in the preceding sections, is increasing because of the metabolic and anatomic similarities to humans and the availability of miniature breeds. Miniature breeds should be used whenever possible, because of the ease of handling smaller animals, which makes them significantly safer for personnel. For instance, a pregnant Yucatan sow may weigh 45–60 kg in contrast to a pregnant domestic farm pig that will probably weigh in excess of 135 kg. In this section, only the general surgical principles and the fetal catheterization techniques will be described. Other models will be described in the systemic sections of this text (Care et al., 1986; Dungan et al., 1995; Jackson and Egdahl, 1960; Jones and Hudak, 1988; Kraeling et al., 1986; Papanna et al., 2014 submitted, Randall, 1986; Rosenkrantz et al., 1968; Sims et al., 1997; Swindle et al., 1996; Wiest et al., 1996). The optimal embryonic age for harvesting of fetal tissues for transplantation has been outlined for the liver, pancreas, and lung (Eventov-Friedman et al., 2005). The embryonic ages at which tissues are produced following subrenal capsular implant of macerated tissues in mice were as follows: liver (21- to 28-day gestation), pancreas (28- to 56-day gestation), and lung (56- to 80-day gestation). Earlier implantation dates resulted in teratoma formation without functional adult tissue. Comparisons of embryonic development with humans and spontaneous congenital defects of minipigs are outlined in Tables 8.1 and 8.2, respectively.

Timing of the surgery may make a difference in the selection of the breed because of size of the fetus. Each trimester of pregnancy is 38 days in a species with a 114-day gestation period. The last trimester of pregnancy (>78 days) is used for many procedures, because the size of the fetus makes it amenable to catheterization and device implantation. The consistency and volume of the amniotic fluid changes as gestation progresses. For the first two trimesters, the amniotic fluid has a watery consistency and occupies a relatively large volume of the amniotic sac. As parturition approaches, the amniotic fluid becomes relatively thick and yellowish in color from the passage of meconium. In the last few days of pregnancy, the fluid volume is substantially decreased. The fluid volume is highly variable between sows, and it is unclear whether replacement of the volume is necessary following fetal surgery. In our experience, the saline drip (described later) used during surgery is generally sufficient to replace the volume lost. Some relative sizes of the fetus at different stages of pregnancy for various breeds are given in Table 8.3.

TABLE 8.1
Embryonic Development in Göttingen Minipigs

	Minipigs		Humans	
Event	**Gestation Day[a]**	**Length (mm)**	**Gestation Day[a]**	**Length (mm)**
Two-cell stage	1		1	
Morula	3		2	
Blastula	5–6		4–5	
Somites appear	14–15	3.0	20–21	2–3
Optic vesicles form	16	3.5–4.5	24–25	3–4.5
Neural groove closes	16–18	4.5–5.5	24–27	3–5
Upper limb buds appear	18	5.5	26–27	3.5–5
Hind limb buds appear	20	8.5	28–30	4–6
Digital rays present	24	14–15	36–42	9–14
Nipples form	28	22.5	43–49	13–22
Eyelids form	35	30–35	43–49	13–22
Palate closes	35	30–35	56	21–31

Source: Reprinted from Jørgensen, K.D., 1998. *Scand. J. Lab. Anim. Sci.,* 25(Suppl. 1): 63–75; as modified from Glodek, P. and Oldigs, B., 1981. *Das Gottinger Miniaturschwein.* Hamburg, Berlin: Verlag Paul Parey, pp. 130–142. With permission.

[a] Fertilization day = 0.

Maintenance of normothermia and homeostasis of the sow is imperative in order to be successful with the procedures described here. This can be achieved by a combination of all or some of the following: (1) keeping the room temperature higher than 29°C, (2) using circulating water blankets both below and above the sow's thorax, (3) using heat lamps suspended near the surgical site, and (4) dripping saline of 37°C into the amniotic cavity during surgery. Use of the warmed saline drip allows lowering the room temperature and discontinuance of the heat lamps that can be desiccating to delicate fetal tissues.

Other perioperative procedures that contribute to the success of these procedures include restricting the fetus from breathing by keeping the head submerged in amniotic fluid, meticulous attention to asepsis, the prophylactic use of systemic antibiotics, delicate handling of tissues, and an anesthetic protocol that does not significantly depress the sow or fetus. Anesthesia is discussed in a separate chapter; however, analgesics and muscle relaxants may be delivered directly to the fetus if necessary. For example, pancuronium or rocuronium may be administered to restrict fetal breathing if the fetal head has to be removed from the uterus during surgery. Hemostasis is essential in any of the procedures performed because of the small blood volume of the fetus. Vessels should be ligated in advance of transection, if possible, and electrocautery on a low setting should be used. Use of microsurgical instruments and microsurgical cannonball and spear-shaped swabs are helpful in some procedures. All fetal tissue is extremely friable and can be readily traumatized by use of adult-sized surgical instruments. Ophthalmic and pediatric instruments are useful in cases in which the smaller microsurgical instruments are not required. All gauze should be wetted, and the fetus and membranes should be bathed regularly in saline warmed to 37–38°C. Monitoring the fetal heart rate is a very helpful indicator of fetal distress and hypothermia. In an 80- to 90-day-old fetus, the heart rate is approximately 180 beats per minute.

The paramedian surgical approach, as described for c-section, is preferred to avoid having undue pressure placed on the aorta when the sow is restrained in dorsal recumbency. This may lead to cardiovascular compromise, uteroplacental ischemia, or both. The line of incision is made in an

TABLE 8.2
Incidence of Congenital Anomalies in Göttingen Minipigs (2001–2005)

Description	Code	2005	2004	2003	2002	2001
Number of live-born minipigs		4957	4957	3916	2887	3021
Male		2519	2569	2049	1490	1544
Female		2438	2388	1867	1397	1477
Stillborn	240,303	230	298	305	212	216
Percentage of total pigs born		4.64	6.01	7.79	7.34	7.15
Cryptorchidism	241,701	95	127	97	—	—
Percentage of male pigs born		3.77	4.94	4.73	—	—
Double cryptorchidism	241,702	9	18	11	1	1
Percentage of male pigs born		0.36	0.70	0.54	0.07	0.06
Scrotal hernia	241,703	101	172	157	—	—
Percentage of total pigs born		2.04	3.47	4.01	—	—
Inguinal hernia	411,801	62	119	175	—	—
Percentage of total pigs born		1.25	2.40	4.47	—	—
Anal atresia	241,801	1	1	3	0	1
Percentage of total pigs born		0.02	0.02	0.08	0.00	0.03
Defect in claw[a]	241,901	2	0	0	0	0
Percentage of total pigs born		0.04	0.00	0.00	0.00	0.00
Polydactyly[b]	241,902	84	154	223	66	42
Percentage of total pigs born		1.69	3.11	5.69	2.29	1.39
Syndactyly	410,301	15	17	18	2	3
Percentage of total pigs born		0.30	0.34	0.46	0.07	0.10
Other deformity, leg[c]	410,401	24	27	36	1	1
Percentage of total pigs born		0.48	0.54	0.92	0.03	0.03
Blind, one eye	410,901	4	9	11	0	0
Percentage of total pigs born		0.08	0.18	0.28	0.00	0.00
Blind, both eyes	410,902	0	2	1	1	0
Percentage of total pigs born		0.00	0.04	0.03	0.03	0.00
Defect in ears[d]	411,201	5	9	8	21	0
Percentage of total pigs born		0.10	0.18	0.20	0.73	0.00
Other deformity, head[e]	411,301	12	12	20	—	—
Percentage of total pigs born		0.24	0.24	0.51	—	—
Hermaphrodite	412,101	0	0	0	0	0
Percentage of total pigs born		0.00	0.00	0.00	0.00	0.00
Defect in jaw[f]	411,101	6	4	—	—	—
Percentage of total pigs born		0.12	0.08	—	—	—

Source: Courtesy of Ellegard Göttingen Minipigs ApS, Dalmose, Denmark.

Note: Dash (—) indicates items not registered.

[a] Process on claw, abnormal nail growth, etc.

[b] Includes front and rear legs.

[c] Stiff knee joint, crooked/gnarled leg, stiff leg aligned with body, etc. In all cases, both forelegs and hind legs, but most commonly forelegs. Can be one or more legs.

[d] Thick or very thick ear, part of ear missing, etc.

[e] Hole in skull, skull not closed, balloon-shaped skull, etc.

[f] Cleft palate, cleft lip and palate, deformed jaw, etc.

TABLE 8.3
Relative Fetal Sizes for Various Breeds at Different Stages of Pregnancy

Breed	Gestation Day	Crown/Rump Length (cm)	Weight (g)
Yucatan	100	15.7–16.9	350–391.3
	90–92	15.8–17.5	318–403.5
Göttingen	111–112	13–16	305.3–370.4
Landrace	80	16.5–17.5	332.5–337

avascular section along the antimesometrial border of the uterus. The uterotomy is best performed using staple surgical devices as described for c-sections (Figure 8.6). If the uterotomy is done using conventional surgical techniques, hemostasis becomes problematic for the fetal membranes. They are too friable for most hemostatic techniques, and electrocautery does not function well. Babcock forceps can be placed on the edges of the incision so as to provide occlusion of the hemorrhaging vessel. Use of surgical staples seems to help prevent the edematous reaction that occurs in the fetal membranes if conventional surgical methods are used.

The position of the fetus in the uterus can be determined prior to performing the uterotomy by careful palpation. The snout tends to be readily identifiable after the middle of the second trimester of pregnancy. Care should be taken to avoid damaging the membranes or the umbilical cord during the palpation and the surgical procedure. Hemorrhage in the membranes can lead to abortion, fetal mummification, or resorption. In the first trimester of pregnancy, pinching the membranes and the fetal head can be used as a technique to reduce the number of fetuses *in utero*.

The smallest incision possible should be used to expose the area of interest in the fetus. Following uterotomy, the fetal surgical site should be gently rotated into the incision with care, so that torsion of the umbilical vessels is avoided. An assistant can retain the fetal surgical site in the uterotomy exposure by applying gentle pressure beneath the fetus. The fetus can also be positioned by exposing a fetal leg and manually restraining it using a wetted gauze sponge. Loss of amniotic fluid is not harmful provided it is replaced with a similar volume of sterile saline. With continuous drip of warmed saline as the method of providing heat to the fetus, this volume is constantly replaced.

Once the fetal incision is made, the edges can be retained by clamping them to the edges of the uterotomy incision with Babcock forceps. Microsurgical or ophthalmic self-retaining retractors can also be applied into the incision and held by an assistant.

The site of the fetal surgical incision will depend upon the technique being performed; however, some general guidelines are useful. The area of the umbilicus and the cranial abdominal midline are problematic for incisions because of the possibility of damaging the umbilical vessels or causing vasospasm. It is best to use either lateral thoracotomies and paramedian or flank incisions to enter the thorax and abdomen rather than median sternotomies or midline celiotomy incisions. The flank region offers the most easily dissected subcutaneous tissue for implantation of devices.

Catheterization of the neck vessels can be done from either a ventral midline or preferably a paramedian incision made over the external jugular vein as described for the adult (Figure 8.8). The femoral vessels can be catheterized by externalizing the rear leg and performing the cut down in the same manner as for adults. The axillary vessels are potentially useful, but difficult to access and catheterize because of the acute angles involved in catheter insertion. The umbilical vessels can be catheterized, but their tortuous course and friability frequently lead to extravasation, especially after they enter the abdomen and turn cranially. When manipulating the fetus without observing the umbilical vessels, as for these procedures, the surgeon must take care to avoid torsion of the umbilical vessels.

A variety of catheterization techniques have been described for fetal swine. The system of using vascular access ports has proved to be reliable and minimizes the opportunity for infection.

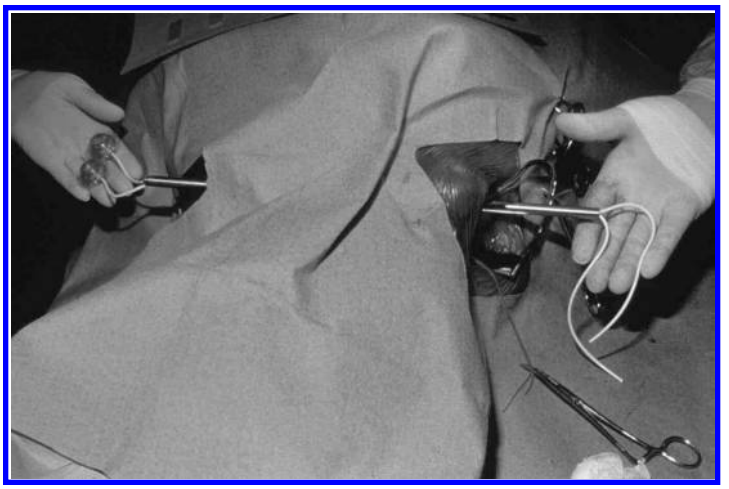

FIGURE 8.8 Trocar placement for catheterization of the neck vessels with the sow in lateral recumbency. (Reprinted from Swindle, M.M. et al., 1996. *Lab. Anim. Sci.,* 46(1): 90–95. With permission.)

The catheter system designed for use in our laboratory is a 4-French (Fr) catheter for the carotid artery and a 2-Fr catheter for the external and internal jugular veins and femoral vessels in a third-trimester fetus. These silicone catheters must be manufactured inside a larger 6-Fr catheter sheath to avoid kinking in the abdomen. The catheters are passed into the abdominal cavity using a trocar from the lateral wings of the lumbar vertebrae. The ports are either sutured to the dorsum of the pig or implanted subcutaneously. If externalized, they should be covered with a pouch or an adhesive plastic sheet (Figure 8.9). When threading the catheters into the blood vessels, it is essential to have a beveled tip and preplace a 2-Fr wire catheter insertion aid into the lumen of the catheter. Polydiaxonone or other monofilament sutures are used to avoid vasospasm caused by braided sutures when tying the catheters in place. Catheter care should be the same as described for adults (Chapter 9).

The closure of fetal incisions should be performed using an atraumatic technique and noninflammatory sutures. Continuous suture patterns are acceptable, and multiple muscle layers can be closed

FIGURE 8.9 Canvas pouch sewn to the skin on the dorsum of the pig to protect externalized catheters. (Reprinted from Swindle, M.M. et al., 1996. *Lab. Anim. Sci.,* 46(1): 90–95. With permission.)

together depending upon the thickness of the layer. If subcuticular sutures are not used, then care should be taken to cut the suture ends close to the knot to avoid irritation of the fetal membranes.

Closure of the uterotomy and fetal membranes may be performed with staples as described earlier for c-section. A technique described by Randall (1986) for suturing the fetal and maternal membranes to reduce the possibility of conjoining the circulations should be used if staples are not available (Figure 8.10). The suture pattern consists of a continuous pattern in which the suture passes from the outside throughout the myometrium, endometrium, and allantochorion into the amniotic cavity. The suture then continues through both sides of the allantochorion passing across the incision in such a manner as to invert the edges of the membranes. The suture then passes completely through all three layers to the outside. The same suture is then reinserted through the myometrium and endometrium close to the edge of the incision, passed across the incision, and passed out through the endometrium and myometrium. After tying the suture, the effect from the outside is to have a vertical mattress-type pattern with inverted edges. This pattern is continuous along the entire edge of the incision. It is then oversewn with continuous or interrupted Lembert sutures. The suture used for these patterns should be a 2/0 or 3/0 synthetic absorbable material. If the goal of the project involves the necessity of preventing conjoinment of the maternal and fetal circulations, then the absence of gamma-globulins in the fetal plasma can be used as confirmation. Staple closures provide better hemostasis and a tighter seal and are more likely to prevent this phenomenon (Figure 8.7). They also greatly reduce operative time. Regardless of which uterine closure method is used, the final suture pattern is a Lembert to oversew the other layers, and catheter flanges are sutured to the uterine wall (Figure 8.11).

Complications of the surgery include sepsis, fetal death, and mummification, which may be followed by abortion. In our experience, the presence of a bloody vaginal discharge within 5 days postoperatively always leads to abortion. If this sign is noted, then an acute physiological experiment should be performed. Fetal death may lead to mummification without abortion. Fetal sepsis may lead to pyometritis and systemic sepsis, but more likely, to abortion. Factors that appear to lead to abortion include animals from herds susceptible to abortion, invasive procedures likely to be life threatening to the fetus, the presence of fetal distress at the time of surgery, and the presence of uterine contractions and other signs of uterine irritation. If abortion occurs as a complication, then the protocol should be reviewed for refinement, and progestins and agents that relax smooth-muscle contractions may be used for prophylaxis.

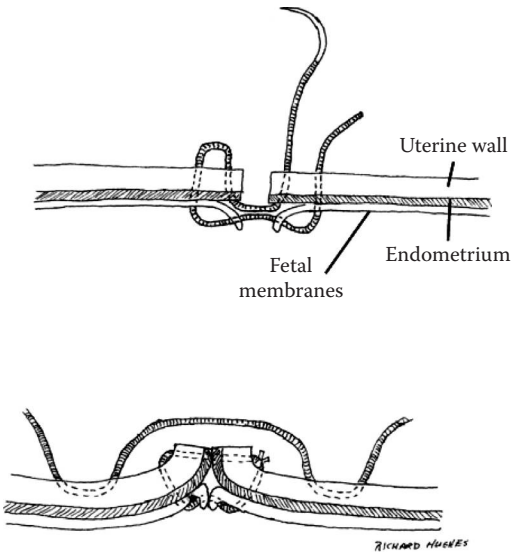

FIGURE 8.10 Manual suturing technique for fetal membranes.

FIGURE 8.11 Lembert sutures oversewing the staple line and catheter flanges sewn to the uterine wall. (Reprinted from Swindle, M.M. et al., 1996. *Lab. Anim. Sci.,* 46(1): 90–95. With permission.)

The progesterone agents include medroxyprogesterone (Depo-Provera®, Upjohn Co., Kalamazoo, MI), 15–50 mg im, 2 days before surgery, the day of surgery, and 2 days after surgery, or an oral progestin (ReguMate®, Hoechst Roussel, Philadelphia, PA), 0.1 mg/kg/day po, for the same time period. Nitroglycerine infusions or infusions with terbutalin may be used intraoperatively to prevent uterine contractions. Pancuronium, 0.02–0.15 mg/kg, is used as a muscle relaxant during surgery to increase exposure of the uterus; however, it is not effective in relaxing smooth muscle. Postoperative and intraoperative analgesia with buprenorphine prevents anxiety and straining by the sow. Nonsteroidal anti-inflammatory drug (NSAID) analgesics are contraindicated because they may induce parturition. Third-generation cephalosporins are useful as prophylaxis against infection, starting the day prior to surgery in order to allow the antibiotic to cross the fetal membranes. They may also be used as a dilute flush in the amniotic sac prior to closing the incision. Tether and harness systems for providing chronic infusions have been developed (Figure 8.12).

Most fetal surgical models that have been applied in other species, notably the lamb, could be modified to be performed in swine. Other than fetal vascular catheterization (Figure 8.13), models that have been created include craniofacial defects, thoracotomy with pacemaker implantation (Figure 8.14), pulmonary arterial banding (Figures 8.15 and 8.16), hydronephrosis, urinary function, vesicoureteric reflux, fetal retardation, and endocrine gland ablation (Care et al., 1986; Dalmose et al., 2000; Dewan et al., 2000; Dungan et al., 1995; George and Fuh, 2003; Jackson and Egdahl, 1960; Jones and Hudak, 1988; Kraeling et al., 1986; Olsen et al., 2001, 2004; Peters, 2001; Randall, 1986; Rosenkrantz et al., 1968; Schmidt et al., 1999; Sims et al., 1997; Swindle et al., 1996; Vuguin 2007; Wiest et al., 1996). Transumbilical catheters have been utilized in interventional procedures in newborns. They could conceivably be used for fetal intervention as well (Divekar et al., 2007).

PROLAPSE OF THE REPRODUCTIVE TRACT

Prolapse of the vagina, uterus, or both may occur during parturition or in the immediate peripartum period. Modifications of the techniques of St-Jean and Anderson (1999), Ladwig (1975), and Markham (1968) are described here. The prolapsed tissue should be examined to determine if it contains the urinary bladder, and a determination made whether fetuses are retained in the uterus. Prolapse of the uterus and bladder or retention of additional fetuses requires a celiotomy. Any

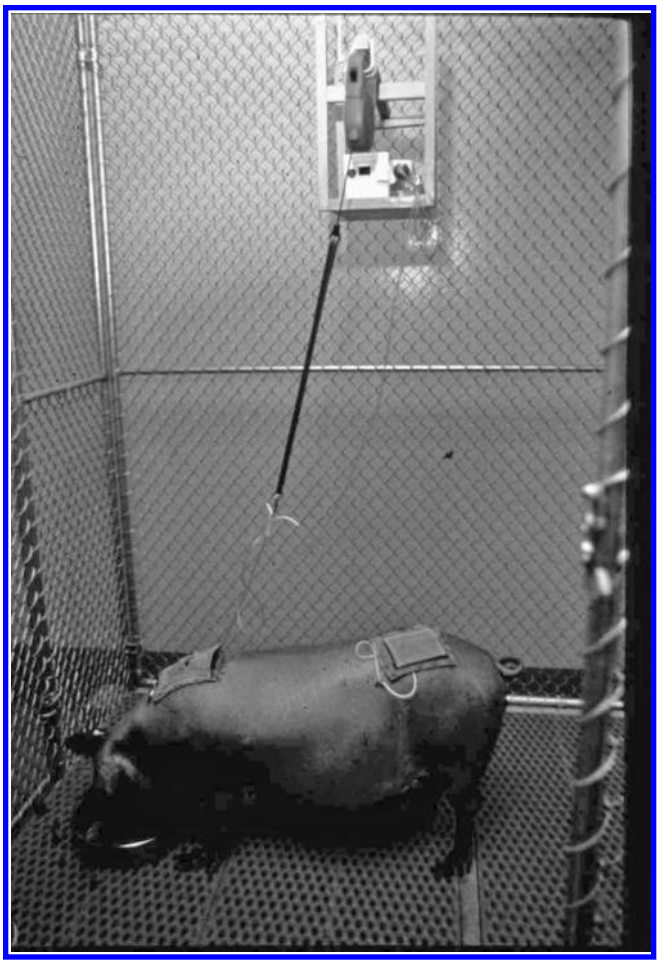

FIGURE 8.12 Harness and tether device for chronic infusion of vascular catheters in the sow and fetus. (Reprinted from Swindle, M.M. et al., 1996. *Lab. Anim. Sci.,* 46(1): 90–95. With permission.)

prolapsed tissue should be examined for necrosis and cleaned carefully with mild surgical soap solutions. Flushing the tissue with antibiotic solutions before reduction is also indicated.

If the vagina is prolapsed, it may be reduced by manual manipulation. The vagina is gently manipulated through the vulva after cleaning. This should not be done with sharp instruments or the extended fingers because the tissue will be friable and easily ruptured. If the prolapsed material can be manipulated into the vulva, it should be extended into the pelvic cavity. A deep subcutaneous purse-string suture should be placed to prevent recurrence, and the bladder should be catheterized.

If the uterus or bladder is involved, then a paramedian flank or midline abdominal incision is performed as previously described. The prolapsed structures are gently reduced, and any remaining fetuses are removed by c-section as described earlier. Oxytocin should be administered to promote uterine contraction. Use of the purse-string suture described previously for the vagina may help prevent recurrence. Use of a modified technique for primates may be useful, however, if the animal is to be retained for the long term (Adams et al., 1983). The technique involves suturing a loop of the ovarian ligament on both horns of the uterus to the abdominal wall on its corresponding side. This provides security against future prolapses by surgical fixation of the cranial poles of the uterus to the abdominal wall. The celiotomy incision is closed in a routine fashion, as previously described.

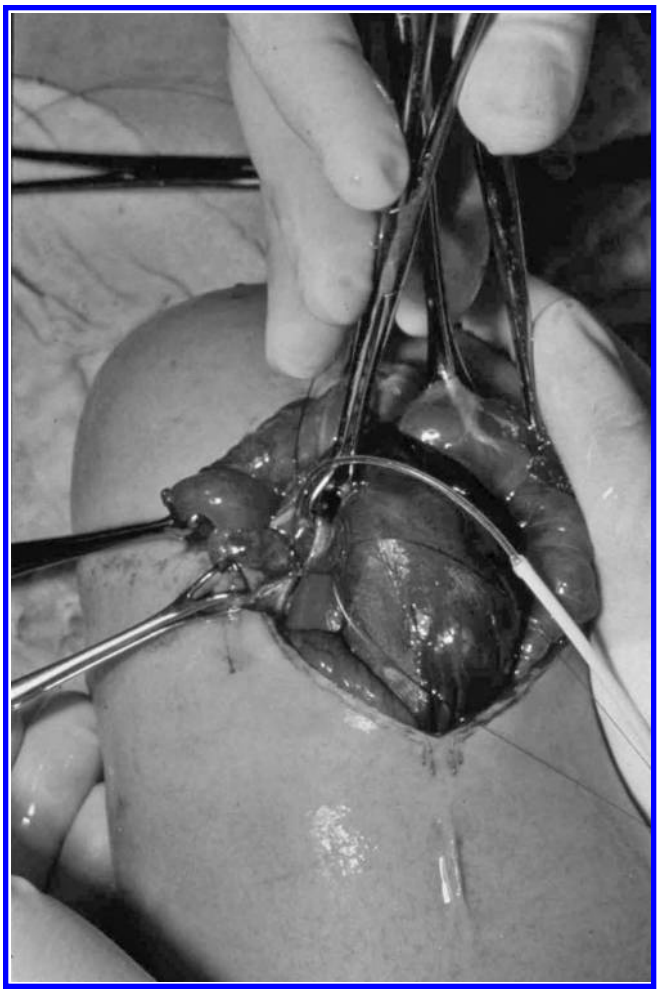

FIGURE 8.13 Catheter implantation into the fetal carotid artery.

If the tissue is necrotic, an amputation of the prolapsed stump may have to be performed. As described earlier, it should be ensured that the bladder or additional fetuses are not involved in the prolapse; a laparotomy should be performed if they are. The vaginal stump is best amputated using staple surgical techniques. If it is performed manually, then a V-shaped incision is made from the perineum to the tip of the viable tissue in the stump. The vessels are ligated, and the edges of the stump are oversewn with a continuous interlocking suture to ensure hemostasis and to provide a patent opening in the reproductive tract. Alternatively, an ovariohysterectomy may be indicated and performed as described previously.

If any of these conditions occur, restriction of future breeding or the use of c-section delivery of fetuses if breeding is necessary for research purposes should be considered. Uterine transplantation in swine and other animals has been reviewed as a model in the human (Hanafy et al., 2011).

ENDOMETRIOSIS

Endometriosis, characterized by the presence of endometrial glands and stroma outside the endometrial cavity, is a naturally occurring condition in humans and nonhuman primates. A model of

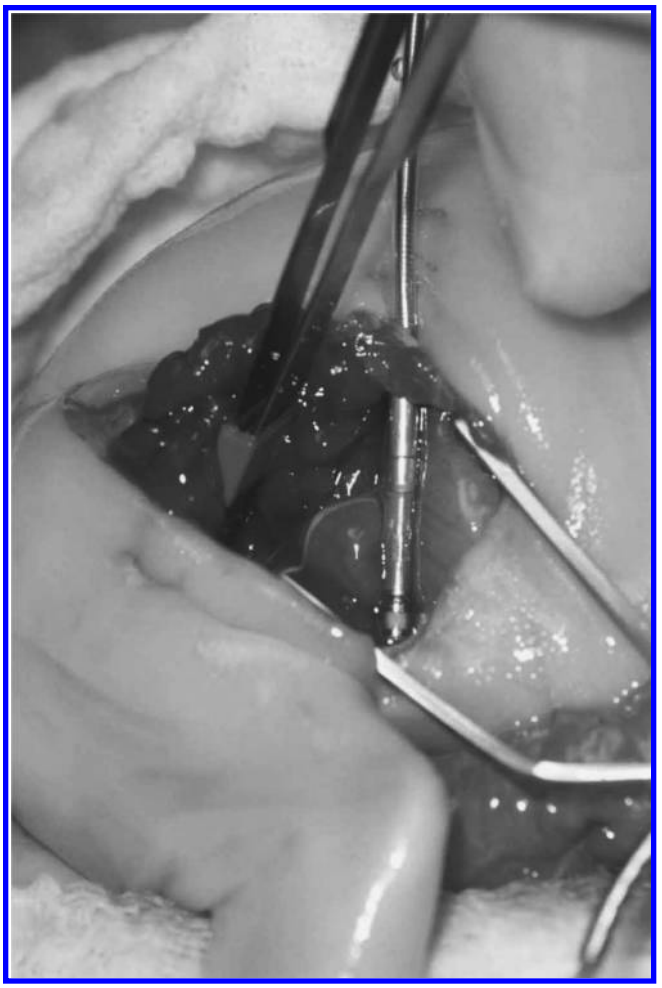

FIGURE 8.14 Left lateral thoracotomy to implant a pacemaker lead in a fetus.

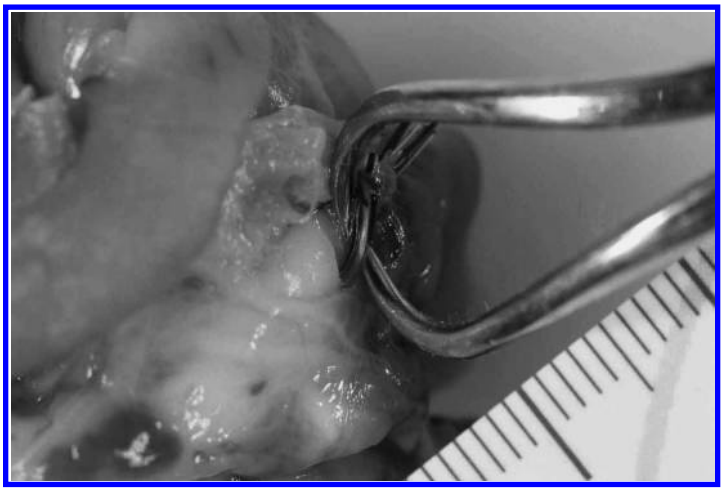

FIGURE 8.15 Silastic band on the pulmonary artery of a fetus.

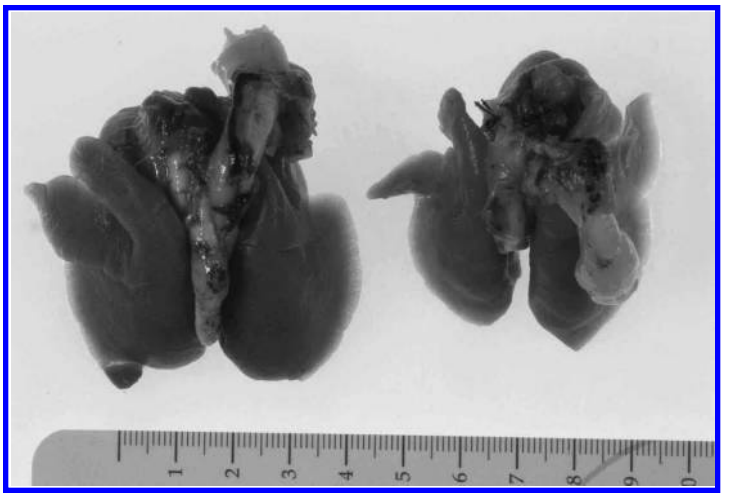

FIGURE 8.16 Heart and lung blocks from littermates at the end of gestation. The heart and lungs on the right have been retarded in growth from pulmonary artery banding in the third trimester.

endometriosis in pigs has been developed using laparoscopy techniques (Siegel and Kolata, 2003). Hormonal therapy is an essential element in the development of the model.

Using standard caudal abdominal laparotomy techniques, the uterine horn is exteriorized through a trocar, the abdomen is desufflated, and the trocar removed, which results in a portion of the uterus being exteriorized. This procedure can also be performed using open incision techniques.

After exteriorizing the uterus and holding it in place with Babcock clamps, a longitudinal incision is made in the uterus. Metzenbaum scissors are used to trim pieces of the endometrial lining. The endometrial pieces are minced with scissors and mixed with heparinized saline. After closure of the uterine incision and replacement in the abdomen, the solution of endometrial tissue is injected into the caudal abdomen using a 60-mL syringe attached to a catheter. The abdominal incision is closed in a routine manner.

The procedure was more effective in producing endometrial lesions in sexually mature Yucatan minipigs than in immature domestic swine. Hormonal injections were administered pre- and postoperatively. In minipigs, these were injections of estradiol cypionate 15 mg im starting at 14 days preoperatively and administered every 7 days, including the day of surgery, followed by one injection 7 days postoperatively. Progesterone 100 mg im was administered on the day of surgery, followed by one postoperative injection 7 days postoperatively. Similar injections of estradiol were given to the immature domestic swine, but progesterone was not administered because of concern that atrophy of the reproductive system may occur.

The lesions that were produced on the surface of the caudal abdominal viscera were generally foci of stroma, tubular glands, and fibrous tissue with inflammatory cells. Postoperative care also included the administration of analgesics. Pigs developed diarrhea, vomiting, and bloody vaginal discharge, which resolved within 4 days. The complications were mild and did not require treatment.

PELVIC ADHESIONS

Pelvic adhesion models to study prevention and treatment of the condition have been established (Christoforoni et al., 1996; Ferland et al., 2001; Montz et al., 1993). The basic technique requires a midline incision from the umbilicus to the pubis causing an injury to the parietal or visceral peritoneum (or both). Some degree of damage may be caused by rubbing the visceral peritoneum with dry gauze sponges. However, the model of pelvic adhesion with uterine horn surgery creates a more reliable model (Ferland et al., 2001). In this model, the parietal peritoneum of the caudal

pelvic wall opposed to the uterine horn is stripped in an area approximately 4 × 5 cm. The uterine horn is transected and reanastomosed with simple interrupted vicryl sutures. The abdomen is closed routinely. Pelvic adhesions will develop within 4–5 days, and the model can be completely evaluated for pelvic adhesions within 14 days.

ARTIFICIAL INSEMINATION AND EMBRYO TRANSFER

There is considerable interest in the development of transgenic and knockout swine for research purposes, agricultural methods of artificial insemination, and semen collection and preservation; embryo collection, culture, and preservation are being modified for the purpose of maintaining genetically engineered lines of pigs (Abeydeera, 2001, 2002; Bazer et al., 2001; Beebe et al., 2005; Holker et al., 2005; Massip, 2001; Niemann and Rath, 2001; Prochazka et al., 2004; Singleton, 2001; Sommer et al., 2002). There are many variables contributing to the success of these techniques, including breed, age, season, stress, and timing (Britt et al., 1999). The methodologies for most of these laboratory techniques are beyond the scope of this text; however, the surgical methods of collection and implantation will be described.

Synchronization of estrus and superovulation are essential techniques for these procedures. A simplified research technique for superovulation and artificial insemination has been described as a modification of common agricultural practice (Sommer et al., 2002). Briefly, gilts (150–160 days old) receive 1500 IU of pregnant mare serum gonadotropin i.m. followed by 500 IU of human chorionic gonadotropin (hCG) im 72 h later. Gilts are inseminated at 42 and 47 h with thawed or fresh semen. This technique is less complicated than administering oral altrenogest followed by injections of PGF_{2a}, eCG, and hCG, the commonly used method (Bazer et al., 2001).

If natural estrus is used, the signs are swelling, reddening, and discharge of the vulva, as well as behavioral signs. The period of an estrous cycle is 17–25 days (average 21 days). During estrus, which lasts 1–3 days, the female is receptive to the male. Behavioral signs include vocalization, riding of other females, and the standing reflex when exposed to pheromones from a boar, which generally occur within 24 h of ovulation. In the standing reflex, the female will stand rigidly when manually pressed on the lower back. Mating or artificial insemination is performed within 24 h after the onset of estrus, which occurs before ovulation. Ovulation is highly variable over 2–3 days, and it is generally best to mate or perform artificial insemination daily during estrus. A detailed discussion of breeding techniques in the agricultural setting has been published (Britt et al., 1999).

Using a porcine artificial insemination catheter, thawed sperm may be delivered into the cervix. The catheter is passed through the vulva and into the vagina at an upward angle to avoid entering the urethra. The catheter is screwed counterclockwise when resistance is encountered at the level of the cervix. By pulling with gentle pressure backward, you can determine if the catheter is locked in place in the cervix. The semen is gently injected into the cervix, and the catheter is screwed clockwise and removed.

Embryo transfer is performed by identifying the ovary and the infundibulum of the oviduct either surgically or laparoscopically (see the earlier section on ovariohysterectomy). Newer techniques have been developed using transcervical catheters and implantation in a retrograde fashion (Bazer et al., 2001; Hazeleger and Kemp, 2001; Li et al., 1996). The fertilized embryos are infused with gentle syringe or pipette pressure into the ampulla of both oviducts at 5–6 days of age. It is necessary to implant embryos in both oviducts to prevent resorption. Uterine implantation occurs approximately on day 13 or 14. Pregnancy can be reliably determined by ultrasound techniques in the middle of the first trimester of pregnancy (>20 days gestation). It is likely that embryo transfer and artificial insemination techniques will continue to evolve and improve because of the importance of the techniques to develop transgenic/knockout models. A review of animal models of implantation which compares the pig to humans and other animals has been published (Lee and DeMayo, 2004). The study compares the mechanical and molecular events that occur in this process.

MAMMECTOMY

Most sows have seven pairs of mammary glands on the abdomen; however, the number can vary substantially depending upon the breed. Surgical removal of a mammary gland may be indicated in chronic infection or trauma. Modifications of the techniques of St-Jean and Anderson (1999) and Mbiuki (1982) are described here. The vascular supply to the mammary glands is derived from branches of the internal thoracic, cranial epigastric, external pudendal arteries, and veins.

A paramedian skin incision is made over the center of the glandular structure to be excised. The skin is bluntly dissected to both edges of the gland. The vascular supply is identified, ligated, and transected before removal of the gland. The subcutaneous dissection is easily performed using Metzenbaum scissors. The vascularity of the gland increases substantially with lactation, and leakage of milk from adjacent glands should be prevented by taking care not to damage them during the dissection. After removal of the gland, the skin is trimmed and the nipple excised with the excised tissue. The subcutaneous pocket must be closed carefully to avoid a seroma. The skin is closed with subcuticular sutures. A circumferential bandage of the torso, including the surgical site, is indicated for the first 24 h to prevent seroma formation.

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9 Cardiothoracic and Vascular Surgery/Chronic Intravascular Catheterization

Daniel D. Myers Jr., Jose Antonio Diaz, Marisa L. Conte, and M. Michael Swindle

CONTENTS

GENERAL PRINCIPLES OF CARDIOTHORACIC SURGERY AND SURGICAL ANATOMY

Swine share important characteristics with humans in anatomy and physiology of the cardiovascular and pulmonary systems, making them useful models in the study of human diseases (Corin et al., 1988; Gardner and Johnson, 1988; Gootman, 2001; Horneffer et al., 1986; Hughes, 1986; Lee, 1986; Lelovas et al., 2014; McKenzie, 1996; Smith et al., 1990, 1994; Stanton and Mersmann, 1986; Swindle, 1983, 1986, 1992; Swindle and Adams, 1988; Swindle and Bobbie, 1987; Swindle et al., 1986, 1988). Colored histologic sections of the organs and tissues in this chapter as well as videos of actual surgical procedures are included in the DVD attached with the textbook.

Swine have been used in teaching and surgery for hundreds of years; the first published account was Vesalius' use in medical school anatomy in 1543. During the eighteenth century, Dr. John Hunter (1728–1793) of Britain recognized the pig as a worthy model in physiological research. Porcine use in biomedical research and preclinical pharmacology evolved greatly over the past 60 years.

Besides the size and morphologic characteristics, there are physiological similarities in the areas of coronary blood flow, growth of the cardiovascular system, and neonatal pulmonary development. The approximate distribution of the coronary arteries is as follows (Gootman, 2001):

- Right coronary artery (RCA): 72% right ventricle, sinoatrial node, and atrioventricular (AV) node, 25% left ventricle
- Left anterior descending (LAD) coronary artery: 28% right ventricle, 49% left ventricle

The coronary circulation of the pig has few subepicardial collateral anastomoses, similar to 90% of the human population. The circulation to the conduction system is predominantly right-side dominant from the posterior septal artery, in contrast to the dog. Consequently, the pig responds in a similar manner to humans with acute myocardial infarction (Bloor et al., 1986, 1992; Gardner and Johnson, 1988; Unger, 2001; Verdouw et al., 1998; White et al., 1986). Anatomic views of the heart are shown in Figures 9.1 through 9.11.

There are also differences from humans in physiological composition of the conduction system. The pig endocardium and epicardium are activated simultaneously because of differences in distribution of the specialized conduction system in the ventricles (Brownlee et al., 1997; Gillette et al., 1991; Hughes and Bowman, 1986; Schumann et al., 1994; Smith et al., 1997; Tong et al., 1995, 1996; Verdouw and Hartog, 1986). Atherosclerotic and coronary occlusion models are readily induced in swine and are discussed in this section; there is also additional information in Chapter 12 (Gal and Isner, 1992; Mitchell et al., 1994; Murphy et al., 1992; Rogers et al., 1988; Rysavy et al., 1986; White et al., 1992). The aorta has a true vaso vasorum unlike many species of animals but similar to humans; this structure leads to a difference in reaction to aortic banding techniques. The growth of the cardiovascular system from fetus to sexually mature adult in swine parallels the growth and development of the cardiovascular system of humans into early sexual maturity (Brutel de la Riviere et al., 1983; Gootman, 2001; Pae et al., 1981). Histology of the heart, great vessels, and conduction system is illustrated in Figures 9.12 through 9.18.

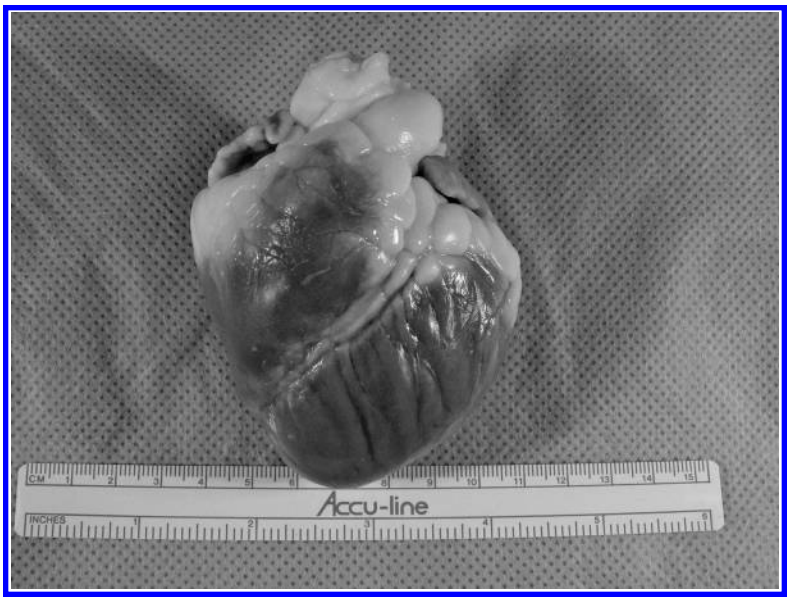

FIGURE 9.1 Ventral view of the heart in a 22-kg male Yucatan.

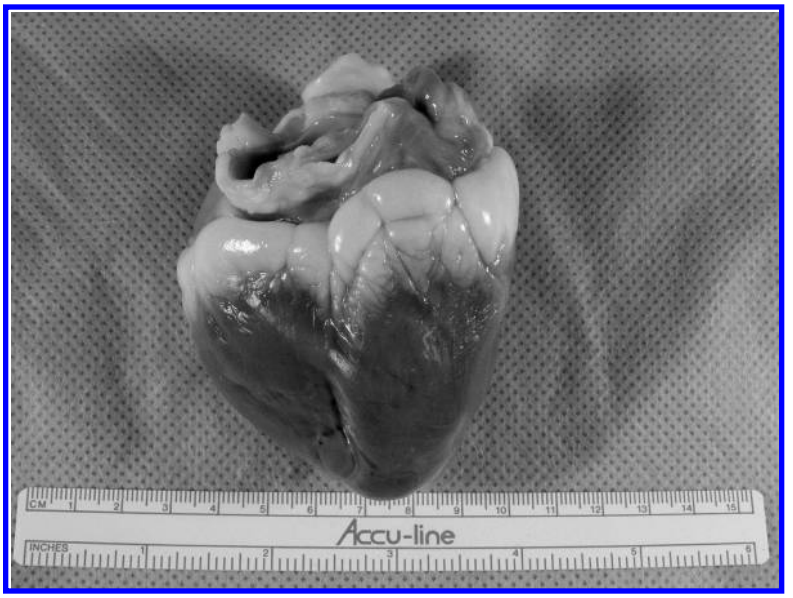

FIGURE 9.2 Dorsal view of the heart in a 22-kg male Yucatan.

Pigs have been described as a translational model for arteriogenesis because of their reactions to stimuli-inducing collateral growth of arteries (Hoefer et al., 2006). This is typically studied in a hind-limb model of femoral occlusion. Peripheral veins in the pigs have valves similar to humans, and this may be a problem with passage of catheters into the small vessels in the extremities.

The pulmonary tissues (Figures 9.19 and 9.20) and pulmonary circulation have been studied from fetal life to 6 months of age and show similarities to humans morphologically and functionally (Ackermann, 2001; Greenberg et al., 1981; Haworth and Hislop, 1981; Sparrow, 1996). Pulmonary function matures by 2 weeks of age, but growth and remodeling continue into adult life. The lungs have six lobes: right cranial (apical), right middle, right caudal, left cranial (apical cranial and caudal

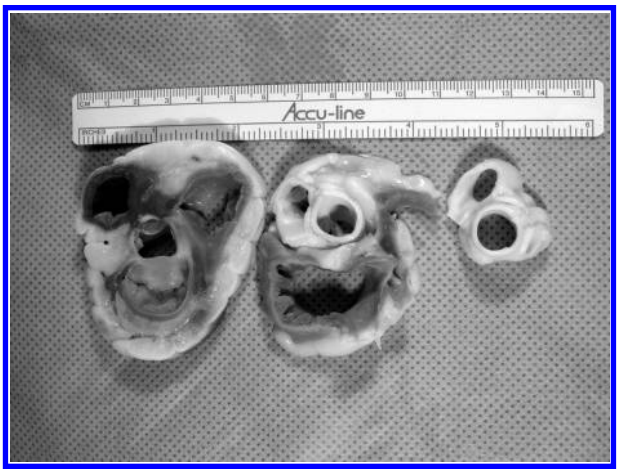

FIGURE 9.3 Cranial (topside) view of the valves of the heart and great vessels in a 22-kg male Yucatan.

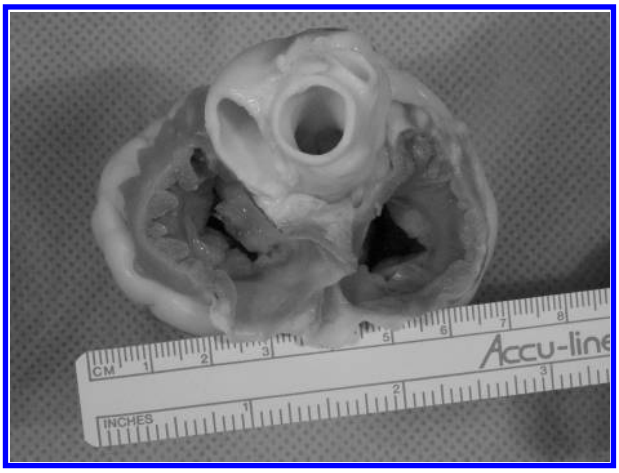

FIGURE 9.4 Cranial (topside) view of the cap of the heart and the valves in a 22-kg male Yucatan.

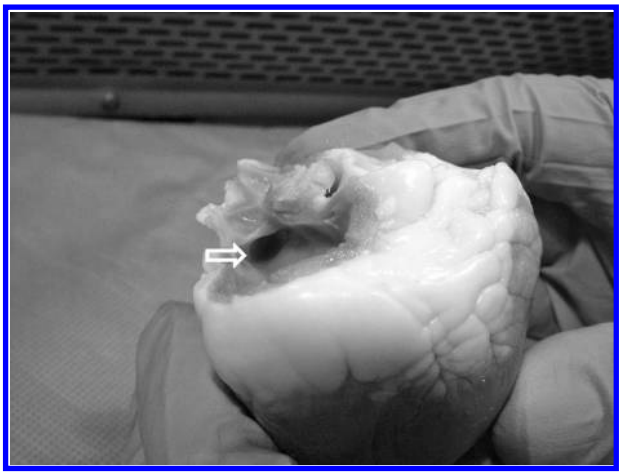

FIGURE 9.5 Coronary sinus (arrow) in the heart of a 22-kg male Yucatan.

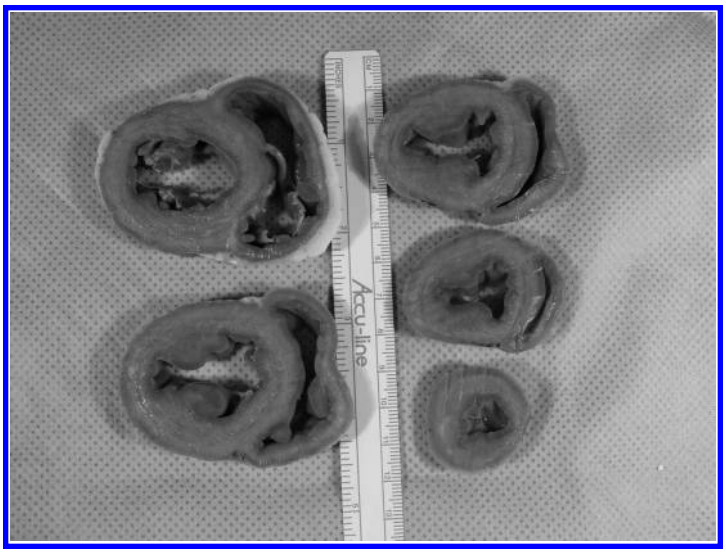

FIGURE 9.6 Serial cross sections of the heart of a 22-kg male Yucatan. The most dorsal (cranial) part of the heart is at the 11 o'clock position and the most caudal portion of the heart is at the 5 o'clock position.

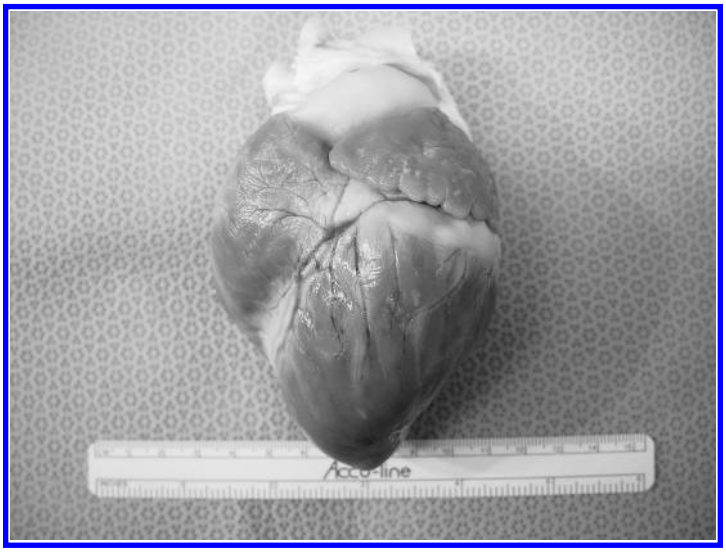

FIGURE 9.7 Ventral view of the heart in a 22-kg male Yorkshire farm pig for comparison with the Yucatan minipig.

segment), left caudal, and an unpaired accessory intermediate lobe from the right side (Figure 9.21). The respiratory rate decreases with age, from approximately 40/min in the neonate to 15/min in the adult. The pulmonary tissues are friable and must be handled gently during thoracic surgery. Overinflation of the lungs with a respirator can cause alveolar rupture and emphysematous bullae, similar to humans. Air bubbles from this trauma can extend into the abdominal mesenteric tissues and create pneumotosis intestinalis. This is especially true of respirators without bellows, such as the Harvard pump. The tidal volume is approximately 10–15 mL/kg and the inflation pressure on a respirator should not exceed 18–20 cm H_2O. Oxygen flow rates will differ between anesthetic machines, but 5–15 mL/kg/min is a general starting range (Swindle, 1983). Structures of the thorax with the lungs removed are illustrated in Figures 9.22 and 9.23.

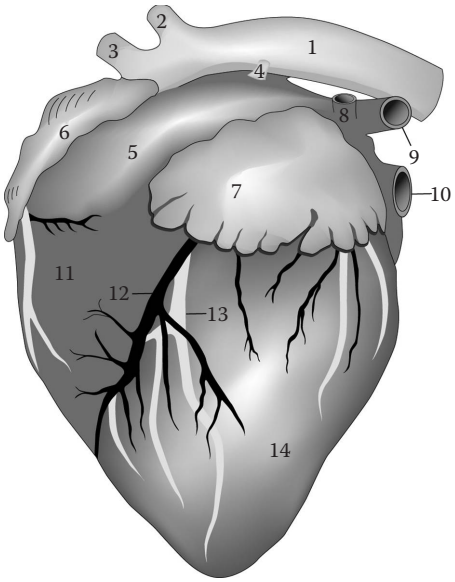

FIGURE 9.8 Heart of pig, left surface: 1, descending aorta; 2, left subclavian artery; 3, brachiocephalic trunk; 4, ligamentum arteriosum; 5, pulmonary trunk; 6, right auricle; 7, left auricle; 8, left azygous (hemiazygous) vein; 9, left pulmonary artery; 10, left pulmonary vein; 11, right ventricle; 12, great cardiac vein; 13, paraconal interventricular branch; 14, left ventricle. (Reprinted from Ghoshal, N.G. 1975. *Sisson and Grossman's The Anatomy of the Domestic Animals*, 5th Ed., Vol. 2. W.B. Saunders Co., p. 1307. With permission.)

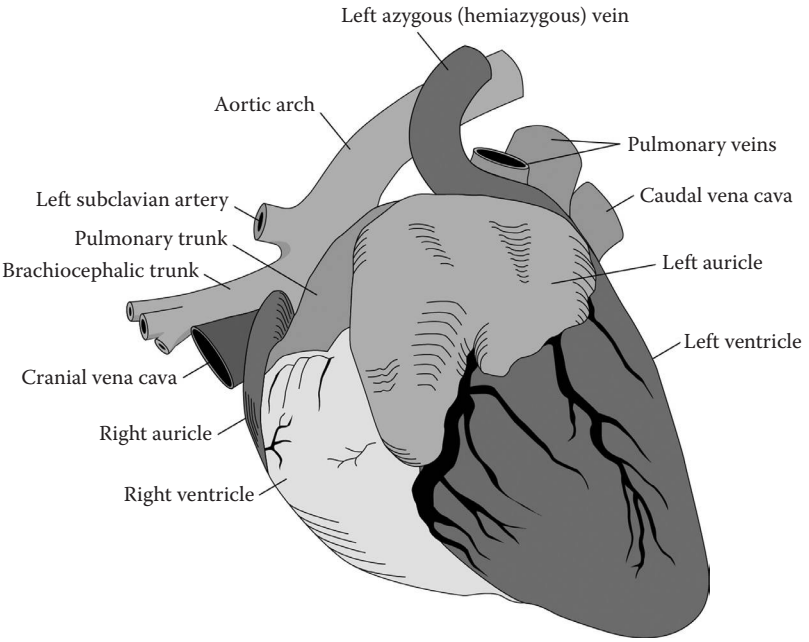

FIGURE 9.9 Heart of the pig; left view. (Reprinted from Ghoshal, N.G. 1975. *Sisson and Grossman's The Anatomy of the Domestic Animals*, 5th Ed., Vol. 2. W.B. Saunders Co., p. 1307. With permission.)

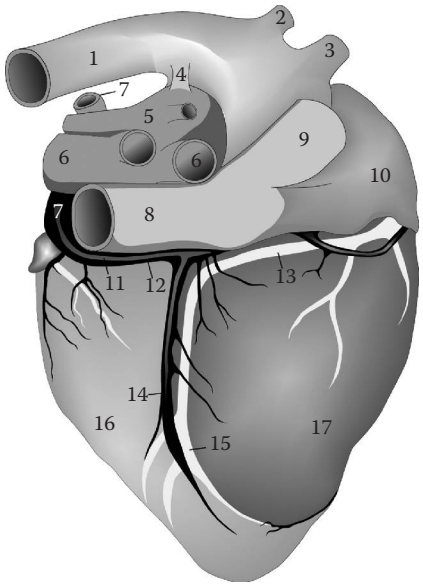

FIGURE 9.10 Heart of pig, right surface. 1, descending aorta; 2, left subclavian artery; 3, brachiocephalic trunk; 4, ligamentum arteriosum; 5, pulmonary trunk; 6, pulmonary vein; 7, left azygous (hemiazygous) vein; 8, caudal vena cava; 9, cranial vena cava; 10, right atrium; 11, great cardiac vein; 12, coronary sinus; 13, right coronary artery; 14, middle cardiac vein; 15, subsinuosal interventricular branch; 16, left ventricle; 17, right ventricle. (Reprinted from Ghoshal, N.G. 1975. *Sisson and Grossman's The Anatomy of the Domestic Animals*, 5th Ed., Vol. 2. W.B. Saunders Co., p. 1308. With permission.)

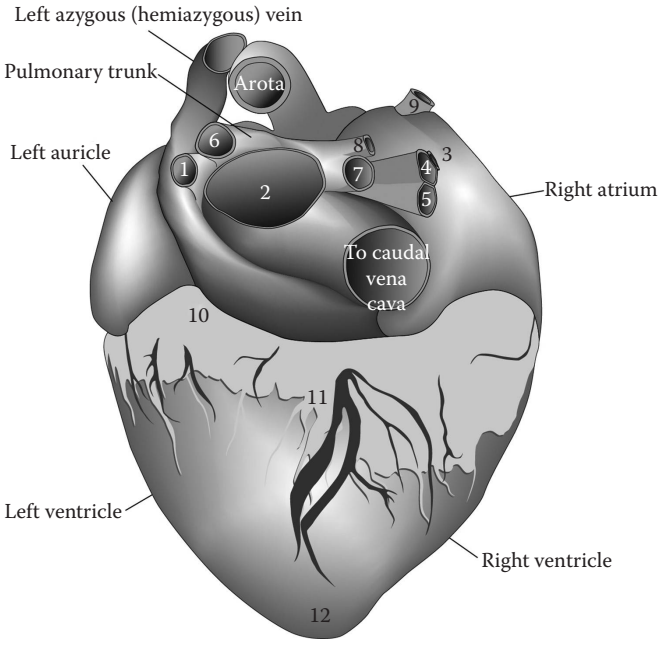

FIGURE 9.11 Heart of pig, atrial surface. 1 to 5, pulmonary veins; 6, left pulmonary artery; 7, 8, branches of the right pulmonary artery; 9, right azygous vein; 10, fat in coronary groove; 11, vessels and fat in subsinuosal interventricular groove; 12 apex. Caudal vena cava. (Reprinted from Ghoshal, N.G. 1975. *Sisson and Grossman's The Anatomy of the Domestic Animals*, 5th Ed., Vol. 2. W.B. Saunders Co., p. 1308. With permission.)

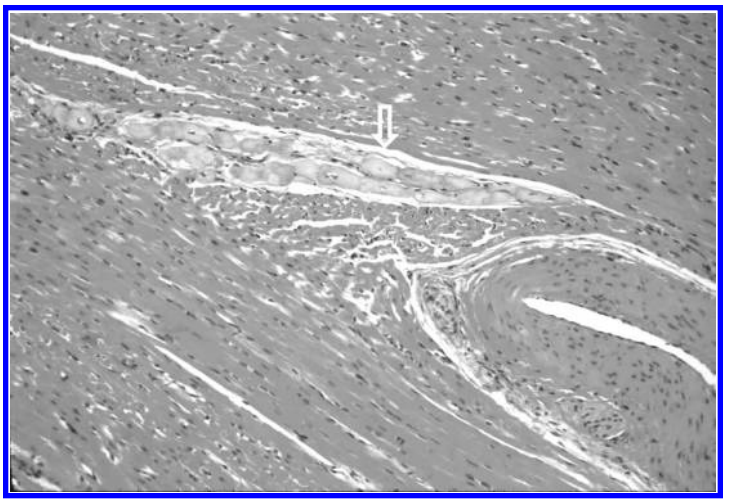

FIGURE 9.12 Histologic section of the heart. Purkinje cells are illustrated with the arrow. H&E, ×100.

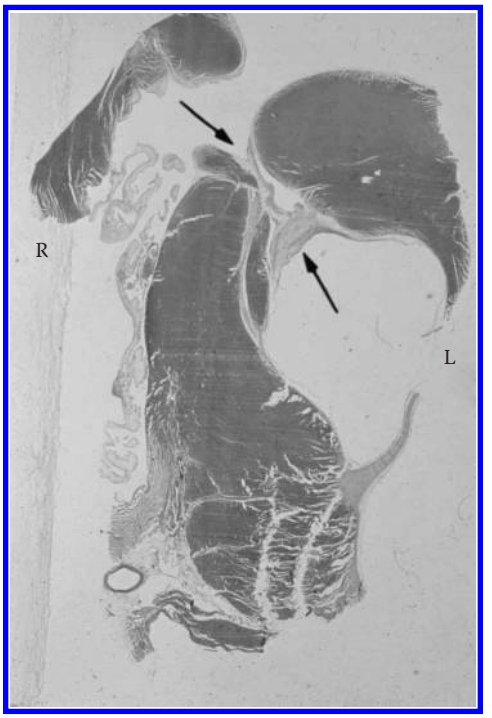

FIGURE 9.13 Subgross microscopy of the heart of a pig with a ventricular septal defect illustrating the close association with the conduction system (arrows). H&E, ×4.

Surgical access to the chest (thoracic, cardiac, and large vessel surgery) requires one-lung ventilation to facilitate surgical manipulation. Common techniques to facilitate one-lung ventilation include placement of a double-lumen endobronchial tube or the use of an endobronchial blocker in conjunction with a single-lumen endotracheal tube (Mirzabeigi et al., 2005). In all cases, the use of a pediatric fiberoptic scope to facilitate and confirm correct placement is strongly recommended. An important limitation of this procedure is the fact that one-lung ventilation in swine is only possible

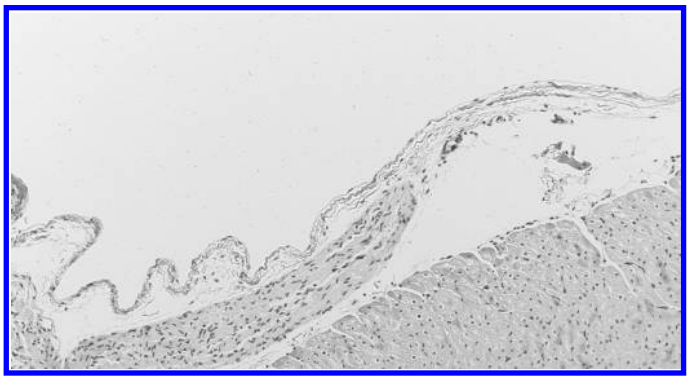

FIGURE 9.14 Histology of a cardiac valve. H&E, ×100.

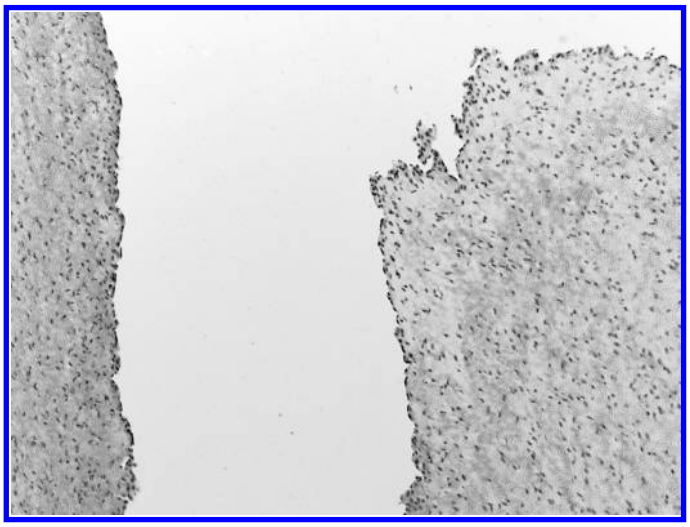

FIGURE 9.15 Histology of the atrium. H&E, ×100.

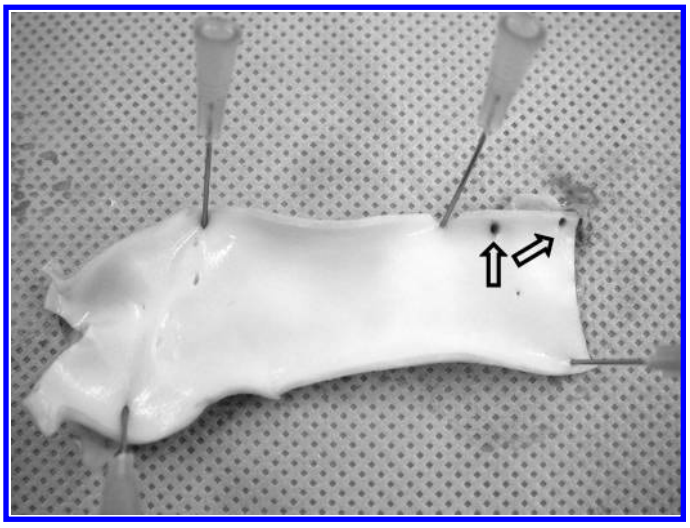

FIGURE 9.16 Aorta of the pig demonstrating vaso vasorum (arrows).

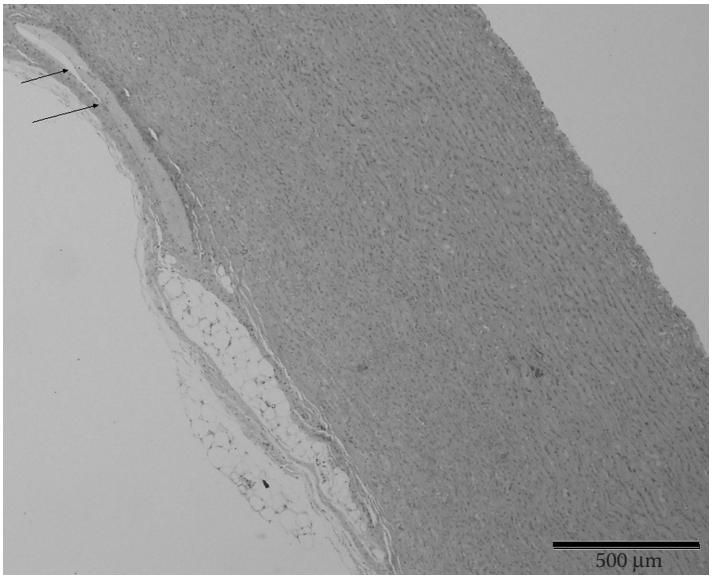

FIGURE 9.17 Histology of the aorta, vaso vasorum (black arrows). H&E, ×4. (Courtesy of Dr. Robert E. Sigler, DVM, PhD IVAC, U-M.)

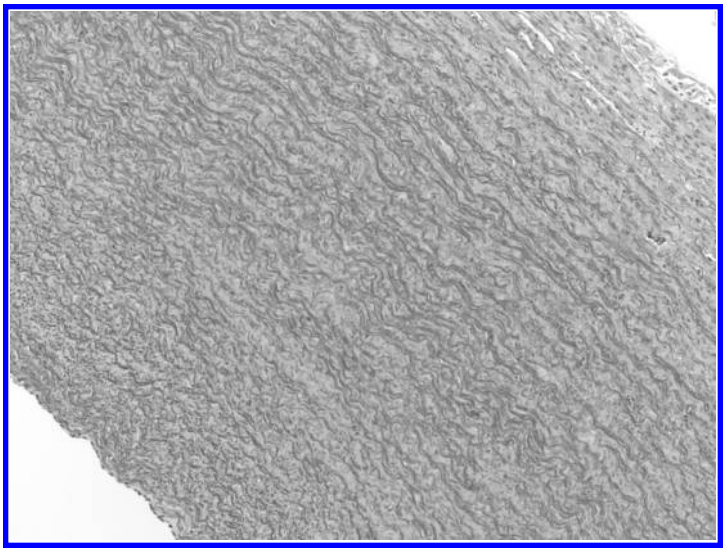

FIGURE 9.18 Histology of the pulmonary artery. H&E, ×100.

in the right bronchus, blocking the left bronchus. Swine possess a unique apical lobe bronchus, most commonly found on the right trachea; yet, it has also been reported to branch from the left side of the trachea (Mouton et al., 1999; Nakakuki, 1994). This unique anatomical variance between swine and humans can be used as landmark for a correct placement of an endobronchial blocker to the left. Use of human double-lumen endobronchial tubes in swine is challenging, especially to prevent occlusion of apical lung lobes or dislodgement of the endobronchial cuff into the carina (Mirzabeigi et al., 2005). Schwarzkopf et al. (2010) developed and utilized a specialized double-lumen endobronchial tube specifically adapted to swine bronchial anatomy in order to achieve effective one-lung ventilation for periods ranging from 4 to 10 h. Commonly used endobronchial blockers include the Arndt

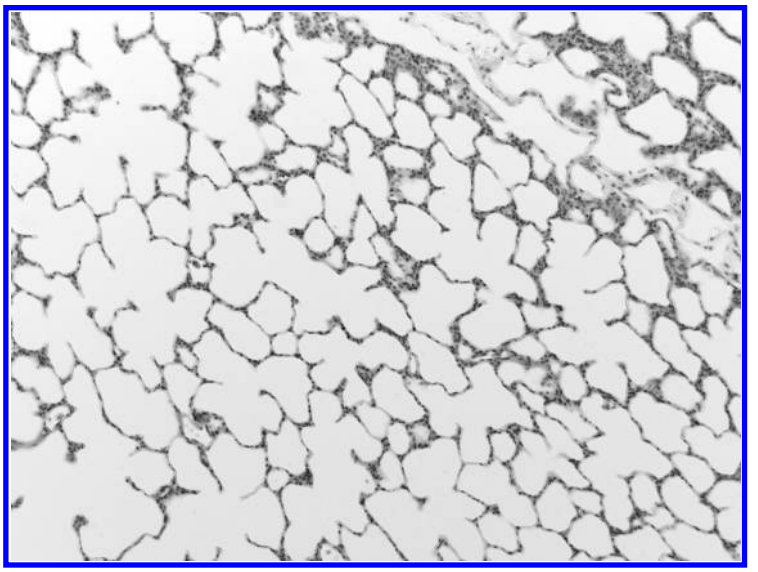

FIGURE 9.19 Histology of the alveoli. H&E, ×100.

wire-guided endobronchial blocker (Cook Medical, Bloomington, IN) available in 5–9 French sizes, and the similar Cohen endobronchial blocker (Cook Medical, Bloomington, IN), which possesses a soft flexible tip that facilitates guidance into the desired bronchus.

The Arndt and Cohen endobronchial blockers are packaged with a three-way adaptor equipped with multiple valves to facilitate a connection to the endotracheal tube and the anesthesia circuit,

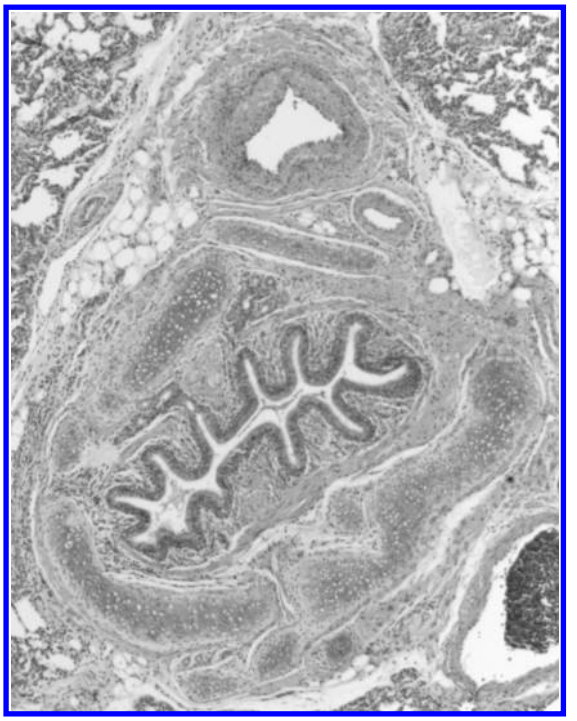

FIGURE 9.20 Histology of a bronchus. H&E, ×100.

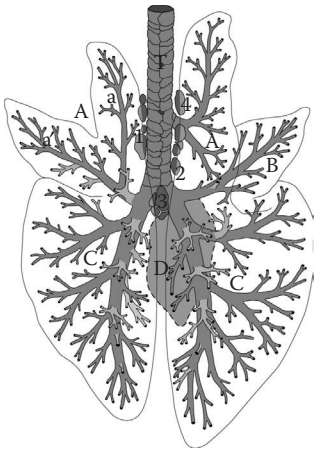

FIGURE 9.21 Teased lungs of pig. Dorsal view: A, apical lobe; a, apical lobe, cranial segment; a′, apical lobe, caudal segment; B, middle lobe; C, diaphragmatic lobe; D, accessory lobe; T, trachea; 1, left tracheobronchial lymph node; 2, right tracheobronchial lymph node; 3, middle tracheobronchial lymph node; 4, cranial tracheobronchial lymph nodes. (Reprinted from Hare, W.D.C. 1975. *Sisson and Grossman's The Anatomy of the Domestic Animals*, 5th Ed., Vol. 2. W.B. Saunders Co., p. 1295. With permission.)

allowing additional access to, for example, insertion of a fiberoptic scope and insertion of the endobronchial blocker. In addition, continuous positive airway pressure or active suction can be applied via the endobronchial blocker. In the authors' experience, the Cohen endobronchial blocker in conjunction with a pediatric fiberoptic scope facilitates rapid and efficient placement of the endobronchial blocker into the left main bronchus of swine (Figures 9.24 through 9.26).

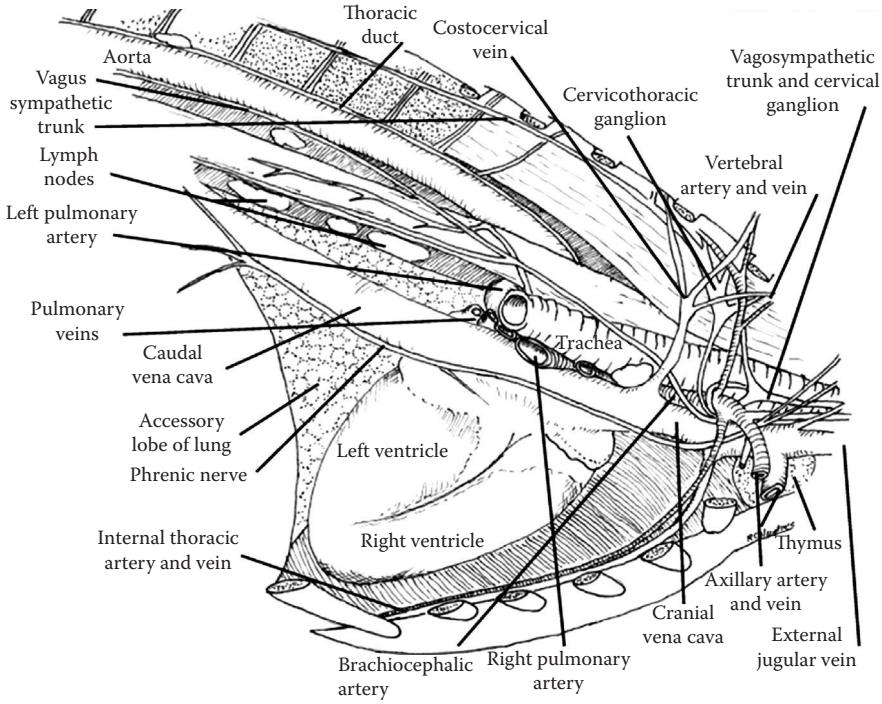

FIGURE 9.22 Right thorax with the right lung removed.

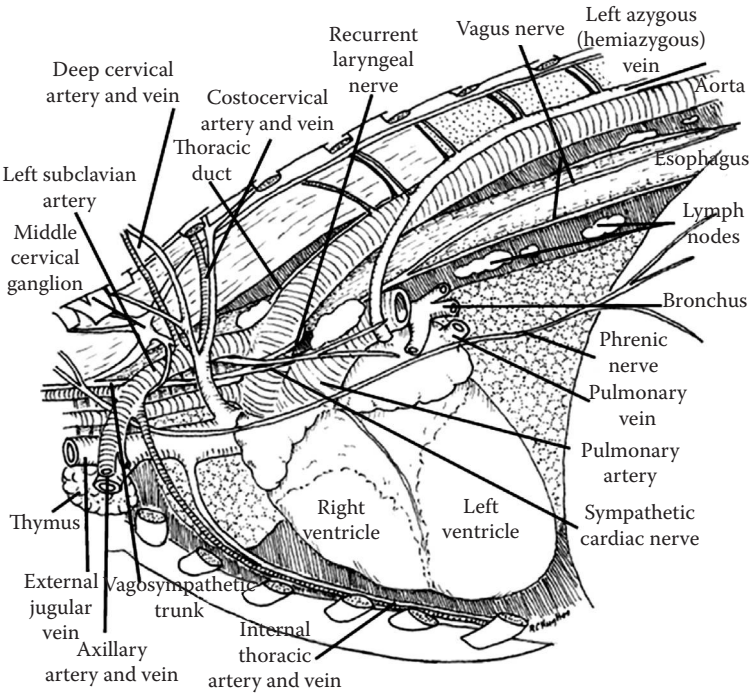

FIGURE 9.23 Left thorax with the left lung removed.

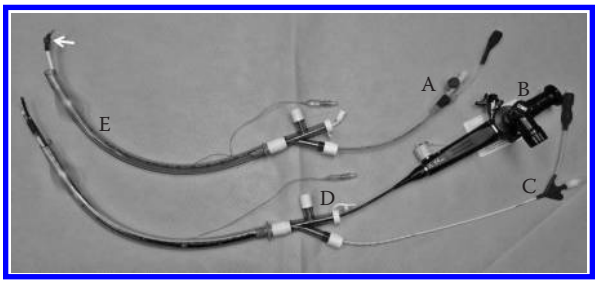

FIGURE 9.24 Cohen endobronchial blocker (A) with dial flexible tip (white arrow) inserted into endotracheal tube (E); Arndt and endobronchial blocker (C) with wire-guide inserted into an endotracheal tube, note port (D) for pediatric fiber optic scope (B) to facilitate to confirm correct placement.

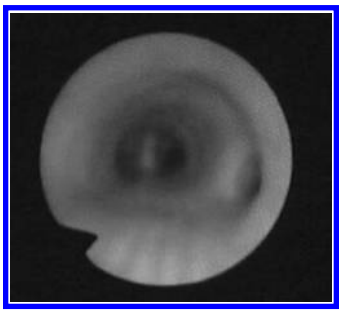

FIGURE 9.25 View in the nonintubated trachea of a pig. On the right side of the trachea the access to the right cranial lobe is visible. The main carina is located in the center. (Reprinted from Schwarzkopf, K. et al. 2010. *Scand. J. Lab. Anim. Sci.* 37(2): 69–72. With permission.)

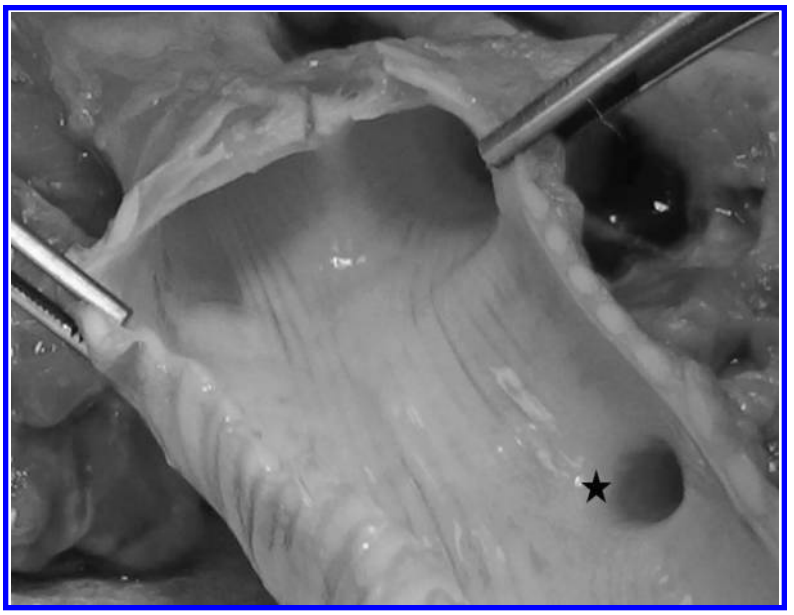

FIGURE 9.26 Gross view into the open trachea of an adult pig. The main carina is located in the center. The tracheal access to the right cranial lobe is visible (black star).

Lateral thoracotomies (Figures 9.27 and 9.28) in the pig can be a challenge because of the width of the ribs and the narrowness of the intercostal spaces, which can result in minimal exposure of the intrathoracic structures. The pig should be positioned in lateral recumbency. Placing a rolled-up towel or sandbag under the thorax, as is used in human thoracotomies, makes the operative side more convex and increases surgical exposure. Thoracic incisions should be performed within the intercostal spaces parallel to the ribs. Lateral thoracotomy incisions will be oblique because of the anatomy of the ribs in swine, similar to humans. When the access to the chest is limited, the thoracotomy may require an osteotomy to the intercostal incision to increase surgical access. The pig usually has 15 pairs of ribs including the floating rib, and they can be counted to locate the appropriate intercostal space. Some miniature pigs may have one rib and one thoracic vertebra fewer than domestic swine (Swindle, 1983; Swindle et al., 1986). Swine have been developed as a model for induced hypothermia for emergency trauma surgery using a thoracotomy approach (Rhee et al., 2000). The left lateral thoracotomy approach is also used for the evaluation of graft and mechanical heart valve implantation in the descending thoracic aorta in swine. In this case, the left azygous (hemiazygous) vein that crosses ventrally to the aorta

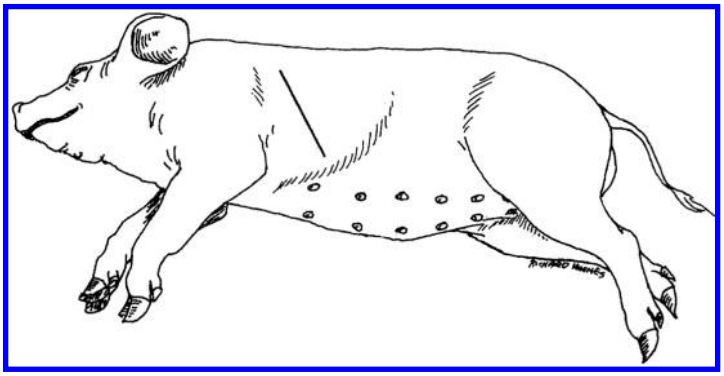

FIGURE 9.27 Position of the incision for a left lateral thoracotomy.

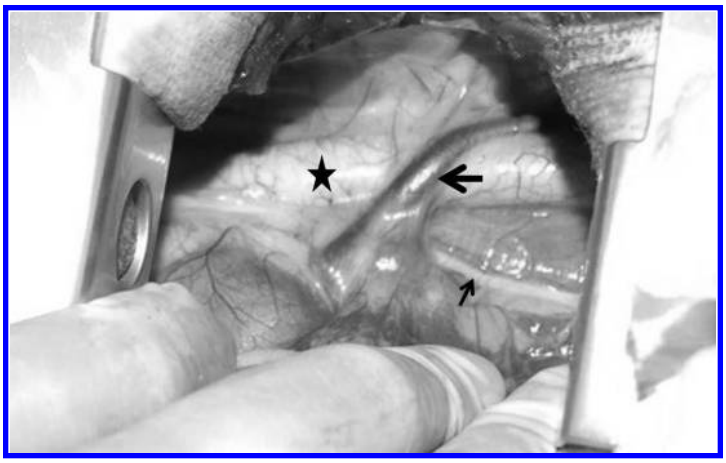

FIGURE 9.28 Left lateral thoracotomy through the sixth intercostal space. The large arrow points to the hemiazygous vein, the star is on the aorta, and the small arrow on the pulmonary trunk points to the phrenic nerve. The left lungs have been deflated using an endotracheal blocker.

should not be damaged or, if not needed, may be sacrificed without significant postoperative complications (Greiten et al., 2014; McKellar et al., 2007, 2008, 2011; Thompson et al., 2007).

Closure of thoracic surgical incisions is comparable to other species. After removal of the rolled-up towel or sandbag under the thorax, lateral thoracotomies are closed with heavy-gauge (0–1) circumferential sutures that have been preplaced around the cranial and caudal ribs adjacent to the incision. Three to five sutures are usually required to close the incision adequately. Sutures may either be absorbable polydiaxonone or nonabsorbable materials. After preplacing all sutures, the central one in the incision is tied first, followed by the peripheral ones, with alternated tying of sutures in both directions. Rib-approximating forceps are helpful for tying the first suture. The latissimus dorsi always requires closure as a separate layer, even in the smallest pig. However, the decision whether or not to close the rest of the muscles in layers varies with the size of the pig. Muscle layers can be closed either with interrupted or continuous sutures of any of the synthetic absorbable materials. The subcutaneous tissues are also variable in thickness depending upon the size of the pig, but this layer is closed, if necessary, using synthetic absorbable materials. The skin layer is best closed with a subcuticular layer of 2/0 or 3/0 synthetic absorbable suture. Other types of closure may be used, if preferred.

Median Sternotomy

With the pig in dorsal recumbency, incisions can be performed successfully in swine as a survival procedure, unlike in many other animals (Figure 9.29). Pigs experience relatively little discomfort with the procedure, especially if the manubrium sterni is left intact, as it can be for many cardiac procedures. Care must be taken when performing this procedure because the heart is in sternal contact between the fourth and seventh costal cartilages in most pigs. The sternum may be bisected using a *Stryker* saw if a scalpel handle or straight ribbon retractor is held along the interior surface of the sternum to prevent cardiac trauma. It may also be bisected using sternal cutters of all the various configurations used in humans. The apex of the heart is in close apposition to the diaphragm at its most cranial attachment to the sternum at the level of the seventh costal cartilage. The heart will remain in a pericardial cradle after this procedure is performed because of the close attachment of the pericardium to the sternum (Swindle et al., 1986, 1988).

Cardiac and pulmonary tissue is friable in swine, especially in the smaller farm pigs commonly used in research. This tissue friability decreases with maturity. Miniature pigs present the great advantage that the rate of growth is decreased compared to farm pigs. This could be beneficial particularly

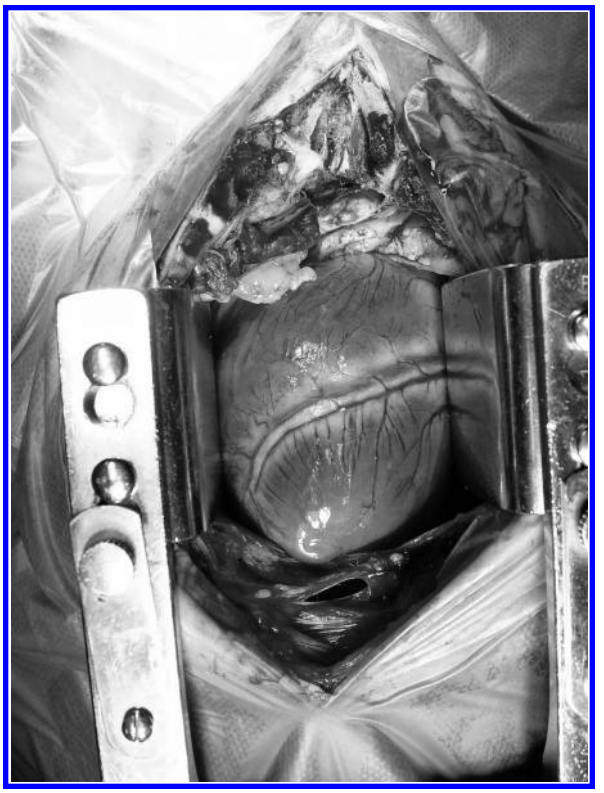

FIGURE 9.29 Median sternotomy.

in cardiovascular research, since they tend to be easier to manipulate surgically at the same weight as farm breeds due to their greater age at the same weight. Gentle handling of tissues using appropriate instrumentation is imperative to prevent complications, such as tearing of atrial tissue and rupture of pulmonary tissue, which may cause emphysematous bullae. Vessels are similar to those in humans except for the presence of the left azygous (hemiazygous) vein (Figures 9.8, 9.9, 9.11, and 9.28) that crosses from the intercostal vessels ventral to the left hilum of the lung to drain into the coronary sinus (Swindle, 1983; Swindle and Bobbie, 1987; Swindle et al., 1986). Retractors are needed in open thoracic surgery; the edges of thoracotomy incisions should be protected with wetted gauze sponges when using retractors. If possible, self-retaining retractors used for most swine need to be pediatric instruments with blunt blades to avoid injuring the underlying pulmonary tissue, which is a potential complication with retraction blades that extend too deep into the thoracic cavity. The mediastinum is thin and easily ruptured in swine. Chest tubes or instruments will readily injure the structure and, as a practicality, the mediastinum should be considered incomplete, because it is likely that fluids or surgical hemorrhage from one side of the thoracic cavity will extend into the other side through inadvertent rents.

A median sternotomy is closed in a similar fashion to the lateral thoracotomy. Nonabsorbable heavy-gauge (0–2) wire, braided nonabsorbable sutures, or plastic lock ties are preplaced in the intercostal spaces from cranial to caudal. Care should be taken not to unnecessarily occlude the interior mammary artery with these sutures; however, they may be sacrificed if necessary. If the manubrium sterni was not transected, the pig will have an easier recovery with less postoperative discomfort, and the surgical incision will be easier to close with proper alignment. If it was transected, then great care should be taken to ensure that closure of the sternotomy is performed in an anatomically correct fashion. The sutures are tied sequentially from cranial to caudal. The pericardium does not need to be sutured as a separate layer. A single-layer closure of muscle and

subcutaneous suture using 2/0–3/0 synthetic absorbable suture should be placed next. The skin is closed in the same fashion as for the lateral thoracotomy.

Postsurgical Pneumothorax Management

Evacuation of air from the chest can be performed by a variety of methods (Figure 9.30). The lungs should be expanded to their maximum capacity to reduce the air volume of the thorax while closing the initial layer of the thoracotomy, whether it is a lateral or median incision. For simple procedures in which postoperative bleeding and leakage of air are not expected, the chest may be evacuated with a needle or preferably a small-gauge catheter and syringe with an attached three-way valve. The insertion is in the dorsocaudal area of the thoracic cavity, care being taken to avoid damage to the lung with the needle or catheter. Evacuation may be performed after closing the internal muscle layers so that a watertight seal exists.

If a chest tube is to be placed, it is inserted while the incision is still open. A skin incision is made in a dorsocaudal position at least two intercostal spaces caudal to the lateral thoracotomy. A flexible chest tube, 14–30 Fr, with fenestrations at the distal end, is passed through the skin after a stab incision is made and then advanced into the thoracic cavity. The chest tube will be attached to a hemostat and tunneled subcutaneously, two to three spaces, and then firmly placed through the chest wall at the junction of the dorsal and middle third of the chest at the seventh or eighth intercostal space. The hemostat will be held close to the cranial aspect of the rib, in order to avoid the intercostal vessels located on the caudal border of the ribs. After the chest tube is inserted into the pleural space, a nonabsorbable purse string suture is placed in the skin incision around the catheter. Upon closure of the thoracotomy site, air will be evacuated through the chest tube until negative pressure is achieved. Then the chest tube is clamped. The chest tube can remain for 24–48 h postoperatively (and up to 5 days if needed) and is removed upon close observation of normal respiration patterns and when minimal volumes of air/liquid are obtained during controlled aspiration.

Local pain management at the thoracotomy site can include infiltration of the surgical site with either lidocaine or bupivacaine. The most commonly used regional anesthetic is lidocaine. The onset of action is rapid and duration of action of lidocaine at 1 mg/kg is 90–180 min. Longer acting local anesthetics, such as 0.5% bupivacaine (+/– epinephrine 5 mcg/mL), 1–5 mg/kg over 1–2 min, can provide 180–300 min of analgesia, but the onset is slow (Skarda, 1987; Swindle, 2007). Wound soaker catheters are a viable means of providing intermittent local analgesia during the postoperative period (Abelson et al., 2009). These wound infusion catheters can be used to deliver continuous infusions of local anesthetics to the surgical site.

For a median sternotomy, single or double chest tubes are placed on the ventrolateral aspects of the midthorax cranial to the attachment of the diaphragm. Tubes may either be the one-way valve Heimlich type for short- or mid-term usage, or the use of water-sealed systems may be required for more involved procedures that have the possibility of increased complications. Chest tubes are placed with purse-string sutures around them in the skin. A three-way stopcock and syringe are

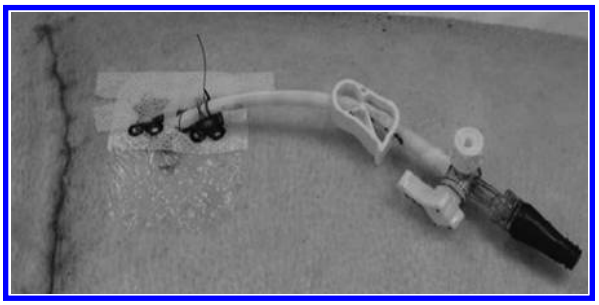

FIGURE 9.30 Chest tube placement in the left thorax caudal to thoracotomy site.

TABLE 9.1
Heart Weight to Body Weight of Various Pig Breeds

Breed	Body Weight (kg)	HW/BW (%)
Farm	25	0.49
	112	0.4[a]
Hanford	26	0.46
Sinclair	26	0.47
Yucatan	26	0.57
Yucatan Micro	26	0.55
Göttingen	11–13	0.46–0.49

[a] The heart weight (HW) decreases as a percentage of body weight (BW) in swine as they grow.

attached to the catheter to remove fluid and/or air and to improve the pigs' breathing ability during the initial postoperative period. The chest tube can remain for 24–48 h postoperatively. Purse-string sutures are tightened during removal of the chest tubes after it has been determined that pneumothorax or hemorrhage is no longer likely.

The major difficulties encountered in thoracic surgical procedures are likely to be cardiac arrhythmias and cardiodepression. Methods of preventing these common complications are detailed in Chapter 2. Likewise, procedures such as graft implantation, which are likely to induce thrombosis, can be effectively prevented with anticoagulant therapy as discussed in Chapters 2 and 12. Inherent in the design of these surgical protocols is the necessity of designing an appropriate anesthetic and analgesic protocol for the procedure being performed. Good surgical technique alone is not sufficient to have a successful outcome for thoracic surgery. Intraoperative and postoperative monitoring of heart rate, electrocardiogram, blood pressures, and blood gases are essential in these cases if survival is expected.

General principles of vascular surgery are discussed later in the sections on vascular cannulation and anastomosis. Some comparative cardiovascular measurements and hemodynamic values are included in Tables 9.1 through 9.4. Additional measurements and weights are given in the tables and appendix in Chapter 12.

PULMONARY SYSTEM

Tracheostomy and Tracheotomy

Tracheostomy can be performed in the pig as a survival procedure either following an emergency or as part of a planned experiment. With the pig in dorsal recumbency, the cricothyroid cartilage can be palpated easily, even in large swine. The caudal end of the cartilage has a blunted tip similar to a bird's beak. The cricothyroid membrane is immediately caudal to this structure and is the preferred location for a tracheostomy (Murphy et al., 2011; Swindle, 1983).

A 1–2 cm incision is made over the membrane on the ventral midline. The cutaneous coli and sternohyoideus muscles are separated longitudinally, and the membrane is identified. This procedure can be performed bloodlessly in seconds in an emergency. The cricothyroid membrane is incised, and either a tracheostomy tube or an endotracheal tube of the appropriate size can be inserted (Figure 9.25). The larynx may be held between the thumb and forefinger of the surgeon's hand for countertraction to aid this procedure. As an option, the membrane between the first and second tracheal rings can be incised and used for this procedure. For a survival procedure, however, this transection does not heal as readily as an incision in the cricothyroid membrane. The larynx of

TABLE 9.2
Vessel Luminal Diameters (ID) in Various Breeds of Swine

Breed	Body Weight (kg)	Coronary Artery (mm)	Thoracic Aorta (mm)	Postrenal Abdominal Aorta (mm)	Carotid Artery (mm)	External Iliac Artery (mm)	Internal Iliac Artery (mm)	Posterior Vena Cava (mm)	Femoral Artery (mm)	Portal Vein (mm)	Renal Artery (mm)
Hanford[a]	15	2.0–2.5									
Hanford[b]	30–35	1–1.5									
Hanford[b]	44		13	5		3	2	14			
Yucatan[a]	10–20	2–3.5				2.5–4		20			
Yucatan[b]	29		12	6		2	1				
Yucatan[b]	48		11		4						4
Yucatan[b]	109		11	6		4	3				2
Yucatan Micro[a]	14–33		12	6		5	1–2		3.5	10	
Yucatan Micro[b]	55–60		9–16	5–7	3–4	2					
Sinclair[a]	45					4.8	3.5		4		
Göttingen[b,d]	20–40	1.5–2.0	14–16		3						
Göttingen[b]	37–50						3–5				
Farm[a,c]	30–39					5.3	3.4		4.8		
Farm[a,c]	40–49					5.8	3.7		4.9		
Farm[a,c]	50–59					5.9	4.5		4.6		
Farm[a,c]	60–69					6.1	4.2		5.6		
Farm[a]	55			12		6					
Farm[b]	70	3	12	5	3.5						
Farm[a]	16	3	13	8.5	4	4	2.1		4		2.5
Farm[b]	25		21	10	5	6	3	12		16	4
Farm[b]	47	3	22	15	5	6	4	18	4		5

[a] *In vivo* measurement using fluoroscopy and contrast material.

[b] *In vitro* necropsy measurement.

[c] Courtesy of Dagan Harris, MindGuard, Israel.

[d] Courtesy of Professor Rainer Schulz.

TABLE 9.3
Cardiac Measurements at Necropsy

Breed	Body Weight (kg)	Heart Length (mm)	Heart Circumference (mm)	Left-Ventricular Chamber Length (mm)	Right-Ventricular Chamber Length (mm)	Mitral Valve Diameter (mm)	Tricuspid Valve Diameter (mm)	Aortic Valve Diameter (mm)	Pulmonary Valve Diameter (mm)	Left-Ventricular Free Wall (mm)	Right-Ventricular Free Wall (mm)	Intraventricular Septum (mm)
Yucatan	47.6	95	230	48	75	63	65	11	11	16	7	15
Farm	47.5	120	190			25	27	22	22	20	6	16
Farm	25	82				20	23	21	21	12	7	7

TABLE 9.4
Cardiovascular and Hormonal Values of Conscious Swine

Heart rate, beats/min	101 ± 2
Right atrial pressure, mmHg	0.8 ± 0.7
Pulmonary capillary "wedge" pressure, mmHg	5.1 ± 0.8
Mean aortic blood pressure, mmHg	96 ± 2
Mean pulmonary arterial blood pressure, mmHg	13.8 ± 0.7
Cardiac output, mL/min/kg	210 ± 6
Systemic vascular resistance, mmHg kg min/L	464 ± 21
Pulmonary vascular resistance, mmHg kg min/L	40.4 ± 2.6
Plasma rennin activity, ng Al/mL/h	0.88 ± 0.17
Plasma vasopressin concentration, pg/mL	2.0 ± 0.4
Temperature (pulmonary artery blood) °C	38.99 ± 0.14
Arterial pH, units	7.458 ± 0.003
Arterial PCO_2, mmHg	44.5 ± 0.7
Arterial PO_2, mmHg	96.2 ± 2.2

Source: Reprinted from Weiskopf, R.B. et al. 1992. *Swine as Models in Biomedical Research.* Ames: Iowa State University Press. With permission.

swine is large, and the tracheal rings will be somewhat obscured by this structure, necessitating a longer incision (Figure 9.31).

The cricothyroid membrane can be sutured with a continuous pattern using synthetic absorbable sutures. Problems have not been noted with the suture material entering the lumen of the larynx; however, some surgeons may wish to use a continuous Lembert pattern instead. The sternohyoideus muscle is closed with a continuous suture pattern and the skin, with a subcuticular pattern, using synthetic absorbable material.

Chronic ostomies can also be maintained by suturing a standard tracheostomy tube to the skin after closing the muscular and subcutaneous tissues around the device. By making the incision in the cricothyroid membrane, complications associated with the vocal cords can be avoided as long as

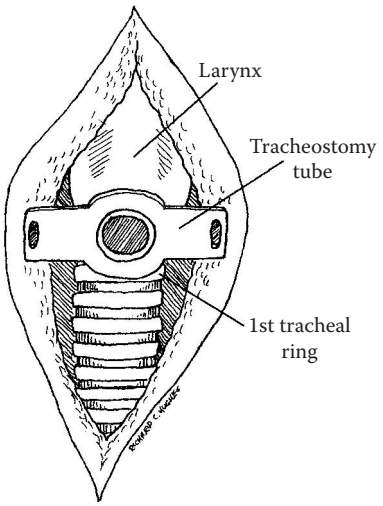

FIGURE 9.31 Tracheostomy.

the surgeon passes the tracheostomy device caudally. An ostomy can also be formed by suturing the edges of a tracheal window to the skin. A tracheal window can be formed by resecting a rectangular section from the first few tracheal rings on the ventral surface. The trachea is too deep in the musculature of the neck to use this procedure beyond the area immediately caudal to the larynx.

Pneumonectomy and Lobectomy

The hilum of either lung is surgically approached through the fourth intercostal space. A total pneumonectomy is more easily performed on the left side and is described here (Swindle, 1983; Swindle et al., 1986). Delayed pulmonary arterial hypertension postpneumonectomy has been described experimentally in swine (Berthet et al., 2013) as well as a nonsurvival model of pulmonary embolism created by injection of blood clots (Beam et al., 2015). With the pig in right lateral recumbency, the left foreleg is drawn cranially, and a sandbag is positioned under the thorax. The incision is made obliquely from the dorsal caudal border of the scapula ventrocaudally toward the first nipple. After making the skin incision, the ribs may be palpated; the fourth intercostal space is the most cranial one, approachable with this skin incision. The muscle layers are successively divided, and the thoracodorsal artery and vein within the body of the latissimus dorsi muscle will be the only major blood vessels encountered. The muscles are easily divided, and the intercostal muscles may be divided along the cranial border of the fifth rib using Metzenbaum scissors. At the dorsal edge of the incision, the superior intercostal vessels may be encountered and at the distal margin of the incision, the internal thoracic vessels should be avoided. Care should be taken to enter the thorax without damaging the lungs. This can be facilitated by underinflating the lungs and cutting the last muscle layer with Metzenbaum scissors.

After placing self-retaining retractors, the pulmonary ligament is identified at the dorsocaudal aspect of the caudal lobe of the lung. This fibrous structure cannot be seen through the thoracotomy and must be cut blindly with Metzenbaum scissors while retracting the tip of the caudal lobe proximally with the fingers. The caudal and apical lobes of the lung can now be exteriorized to expose the hilum of the lung. The lobes of the lung are retracted dorsally using wetted gauze pads.

The hilum of the lung (Figure 9.32) is dissected, and the blood vessels are identified and surgically transected in this order: cranial ventral branch of the pulmonary vein, left pulmonary artery, apical branch of the pulmonary vein, and caudal diaphragmatic branch of the pulmonary vein. The first branch of the pulmonary vein has to be transected to adequately expose the pulmonary artery branch. If these transections are being performed manually, then it is best to use transfixion sutures in the pulmonary artery. During this dissection, the left azygous (hemiazygous) vein that crosses ventrally to these vessels should not be damaged. However, it may be ligated and removed if necessary without significant postoperative complications. The lung is then retracted ventrally to expose the bronchial artery on the dorsal aspect of the bronchus in order to transect it. If vagal stimulation resulting in bradycardia, cardiac standstill, or both is problematic during the deep dissection of the hilum, atropine should be administered.

After all the vessels have been ligated, then the bronchus is cleared of fascial tissue and transected. This transection is best performed with staples. However, if it must be performed manually, the bronchus is cross-clamped distally, and the bronchus is cut on the proximal end of the clamp a few millimeters at a time while placing simple interrupted sutures using nonabsorbable suture material. As an alternative, a continuous horizontal mattress pattern can be used. This cut-and-suture technique is performed from the caudal end to the cranial end of the bronchus. The bronchus is checked for leaks with saline, and any leak noted is repaired with additional sutures. Leaks are most likely to occur at the middle of the bronchus where there is an indentation in the dorsal border. The leak can usually be repaired by placing a horizontal mattress suture in this area.

The right lung can be removed in a similar manner. However, the right cranial lobe bronchus branches directly from the trachea separate from the bronchus, which branches to supply the caudal and accessory lobes. Consequently, both bronchi have to be divided surgically. Access to the

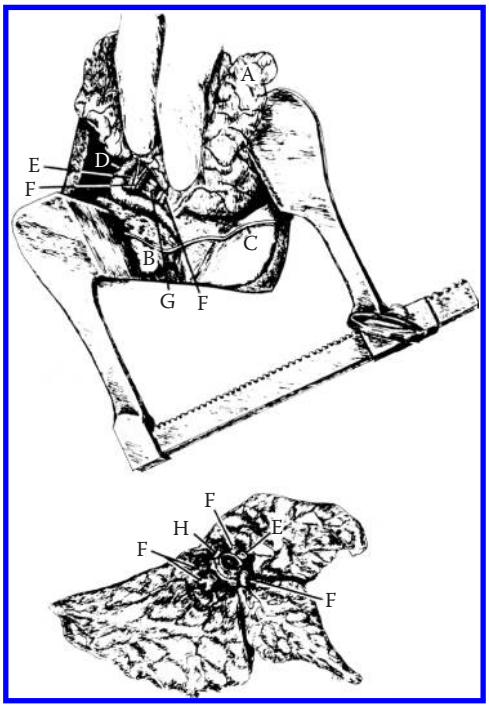

FIGURE 9.32 Hilum of the lung during left pneumonectomy. A, Left lung; B, heart; C, phrenic nerve; D, left azygous vein; E, pulmonary artery; F, pulmonary veins; G, bronchus; H, bronchial artery. (Redrawn from Swindle, M.M. et al. 1986. *Lab. Anim. Sci.* 36(4): 357–361. With permission.)

accessory lobe is difficult because of the location of the vena cava, which makes dissection of the pulmonary veins difficult.

Lobectomies and partial lobectomies of the lung in swine are technically feasible but difficult. Jangra et al. (2005) have described a thoracoscopic technique of partial lobectomies. In that model, platinum coils were implanted into the lung parenchyma to simulate cancerous nodules, which could be located using CT imaging. Division of the friable lung tissue always leads to leakage of air and, frequently, the formation of emphysematous bullae. The two best locations for attempting the procedure are the right cranial lobe followed by the left cranial lobe at the caudal interlobular fissure. These two locations provide the deepest fissure between lung tissue and have a single branch of the bronchus to transect.

The lung is divided using staples at the identified location. If staples are not available, manual suturing using a horizontal mattress suture pattern can be used; however, the pulmonary tissue frequently ruptures with this technique. The branches of the pulmonary artery and veins supplying the resected section are identified and surgically transected. The branch of the bronchus is then dissected free and transected with staples. The transected edge of the lung remaining in the animal will have to have the leaks sealed in the tissue following removal of the resected lobe. Various tissue glues and oxycellulose patches have been used to seal these leaks in the past with variable success. Tissue glues are probably more effective than patches. The complication rate is high with this procedure because of the friability of the pulmonary tissue and the high potential for air leaks. Development of better methods of sealing the cut edges of the lung would help in this species. The lateral thoracotomy is closed as described earlier.

Pulmonary Transplant

Swine have been used for single-lung transplantation and heart-and-lung transplantation (Baumgartner et al., 1988; Calne et al., 1976, 1978; Hall et al., 1986; Harjula and Baldwin, 1987;

Hillinger et al., 2000; Qayumi et al., 1990, 1991, 1993; Saito and Waters, 1994; Salminen et al., 2000; Swindle et al., 1986). A left single-lung transplant is more applicable than a right-lung transplant because of the presence of the right cranial bronchus, which makes it substantially different from humans. Consequently, a left single-lung transplant is described here. Pulmonary denervation can lead to respiratory insufficiency, which can be a problem for long-term studies. This model develops obliterative lesions in small airways during chronic rejection, similar to humans (Salminen et al., 2000).

The donor is prepared for a complete median sternotomy. The left lung is removed following heparinization and subsequent transection of the following structures: left main bronchus, pulmonary artery, and atrial cuff containing the pulmonary veins. All structures should be transected so that the longest possible segment remains attached to the donor lung. It is perfused with cold preservation solution through the pulmonary artery while awaiting transplantation.

The recipient is prepared in a similar manner. The donor lung is sutured in place in the following order: atrial cuff with pulmonary veins, pulmonary artery, and bronchus. Suture selection depends upon the size of the animal and the experience of the surgeon. However, a continuous suture pattern with nonabsorbable suture is the most common technique.

Immunosuppressive therapy typically includes such pharmaceuticals as methylprednisolone (2–20 mg/kg/d), cyclosporine (10 mg/kg/d), azathioprine (2 mg/kg/d), and SDZ RAD (1.5 mg/kg/d), all administered postoperatively following an intravenous (IV) loading dose at surgery. In many instances, the goal of the research is to study immunosuppressive therapies, and new compounds are studied with combinations of the standard immunosuppressive therapies. Preventive therapies with compounds such as prostaglandin E_1 (250 mg, IV) are also typically used. Postoperative complications include increased extravascular lung water due to reperfusion injury and obliterative bronchitis (Hillinger et al., 2000; Salminen et al., 2000).

CARDIOVASCULAR SYSTEM

Peripheral Vascular Cannulation and Chronic Catheterization Techniques

General Principles

Several sites of peripheral intravascular catheterization available in swine are the auricular artery and vein, external and interior jugular vein, carotid artery, cephalic vein, femoral artery and vein, and medial saphenous artery. Internal sites include pulmonary artery, vena cava, hepatic artery and vein, portal vein, renal artery and vein, aorta, and internal and external iliac artery and vein. Regardless of the site, it is important that the tip of the catheter, when used for infusion or blood draw, be in a high-flow, turbulent area and that the tip not be in contact with a vessel wall to help prevent thrombosis, wall damage, and catheter malfunction. Videos of the procedures in this section are included on the DVD attached with this textbook.

Except for the carotid artery, these vessels may be ligated and sacrificed bilaterally instead of being surgically repaired without significant postoperative complications. Both carotid arteries can be sacrificed with staged surgeries to allow collateral circulation to develop. Collateral circulation is sufficient with one intact carotid artery, and no brain damage is observed. One of either the external or internal jugular veins on each side of the neck should also be left intact, but this is not essential. Other vessels that are chronically cannulated for specific research purposes include portal vein, pulmonary artery, and aorta. These vessel approaches will be discussed in this section. Using these vessels as an example, many other blood vessels can be cannulated if indicated by the research protocol. Superficial access to some veins makes it possible to cannulate the central venous system either percutaneously or with minor skin incisions. These veins include the auricular vein, the cephalic vein at the level of the thoracic inlet, and the cranial epigastric vein on the ventral abdomen.

General Surgical Principles for Catheterization

The general principles of surgery apply to these procedures. They are asepsis, closure of dead space, hemostasis, gentle handling of tissues, careful approximation of the wound, avoiding wound tension, and minimizing foreign material. In addition, there are specific principles that apply to the implantation of catheters: immobilization of the catheter, an atraumatic tunneling pathway, securing the exit site, antibiotic prophylaxis at the time of surgery, and use of anticoagulant therapy. It is very important to immobilize the catheter at the site of insertion into the blood vessel and at the site of exit from the skin. This can be performed by leaving a coil of the catheter subcutaneously and putting a subcutaneous purse-string suture around the catheter before closing the skin. At the exit site, the catheter should have a cuff that allows tissue ingrowth or fibrosis around it to ensure a permanent seal against infection. Antibiotic prophylaxis is not a substitute for aseptic technique. The blood level of antibiotic present at the time of skin incision is the most important dose if such therapy is indicated. Long-term administration is not necessary unless contamination has occurred. Catheter exteriorization should be avoided due to normal swine behavior when appropriate. An alternative option is the use of implantable (under the skin) vascular access devices, as described later. Swine of the same breed and age tend to have the same measurement from the catheter insertion point to the site of interest for the tip of the catheter. Therefore, catheters can be manufactured in advance with suture retention beads fixed in place (Figure 9.33).

The technique of incising the vessel for insertion of the catheter depends upon the catheter design and the experience of the surgeon. Systems with needles and guidewires are reliable in swine (see Chapter 12). The vessel should be occluded with elastic vessel loops (Figure 9.34) cranial and caudal to the site of entry and the vessel allowed to fill with blood. The vessel may be entered with a no. 11 surgical blade, iris scissors, or a needle tip. The authors prefer to use iris scissors, because they tend to be less traumatic in small vessels and serve to prevent vasospasm in arteries. Suture material for tying the catheters in place for chronic cannulation depends upon the length of time that the animal will survive. Polydiaxonone is a good first choice if 3–6 months of suture retention is sufficient. Other synthetic absorbable or nonabsorbable materials may be more appropriate for some projects. Silk and surgical gut should not be used because of their inflammatory characteristics in swine. Both arteries and veins may be repaired surgically following catheterization using a nonreactive 5/0–6/0 cardiovascular suture material in a simple interrupted pattern.

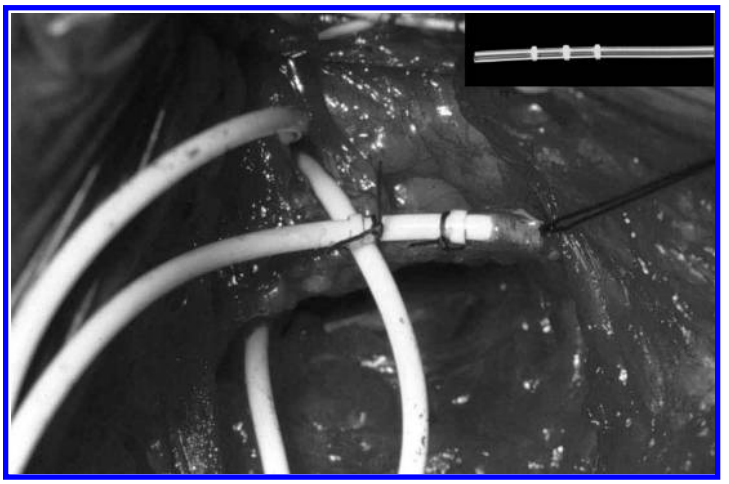

FIGURE 9.33 Insert shows three retention beads glued on the catheter. The surgical illustration demonstrates the proper method of suturing the catheter in place.

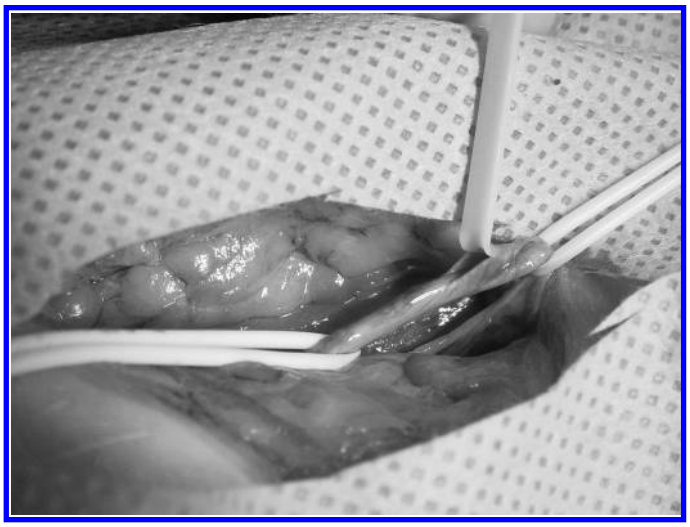

FIGURE 9.34 Elastic vessel loops are used to provide hemostasis during venotomy, in which a vessel pick is used to enlarge the lumen for catheter insertion.

Catheters for chronic implantation should have retention beads permanently fixed to the catheter at the site of vascular insertion (Figure 9.23). These can be simply beads of silicone glue instead of manufactured structures. Placing sutures in between the beads provides some security that the catheter will not move after implantation. A loop of the catheter should also be coiled in the area of venous access to prevent tension on the catheter as a result of movement or growth. Retention beads or velcro cuffs may be necessary in the subcutaneous tissue to prevent the catheter from moving into and out of the skin causing contamination. Nylon velour cuffs or cuffs (Figure 9.35) of other porous material allow tissue ingrowth to form a barrier against contamination. When closing the skin around a catheter exit site, synthetic absorbable materials should be used in subcuticular purse-string fashion. Catheters for vascular access should never be exited through the surgical site, because of the stress on the suture line and the high probability of contamination. Monofilament sutures may be used on the surface of the skin for a tight closure. Tissue glues may be helpful for the purpose of sealing the exit site.

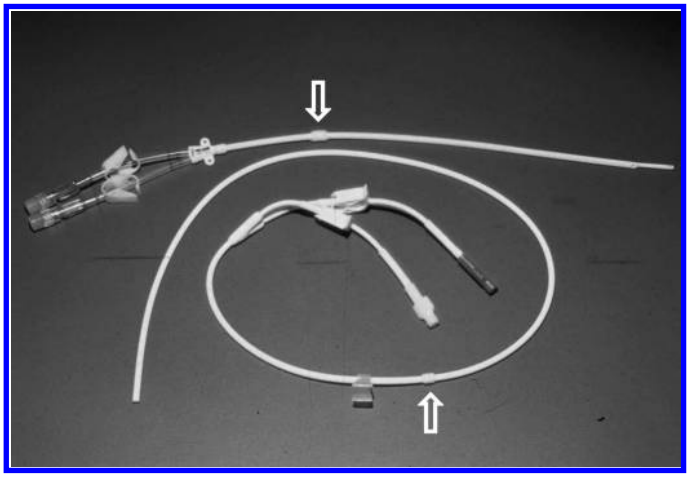

FIGURE 9.35 Hickman and Broviac catheters with cuffs (arrows) to allow tissue ingrowth.

Many of the vascular access sites in swine are relatively deep in the tissues. Venipuncture techniques have previously been described and the anatomic locations are illustrated in Chapter 1 (Bobbie and Swindle, 1986; Swindle, 1983). The Seldinger technique for vascular access for cardiac catheterization studies is discussed in Chapter 12 (Gaymes et al., 1995; Namba et al., 2013; Smith et al., 1989).

A technique of using ultrasound guidance for vascular access has been described (Wallace et al., 2003). In this section, the surgical principles of vascular access to the main blood vessels will be described (Brederlau et al., 2008; Hand et al., 1981; Nicolau et al., 1996; Purohit et al., 1993; Smith et al., 1989; Swindle, 1983; Swindle et al., 1986, 1996, 1998, 2005).

Peripheral arterial vessels of swine are prone to vasospasm and are relatively easy to rupture during catheterization techniques. Good surgical technique and gentle handling of tissues cannot be replaced by use of topical antispasmodic agents such as lidocaine or papaverine. When performing vascular access surgery, the division of muscular tissues should be performed in fascial planes between muscle bodies and not by dividing musculature. Blood vessels should be handled gently, and suture material used around the blood vessels should not be abrasive; for example, silk should not be used. The use of elastic vessel loops, especially the rounded type, is the preferred method of isolating and occluding blood vessels during catheterizations. Experience has shown that they do not have the sawing action that braided suture materials have on vessels, which frequently leads to vasospasm. When using gauze, it should be wetted with warm saline to prevent hypothermic vasoconstriction. Gauze should be pressed and held on a bleeding site, never rubbed. The use of electrocautery for hemostasis and ablation of collateral branches works well, as long as the power settings are not high enough to cause collateral cauterization and vasoconstriction.

Catheters should be exteriorized on the dorsal surface of swine (Chapter 8, Figures 8.8 and 8.12). Pigs do not scratch or bite at catheters because of their body conformation. Rather, they rub sites that irritate them. The cage design should be free of materials that a pig could reach by rubbing. Exteriorized catheters can be protected by pouches or covers sutured to the skin or secured with adhesives. Use of nylon jackets and vests designed for research purposes can also be used as well as tether and harness systems. For continuous infusion catheters, such as those attached to portable infusion pumps, flow rates of 3–5 mL/h are generally high enough to prevent occlusion. Catheters should be filled with full-strength heparin (1:1000 solution) or other catheter lock solution at the time of surgery, if they are static. The amount of heparin solution required to fill the catheter should be measured and recorded. Some prefer to add hypertonic solutions, such as 50% glucose, to the heparin solution to aid in prevention of thrombosis or antibiotic solutions to aid in the prevention of infection. These are unnecessary if meticulous attention is paid to aseptic handling of the catheters during sampling and if the catheter solutions are withdrawn, flushed with sterile saline, and refilled with heparin two to three times a week. Closed systems, such as the vascular access ports (VAPs), are more reliable in the prevention of complications than systems using three-way valves attached to the ends of open catheters (Swindle et al., 1998, 2005). Additional discussion of catheter lock solutions and postoperative maintenance techniques are discussed later in the subsection on catheter maintenance.

Catheter Design

Catheter selection is based on the technique and purpose of the experiment and the surgeon's preference and experience. Many laboratories manufacture their own catheters for saving costs; however, inappropriate designs and flaws can lead to problems more readily than if commercial catheters are used. Consequently, it is not recommended that investigators manufacture their own catheters for saving costs alone. There either should be an experimental reason to do so or the laboratory should be experienced in design and manufacture of catheters without sharp edges, surface flaws, or contaminants (Dougherty, 1981; Hand et al., 1981; Harvey and Jones, 1982; Swindle et al., 1988, 1998, 2005).

Many problems can be minimized by proper catheter design, which avoids traumatizing the vessel walls, thus initiating a thrombogenic response or erosion. For most procedures, biocompatible

silicone or polyurethane catheters are appropriate, although other materials such as polyethelyne, teflon, or polyvinyl chloride may sometimes be indicated (Tables 9.5 and 9.6). Silicone has the advantage of being soft, flexible, atraumatic, and autoclavable, although it is porous and is more difficult to insert than materials that are stiffer. Polyurethane is firm and easy to insert, but it is more traumatic to blood vessels and is not autoclavable. Trauma from this material on the vessel wall can lead to erosion or perforation of the vessel. Tips of the catheters should be tapered and have smooth, rounded edges (Figure 9.36). Part of the length of a silicone catheter can be partially covered with polyurethane to give stiffness to the body of the catheter and avoid kinking. This technique leaves only the length of rigid material exposed in the body cavity, with the softer silicone tip able to

TABLE 9.5
Comparison of Silicone versus Polyurethane

Polyurethane		Silicone	
Benefits	**Disadvantages**	**Benefits**	**Disadvantages**
Material is stronger and less likely to tear	More difficult to modify than silicone	Softness renders it less traumatic to vessel	Less strong and thus more prone to tear
Because of its strength, thinner catheter walls are needed, thereby yielding larger internal lumens and increased flow	Initial stiffness may be more damaging to the vessel	More easily modified because of reliable silicone adhesive	More difficult to insert and advance because of softness
Easier to insert and advance because of initial stiffness	Cannot be autoclaved	Smaller lumen yields less dead space, which is advantageous in certain applications	Difficult to coat with specialized coatings
Softens upon warming to body temperature, which may reduce vein trauma	Poor memory may result in loose-fitting connections over time	Can be autoclaved	
Can be coated with a variety of specialized coatings		Excellent memory may maintain tight-fitting connections	

Source: From Swindle, M.M. et al. 2005. *Contemp. Top. Lab. Anim. Sci.* 44(3): 7–17. With permission.

TABLE 9.6
Catheter Sizes: Silicone and Polyurethane

		Silicone Catheters				Polyurethane Catheters			
		Inner Diameter		Outer Diameter		Inner Diameter		Outer Diameter	
French Size	Approximate Gauge	in.	mm	in.	mm	in.	mm	in.	mm
1	27	0.007	0.2	0.016	0.4	0.008	0.2	0.017	0.4
2	23	0.012	0.33	0.025	0.6	0.012	0.3	0.025	0.66
3	20	0.020	0.5	0.037	0.9	0.023	0.6	0.037	0.9
4	18	0.025	0.6	0.047	1.2	0.025	0.6	0.044	1.1
5	16	0.030	0.7	0.065	1.7	0.040	1.0	0.065	1.7
7	13	0.050	1.3	0.095	2.4	0.058	1.5	0.095	2.4
9	11	0.062	1.6	0.125	3.2				

Source: From Swindle, M.M. et al. 2005. *Contemp. Top. Lab. Anim. Sci.* 44(3): 7–17. With permission.

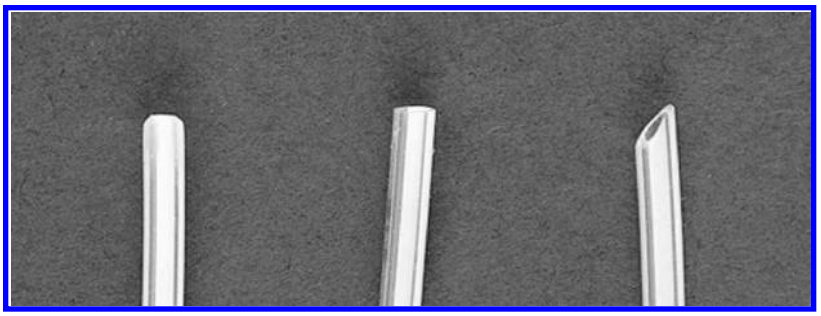

FIGURE 9.36 Catheter tip on the left is rounded and tends to be atraumatic to vessel walls as compared to cut (center) or beveled (right) tips. (Photo courtesy of Instech Solomon, Plymouth Meeting, PA.)

contact the vessel wall. Careful handling of silicone to prevent absorption of contaminants such as tissue glues, which can be toxic, should be ensured. A variety of implantable port devices (Figure 9.37) are available to avoid having to exteriorize the catheter from the skin.

Hickman (Figure 9.35) and Broviac catheters are excellent catheters if large-bore or multiple-channel cannulae are required. Subcutaneously implantable catheters, referred to as VAPs (Figure 9.37), are manufactured by several companies (e.g., Access Technologies, Da Vinci, Instec-Soloman, Phamacia-Deltec, and Uno). There are a variety of designs for the ports, which need to be considered for the particular protocol for which they are being considered. These catheters may be modified or designed by the manufacturers to specifications for particular experimental purposes (Bailie et al., 1986; Swindle et al., 1986, 2005). These require special Huber point needles and placement techniques but have the advantage of reducing risk of infection to exit sites (Figures 9.37 and 9.38). They may also be sutured onto the surface of the skin (Swindle et al., 2005). They offer

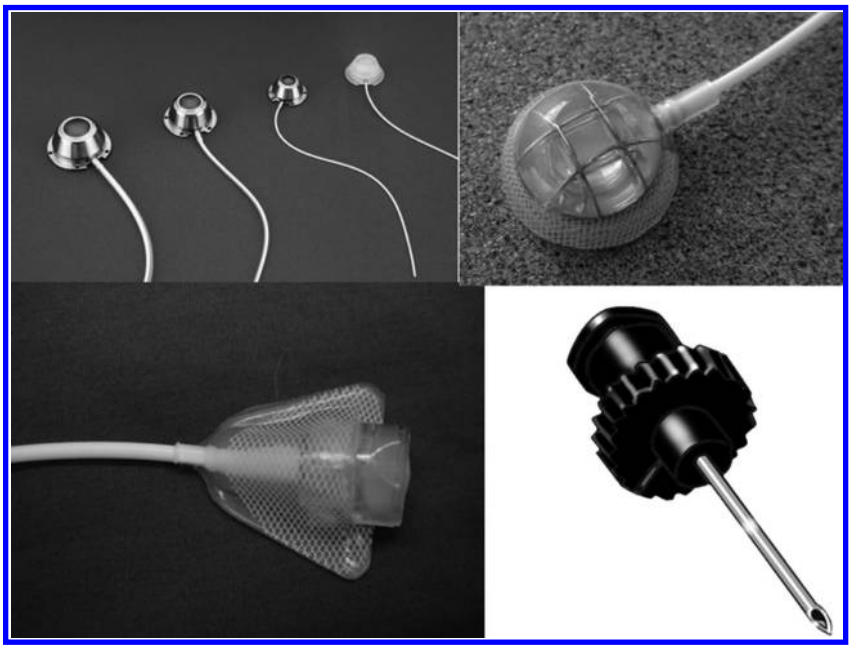

FIGURE 9.37 The composite shows a variety of designs of vascular access ports and a Huber point needle (lower right) for accessing them. (Photos courtesy of Access Technologies and Instech Solomon.)

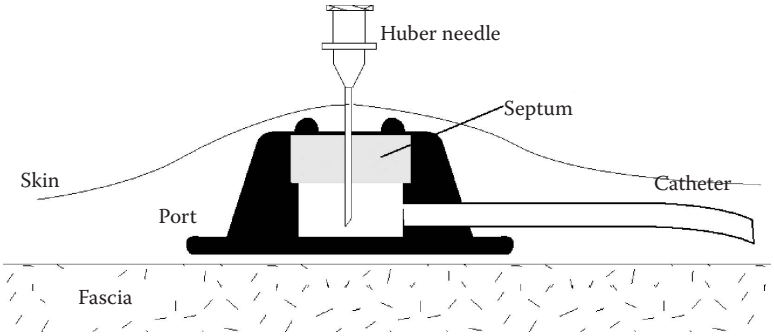

FIGURE 9.38 Schematic drawing of the use of a subcutaneously implanted vascular access port. (Photo courtesy of Access Technologies, Skokie, IL.)

an advantage of improved asepsis because of the closed nature of the design system. These catheters can also be procured with a tapered edge on the tip to help prevent vascular damage and thrombosis. Small catheters for short-term catheterization include the use of IV catheters available from any hospital supply.

Catheter Patency: Maintenance

To prevent blood from clotting within the catheter, a small volume of anticoagulant should be used. Catheters used for continuous infusion do not need anticoagulant use, but an infusion rate of 3–5 mL/h is necessary to prevent intracatheter coagulation. Systemic coagulation or anticoagulant therapy is not required for intravascular catheters to conserve the patency of the catheter.

No consensus exists on a postoperative protocol for every type of intravascular catheterization. However, some principles utilized by our laboratories have proven effective to maintain long-term catheter patency. It is essential to ensure complete asepsis with iodine preparation of the catheter and use of sterile gloves to handle an implanted catheter. At the time of implantation, the volume of the catheter should be determined, and the correct amount of undiluted 1:1000 heparin is injected to completely fill the catheter. Flushing of the catheter is necessary every time it is accessed or once or twice per week when not accessed for sample administration and collection. The procedure is to withdraw all the old heparin until blood is visualized, flush copiously with saline, and then reinject the predetermined amount of heparin required to fill the catheter. Generally, 10%–20% more than the predetermined fill amount of catheter lock solution is injected to ensure that the total catheter length is filled to preserve patency.

Other catheter locking solutions that have been used include 100–1000 U/mL heparinized saline, hypertonic 50% dextrose, enzymatic solutions, and antibiotics within the anticoagulant solution. The use of antibiotics is discouraged (microbial resistance), and unnecessary, if meticulous aseptic technique is used. Taurolidine citrate is an antimicrobial solution that has been developed as a substitute for antibiotics in catheter solutions. It has a wide range of activity against most types of infectious organisms, and the citrate provides some anticoagulant activity (Betjes and van Agteren, 2004; Jurewitsch and Jeejeebhoy, 2005; Koldehoff and Zakrzewski, 2004; Moroni et al., 2011; Swindle et al., 2005).

Neck Vessels: External and Internal Jugular Veins and Carotid Artery

The neck vessels can all be approached from an incision in the jugular furrow (Figures 9.39 through 9.41). By retracting one leg caudally with the pig in dorsal recumbency, the jugular furrow can be seen along a line drawn slightly medial from the point of the jaw to the point of the shoulder. An incision made in this plane will provide access to the external jugular vein, which is deep in the intermuscular plane between the brachiocephalic and sternocephalic muscles at the same level as

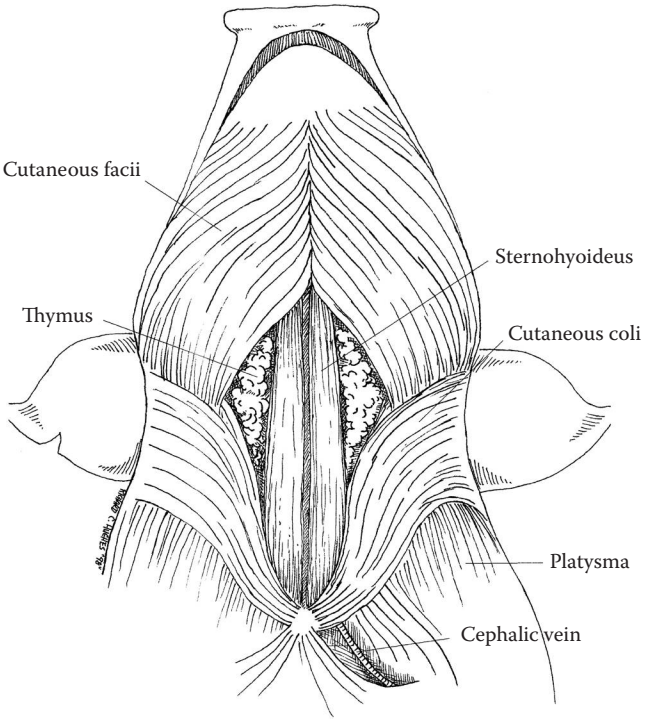

FIGURE 9.39 Superficial dissection of the neck (ventral aspect).

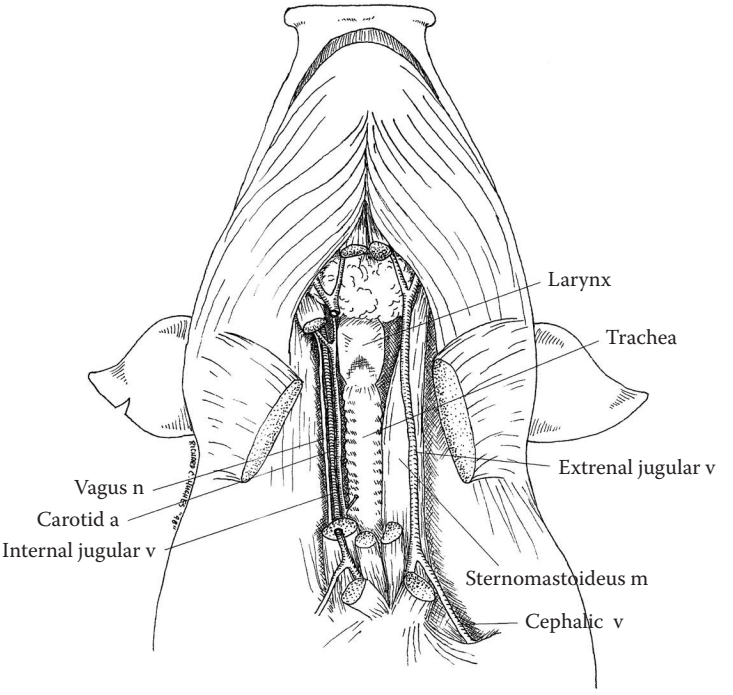

FIGURE 9.40 Deep dissection of the neck, which exposes the blood vessels. The right side has had the sternomastoideus muscle removed to expose the carotid sheath.

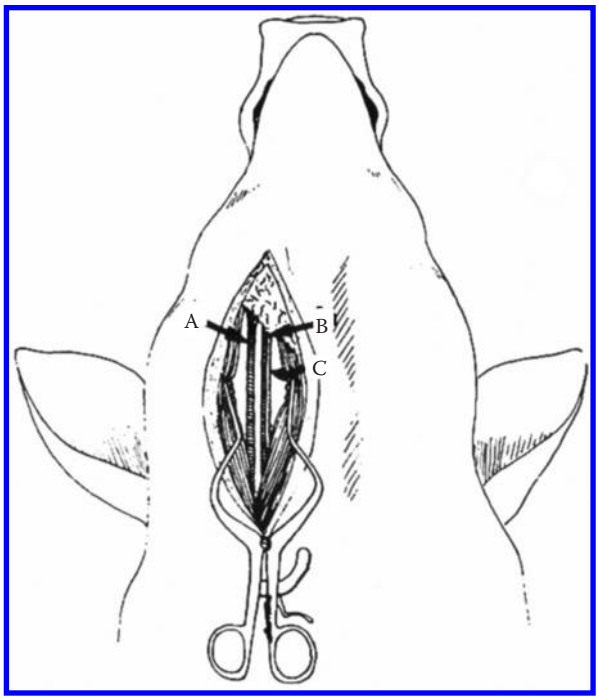

FIGURE 9.41 Surgical approach to the neck. (A) external jugular vein; (B), common carotid artery; (C) internal jugular vein. (Reprinted from Smith, A.C. et al. 1989. *J. Invest. Surg.* 2(2): 187–194. With permission.)

the trachea. After incising the skin, subcutaneous tissue, and cutaneous coli muscle, the external jugular vein can be isolated by blunt dissection. From this incision, the two mandibular branches of the vein and the main trunk can be easily isolated.

The internal jugular vein, carotid artery, and vagus nerve can be isolated from this same incision (Figure 9.42), or they can be approached by a ventral midline or paramedian incision followed by dissection of the fascial plane parallel to the trachea. They are located at the same depth as the external jugular but are medial and lie along the ventral surface of the cervical vertebrae parallel to the trachea. They are exposed from the jugular furrow incision by dissecting the fascial plane on the dorsal surface of the sternocephalic muscle. After dissecting this fascia, the floor of the vertebrae and the carotid pulse can be palpated easily. The blood vessels can be retracted carefully into the area of the external jugular vein with a right-angle forceps and isolated with vessel loops for cannulation.

From this location, all three vessels can be cannulated, and the catheters exteriorized if desired. Placement of the tip of the catheter in the correct location is important, and premeasurements in similar-sized pigs can be made to determine the length and premark the catheter with retention beads. Alternatively, the catheter placement can be checked radiographically, or pressure wave tracings can be used for confirmation. Generally, catheters in the jugular veins are meant for chronic infusion and sample withdrawal. Placement of the tip at the entrance of the precava to the right atrium (approximately at the second intercostal space) is optimal, because the turbulence and velocity of venous blood in this location help prevent thrombosis. Placement into the atrium or ventricle can lead to cardiac arrhythmias, valvular damage, or atrial appendage thrombosis, rupture, or both.

Exteriorization of the catheters is best done on the dorsal surface of the caudal neck between the scapulas. If the pig was positioned in dorsal recumbency for access to the cervical vessels, the

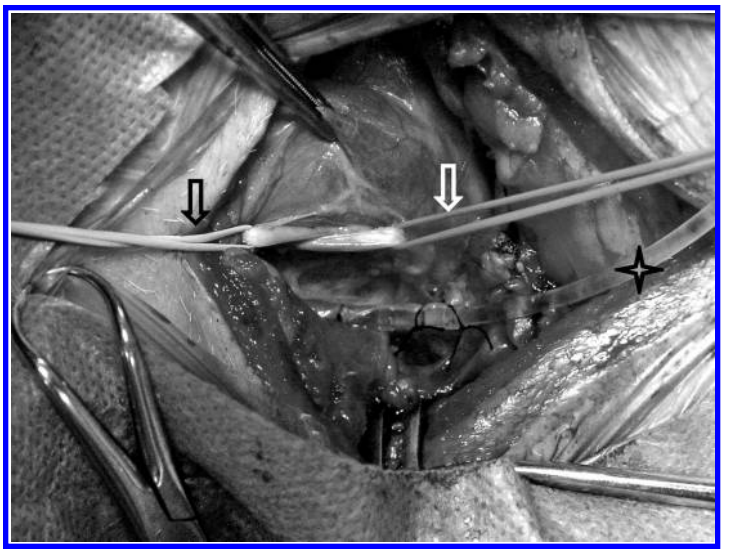

FIGURE 9.42 The external jugular vein has been catheterized (star). The internal jugular vein (black arrow) and the carotid artery (white arrow) have been retracted with elastic vessel loops after medial retraction of the sternothyrohyoideus muscle with dissection over its dorsal surface.

pig will need to be repositioned to lateral recumbency in order to implant the port dorsally (see Chapter 8, Figure 8.8). If a subcutaneously implanted VAP is used, the tunneling rod is passed caudoventral to the axillary space to the lateral aspect of the thoracic wall. The port is implanted subcutaneously in the dorsal region of the chest wall. The port may also be implanted on the dorsum of the neck or the lateral aspect of the scapula. The subcutaneous pocket incision should be made either cranial or caudal to the site of the device implantation. The subcutaneous tissues are carefully dissected, and complete hemostasis must be ensured to prevent hematomas and seromas. The device should be anchored to the muscle fascia, and the dead space closed adequately with subcutaneous and subcuticular sutures. The suture line should not be under tension and should not overlap the device; this prevents the complications of suture line dehiscence and necrosis. Skin sutures are placed as required. The steps of implanting a VAP are illustrated in Figures 9.43 through 9.49.

In order to avoid repositioning the animal on the table, an exception to the conventional technique described above can be made. The swine is initially positioned in lateral recumbency to access both neck veins and port implantation area (see Figure 9.27). This requires the development of extra surgical skill sets by the surgeon/researcher in order to dissect the neck vessels in the lateral position. However, it is possible to perform this procedure without complication and has the advantage of avoiding reposition of the pig intraoperatively.

Since the ports are implanted subcutaneously, the risk of infection is lower than having a catheter exiting through the skin. Catheters of any size can easily be connected to access ports to facilitate serial blood sampling or IV infusion. Advantages of VAPs include: low maintenance; reduced infection rates; decreased animal stress; group housing is enabled; and exteriorized components are unnecessary.

The neck incision is closed in three layers in most swine: muscles, subcutaneous tissues, and skin. In larger swine, dead space in the deep fascial plane of the incision may also have to be closed.

The cephalic vein can be percutaneously catheterized on the cranial aspect of the foreleg as well as the ventral surface of the neck at the thoracic inlet, as described in Chapter 1. The vein can be identified by applying digital pressure at the thoracic inlet and watching it fill with blood

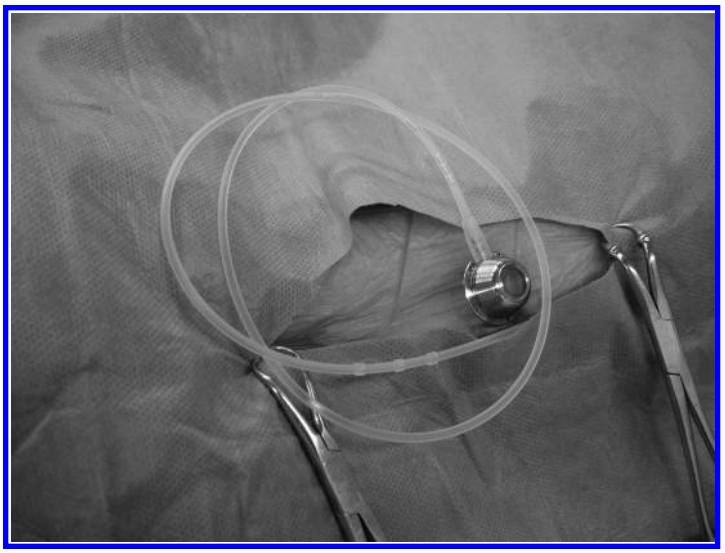

FIGURE 9.43 A vascular access port (VAP) with fixed vascular retention beads is sized to surgically produce an implantation pocket on the dorsolateral surface of the thoracic wall.

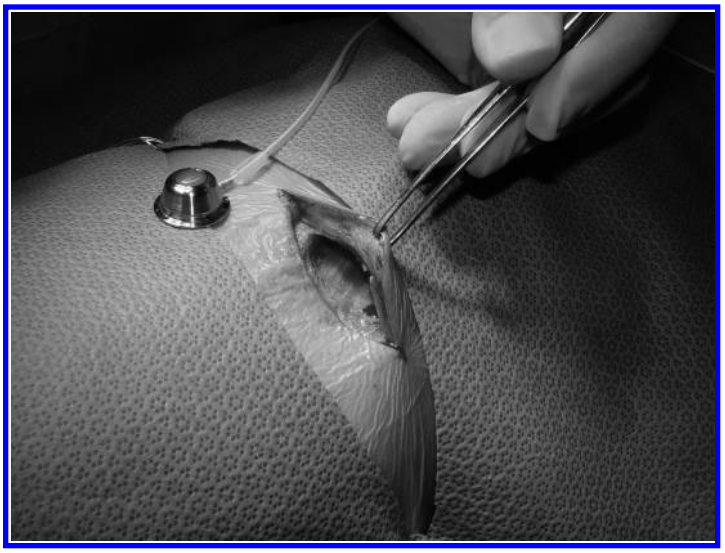

FIGURE 9.44 A curvilinear incision slightly larger than the vascular access port (VAP) has been made into the subcutaneous tissues.

along its course from the leg through the neck. An incision over the vessel is made, and the dissection is continued through the skin, subcutaneous tissue, and thin body of the cutaneous coli muscle. The vein may be cannulated and the catheter tunneled subcutaneously as described for the other neck vessels. For short-term catheterization, exteriorization may be done on the ventrolateral aspect of the neck. However, the pig is likely to traumatize this site unless it is protected with a bandage.

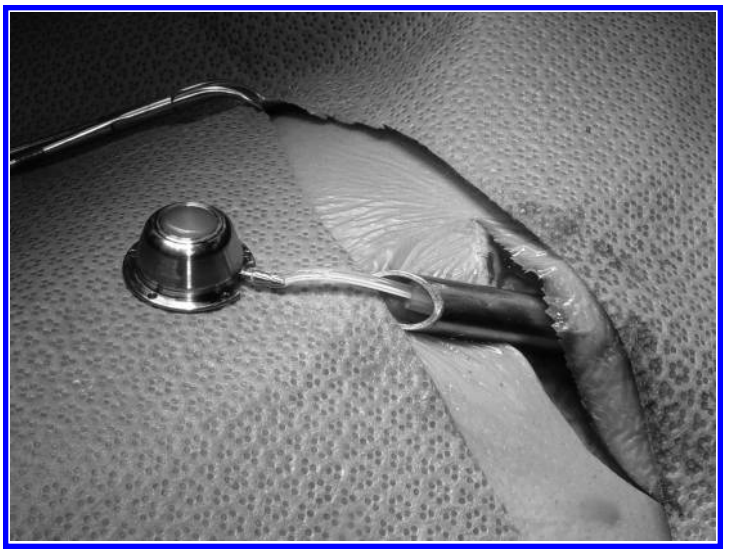

FIGURE 9.45 A trocar has been passed from the neck incision (see Figure 9.41) and the catheter has been passed through it.

FIGURE 9.46 Trocar has been removed, and the neck vessel has been catheterized. The port has been filled with heparinized saline. Sutures are being placed through the suture holes of the port into the deep subcutaneous tissues. These sutures will ensure that the port does not migrate or flip in the pocket.

Leg Vessels

The femoral vessels and the medial saphenous vessel are all approached (Figures 9.50 and 9.51) with the pig in dorsal recumbency and the rear leg retracted caudally. The pulse of the medial saphenous artery may be consistently palpated over the medial aspect of the stifle joint. This pulsation may be followed cranially to the level of the thigh, where the artery courses deeply into the musculature of the medial aspect of the leg. The division of the musculature where the arterial pulse disappears is the fascial division of the sartorius and gracilis muscles. The femoral artery, vein, and

FIGURE 9.47 Vascular access port (VAP) has been anchored in the pocket, and a row of subcutaneous sutures is being placed to close the pocket and eliminate dead space.

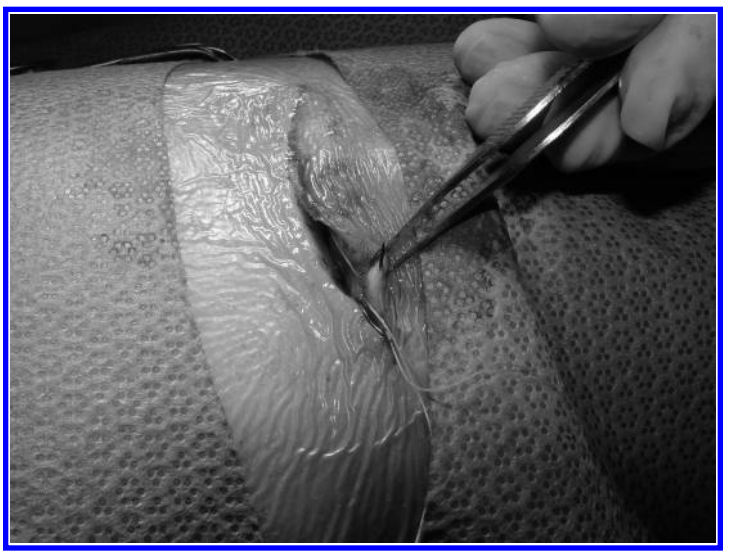

FIGURE 9.48 A subcuticular suture is used to close the port pocket.

nerve are located below the edge of the body of the gracilis muscle, and the pulse in this location is frequently difficult to palpate (Bismuth et al., 2011; Magovern et al., 2005).

The surgical incision for the medial saphenous artery may be made directly adjacent to the arterial pulse along the medial aspect of the stifle joint or tibia. The artery is superficial and may be isolated after dissection of the subcutaneous tissue. The vein is usually a plexus in this region and not useful for cannulation. The artery may be cannulated as a superficial site for measuring arterial pressures and taking samples, or catheters may be passed into the femoral artery from the access site. Most small swine can accommodate 18- to 20-ga catheters in this vessel. As mentioned previously, arterial spasm can occur, so careful manipulation is required.

FIGURE 9.49 Fluid is withdrawn from the port using a syringe and Huber point needle. The catheter is flushed with saline and then filled with heparin.

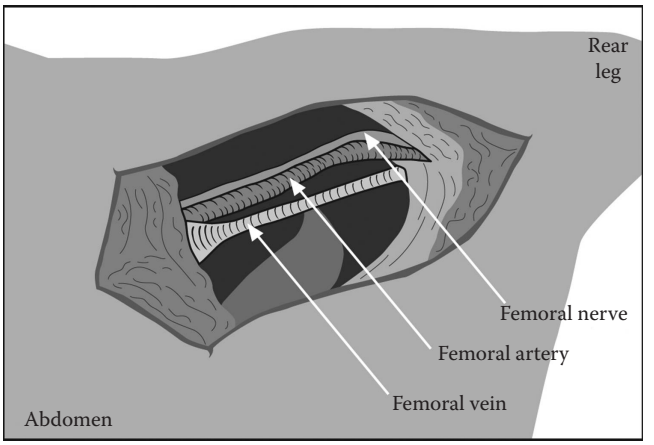

FIGURE 9.50 Surgical cutdown on the femoral nerve, artery, and vein. (Redrawn from Swindle, M.M. 2007. *Swine in the Laboratory: Surgery, Anesthesia, Imaging and Experimental Techniques*, 2nd Ed. Boca Raton, FL: CRC Press, p. 227. With permission.)

The femoral vessels may be approached by making either a longitudinal or a transverse incision over the fascial muscular division described previously on the medial aspect of the rear leg. There are lateral and deep branches of both the artery and vein that should be ligated or cauterized during the isolation of the vessels. Rupture of these branches usually leads to bleeding in the sheath of the vessels and vasoconstriction.

Portal Vein

The portal vein may be catheterized through a ventral midline incision from the xiphoid process to the umbilicus (Maleux et al., 2010; see also Chapter 5). Access to the region is difficult, especially in large swine, and all the principles of abdominal surgery previously described should be followed. The liver needs to be retracted cranially and the stomach and duodenum, caudally. The intestinal mass will have to be excluded from the surgical area by packing with wetted laparotomy sponges.

FIGURE 9.51 Catheterization of the femoral artery.

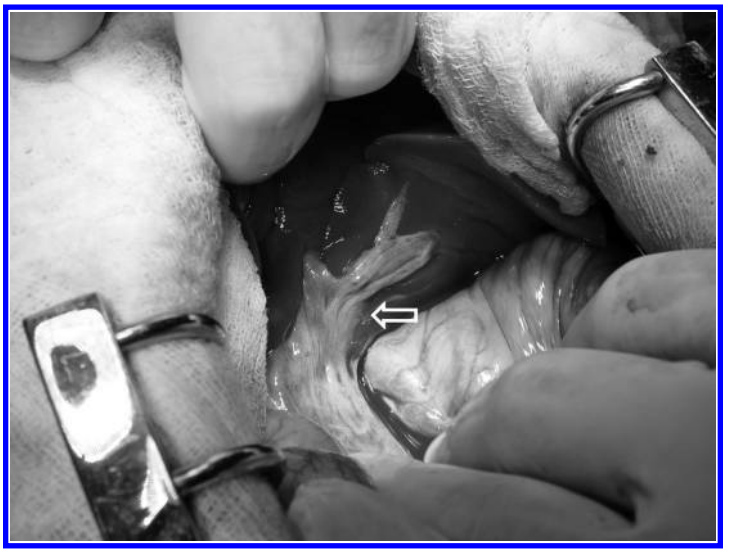

FIGURE 9.52 View of the hilum of the liver in the region of the branching of the portal vein (arrow).

The portal vein can be identified at the hilum of the liver as it passes through the pancreas dorsal to the duodenum (Figure 9.52; see also Chapter 5, Figure 5.7). The common bile duct runs on its ventral surface (Hand et al., 1981).

The portal vein may be carefully dissected around its dorsal surface above and below the area where the cannula is to be inserted. Elastic vessel loops are placed around the vessel to provide occlusion during the venotomy. The portal vein is thin walled, and extensive hemorrhage can occur if it is damaged. A preplaced purse-string suture is placed around the venotomy site. The best location for access is usually in the ventrocaudal portion close to the duodenum.

The portal vein is incised with either a no. 11 scalpel blade or iris scissors after cranial and caudal occlusion of the vessel with the vessel loops. A catheter with a retention bead is fed into the venotomy toward the liver (Figure 9.53). If the catheter actually passes into the liver, it is more likely to occlude. After placing the tip in the desired location, the purse-string suture is tightened so that a

FIGURE 9.53 Direct catheterization of the portal vein through a purse-string suture.

retention bead remains within the vessel. The cranial and then the caudal occlusion loops are loosened. The catheter should either be sutured to the side of the portal vein wall between two retention beads or to some other structure in the region to prevent kinking. The catheter should be checked for patency at this stage. Experience from liver transplantation experiments has shown that more than 15 min of occlusion of the portal vein leads to irreversible portal hypertension. The catheter may be exteriorized through the abdominal wall caudal to the ribs. If a VAP is used, it is placed on the lateral surface of the ribs. Experience from many laboratories does not give a consensus on the best type of catheter or technique to use for catheterization of this vessel. Most would consider 6 weeks of patency to be exceptional. Postoperative problems include thrombosis and erosion of the portal vein because of the low velocity in a thin-walled vessel.

The left branch of the portal vein may sometimes be located within the visceral surface of the left lateral liver lobe by palpation of a groove that corresponds to the entry of the branch into the liver parenchyma (Figure 9.52) (Drougas et al., 1996; Svendsen and Rasmussen, 1998). The vein may be catheterized in a retrograde manner by passing a trocar into the vessel and then inserting the catheter. The tip of the catheter should be passed into the main part of the vein where it can be palpated to determine that it is in the correct location. A catheter inserted in this manner must be sutured in place to the liver capsule, and care should be taken to prevent kinking.

The portal vein can also be cannulated retrogradely from the splenic vein (Figure 9.54) or pancreatic vein (Figure 9.55) (Drougas et al., 1996; Kaiser et al., 2005). These approaches offer the advantage of ease of exposure, although it is more difficult to determine proper placement of the catheter tip with the splenic vein approach. This must be confirmed by either direct visualization or radiographic techniques. It is also possible for the catheter to advance into the wrong vein or kink or puncture the portal vein during placement. More rigid material than silicone may be required to advance the catheter, but this may cause postoperative erosion of the vessel wall. The pancreatic vein approach is relatively atraumatic and close to the hilus of the liver, so that catheter tip placement can be accurately determined (Kaiser et al., 2005).

In this method, the spleen is retracted caudally out of the abdomen, and the splenic vein is dissected free from the splenic artery close to the hilum of the organ (Drougas et al., 1996). This is the middle of the three major veins draining the spleen. The catheter must be passed slowly and guided with a finger on its tip to ensure that it follows the correct pathway into the portal vein. The catheter is fixed in place and exteriorized in the same manner as other abdominal catheters. The distal

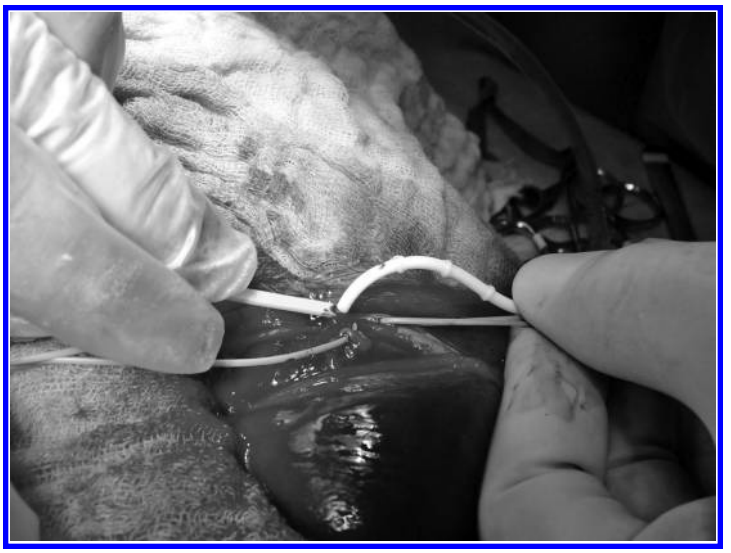

FIGURE 9.54 Catheterization of the portal vein through the splenic vein.

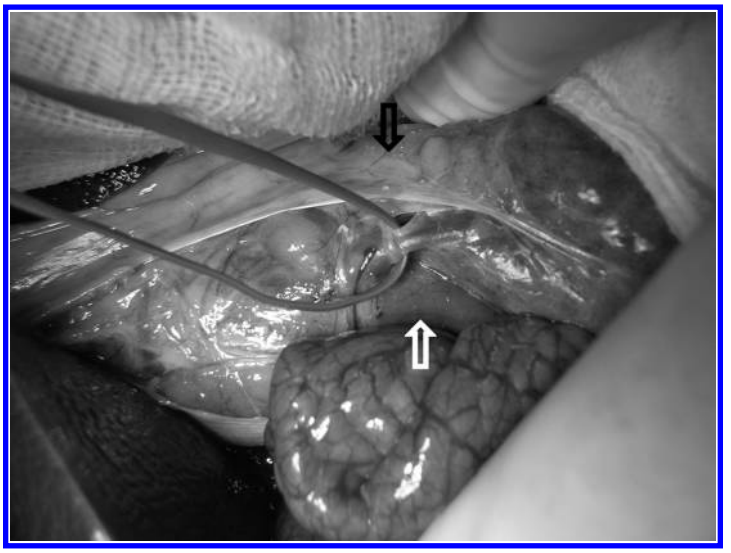

FIGURE 9.55 Isolation of a pancreatic vein entering the portal vein (white arrow). The pancreas is identified by the black arrow.

portion of the pancreatic vein can be isolated near the portal vein, which it enters as a side branch. It is cannulated in the same manner (Kaiser et al., 2005).

Other Blood Vessels

The cephalic vein may be used for chronic cannulation (Chapter 1, Figure 1.29) by locating the vein as it crosses the neck superficially from the point of the shoulder to the thoracic inlet. With the pig in dorsal recumbency, digital pressure is applied in the thoracic inlet, and the dilated vessel becomes apparent. It has the advantage of being more superficial in its location than the jugular veins; however, it has a much smaller diameter.

The external mammary vein is located along the lateral aspect of the mammary glands on the abdomen (Chapter 1, Figure 1.30). This vein may be located by putting digital pressure at either side

of the sternum with the pig in dorsal recumbency. This vein is best utilized for short-term catheterization procedures because of its superficial location in an area easily traumatized by the pig. It is relatively small in immature pigs.

The medial saphenous artery (Figure 9.50; see also Chapter 2, Figure 2.18) is located over the medial aspect of the stifle (knee or femoral or tibial joint). Its pulse can be located superficially in all ages of animals. Namba et al. (2013) have described a percutaneous medial saphenous artery catheterization technique for femoral access in swine using the Seldinger technique. The artery is best utilized for short-term catheterization because of its location. Smaller catheters can be advanced into the femoral artery from this location. The vein is usually a plexus and is unreliable for cannulation.

Internal blood vessels may be cannulated by following the surgical approach for exposure of the organ or structure of interest. For instance, the left hemiazygous vein can be cannulated and the cannula threaded to the coronary sinus to measure venous coronary blood flow by following the applicable surgical instructions for the left total pneumonectomy described previously. Using these principles of catheter placement, reasonable success rates can be expected.

Postoperative Considerations

For survival procedures, meticulous aseptic techniques are obligatory not only intraoperatively, but also postoperatively. Every aspect of the use of chronic catheters must include attention to aseptic techniques. The outside of the catheter and the skin site above the subcutaneous VAP should be prepped with iodine solution, and personnel should wear gloves when injecting or withdrawing samples. Therapeutic levels of antibiotics at the time of surgery for device implantation have been shown to be useful, but chronic administration of antibiotics is not a reliable replacement for aseptic techniques. Infected implants will have to be surgically removed to resolve a chronic infection (Swindle et al., 1998, 2005). Analgesics help control the urge of the animal to rub the incision site and should be administered preemptively. When subcutaneous VAPs are implanted, the use of lidocaine or prilocaine (or both) topical adhesive patches provides topical anesthesia over the injection site for up to 12 h and should be used to prevent needle sensitivity when multiple injections are required over a short period of time.

Vascular Anastomosis

Anastomosis of blood vessels (Figures 9.56 and 9.57) is a routine procedure required for experimental procedures such as organ transplantation or implantation of vascular grafts (Budde et al., 2005;

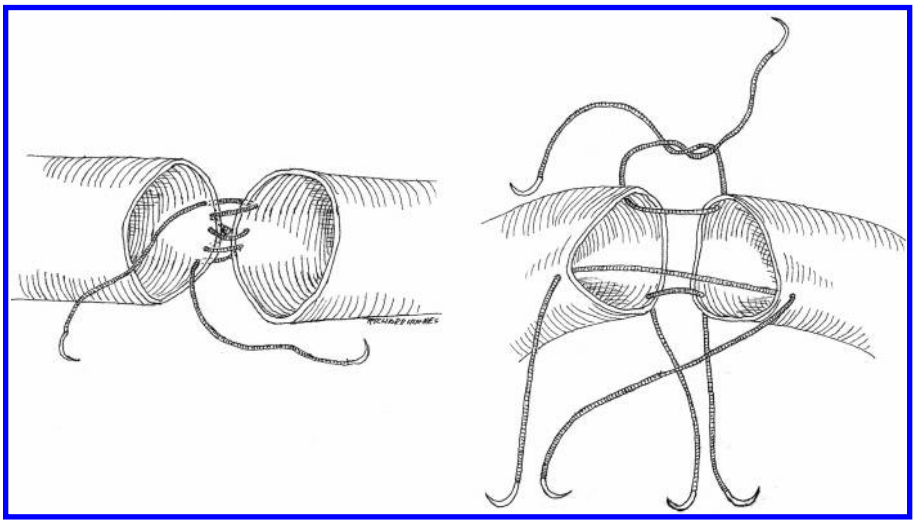

FIGURE 9.56 Anastomosis of the aorta using triangular and continuous suture patterns.

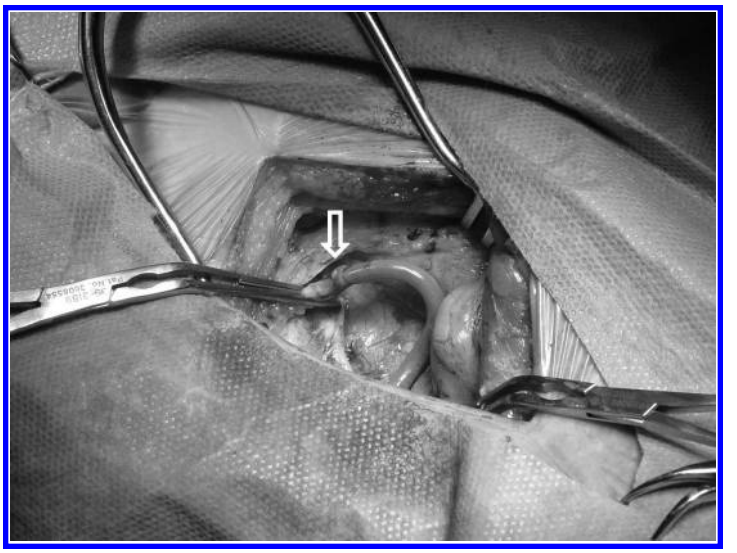

FIGURE 9.57 End-to-end anastomosis (arrow) of a graft to the carotid artery with a continuous pattern.

Jahrome et al., 2011; Lovstakken et al., 2008). It may also be required clinically for trauma. The basic techniques of suturing are the same as for other species. Two characteristics of the porcine vascular system are important considerations. These are the tendency for vasospasm and the pig's rapid clotting time, which necessitate frequent administration of heparin for some procedures. The IV dosage of heparin is 100–300 units/kg as a priming dose with maintenance doses of 100–200 units/kg given approximately every 45 min (Gaymes et al., 1995; Smith et al., 1989; Swindle, 1983). Porcine graft implants have been shown to have a smooth fibrin surface and be relatively resistant to experimental infection; these characteristics may make them useful for some preclinical studies. Endothelialization of implanted biomaterials usually occurs. The pig is a definitive model for development of neointimal hyperplasia at the site of graft anastomosis, which occurs within a few months following surgery (Johnson et al., 2001; Kelly et al., 2002; Mehran et al., 1991; Rashid et al., 2003; Ricci et al., 1991a,b; Rotmans et al., 2003).

The surgical approach to the blood vessels will be dictated by the procedure being performed. For instance, the abdominal aorta can be approached from either a retroperitoneal flank incision or a midline incision. If the procedure being performed is a simple anastomosis or implantation of a short graft segment, then the retroperitoneal approach should be considered because of the better exposure without the intestinal mass having to be retracted. On the other hand, if the aortic anastomosis is to be performed in conjunction with another procedure on the vena cava, then the midline incision may be more appropriate. Other vessels, such as the femoral or neck vessels, may be approached from the standard incisions described under vascular cannulation.

Isolation of the vessel and ligation or clamping of branches in the proximity of the anastomotic site should be performed gently to avoid vasospasm. The blood vessels to be anastomosed are cross-clamped with atraumatic vascular forceps, such as bulldog clamps for small vessels and Satinsky or DeBakey clamps for larger vessels. Cross-clamping resulting in total occlusion of blood flow is problematic in some regions, notably the aorta. When cross-clamping the aorta or other major vessels without substantial collateral circulation, heparin should always be administered previous to the clamping. The total cross-clamp time of the suprarenal descending aorta that is tolerated without ischemic damage to the spinal cord is approximately 15 min. In order to avoid spinal cord ischemia in models, where bypasses are implanting in the descending suprarenal aorta, partial clamping (allows blood flow) of the suprarenal descending aorta can be used to perform anastomosis to protect the spinal cord. Early ischemic preconditioning of 20 min followed by ischemia 80 min later

has been demonstrated to provide some protection to spinal cord damage in a model of thoracic aortic occlusion (Toumpoulis et al., 2004). Likewise, mild hypothermia (32°C) has been shown to protect against up to 50 min of thoracic aortic occlusion (Strauch et al., 2004). Hypothermia with vena cava to left atrium bypass has also shown to be effective in prevention of spinal cord damage (Doty et al., 2002). Infrarenal cross-clamping results in the same syndromes of decreased cardiac index and increased arterial pressure, which occur in humans (Alric et al., 2003).

The number of branches to the psoas musculature that are ligated during isolation of the aorta should be limited to the minimal number required for the procedure. Ligation of substantial numbers of these branches, that is, four to six continuous pairs in most regions, can also result in spinal cord ischemia. Lymphatic vessels in the region of the aorta should be either circumvented or ligated to avoid leakage of chyle. Consequently, it is preferable that vascular surgery is performed using tangential clamps that only partially occlude the aorta. Bypass procedures can also be used as for some types of organ transplantation. Peripheral vessels are less of a problem when cross-clamped, but heparinization should be used if the goal of the procedure is to provide a patent vessel (Qayumi et al., 1993; Swindle, 1983).

Synthetic nonabsorbable 5/0–6/0 cardiovascular suture is indicated for most vascular anastomoses in swine. Larger vessels, such as the aorta, are sutured in a continuous pattern, and smaller vessels are sutured using simple interrupted sutures. For large vessels, the wall of the aorta most distal from the surgeon is the starting point. A double-armed cardiovascular suture is passed from inside the lumen to outside, and a knot is tied at the middle of the suture length to ensure that the knot is outside the lumen. Suture bites of approximately 2–3 mm are necessary to avoid tearing the vessel. The strands are tied one at a time in a continuous pattern to a position opposite the first knot toward the surgeon. The distal clamp may be partially released at this point to allow the vessel to fill and to remove air bubbles. The knot is tied, and the distal clamp is released slowly to check for leaks. This is followed by gradual release of the proximal clamp one notch at a time. If substantial leakage occurs, then the leaks can be repaired with simple interrupted sutures. If leakage is minor, then the anastomotic incision may be packed with gauze for 3–5 min. This is usually sufficient for clotting to occur at the needle puncture sites. Longitudinal incisions in blood vessels, such as the aorta, may also be utilized. They may be closed in a similar fashion or, if extra security is required, a continuous mattress suture with buffering tags at the knots may be used. This suture pattern is oversewn with either a simple continuous or continuous Lembert pattern.

When smaller vessels are repaired with simple interrupted patterns, the basic procedure is the same. The wall of the vessel distal from the surgeon is sutured first, and the sides are sutured alternately until the portion of the wall most proximal to the surgeon is closed last. A triangular pattern may also be used for appropriately sized vessels with the first sutures placed distal to the surgeon as for the other patterns. Vascular picks are helpful in positioning the walls of the smaller vessels for proper alignment during suturing.

Veins may be sutured in the same manner as arteries; however, they are much more friable and easily torn with sutures. Closure of the surgical incision is routine for the area in which the vessel is located.

Femoral arterial ligation, excision, or both have been utilized as a model of arteriogenesis (Hoefer et al., 2006). Collateral arterial growth simulates the same phenomena in humans. With some treatments, rodent and rabbit models are typically hyper-responders compared to the pig. Postoperative anticoagulant and antimicrobial therapy may be required for some procedures, such as the implantation of synthetic grafts. Oral coumarin and aspirin (25 mg/kg once daily) are readily administered to swine in their food (Schomburg et al., 2012). Newer anticoagulants and injectable low-molecular weight heparins (2 mg/kg, subcutaneous) twice daily may also be administered but require therapeutic index monitoring (Greiten et al., 2014; McKellar et al., 2011). Individual sensitivity to these agents, including protracted bleeding, GI bleeding, and anaphylaxis, may be encountered. These supportive agents are discussed in Chapter 2. Antibiotics are given intraoperatively for graft implantation and postoperatively if contamination is suspected (Rogers et al., 1988).

Arteriovenous and Venovenous Fistulas and Vascular Shunts

Arteriovenous fistulas (Figure 9.58) and shunts are usually created in swine to produce a model of volume overload heart failure (Randsbaek et al., 1996; Wittnich et al., 1991) that leads to cardiac dilatation and subsequent eccentric cardiac hypertrophy (Carroll et al., 1995; Gardner and Johnson, 1988). They have been used to create a dilated vascular space for enhanced vascular access as for human dialysis patients (Johnson et al., 2001; Kelly et al., 2002; Rotmans et al., 2003). The technique involves performing a side-to-side anastomosis between an artery and a vein or the suturing of a vascular prosthesis in an end-to-side vascular window technique between an artery and a vein to produce a left-to-right shunt. The more peripheral the location (femoral vessels), the longer the time for production of volume overload. Fistulation between the aorta and pulmonary trunk will have significant immediate effects. This is in contrast to a period of months for clinical effects if peripheral vasculature is used. A fistulation between the carotid artery and the internal jugular vein would be intermediate in time for development of clinical effects. The size of the shunt required differs with the age and breed of the pig and location of the shunt. Generally, the fistulation needs to be 2.5 times the outside diameter of the blood vessels in length for peripheral vessels. If a fistula of this size were used in an aorta-pulmonary shunt of a neonatal pig, it would die acutely. Miniature pigs are less susceptible to the effects of arteriovenous fistulas and shunts, at least in the acute and short-term phases. Because there are relatively few references in the literature to creation of this model in swine, no consensus on the best surgical technique or breed and age of pig has been reached. A model of cyanotic heart disease can be produced by creating a fistula between the pulmonary artery and the left atrial appendage (Zhang et al., 1998). In a 12-kg swine, a fistula of 4×6 mm created by partial occlusion of the pulmonary artery with vascular clamps and suturing to a similar incision in the atrial appendage resulted in the development of cyanotic heart disease over 4–6 weeks. It may be enhanced by placement of a nonrestrictive pulmonary arterial band distal to the fistula.

A portocaval shunt may be created by side-to-side or end-to-side anastomosis of the portal vein and the posthepatic caudal vena cava. This model has been used to study the effects of the portocaval shunt on the metabolism of lipids and lipoproteins. Attempts to produce a model of portal hypertension with gastric ulcers and esophageal varices have been made in swine. This involves creation of an end-to-side anastomosis of the vena cava to the portal vein and gradual constriction of the portal vein with devices such as an ameroid constrictor. Collateral circulation in the region helps lower the portal pressure in swine. Irreversible portal hypertension has also been reported as a complication in hepatic transplantation with prolonged portal vein occlusion, and should be avoided

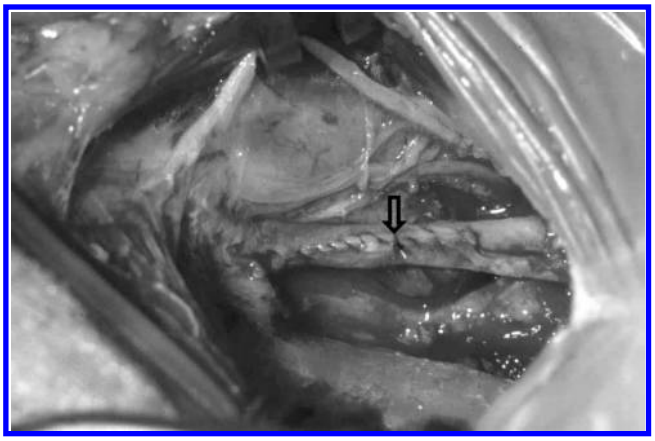

FIGURE 9.58 Femoral arteriovenous fistula (arrow).

during portal vein surgery (Carew et al., 1976; Dupont et al., 1985; Gadacz, 1988; Jensen et al., 1983; Kahn et al., 1994; Nestruck et al., 1977).

An arteriovenous malformation model has been developed in swine by using a transorbital puncture in the cavernous sinus to create an arteriovenous communication between the rostral rete and the cavernous sinus. The model is possible because of the presence of a rete mirabile in the cavernous sinuses of the skull base (Chaloupka et al., 1994).

The surgical approaches to the vessels will be the routine approaches described for isolation of the vessels in the other sections, that is, vascular cannulation for femorals or caudal celiotomy for the iliacs. The techniques for suturing the vessels are described in the section on vascular anastomosis.

Atriotomy and Ventriculotomy

The atria on either side can be approached through the fourth or fifth intercostal space or a median sternotomy using the standard thoracotomy incisions described earlier. Indications would include cannulation procedures and surgical approaches to the foramen ovale, AV valves, and high membranous ventricular septal defects. Cannulation and other procedures should be performed in the atrial appendage, if possible (Figure 9.59). However, this approach would not be useful for the valvular rings. Cardiopulmonary bypass (Chapter 2) would be required for most procedures except simple cannulations. The atria are extremely friable in swine and should be handled gently with appropriate instruments (Ehler et al., 1990; Hall et al., 1986; Horneffer et al., 1986; Smerup et al., 2004; Swindle et al., 1986).

The appropriate portion of the atrium is incised or punctured with a scalpel and the incision extended with scissors. Gentle handling of the tissues using cardiovascular instruments is essential to prevent tearing of the atrial incision. Care should be taken to avoid traumatizing the conduction system, especially in the region of the sinoatrial (SA) node, AV node, and bundle of His in the right atrium.

After the procedure is performed, closure of the incision should be undertaken with great care. Synthetic monofilament, nonabsorbable cardiovascular suture material, 5/0–6/0, is usually indicated. A purse-string suture may be sufficient for the implantation of a catheter into the atrial appendage. However, such simple suturing techniques for the atria will probably be insufficient, especially in immature animals. Continuous suture patterns can be used if care is taken with their placement and tension on the suture line. The use of continuous horizontal mattress suture patterns with buttress pledgets at both ends of the suture line offers more security. This pattern may be oversewn with a simple continuous pattern for control of leaks. Before tying the suture, the usual precautions for closure of cardiac incisions on bypass should be taken. This would include such procedures as the removal of air from the atrium and filling it with blood. If leakage occurs from

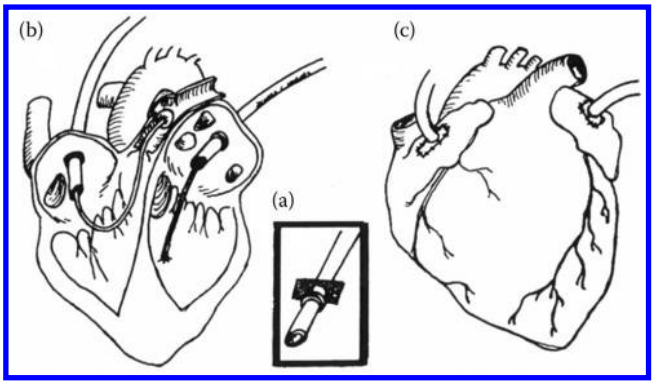

FIGURE 9.59 Atrial cannulation. (a) tip of silastic catheter showing placement of silastic ridge and sheeting; (b) cutaway view of heart showing a balloon-tipped catheter in the main pulmonary artery and a biopsy catheter in the left ventricle; (c) external view of the heart with left and right artial catheters sutured in place. (Reprinted from Smith, A.C. et al. 1989. *J. Invest. Surg.* 2(2): 187–194. With permission.)

the atrium, it may be repaired by either packing it with gauze sponges or the judicious placement of simple interrupted sutures. A small amount of leakage can be tolerated in a heparinized animal and controlled postoperatively with chest tube drainage. The use of protamine to counteract heparin is not recommended as a routine in swine; however, it can be administered slowly with monitoring of blood pressure, if indicated.

Intracardiac surgery should be limited to atrial approaches if at all possible, because of the potential complications with ventricular incisions. The surgeon must take care to avoid compromising the coronary circulation and the conduction system. There is also the potential of developing a surgical scar causing arrhythmias. Consequently, most procedures are limited to small puncture wounds or windows with internal instrumentation except for outflow tract approaches and aneurysm repair. Examples would include cutting tendinous chordae or dilation of valves. When incising the ventricle without cardiopulmonary bypass, a purse-string suture should be preplaced around the entry site. Following the removal of the instrument, the purse-string suture is tightened.

Epicardial implantation of devices is easily performed if the same precautions concerning the blood supply and conduction system are taken. The ventricle is easily catheterized from peripheral vessels with or without the use of fluoroscopy, this being the preferred method of placing internal catheters. Cardiac catheterization is discussed in Chapter 12. Closure of the incision and postoperative care techniques are the routine ones described for thoracotomy.

Valvular Surgery

The AV valves, the pulmonary valve, and the aortic valve are approached for surgical replacement during cardiopulmonary bypass (Wendt et al., 2013). The AV valves are usually approached using the appropriate atriotomy incision after a lateral thoracotomy at the fourth to fifth intercostal space on the appropriate side. A median sternotomy approach can also be used, as in humans, particularly for the right atrium. The mitral valvular approach is problematic regardless of the incision site selected and may require slight rightward rotation for visualization. The pulmonary and aortic valves are approached by longitudinal or transverse incisions over the site of the valve from a median sternotomy or, alternatively, a left lateral thoracotomy may be used for the pulmonary artery (Gallegos et al., 2005; Grehan et al., 2000; Gross et al., 1997; Hasenkam et al., 1988; Hazenkamp et al., 1993; Litzke and Berg, 1977; Lomholt et al., 2002; Nguyen et al., 2004; Shimokawa et al., 1996; Smerup et al., 2004; Smith et al., 1994; Swindle et al., 1986). Bioprosthetic valves of 15- to 23-mm diameter have been percutaneously placed in the aorta in 64- to 76-kg pigs using an expandable stent with porcine valves obtained at slaughterhouses (Lutter et al., 2002).

The selected valve is sutured in place using the manufacturer's instructions (Figure 9.60). Most of the valves used will require sizing of the orifice and preplacement of a specified number of sutures using synthetic nonabsorbable suture material. The sutures are passed through the AV valvular tissue into the valve sleeve and then tied in turn. Experimentally, this procedure is usually performed to test new valves using a mitral valve implantation site. The porcine bioprosthesis valves prepared from glutaraldehyde fixed aortic valves collected at slaughterhouses are the preferred valvular implant if there is a choice. These valves offer the advantages of being of porcine origin and have minimal problems with thrombosis and emboli, thus eliminating the need for anticoagulant therapy. Sizing of the orifice for selecting a valve can be performed using two-dimensional echocardiography or may be performed intraoperatively. As a guideline, 60-kg farm pigs have been determined to require 29-mm mitral valves and have a similar mitral valvular leaflet and tendinous chordae anatomy (Smerup et al., 2004). If mechanical valves of synthetic material are selected, lifetime anticoagulant therapy will be required.

Cutting of the valves to produce valvular regurgitation and creation of volume overload models of heart failure and eccentric ventricular hypertrophy can be performed from peripheral vessels without the use of thoracotomy or cardiopulmonary bypass. Chordal cutting (Figure 9.61) has been proposed as a treatment for ischemic mitral regurgitation, causing distortion of the basal leaflet (Messas et al., 2001). Using fluoroscopic guidance, an instrument, such as urologic grasping forceps,

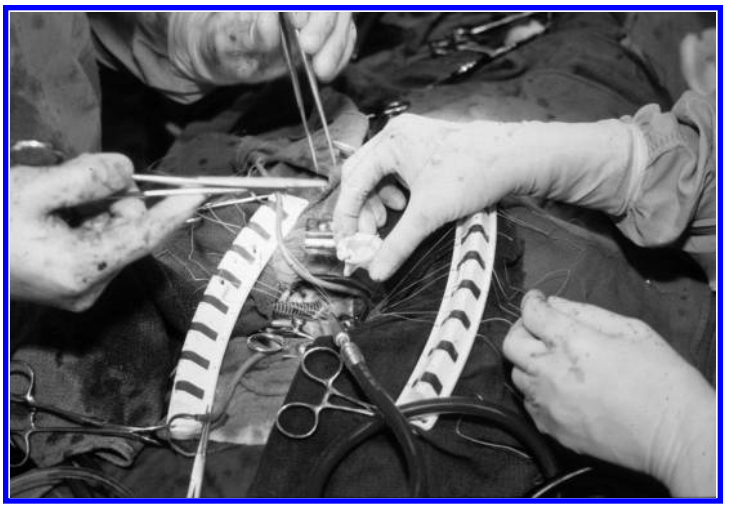

FIGURE 9.60 Replacement of a mitral valve with a porcine bioprosthesis.

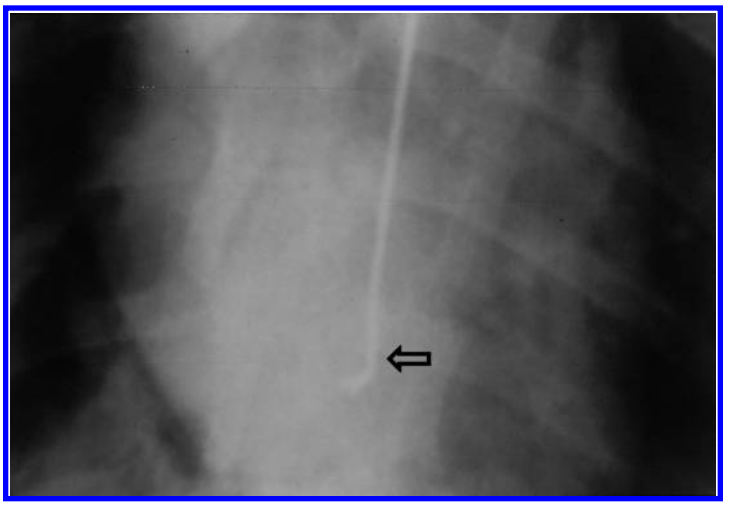

FIGURE 9.61 Urologic grasping forceps (arrow) being used to cut tendinous chordae using fluoroscopic guidance.

is advanced into the ventricle; tendinous chordae are grasped and then torn. Cardiac catheterization or echocardiography can be utilized to judge the degree of regurgitation.

Closures of the atriotomy or blood vessel (or both) and the thoracotomy are routine. Emerging technology provides the opportunity to study minimally invasive intracardiac procedures such as robotics (Chitwood et al., 2001) or three-dimensional echocardiography guidance (Suematsu et al., 2004) in porcine models. Postoperative care should include antimicrobial therapy as well as the usual analgesic and cardiothoracic precautions.

Aortic and Pulmonary Artery Banding, Ligation of the Ductus Arteriosus, and Chronic Instrumentation

The aorta and pulmonary artery are best approached through a left lateral thoracotomy in the third intercostal space. The pulmonary artery can also be approached throughout the fourth intercostal

space in most animals. Both vessels can also be approached through a median sternotomy that does not bisect the manubrium. The ductus arteriosus also can be approached through the same lateral incision.

Indications for these surgical approaches to the aorta and pulmonary artery include cannulation for bypass procedures, implantation of devices or grafts, and constriction banding to produce pressure overload of the ventricles (Carroll et al., 1995; Dougherty, 1981; Gardner and Johnson, 1988; Harvey and Jones, 1982; Kaplan et al., 1995; Rabkin et al., 2004; Swindle et al., 1986). The ductus arteriosus can be ligated through the same approach if it is a patent ductus arteriosus (PDA). Banding of the great vessels produces a model of concentric ventricular hypertrophy as compared to the models of eccentric hypertrophy produced by AV fistulas, as described previously. Banding of the aorta produces more severe postoperative consequences than banding of the pulmonary artery. The left pulmonary artery has been ligated and later reanastomosed to the pulmonary arterial trunk as a model to study pulmonary thromboendartectomy as a treatment for vasculopathy (Fadel et al., 2004). Normal function was shown to return after 5 weeks of reperfusion. Hypertrophic cardiomyopathy occurs spontaneously in both farm and miniature breeds (Lin et al., 2002; Swindle, 1992). The condition is characterized by increases in heart weight:body weight ratio and increased thickness of the left-ventricular free wall and interventricular septum with myocardial fiber disarray, intramural arteriosclerosis, and interstitial fibrosis (Figure 9.62). Attempts to produce a genetic model of this condition have been frustrating because many of the animals die prior to sexual maturity.

The surgical approach is routine, and the pericardium is incised, care being taken to avoid damage to the phrenic nerve. The most difficult part of the surgery is dissection and isolation of the blood vessels, especially in immature animals. Most models require that the dissection be proximal to the ductus arteriosus close to the base of the heart. The tissue is dissected bluntly using either right-angle forceps or Metzenbaum scissors. In older animals, there may be a fat pad present in this location. In younger animals, the thymus gland may also extend to this region. The most common problems during the dissection are tearing of the right atrium, tearing of the ligamentum arteriosus, or tearing of the great vessels. The pulmonary artery is more easily dissected than the aorta from the lateral incision; however, for complex instrumentation, the surgeon may prefer the sternotomy approach.

If the ductus arteriosus is to be ligated, the dissection is similar to that done to isolate the pulmonary artery. The vagus nerve crosses the region and should be retracted dorsally without damaging the structure. Atropine should be administered before this procedure. The ductus can be doubly ligated using nonabsorbable suture material. If a rupture occurs during this process, the aorta or pulmonary artery (or both) may have to be repaired by tangential clamping and suture closure of the tear. The method of reopening a closed ductus and using it as a model of PDA is discussed in Chapter 12.

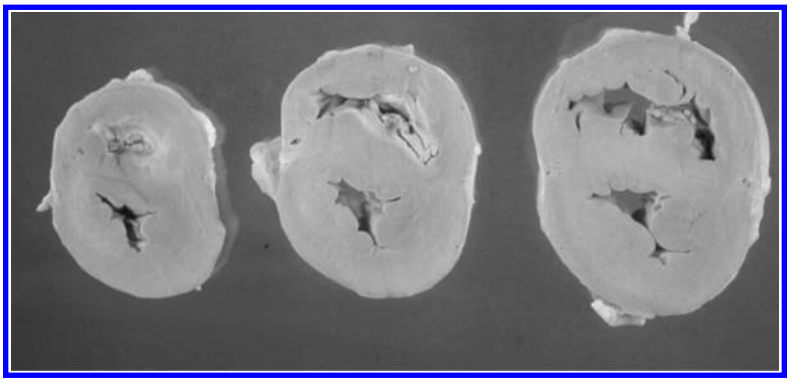

FIGURE 9.62 Hypertrophic cardiomyopathy.

Various methods have been utilized to band these blood vessels to produce a pressure gradient (Figures 9.63 and 9.64). The method and type of banding material will be dictated by the research application. Generally, the bands are placed with a pressure gradient in mature animals, or they are placed loosely around the vessel in immature animals, allowing gradual constriction as the animal grows. When bandings that result in an acute pressure gradient are made, it should be ensured that the constriction produces a gradient of less than 20 torr. This varies with the age, weight, and breed of the animal but may be used as a general guideline. Bradycardia will be readily detectable with a tight banding procedure. Making too tight a constriction acutely can result in heart failure. Procedures using angioplasty balloon implantation between the band and the blood vessel to produce a gradual constriction by periodic inflation of the balloon have been used in the dog and could possibly be applicable in this species (Keech et al., 1997). A telemetry device has been developed (FloWatch®, EndoArt SA, Lausanne, Switzerland), which allows adjustable pulmonary

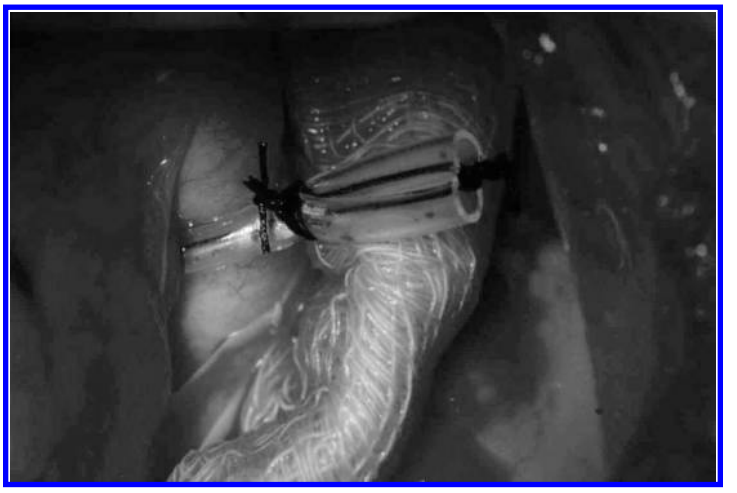

FIGURE 9.63 Pulmonary arterial banding technique.

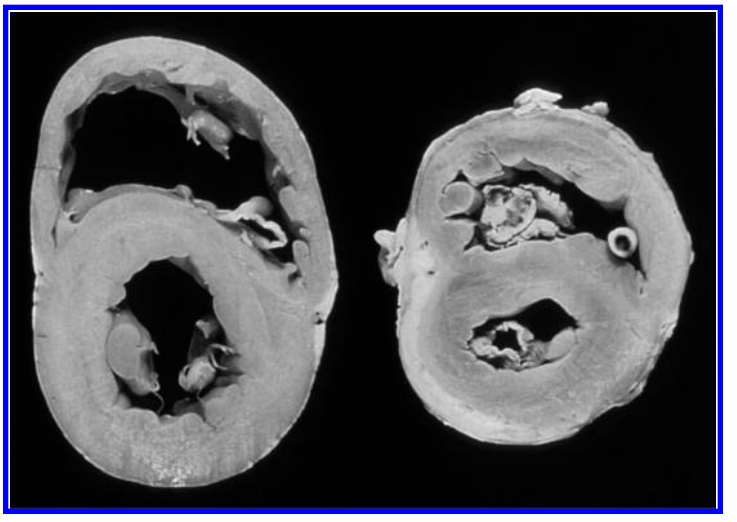

FIGURE 9.64 Cross section of the hearts of a Hanford control (left) and pulmonary arterial banded pig (right) after 4 months.

arterial banding. The pressure gradient can be increased or decreased over time, and the device has proven to be functional for at least 6 months (Corno et al., 2003).

Erosion of the blood vessel and resultant fatal hemorrhage occur to some degree with virtually all banding techniques. Erosion tends to be less than in some species because of the presence of a true vaso vasorum in the aorta. However, the incidence may be minimized by the design of the band. The same principles of constriction banding are true for both the aorta and the pulmonary artery. The band should be wide and without sharp edges. Constricting the vessel with either suture material or umbilical tape tends to have a high incidence of erosions. Polytetrafluoroethylene (PTFE) or teflon bands used clinically have fewer problems. Placing a piece of nonabsorbable suture material inside silicone tubing also has a low incidence of erosion and is the preferred technique. In this technique, the ends of the suture material can be tied after passing it through a piece of tubing that is long enough to encircle the blood vessel with some overlap. The ends of the tubing are then clamped together at a location to produce the appropriate amount of constriction. Enough overlap of the ends of the tubing to suture around it twice is needed to provide security against slippage. With this technique, only curved pieces of silicone tubing are in contact with the blood vessel, and neither the cut ends of the tubing nor the knots in the suture material are in contact with the vessel walls.

A band that can be ruptured with balloon angioplasty techniques to reverse the stenosis can be developed by the use of absorbable suture material within the silicone tubing. Inside a silicone constriction band, 3/0 polydiaxonone will lose its tensile strength within 2 months. The period of time it takes a band to lose enough tensile strength to be ruptured with balloon expansion can be varied with the type and size of absorbable suture material used. For instance, 3/0 polyglactin will lose its tensile strength in approximately one-half of the time as polydiaxonone. If these bands are not ruptured mechanically, they will eventually rupture from the pressure of the blood vessel. These same principles can be used to develop a model of coarctation of the aorta or peripheral vascular constriction in addition to the pulmonic and aortic stenosis models described here.

Closure of the incision is routine for thoracotomies as described previously. In addition to the usual analgesic and antimicrobial administration postoperatively, treatment for congestive heart failure may have to be administered. Generally, in neonates, bands that do not immediately cause constriction (loose banding) will require 2–3 months before significant constriction occurs. Banding in adults with a pressure gradient usually produces significant cardiac hypertrophy in the same period of time. Respiratory distress is usually the first clinical symptom detected. Monitoring with echocardiography should detect changes before the development of clinical symptoms. Furosemide should be the initial agent used in cases of congestive heart failure.

Myocardial Infarction and Associated Arrhythmias

Swine have been extensively used as animal models of myocardial infarction for the reasons discussed in the preceding text. These models have included acute and gradual onset occlusion of all the various coronary arteries and the study of arrhythmias associated with infarction (Bloor et al., 1986, 1992; Gardner and Johnson, 1988; Lee, 1986; Pak et al., 2003; Roberts et al., 1987; Stanley, 2000; Terp et al., 1999; Verdouw and Hartog, 1986; Verdouw et al., 1998; Watanabe et al., 1998; White et al., 1986).

The surgical approach depends upon the vessel being instrumented. The LAD (or anterior interventricular) artery is best approached through a median sternotomy. The left circumflex (LCX) is best seen using a left lateral thoracotomy in the fourth intercostal space after lifting the left auricle. The proximal region of the LAD can also be seen through this approach. The RCA and its posterior interventricular branch are best seen from a right thoracotomy in the fourth intercostal space. The proximal portion of the RCA is also observed using a median sternotomy. All coronary vessels can be observed using a median sternotomy if the heart is gently lifted from the pericardial cradle. However, prolonged lifting of the heart to see its dorsal aspect leads to compromise of the systemic circulation secondary to compression of the major blood vessels.

Occlusive techniques may either be intravascular or extravascular. Extravascular techniques include the use of snares, clips, and suture ligations for acute occlusion (Figures 9.65 through 9.67). Gradual occlusion with chronic models can be performed by encircling the vessel with an ameroid constrictor or inflatable cuffs. Intravascular techniques would include occlusion with angioplasty balloon catheters, injection of embolic substances or coils (Pak et al., 2003), such as 20- to 80-μm

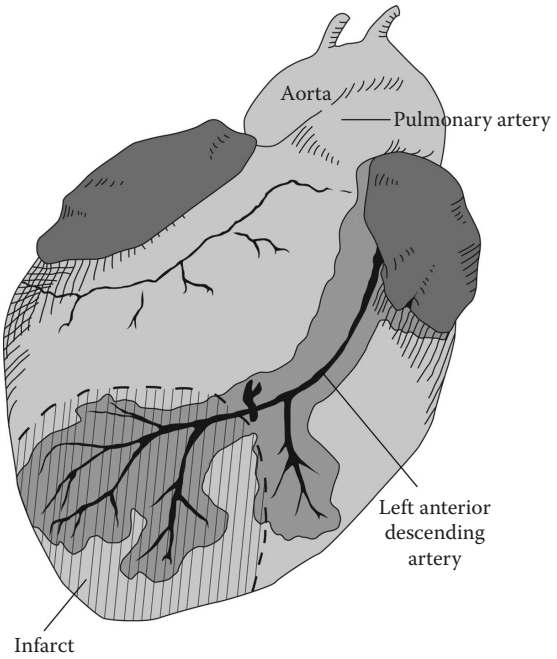

FIGURE 9.65 Schematic of the occlusion of the left anterior descending coronary artery. (Redrawn from Swindle, M.M. 2007. *Swine in the Laboratory: Surgery, Anesthesia, Imaging and Experimental Techniques*, 2nd Ed. Boca Raton, FL: CRC Press, p. 241. With permission.)

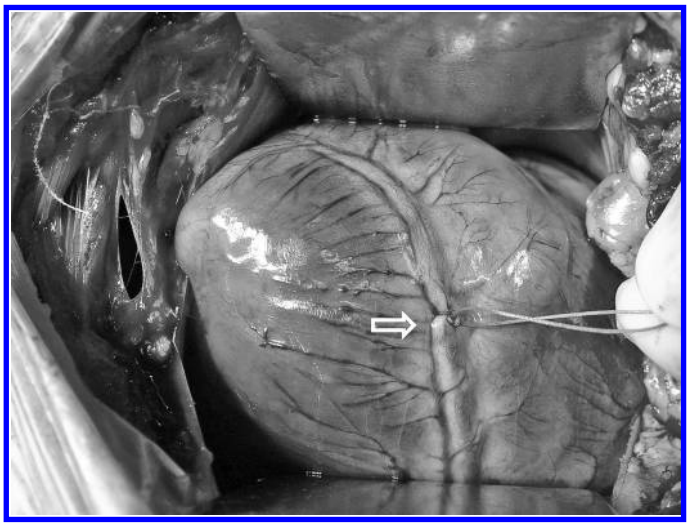

FIGURE 9.66 Ligation (arrow) of the left anterior descending (LAD) coronary artery using a midsternotomy approach.

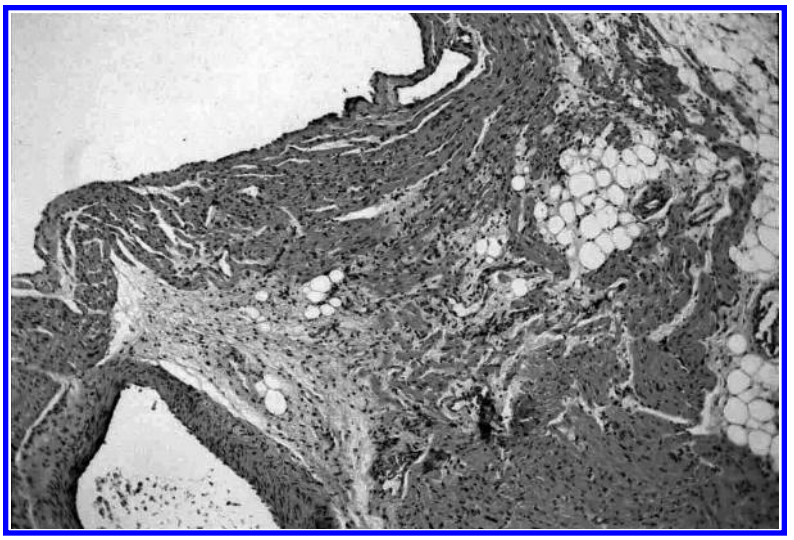

FIGURE 9.67 Histology of an infarct following 30 min of complete occlusion.

microspheres (Borrego et al., 1996; Terp et al., 1999) or sponge-type materials (Reffelmann et al., 2004) to block the vessel, or gradual occlusion by creating an atherosclerotic plaque. Atherogenic occlusion can be created after initiating endothelial damage with an inflated angioplasty balloon in conjunction with feeding an atherosclerotic diet. Global ischemia is produced by administering cardioplegic agents during cardiopulmonary bypass. The midsternal approach may be used for coronary artery bypass procedures between the internal thoracic artery and the LAD or by means of grafts between the aorta and LAD. Additional information on the intravascular procedures may be found in Chapter 12.

Because of the paucity of collateral circulation, swine develop a high incidence of ventricular fibrillation following acute occlusion. This is especially true of the LAD, which has less collateral circulation than other vessels, such as the LCX. The incidence of arrhythmias can be decreased in several ways. Using the gradual occlusion methods in miniature swine allows the collateral circulation to develop as it does in humans.

In acute infarctions, the incidence of fatal arrhythmias can be decreased by limiting the time of occlusion, limiting the region of occlusion, or administering antiarrhythmics (Bloor et al., 1986; Horneffer et al., 1986; Swindle, 1994; Verdouw and Hartog, 1986; White et al., 1986). Generally, the incidence of arrhythmias increases substantially after 15 min and approaches 100% after 30 min. The survival rate for acute occlusion of the LAD can be increased by performing the occlusion distal to the main lateral branch at approximately two-thirds of the length of the artery. Ligation of the posterior vessels of the RCA creates a high incidence of heart block and other damage to the conduction system in contrast to ligation of the LAD. Partial occlusion of blood flow also increases the survival rate. Use of antiarrhythmics, such as bretylium or amioidarone as a slow infusion, substantially decreases the risk of fatal arrhythmias. Pharmacological agents and anesthetic recommendations are discussed in Chapter 2. Miniature swine appear to be more resistant to the development of arrhythmias than farm animals. Arrhythmias in farm animals may vary in incidence among herds, possibly because of heritable factors. However, there is published data indicating that there is no difference in mortality rate between Yucatans and farm pigs in one laboratory (De Leon et al., 2003) (Figure 9.68).

The predominant surgical problem associated with instrumenting the coronary vessels is the development of vasoconstriction. The incidence of vasospasm can be decreased by careful surgical technique and the infusion of lidocaine, IV, following a bolus injection. Care should

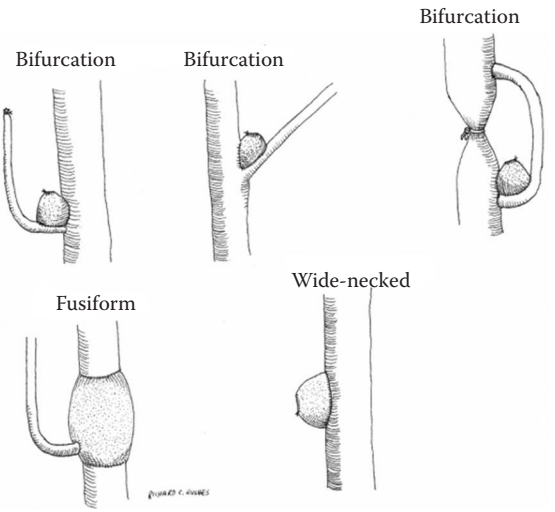

FIGURE 9.68 Examples of surgically created bifurcation, fusiform, and wide-necked aneurysms.

be taken not to damage the atria during manipulation of vessels such as the LCX (Rogers et al., 1988; Swindle et al., 1986).

Following manipulation of the coronary artery, the thoracotomy incision is closed in a routine manner. Animals should be monitored postoperatively with electrocardiograms, as well as the usual procedures recommended for cardiac surgery. However, experience indicates that minimal handling and manipulation of the animals for the first three postsurgical days reduces the mortality, which can occur in the first week, by up to 30%. Keeping the animals in a warm quiet location and using gentle handling and injection techniques are essential.

Vascular Aneurysms

Models of aortic aneurysm have been created in swine by making windows of various sizes in the aorta and suturing in graft materials such as bovine pericardium. These can be variable in their effectiveness in producing aneurysmal sacs. A variety of aneurysms have been created using the neck vessels as a model of intracranial aneurysms. True aneurysms, as they occur in humans, have not been reported in swine (Chaloupka et al., 1994; Dawson et al., 1995; Dion et al., 2003; Guglielmi et al., 1991; Marinov et al., 1997; Massoud et al., 1994, 1995; Maynar et al., 2003; Milner et al., 2004; Turjman et al., 1994; Uflacker and Brothers, 2006).

The classical work of Massoud et al. (1994, 1995) and Turjman et al. (1994) provides a detailed comparison of the various types of aneurysms (Figure 9.62), and their surgical creation in porcine models will be summarized here. Using a combination of procedures on unilateral external jugular veins, carotid arteries, and ascending cervical arteries, three types of bifurcation aneurysms, two types of terminal aneurysms, wide-necked aneurysms, and fusiform aneurysms were created. The surgical approach is the same as that described for access to the cervical vessels. Blood vessels were treated with heparinized saline to prevent coagulation and papaverine to prevent vasospasm.

Bifurcation Aneurysms

One type was created by harvesting a 5-cm segment of ascending cervical artery and a segment of the external jugular vein. One end of the external jugular vein was ligated to form a venous pouch. End-to-side anastomosis of the arterial graft was performed on a segment of the carotid artery. At the caudad anastomosis of the ascending cervical artery, the venous pouch was included in the anastomosis at the V-shaped notch created between the vessels. The cephalad portion of the arterial

graft was looped around the venous pouch and anastomosed to the carotid artery. Blood flow to the carotid artery was restricted between the two arterial anastomoses with an incomplete ligature to increase blood flow through the bypass loop.

A variation of the technique involved ligation of the ascending cervical artery at the caudal end and anastomosis as described previously. This created a bifurcation aneurysm with flow continued in both the carotid and ascending cervical artery. A third type was created by suturing a venous pouch at the bifurcation of two cranial branches of the ascending cervical artery. This resulted in a smaller aneurysm in a smaller blood vessel.

Terminal Aneurysms

As stated, a segment of the external jugular vein is isolated to be used as an aneurysmal sac. An additional 5-cm segment is isolated to be sewn to the carotid artery as a bypass loop. The venous pouch is sewn in the caudad notch of the bypass loop. When distended with blood, the aneurysm compresses the carotid artery, obviating the need for an incomplete ligature.

Another type is formed by the production of a carotid-bijugular fistula with an aneurysmal sac at the T junction. For this procedure, the external jugular vein is harvested for the aneurysm as before. The carotid artery on the same side has a 5-cm segment harvested, and two opposing elliptical arteriotomy sites are incised halfway along the segment. The carotid graft is sutured in an end-to-side manner to the carotid artery stump, and the aneurysmal pouch of the external jugular vein is sutured to the opposite arteriotomy. The ends of the carotid artery graft are then sutured end-to-side to the external jugular vein and the internal jugular vein. This will result in a rapid flow aneurysm with an arteriovenous fistula.

Fusiform Aneurysms

Fusiform aneurysms are produced by end-to-end anastomosis of the isolated external jugular vein segment to the carotid artery. A side branch may be added by anastomosing a segment of the anterior cervical artery in an end-to-side fashion with the external jugular vein segment on one end and ligating the other. The external jugular vein graft will dilate circumferentially, and the side branch will fill with blood as well if it is added.

Wide-Necked Aneurysms

For this model, the external jugular vein segment is anastomosed in an end-to-side fashion to the common carotid artery and the opposite end ligated. The model has had variations performed by suturing the venous pouch obliquely 45° through the carotid artery to give a more acute angle and improve flow to prevent spontaneous thrombosis and minimize the Venturi effect (Dawson et al., 1995; Guglielmi et al., 1991).

Similar types of aneurysms can be produced in the aorta using bovine pericardium, various biomaterials, or venous grafts sutured over oval windows in the distal aorta (Figures 9.69 and 9.70). Infrarenal patches with peritoneum measuring 6–12 cm in length and 2–3 cm in width have been shown to grow and potentially rupture. Generally, shorter patches rupture less than longer patches and most ruptures occur within 3 weeks (Maynar et al., 2003; Uflacker and Brothers, 2006). All the various aneurysms are prone to spontaneous thrombosis but may be used for acute and chronic evaluation of various endovascular devices to occlude them. Use of antiplatelet therapy is useful in maintaining patency as discussed in Chapter 2.

Coarctation of the Aorta

Coarctation of the aorta may be created by surgically decreasing the size of the lumen of the aortic arch or descending aorta (Fossum et al., 2003; Lock et al., 1982; Morrow et al., 1994). Following a left thoracotomy in the fourth intercostal space, vascular clamps are applied to partially occlude the lumen proximally and distally to the ligamentum arteriosus. An elliptical excision of a portion

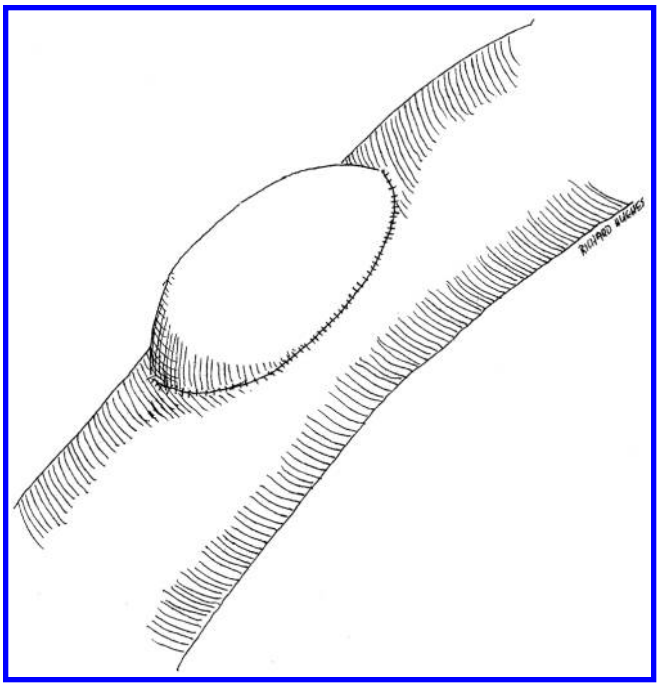

FIGURE 9.69 Schematic of an aortic aneurysm.

of the aorta equal in length to one-half of the circumference of the aorta is performed. Continuous cardiovascular sutures are placed to close the edges of the ellipse (Figure 9.71). After the clamps are removed, a defect that reduces the diameter of the aorta approximately 50% should be formed. Large-sized absorbable sutures, such as 1 surgical gut or polydiaxonone ligatures, are placed around the narrowing of the wedge. The sutures are tied loosely, so that additional constriction of blood flow does not occur. The model may be used for angioplasty or stent placement.

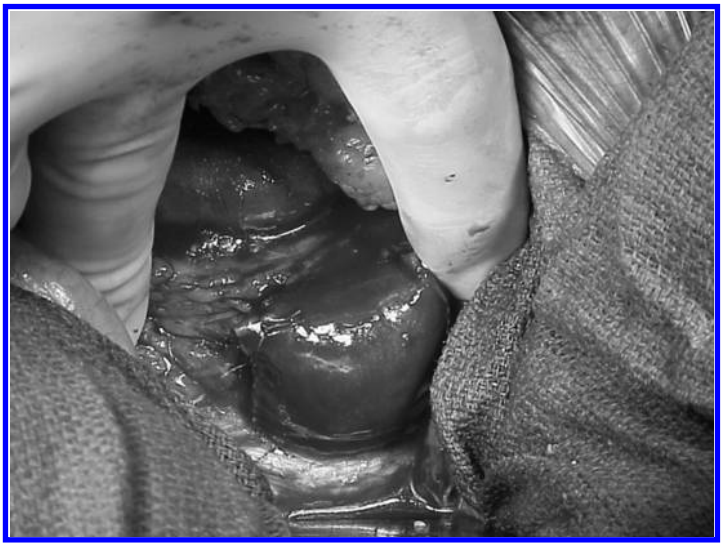

FIGURE 9.70 Surgical creation of an aortic aneurysm. (Courtesy of Renan Uflacker, Department of Radiology, Medical University of South Carolina.)

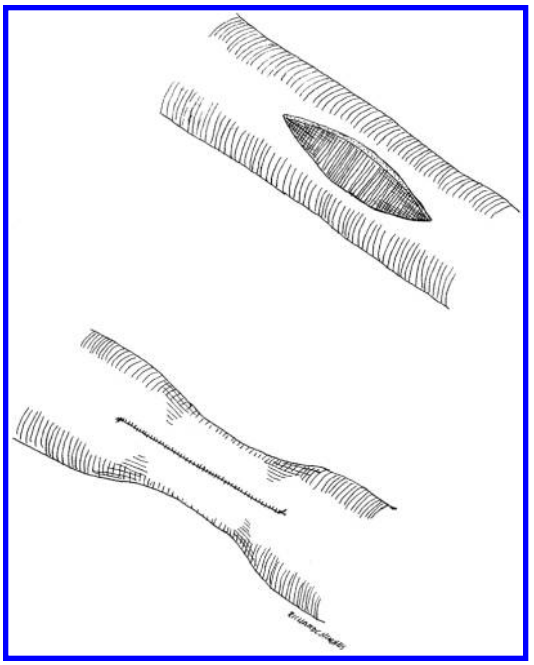

FIGURE 9.71 Coarctation of the aorta.

A technique of implanting an inflatable occluder over a polytetrafluethylene soft tissue patch on the aorta has been developed to provide a model which can be gradually occluded in an animal that is awake. It offers the advantage of being reversible if clinical signs of heart failure occur. It was used to produce renal hypertension but has applications in other studies requiring coarctation (Fossum et al., 2003).

Cardiomyoplasty

Cardiomyoplasty procedures are utilized as interim cardiac assist procedures for heart failure. The procedure involves isolating and wrapping the latissimus dorsi muscle around the heart and stimulating it to contract like cardiac muscle (Figure 9.66) (Borrego et al., 1996; Hansen et al., 1998; Kratz et al., 1994).

In swine, the latissimus dorsi muscle is large and thick and receives its primary blood and nerve supply from the thoracodorsal artery and nerve that enter the muscle close to its insertion at the second rib. The origin of the muscle is broad and extends caudally to the last ribs. Because of the size of the muscle and the thoracic wall in swine, the procedure would be difficult to perform except in smaller animals. A long oblique incision over the fifth intercostal space is made, and the skin is either undermined or an additional transverse incision is made to expose the origin of the muscle, which is transected. The muscle body is isolated to the area of insertion at approximately the 10th–11th rib while preserving the neurovascular pedicle. After isolating the muscle, the third, fourth, and fifth ribs (or such of these as required) are resected to allow space for the muscle to be inserted into the thoracic cavity. The pericardium is incised in a U-shaped opening in such a manner as to allow the muscle to be wrapped around the heart. An epicardial sensor electrode is placed over the ventricle and muscular myostimulators are attached to the insertion of the muscle. The muscle is sutured with mattress sutures in such a way as to form a pocket around the ventricular region of the heart while preserving the blood and nerve supply to

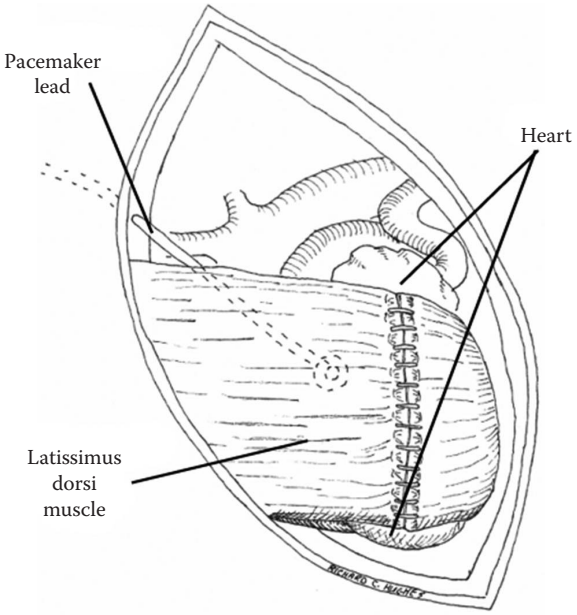

FIGURE 9.72 Cardiomyoplasty procedure.

the muscle (Figure 9.72). It can also be anchored to the retracted pericardium to prevent slippage during the healing phase.

Either before or after performing the procedure, the skeletal muscle must be trained to develop a predominance of type I muscle fibers, which are more resistant to fatigue. This is accomplished by resting the muscle for several weeks and then progressively stimulating it over several weeks to accomplish the task. Performing the procedure in a two-stage progression would involve isolating the muscle first and stimulating it before implanting it into the thorax.

This procedure impairs the mobility of swine and requires combination analgesic therapy with opioids and nonsteroidal anti-inflammatory drugs to control the pain. The thoracic incision is closed in the usual manner.

Epicardial Pacemaker and Device Implantation

Epicardial pacemaker implantation may be performed to test pacemaker devices and leads (Brownlee et al., 1997; Gillette et al., 1991; Hughes and Bowman, 1986; Schumann et al., 1994; Smith et al., 1997; Tong et al., 1995, 1996) or used to create a model of dilated cardiomyopathy and congestive heart failure from rapid epicardial pacing (240 beats per minute) (Caparas et al., 2000; Eble and Spinale, 1995; Hendrick et al., 1990; LeGrice et al., 1995; Spinale, 1995; Spinale et al., 1990; Yarbrough and Spinale, 2003). The model of dilated cardiomyopathy results in congestive heart failure developing over several weeks and requires intensive postoperative care with diuretics for medical management. Endocardial pacemaker lead implantation and electrophysiology are discussed in Chapter 12. Other devices that may be required for implantation on the epicardium include sonomicrometry crystals for measurement of cardiac dimensions and flow transducers on the coronary arteries (Figure 9.73).

The surgical approach to the heart may be either through a left lateral thoracotomy in the fourth to fifth intercostal space or via a substernal or median sternotomy. The approach is dictated by the desired placement of the electrode or device. The left lateral thoracotomy allows access to

FIGURE 9.73 Pacemaker with epicardial lead for a fetal pig.

the left coronary artery and its branches, as well as both the left and right ventricles. The median sternotomy allows access to different regions of the same structures and improved access to the epicardium. A substernal approach through the ventral midline of the abdomen and the ventral attachment of the diaphragm and pericardium allows access to the apex of the heart only. The relatively noninvasive substernal approach allows more rapid recovery without many of the complications associated with a thoracotomy.

Two basic types of pacemaker electrodes with some variations are used. They are generally either screw-in leads or sutured leads (Figure 9.67). The manufacturer's recommendations should be followed. Regardless of which lead is used, the coronary artery and its branches should be avoided during implantation. Because of the characteristics of the coronary circulation discussed earlier, damage of these vessels could result in an infarct. Pacemaker leads are implanted subcutaneously using the same principles of surgery that apply to chronic catheterization. In general, this means that the lead is tunneled through the subcutaneous tissue from the thorax to the subcutaneous site where the pulse generator is to be implanted. Pacemaker pockets can be made in the subcutaneous tissues of the side of the chest or preferably the neck or flank if the lead is long enough. The subcutaneous pockets need to be dissected carefully to avoid having seepage of blood from small vessels, which will consistently lead to hematomas and seromas. The subcutaneous pocket needs to be large enough so that the device to be implanted is easily placed and does not cause tension leading to necrosis and dehiscence. This is especially a problem on the chest wall. The implantation of pacemakers with endocardial pacemaker leads in the jugular furrow of the neck is discussed in Chapter 12. The skin incision should never be directly over or ventral to the implanted device. Implanted devices tend to gravitate ventrally in animals, and the incision is best made either cranial or caudal to the device to be implanted. The pacemaker pocket needs to be closed with subcutaneous and subcuticular sutures, ensuring that the dead space is closed adequately. Skin sutures are placed as required.

Implantation of sonomicrometry crystals and flow transducers follows the principles of surgery discussed previously for ventriculotomy and coronary vessel procedures in myocardial infarction models. The model of epicardial pacing to produce congestive heart failure requires intensive postoperative observation and care, especially for pulmonary edema. The use of furosemide as a diuretic is usually indicated. If high pacemaker rates (240 beats per minute [bpm]) are used, the pacing rate may have to be reduced or the pacemaker turned off in 14–21 days (Caparas et al., 2000; Hendrick et al., 1990; LeGrice et al., 1995; Spinale, 1995; Spinale et al., 1990).

Heart and Heart–Lung Transplantation

The pig has been utilized for all of the various forms of heart (Calne et al., 1976, 1978; Hall et al., 1986; Martin et al., 1999; Qayumi et al., 1991; Saito and Waters, 1994; Swindle et al., 1986; White and Lunney, 1979), heart–lung (Baumgartner et al., 1988; Hansen et al., 1987; Harjula and Baldwin, 1987; Qayumi et al., 1990, 1993; Saito and Waters, 1994), and lung (see preceding text) transplantation. However, swine are susceptible to apnea following total cardiopulmonary denervation and are usually reserved for acute and chronic heart and single-lung transplantation and acute heart-double lung transplantation. The growth of the pig makes it useful for the growing heart model as described previously (Brutel de la Riviere et al., 1983; Haworth and Hislop, 1981; Pae et al., 1981). A growing interest in the porcine model has developed because of the progress with xenotransplantation. It is likely that the pig will be the donor animal of choice for this procedure for multiple organs including the heart (Swindle, 1996).

The donor and recipient are operated on using a complete median sternotomy with a pericardial cradle for all these procedures. Bretylium or amiodarone are useful as a preventative for cardiac arrhythmias in both the donor and the recipient (see Chapter 2).

Heart Transplant

Donor

The donor is prepared by dissection of the cranial and caudal vena cava. The pericardium between the aorta and pulmonary artery is dissected completely. The donor is heparinized (300 IU/kg), and cardiectomy is initiated. The cranial vena cava is ligated and divided caudal to the azygous vein. The caudal vena cava is ligated, and the aorta is cross-clamped. The animal is exsanguinated, and the blood may be used for priming the bypass pump. Chilled cardioplegia solution (4°C) is infused into the aortic root, and the caudal vena cava is opened to allow drainage of the cardioplegia solution. The aorta and pulmonary arteries are transected distal to their first branches. The posterior wall of the left atrium is opened between the pulmonary veins and immersed in chilled preservation solution awaiting implantation.

Recipient

The recipient is prepared in a similar manner except that the animal is placed on cardiopulmonary bypass. Venous bypass cannulae are passed through the right atrial appendage and snared in the cranial and caudal vena cavae. The arterial line is placed in the aorta or femoral artery. Bypass parameters may vary among experiments; however, cooling to 28–30°C and flow rates of 50–80 mL/kg have been recommended.

The recipient cardiectomy is performed following cross-clamping of the aorta with an incision that follows the AV groove. The incision should preserve the coronary sinus. The pulmonary artery and aorta are transected at the level of the commissures of the valves.

Implantation of the donor heart is performed with a series of continuous sutures with 5/0–6/0 nonabsorbable cardiovascular suture. The atria are anastomosed first. Starting the anastomosis with either the left or right atrium has been described (Baumgartner et al., 1988; Saito and Waters, 1994). The heart must be rotated caudoventrally if the right atrium is anastomosed first, in order to accomplish anastomosis of the left atrium. The pulmonary artery and aorta are anastomosed next. Air is removed from the aorta before removing the cross-clamp.

The cardiac rhythm is restored with internal defibrillation if it does not return spontaneously during rewarming and weaning from bypass. Isoproterenol infusion to effect is used postoperatively to provide a regular heart rate and blood pressure as the catecholamine-induced heart rate recovers. Protamine may be used judiciously at a slow infusion rate of 1 mg/100 U heparin if clotting is a problem. A double chest tube with a Y connection should be used postoperatively (Figure 9.74).

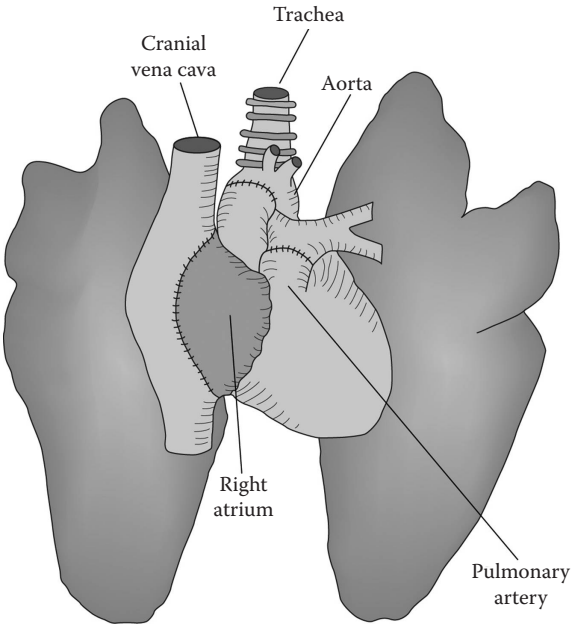

FIGURE 9.74 Heart and lung transplantation. (Redrawn from Swindle, M.M. 2007. *Swine in the Laboratory: Surgery, Anesthesia, Imaging and Experimental Techniques*, 2nd Ed. Boca Raton, FL: CRC Press, p. 250. With permission.)

Heart–Lung Transplant

The donor and recipient are prepared in a similar manner as for heart transplantation, except that the trachea is divided cranial to the bifurcation and the recipient is left with the right atrium with a portion of the interatrial septum. The phrenic nerves are preserved on pedicles during the dissection. The pulmonary artery and veins remain intact with the transplantation block. With this technique, the anastomosis of the right atrium, trachea, and aorta are required for implantation into the recipient. The right lung is passed dorsally to the vena cava and phrenic nerve (Figure 9.68). The donor vena cava is ligated.

Single-lung transplant (described earlier) provides an experimental model for evaluation of pulmonary transplant that is better than isolated double-lung transplant because of the physiological problems associated with respirator apnea postoperatively in the pig. Consequently, double-lung transplant is not described.

MISCELLANEOUS THORACIC PROCEDURES

Thoracic Duct Cannulation

Cannulation of the thoracic duct is performed to collect chyle for experimental studies (Figure 9.75 and Chapter 6, Figures 6.12 through 6.16). The most frequent complication associated with collection of chyle is failure of the cannulation due to clotting shortly after the procedure. The reentry cannulation method described here may provide a longer collection period (Mendenhall et al., 1996). Lymphatic ultrasonography has been performed to study the lymphatic system in the Sinclair malignant melanoma model and compared to the use of vital blue dyes and radiopharmaceuticals (Goldberg et al., 2004) (Figure 9.75).

The thoracic duct courses dorsal to the aorta and ventral to the hemiazygous vein from the base of the heart caudally to the cisterna-chyli, which it drains. The duct is dorsolateral to the

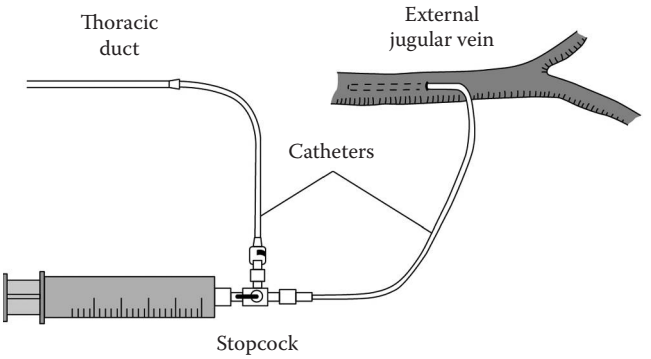

FIGURE 9.75 Cannulation of the thoracic duct with reentry into the external jugular vein. (Redrawn from Swindle, M.M. 2007. *Swine in the Laboratory: Surgery, Anesthesia, Imaging and Experimental Techniques*, 2nd Ed. Boca Raton, FL: CRC Press, p. 251. With permission.)

aorta in the left side of the thorax until approximately the fifth rib; then it is more accessible from the right thorax. Cannulation is usually performed in the right seventh to eighth intercostal space. Variations in ductal anatomy may occur, such as branching ducts, which make the procedure more difficult.

Cannulation of the duct is performed in the same manner as cannulation of blood vessels described earlier. However, the duct is easily damaged and difficult to locate in fat animals and animals that have been fasted for a prolonged period of time. Feeding of liquids containing a high proportion of fatty substances before anesthesia may help identify the duct.

The catheter needs to be small bore and composed of silicone or polyethylene. Coating with silicone or the use of heparin-impregnated catheters may be helpful to prevent catheter failure due to clotting. After cannulation, the catheter is passed through an intercostal space cranial to the cannulation site. It is tunneled subcutaneously in a cranial direction along the dorsum of the rib line. It is exteriorized several intercostal spaces cranial to the exit from the thorax. Chyle may be collected in this location, or the cannula can be placed into a reentry circuit to drain into the right external jugular vein. If it is drained back into the jugular vein, then a three-way valve is placed into the system at the exit site. The cannula is then passed subcutaneously to an incision into the right jugular furrow for cannulation into the jugular vein as described previously for vascular cannulation. The incisions are closed in a routine manner. The cannula tract is kept open until collection of chyle is desired, and then the three-way valve is closed on the cranial side. Meticulous aseptic handling of the exteriorized catheter is indicated. In a closed system, the catheter should be heparinized if continuous flow into a collection site is not provided. Postoperative care is routine.

Esophageal Surgery

Esophageal surgery is usually performed in the thorax or the neck. The techniques are described in this section, because, experimentally, it is likely that the thoracic approach will be the one most likely used (see Chapter 4).

The esophagus courses from the pharynx dorsal to the larynx. It begins to course to the left dorsolateral aspect of the trachea at approximately the fourth cervical vertebra. In the thorax, it passes dorsal to the tracheal bifurcation and to the right of the aortic arch. It enters the esophageal hiatus of the diaphragm and joins the stomach shortly after entering the abdominal cavity.

The esophagus can be surgically approached in the neck through a ventral midline incision with lateral retraction of the trachea. In the cranial thorax, it is best approached using a median sternotomy. It may also be approached using a lateral thoracotomy in the cranial thorax; however, access

and dissection are more difficult. In the caudal abdomen, it is approached through either a right or left lateral thoracotomy from the fourth to the ninth intercostal spaces. The esophageal sphincter can be approached using a midline celiotomy incision with caudal retraction of the stomach and cranial retraction of the liver. At any location, care should be taken to avoid injury to the recurrent laryngeal nerves and vagal trunks, which are located in proximity to the esophagus. Striated and smooth muscles are included in the tunica muscularis of swine.

The esophagus may be fistulated in the neck, have a pharyngostomy tube placed into the cervical region, have an esophagotomy, or be anastomosed with or without a graft. The main problem associated with surgery of the esophagus is the retarded healing process because of the constant tension and peristalsis of the structure and the lack of a strong serosal layer to maintain sutures. The esophagus is usually closed in two layers. The inner layer is placed in the tunical mucosa and submucosa with continuous sutures using synthetic absorbable sutures. The outer muscular layers and adventitial layers are closed with simple interrupted or horizontal mattress sutures. For some chronic procedures, such as graft implantation, synthetic nonabsorbable sutures should be utilized. The surgical incisions are closed in the routine manner described for the region.

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10 Head and Neck Surgery/ Central Nervous System

M. Michael Swindle

CONTENTS

GENERAL PRINCIPLES OF SURGERY AND SURGICAL ANATOMY

The use of swine in dental and neurologic research has been relatively uncommon, probably because of the anatomy of the head, neck, and oral cavity (Figures 10.1 through 10.10). The skull in Figures 10.3 through 10.10 is from a sexually mature boar. Its use in oral and maxillofacial surgery has recently increased (Bermejo et al., 1993; Bredbenner and Haug, 2000; Cheung et al., 2007; Curtis et al., 2001; Donovan et al., 1993; Drisko et al., 1996; Navarro et al., 2007; North, 1988; Oltramari et al., 2007; Ouhayoun et al., 1992; Ruehe et al., 2009; Sims et al., 1997; Wang et al., 2007). Detailed surgical anatomy of the porcine facial structures has been published (Sasaki et al., 2010). Colored histologic sections are contained on the textbook DVD.

The dental formula for deciduous teeth in swine is 2(I3/3, C1/1, P4/4) = 32. Permanent teeth have a dental formula of 2(I3/3, C1/1, P4/4, M3/3) = 44. Swine are born with the last incisors and canine teeth. There are slight differences between breeds and between miniature pigs and domestic swine. Some literatures refer to the deciduous molars as deciduous premolars and there can be a late eruption of M_1 which can also be absent in miniature pigs giving a deciduous formula of 2(I3/3, C1/1, M4/4) = 32. Since the nomenclature of the molars and premolars varies in the literature, the formula can be confusing. The remainder of the incisors erupts between 2 weeks and 2 months of age. The premolars erupt between 4 days and 5 months. The molars are the first permanent teeth to erupt and appear between 4 and 20 months of age. The incisors change between 8 and 20 months, the canines between 9 and 10 months, and the premolars between 12 and 15 months. The tooth eruption sequence of adult permanent teeth is P1/M1/I3/C/M2/I1/P3/P4/P2/I2/M3. In the boar, the canine teeth become the tusks and require trimming two to four times a year in the adult. Growth of the

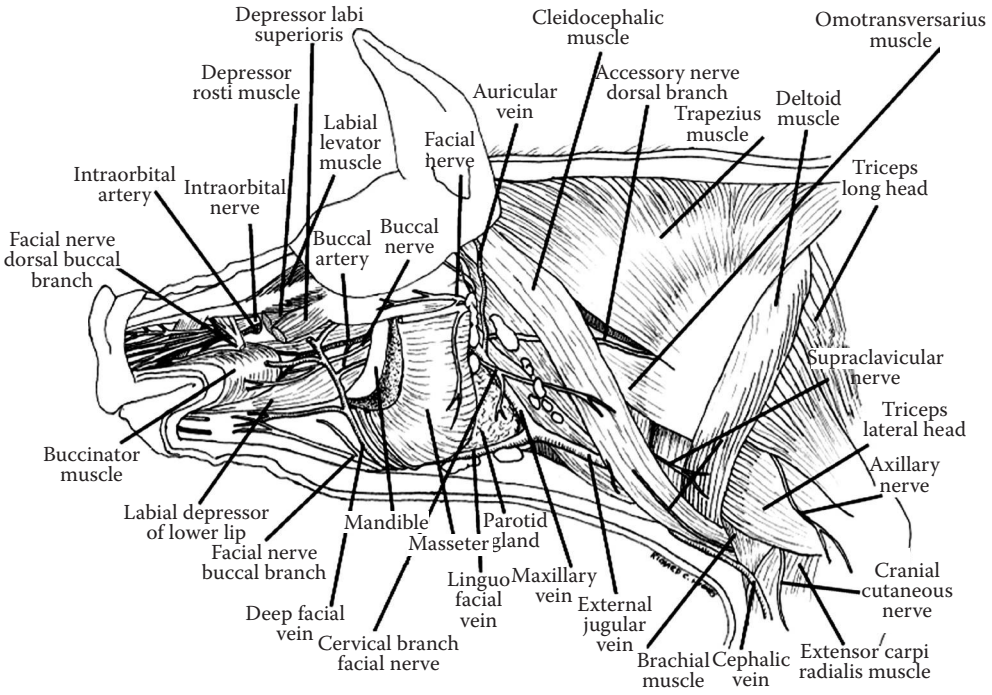

FIGURE 10.1 Superficial dissection of the head and neck.

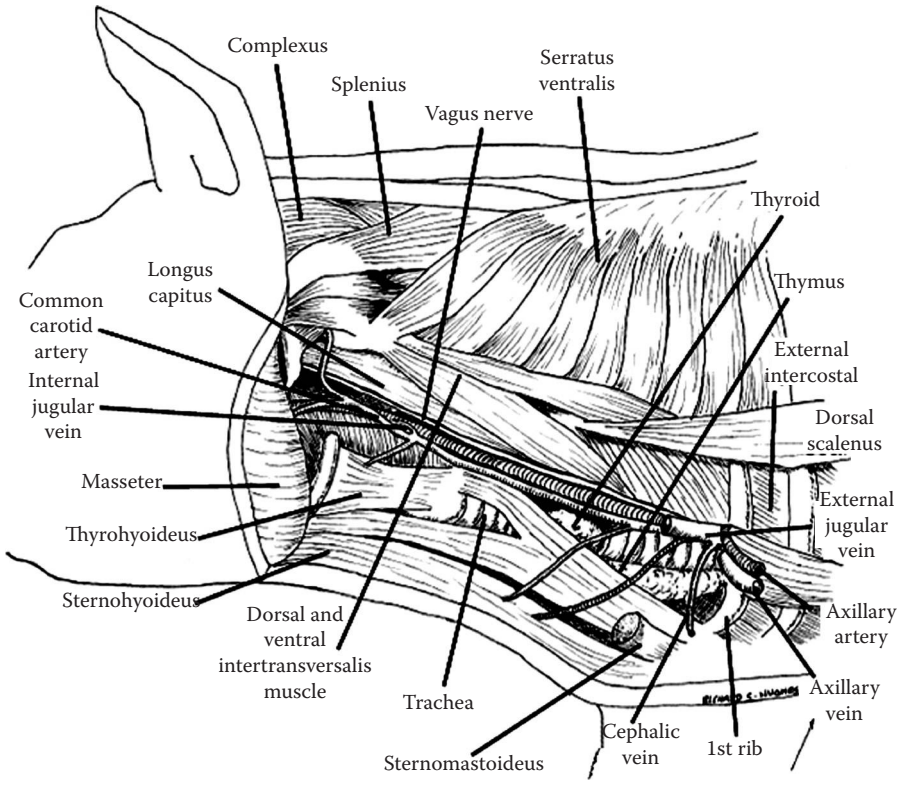

FIGURE 10.2 Deep dissection of the head and neck.

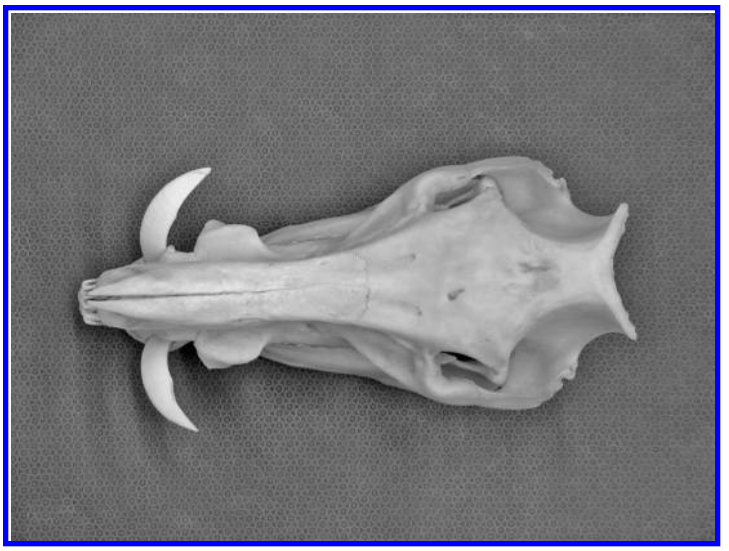

FIGURE 10.3 Dorsal view of the skull. (Courtesy of Mark Rodenberg and Joe Roberts, Elite Barber Shop.)

tusks is delayed in females and castrated males. The newborn's canines are called needle teeth and are usually trimmed shortly after birth to prevent damage to the sow during nursing. The dental formulas are identical among farm and miniature breeds, and eruption dates are similar. A full set of permanent teeth is usually present around 18 months of age. The size of the teeth is similar to human, and the tooth eruption and exfoliation of the primary teeth can be followed in a normal time sequence (Gier, 1986; Hargreaves and Mitchell, 1969; Jump and Weaver, 1965; Oltramari et al., 2007; Sack, 1982; Schantz et al., 1996; Sisson and St. Clair, 1975a,b; Wang et al., 2007; Weaver et al., 1962). Breeders of miniature swine will generally be able to provide more exacting information on the dentition and dental eruption for their particular breed.

There is a substantial difference in the conformation of the head and neck among different breeds; the breeds are illustrated in Chapter 1. The snout of the Yucatan, Göttingen, Sinclair, and most other

FIGURE 10.4 Left lateral view of the skull.

FIGURE 10.5 Right lateral view of the skull.

miniature pigs is considerably shorter than that of domestic farm breeds and the Hanford miniature pig. The heads of miniature breeds tend to be more rounded than that of the farm breeds and the Hanford; the latter has a head and snout shape similar to wild pigs. Selection of a breed for oral and maxillofacial surgery should include a consideration of the differences in head and neck conformation. In the author's experience, the oral cavity of a sexually mature Yucatan miniature pig is satisfactory for testing of intraoral devices. When performing surgery in the oral cavity, the author has utilized several preoperative techniques to prevent infection of implanted devices. Amoxicillin is administered the day prior to surgery and on the surgery day. After endotracheal intubation and inflation of the cuff, the oral cavity is rinsed with dilute betadyne solution. After 5 min, the oral cavity is then flushed with sterile saline. If there is evidence of calculus and/or periodontal disease, the teeth are cleaned prior to using the betadyne flush. The oral flora of LEWE minipigs was studied

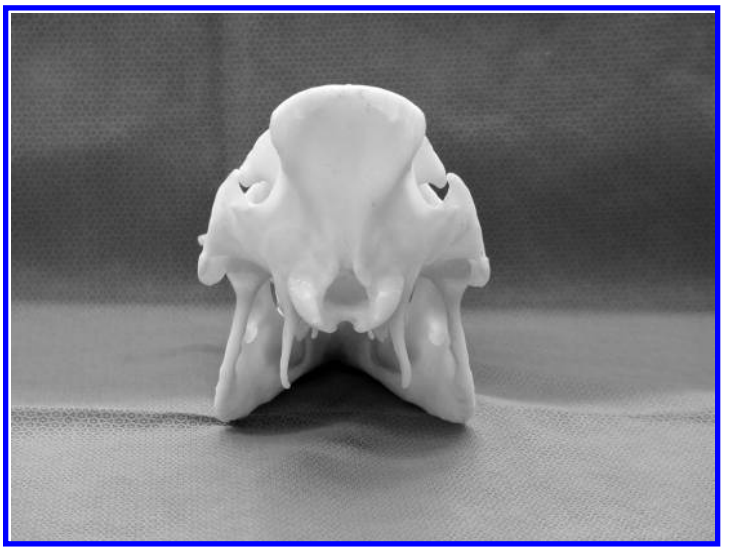

FIGURE 10.6 Caudal view of the skull.

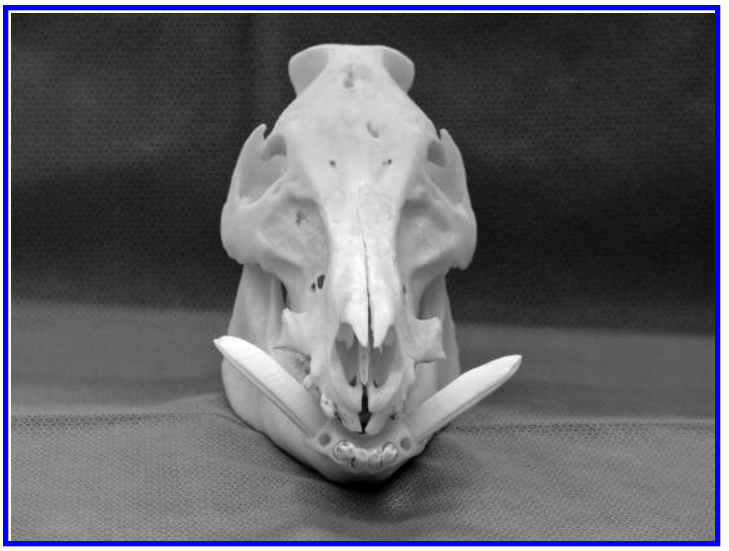

FIGURE 10.7 Frontal view of the skull.

and the predominate organisms were staph, strep, *Fusobacterium*, *Bacteroides*, and *Prevotella* out of 61 organisms that were identified (Becker et al., 2011). There was significant individual variation and it is likely that there will be differences between herds because of differences in feed and environmental conditions.

The bones of the cranium and the mandible are massive. The temporomandibular joint has been compared to those of other species and humans in a detailed anatomic study (Bermejo et al., 1993). The authors concluded that the pig was an appropriate animal model to study temporomandibular joint abnormalities because it was most similar to humans. The pig has a reciprocally fitting meniscotemporal joint and a condylomeniscal joint of the condylar type. The size of the articular structures, the shape of the meniscus, and the omnivorous chewing characteristics of swine provided additional justification for the use of this model over that of rodents, rabbits, carnivores, and

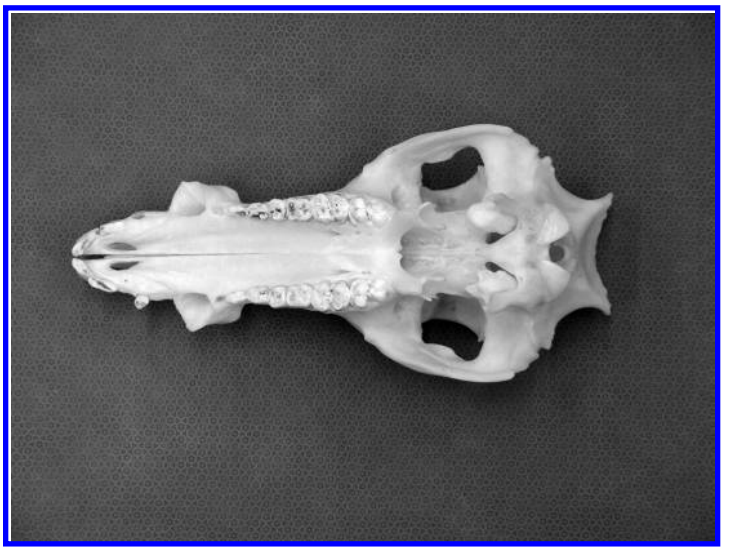

FIGURE 10.8 Ventral view of the maxilla and skull with the mandible removed.

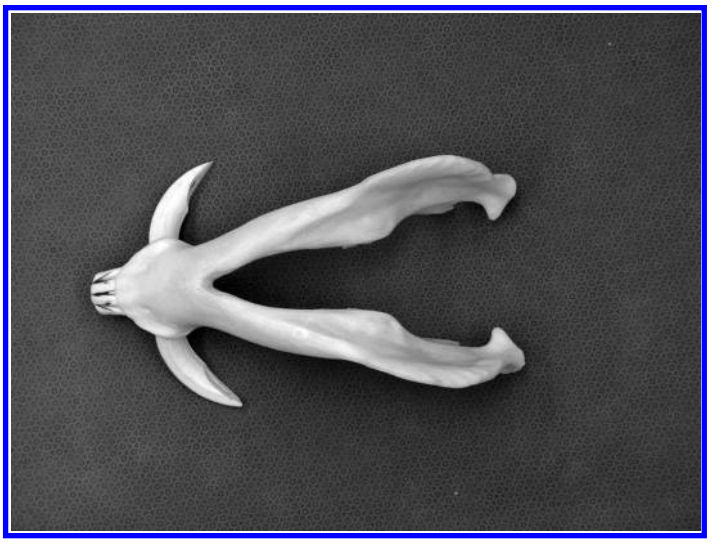

FIGURE 10.9 Ventral view of the mandible.

herbivores that were examined. Maxillofacial bone-healing studies have been performed in swine (Bredbenner and Haug, 2000; Cheung et al., 2007; Mardas et al., 2014; North, 1988; Ruehe et al., 2009; Wang et al., 2007). Among these authors, the critical sized defect has been reported to be 1.9–10.1 cm^3. It is likely that there will be a variation between breeds and ages of pigs and an average of 5 cm^3 may be a good starting point. Circular trephinated defects of 10–25 mm have also been reported. Minipig bone regeneration in the adult mandible has been determined to range from 1.2 to 1.5 μm/day (Mardas et al., 2014). In a comprehensive review (Mardas et al., 2014), the complexities of determining a critical sized defect which differ between breeds, shape of the defect and location of the defects have been discussed. When starting a project of craniofacial defects without previous experience, this review is highly recommended as a starting point.

Swine have also been found to have fluxes of autonomic tone in the nasal airway, identical to humans (Campbell and Kern, 1981). Swine have been studied as a model of laryngeal injury

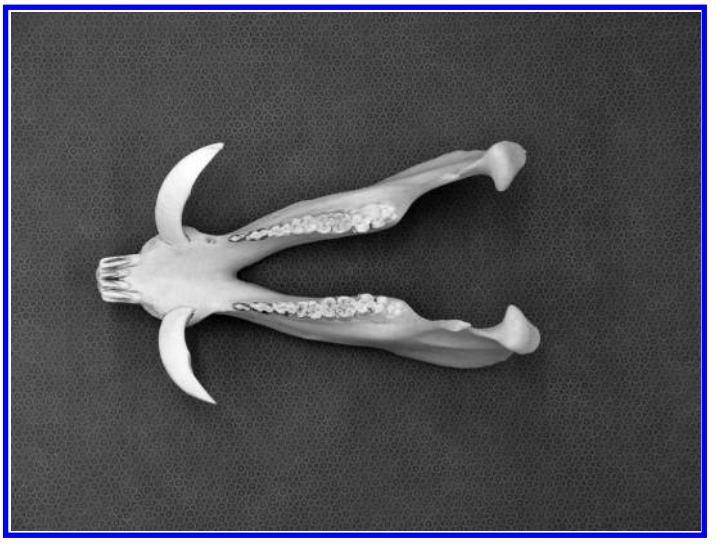

FIGURE 10.10 Dorsal view of the mandible.

due to damage from endotracheal tubes and cuffs (Chadha et al., 2011; Gordin et al., 2010a,b; Shah et al., 2007)

The salivary glands of the pig are the parotid, the mandibular, and the sublingual. The parotid gland enters the oral cavity opposite the upper fourth or fifth cheek tooth and may have accessory glands along the duct. The mandibular gland enters near the frenulum. The sublingual glands are located in bilateral chains and have multiple openings into the floor of the mouth. A series of minor buccal glands are located opposite the upper and lower cheek teeth. In the pig, the parotid gland is serous, the sublingual gland is mucoid, and the mandibular and buccal glands are mixed in secretions.

Tonsils analogous to the palatine tonsils are embedded in the soft palate rather than in the lateral wall of the oropharynx as in other species. There is a pharyngeal diverticulum dorsal to the larynx in the caudal aspect of the nasopharynx; this structure may be damaged during the passing of gastric or endotracheal tubes. The thymus (Figure 10.11) extends from the thorax to the level of the larynx in the pig. The thyroid gland (Figure 10.12) has paired lobules that are fused on the ventral

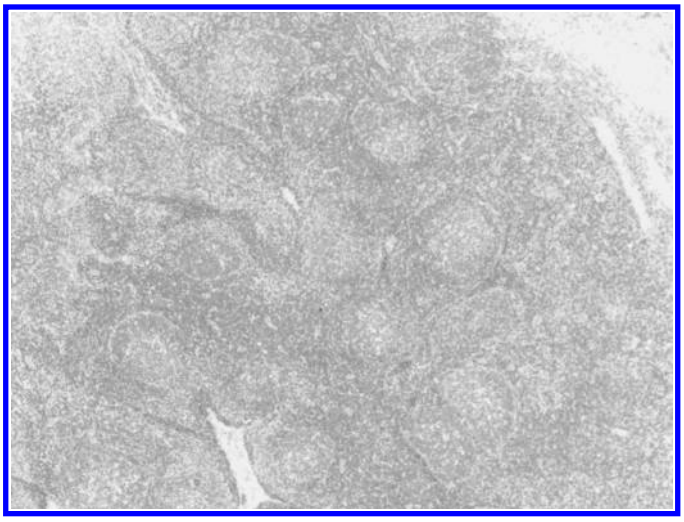

FIGURE 10.11 Histologic section of the thymus. H&E, ×40.

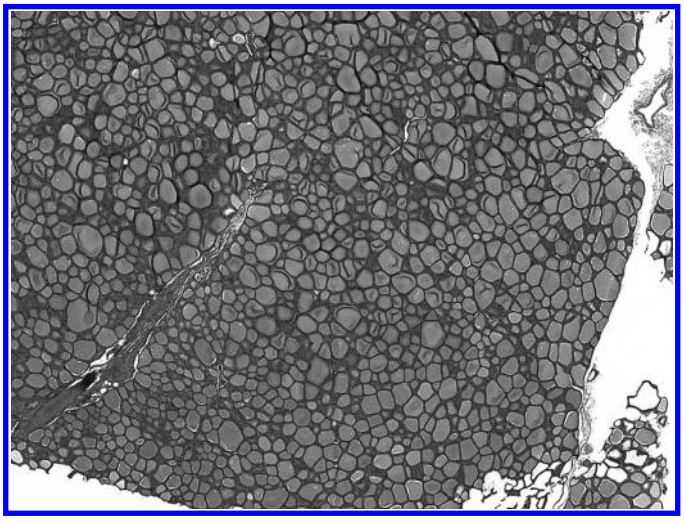

FIGURE 10.12 Histologic section of the thyroid. H&E, ×40.

surface of the trachea near the thoracic inlet. The single pair of parathyroid glands are minute and are associated with the cranial end of the thymus (Sack, 1982; Schantz et al., 1996; Sisson and St. Clair, 1975a,b; Swindle and Bobbie, 1987). The dissection of the glands of the neck is illustrated in Figure 1.44.

The ophthalmic system of the pig has been previously reviewed and compared with other species of laboratory animals (Adams, 1988), and the pertinent comparisons are summarized here. The pig has an open field of vision with a pupil and retina similar to human. The seven extraocular muscles (humans have six) are attached to the orbital wall in deep fossae. A nictitating membrane, Bowman's membrane (rudimentary and different in structure from humans), and Descemet's membrane are present. A tapetum and annulus of Zinn is absent. There may be either one or two puncta for lacrimal drainage. There is a deep gland of the third eyelid, Harder's gland. Pigs usually have brown or blue irises with a small amount of heterochromacity. The retina (Figures 10.13 and 10.14) is completely vascularized (holangiotic). Spontaneous conditions found in the eyes of various miniature pigs are outlined in Tables 10.1 through 10.3.

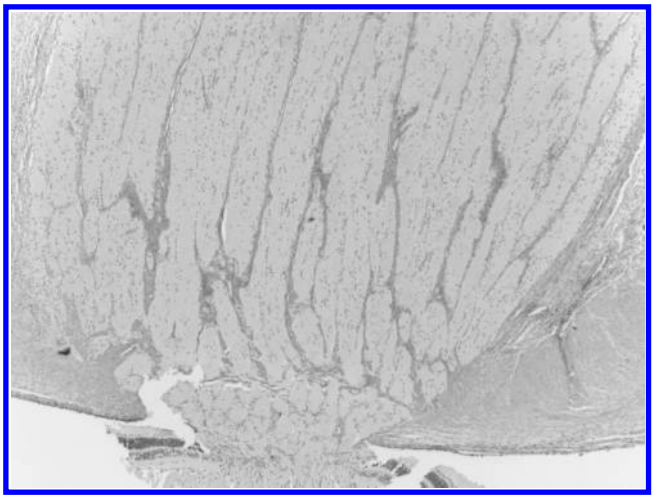

FIGURE 10.13 Histologic section of the optic nerve and retina. H&E, ×40.

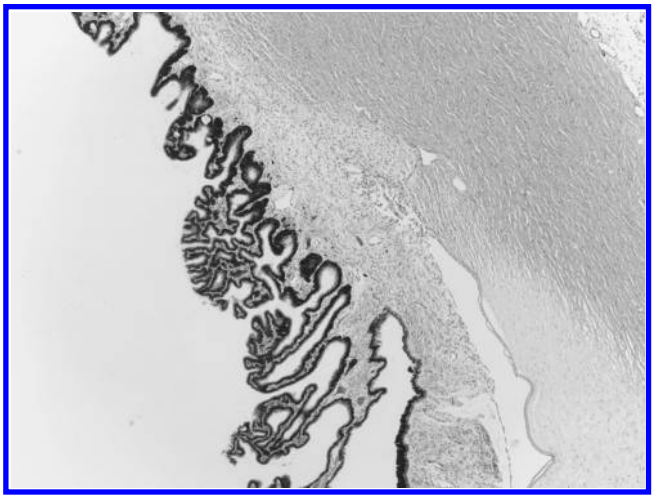

FIGURE 10.14 Histologic section of the iris. H&E, ×40.

TABLE 10.1
Ophthalmologic Findings in 6- to 8-Week-Old Göttingen Minipigs

	Males (*N* = 18)		Females (*N* = 18)		Both Sexes (*N* = 36)	
Observations	***n***	**(%)**	***n***	**(%)**	***n***	**(%)**
		Gross Findings				
Blepharitis	15	83.3	15	83.3	30	83.3
Conjunctivitis	8	44.4	8	44.4	16	44.4
Palpebral papilloma	–	–	1	5.6	1	2.8
Slight brown coloration of the sclera	–	–	1	5.6	1	2.8
		Cornea				
Pinpoint opacities	1	5.6	–	–	1	2.8
Opalescence of the stroma	–	–	–	–	–	–
		Iris				
Blue color	2	11.1	4	22.2	6	16.7
Blue/brown color	2	11.1	2	11.1	4	11.1
Brown color	14	77.8	12	66.7	26	72.2
Pupillary membrane remnants	6	33.3	6	33.3	12	33.3
		Lens				
Suture line abnormality	1	5.6	–	–	1	2.8
Focal nuclear opacity	2	11.1	1	5.6	3	8.3
Posterior cortical pinpoint opacities	4	22.2	3	16.7	7	19.4
Posterior capsular opacities	–	–	3	16.7	3	8.3
Posterior capsular cataract	–	–	–	–	–	–
		Vitreous				
Refringent points	2	11.1	–	–	2	5.6
Hyaloid artery remnants	14	77.8	16	88.9	30	83.3
		Fundus				
Tigroid fundus	12	66.7	14	77.8	26	72.2
Retinal vascular abnormality	–	–	–	–	–	–
Retinal hemorrhage	–	–	–	–	–	–
Retinal degeneration	–	–	–	–	–	–
Optic disc abnormality	–	–	–	–	–	–

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Note: *N* = number of animals examined, *n* = number with finding, % = percentage with finding.

The diameter of the adult eye is approximately 24 mm with an ocular power of 78 diopters, a binocular field of vision of 12°, and peripheral vision of 310° (Curtis et al., 2001). Specific eye measurements have been made in the Göttingen minipigs (Nielsen and Lind, 2005). In the adult pigs they measured, the following values were obtained: mean refractive error (+1.3 diopters), mean corneal power (44.1 diopters), and mean axial length (<19 mm). Additional comparisons of intraocular pressure measurements in 23 male Göttingen minipigs are illustrated in Figure 10.15.

The cornea is horizontally oval in shape and is thicker at the dorsal and ventral limbic area than in the center. Corneal thickness measurements vary widely depending upon the breed and age of the animal as well as the methodology and area of measurement. Values ranging from 600 to 1700 μm,

TABLE 10.2
Ophthalmologic Findings in 2- to 10-Month-Old Göttingen Minipigs

	Males (*N* = 70)		Females (*N* = 92)		Both sexes (*N* = 162)	
Observations	***n***	**(%)**	***n***	**(%)**	***n***	**(%)**
		Gross Findings				
Blepharitis	3	4.3	2	2.2	5	3.1
Conjunctivitis	12	17.1	2	2.2	14	8.6
Palpebral papilloma	–	–	–	–	–	–
Slight brown coloration of the sclera	–	–	1	1.1	1	0.6
		Cornea				
Pinpoint opacities	1	1.1	2	2.2	3	1.8
Opalescence of the stroma	1	1.4	–	–	1	0.5
		Iris				
Blue color	5	7.1	9	9.7	14	8.6
Blue/brown color	8	4.7	21	22.8	29	15.9
Brown color	57	81.4	62	67.4	119	73.4
Pupillary membrane remnants	9	12.8	12	13.0	21	13.0
		Lens				
Suture line abnormality	1	1.4	1	1.1	2	1.2
Focal nuclear opacity	2	2.8	6	6.5	8	4.9
Posterior cortical pinpoint opacities	8	11.4	11	11.9	19	11.7
Posterior capsular opacities	8	11.4	4	4.3	12	7.4
Posterior capsular cataract	–	–	–	–	–	–
		Vitreous				
Refringent points	4	5.7	4	4.3	8	4.9
Hyaloid artery remnants	48	68.5	66	71.7	114	70.4
		Fundus				
Tigroid fundus	50	71.4	64	69.5	107	70.4
Retinal vascular abnormality	–	–	–	–	–	–
Retinal hemorrhage	1	1.4	–	–	1	0.6
Retinal degeneration	–	–	1	1.1	1	0.6
Optic disc abnormality	1	1.4	1	1.1	2	1.2

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Note: *N* = number of animals examined, *n* = number with finding, % = percentage with finding.

which are greater than values for humans, have been reported. The anterior radius of curvature is 10–11 mm. The mean nerve density in swine is 4331 μm/mm^2 compared to a mean of 5867 μm/mm^2 in humans. The cornea and conjunctiva contain similar muscarinic receptor subtypes as the human m1–m5 (Adams, 1988; Curtis et al., 2001; Lagali et al., 2007; Liu et al., 2007; Pan et al., 2007; Tong et al., 2006).

Instead of a single canal of Schlemm, there are multiple small channels making up part of a scleral venous plexus. The sclera and iris are usually pigmented. Lens diameter has been reported to be approximately 12.5 mm with a thickness of 50–66 μm and a volume of 0.4–0.8 mL all of which may vary between breeds and sizes of the pig (Adams, 1988; Curtis et al., 2001; Ruiz-Ederra et al.,

TABLE 10.3
Ophthalmologic Findings in 7- to 12-Month-Old Yucatan Micropigs

	Males (*N* = 112)		Females (*N* = 112)		Both Sexes (*N* = 224)	
Observations	***n***	**(%)**	***n***	**(%)**	***n***	**(%)**
		Gross Findings				
Blepharitis	–	–	–	–	–	–
Conjunctivitis	2	1.8	–	–	2	0.9
Palpebral papilloma	–	–	–	–	–	–
		Cornea				
Corneal pigmentation	4	3.6	4	3.6	8	3.6
Pinpoint opacities	–	–	–	–	–	–
Opalescence of the stroma	–	–	–	–	–	–
		Iris				
Brown color	112	100.0	112	100.0	224	100.0
Pupillary membrane remnants	70	62.5	78	69.6	148	66.1
		Lens				
Suture line abnormality	–	–	8	7.1	8	3.6
Focal nuclear opacity	4	3.6	14	12.5	18	8.0
Nuclear cataract	2	1.8	8	7.1	10	4.5
Posterior cortical pinpoint opacities	26	23.2	20	17.9	46	20.5
Corticonuclear opacities	2	1.8	–	–	2	0.9
Posterior capsular pinpoint opacities	18	16.1	14	12.5	32	14.3
Posterior capsular cataract	6	5.4	2	1.8	8	3.6
		Vitreous				
Refringent points	4	3.6	14	12.5	18	8.0
Hyaloid artery remnants	90	80.4	94	83.9	184	82.1
		Fundus				
Tigroid fundus	58	51.8	54	48.2	112	50.0
Retinal vascular abnormality	–	–	2	1.8	2	0.9
Retinal hemorrhage	4	3.6	2	1.8	6	2.7
Retinal degeneration	2	1.8	4	3.6	6	2.7
Optic disc abnormality	–	–	4	3.6	4	1.8

Source: Reprinted from Loget, O. and Saint-Marcary, G. 1998. *Scand. J. Lab. Anim. Sci.* (Suppl. 1): 173–179. With permission.

Note: *N* = number of animals examined, *n* = number with finding, % = percentage with finding.

2004). Cadaver pig eyes have been injected with a solution of formalin:ethanol:2-propanol in a ratio of 4:3:3 to create a cataract similar to humans for training of surgeons (Sugiura et al., 1999).

In sexually mature 100 kg domestic swine, the physical properties of the lens have been measured as part of studies to develop artificial lenses and vitreous humor (Rapp et al., 2006; Ravi et al., 2005; Reilly et al., 2008; Shafiee et al., 2008; Swindle and Ravi, 2007; Swindle et al., 2006a, b). Their measurements were: refractive index 1.405, specific gravity 1.09, transmission 0.95, elastic modulus 1.2 kPa, and relaxation time constants 50–500 ms. Specific comparisons of the anterior lens capsule indicated that porcine lenses are thicker (50–66 μm) with a smaller accommodative

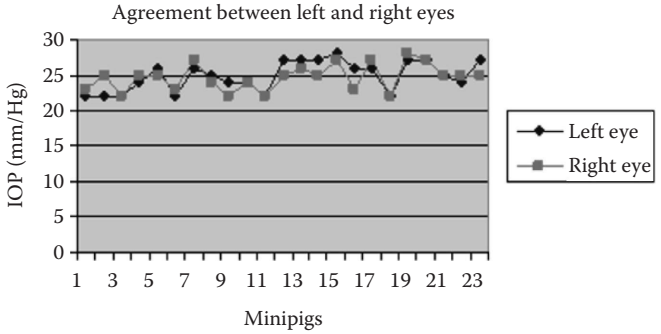

FIGURE 10.15 Intraocular pressure (IOP) agreement in measurements in 24 Göttingen minipigs. (Courtesy of Ellegaard Göttingen Minipigs.)

amplitude than humans (Reilly and Ravi, 2009; Reilly et al., 2008, 2009). In a recent study concerning the use of *ex vivo* eyes for laser safety testing (Fyffe et al., 2005) using 4- to 6-month-old Yucatan and Yorkshire pigs, differences from other published values were noted. They reported that a true Bowman's membrane is absent and the globe diameters were 30 mm, corneal thickness 1063 µm, and corneal epithelial thickness 47 µm, as compared to human values of 24 mm, 770 µm, and 35 µm, respectively. They reported an ED_{50} of 6.7 J cm^2 with infrared lasers. Differences between the minipigs and the farm pigs are likely because of the differences in body size. The ratio of body weight (BW) to eye weight is 2733:1. The extent of their color vision is uncertain, but they can distinguish wide wavelength differences.

Ultrastructural and other detailed studies of the retina have been performed (Chandler et al., 1999; Czajka et al., 2004; Garca et al., 2005; Hendrickson and Hicks, 2002; Jackson et al., 2003; Jacobs et al., 2002; Kyhn et al., 2008; Landiev et al., 2006; Petters et al., 1997; Ruiz-Ederra et al., 2004; Sachs et al., 2005; Shafiee et al., 2008). Pigs have a high density of both rods and cones with a photoreceptor density (200,000 cells/mm) similar to humans. They lack a tapetum cellulosum and foveolar specialization. The retinal vascular pattern is holangiotic with the retinal vessels arising from central arteries. The lamina cribrosa through which the optic nerve passes the sclera is approximately 0.4–0.6 mm^3. Overall, the porcine retina compares favorably with the human, as do the viscoelastic properties of the vitreous humor which has an approximate volume of 3.5 mL. Porcine eyes have been used to test development of artificial vitreous humor (Swindle et al., 2006a,b, 2007, 2008).

The visual characteristics of domestic swine have been summarized as being binocular with a sensitivity to radiation wavelengths of 465–680 nm and can distinguish wavelength differences of 20 nm. They probably have some degree of color vision and an ocular power of 78 diopters. The lens has a refractory index of 1.405, specific gravity of 1.09, transmission of 0.95, elastic modulus of 1.2 kPa, and relaxation time constants of 50–500 ms. The normal intraocular pressure range is approximately 15–27 mmHg (Curtis et al., 2001; Hernandez-Verdejo et al., 2007; Reilly et al., 2008; Swindle and Ravi, 2007).

Pigs have highly developed auditory and olfactory systems (Curtis et al., 2001). Their hearing frequency range is 40 Hz–40 kHz, and they are able to localize sound very well. They are sensitive to sudden loud noises and may stampede if startled. They are capable of vocalizing distress at up to 5000 Hz and will vocalize loudly during feeding and cage cleaning, consequently, personnel should be provided with ear protection.

Stereotaxic atlases of the pig brain (Figure 10.16) have been published (Felix et al., 1999; Saikali et al., 2010; Salinas-Zeballos et al., 1986; Sauleau et al., 2009). A method of making direct comparison between MRI digital images and histologic sections using a common coordinate has been published

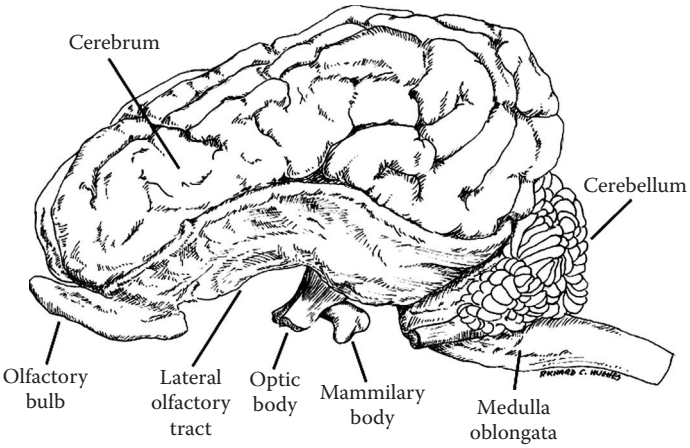

FIGURE 10.16 Lateral view of the brain.

on the pig brain (Sørensen et al., 2000). Functional MRI (fMRI) was performed on domestic piglets 3 weeks to 3 months of age corresponding to neurodevelopment in humans from late infancy into early childhood (Duhaime et al., 2006). The technique involved stimulation of sensory areas of the snout which stimulated the somatosensory cortex of the pigs in specific areas. These piglets were also used in studies of cortical injury secondary to focal impact. The Göttingen minipigs were used in a study to define a stereotaxic coordinate system to clarify studies of radiotracer uptake with PET scanning. The study also defined the mean volumes of the main structures in the brain (Watanabe et al., 2001). The brain of a domestic pig weighs approximately 35 g at birth and 120 g in an adult (approximately 0.35% of BW). The spinal cord weighs approximately 30–40 g, which is approximately 0.14% BW (Curtis et al., 2001). Normal intracranial pressure is usually <10 mmHg, brain blood flow is approximately 1 mL/min/g, and cerebral perfusion pressure is similar to the mean arterial pressure, which varies depending upon the anesthetic protocol (Cameron et al., 1992). Perinatal development of the brain in domestic swine was studied between 70 days of gestation and 140 days of postconception (3.5 weeks of age) (Pond et al., 2000). There were peaks of growth velocity for cerebral weight at 90 and 130 days. Peaks in total protein occurred between 90 and 130 days and total DNA between 90 and 110 days postconception. Myelination continues even after the growth spurts.

Comparisons in morphology and function of the porcine central nervous system (CNS) have been made (Felix et al., 1999; Larsen et al., 2004; Lind, 2005). Briefly, the similarities to humans are as follows: gyrencephalic morphology, distinct caudate and putamen structures in the striatal portion, cytoarchitectonic similarities in the cortex and hippocampus, and some dopanergic nuclei functions. In general, the patterns of distribution of gray and white matter, cerebral blood flow parameters, the size, and brain growth during development are similar. A recent MR atlas of 4-week-old domestic pigs has been published online (Conrad et al., 2014).

A detailed description of the blood supply to the spinal cord and brain has also been published (Stodkilde-Jorgensen et al., 1986). The common carotid arteries leave the brachiocephalic trunk at the level of T1. The internal carotid artery provides most of the circulation to the brain. Vertebral arteries branch off the subclavian arteries at the level of C6–C7 and supply the vertebrae, muscle, and spinal cord at each level. The cranial portion of the vertebral artery provides some blood supply to the medulla as well. In studies that interrupted the blood supply of the branches of the vertebral artery at C1–C2, C4–C5, and C7–T1, the latter produced the least hemodynamic and systemic changes. Venous plexi from the brain and plexus enter the internal jugular vein at the level of C1–C2. The internal vertebral plexus enters the vertebral veins and drains into the azygous system at the level of C6. Venous drainage from the thoracic vertebrae enters the azygous veins cranial to the heart.

Principles of performing surgery on the head and neck of swine are the same as for other species. The principal problems involve the thickness of the cranium and the massive nature of the bones of the mandible and maxilla. Following intracranial procedures, cerebral edema and swelling must be controlled, usually by the use of diuretics such as 50% dextrose or furosemide. Laryngeal and tracheal procedures are discussed in Chapter 9.

A comprehensive review detailing the anatomic and physiologic justification for using the pig as a translational model in neuroscience has been published (Lind et al., 2007). The DVD attached to this book contains images of the head, neck, and brain.

DENTAL PROCEDURES AND TUSK TRIMMING

The principles of performing oral and dental surgery are the same as for other species, except that exposure is limited for the premolars and molars because of the narrowness of the oral cavity opening. Retractors are necessary to keep the mouth open for procedures on these teeth.

The tusks of the pig need to be trimmed periodically in adult animals, especially in boars, for personnel safety (Eubanks and Gilbo, 2005). To perform this procedure, pigs should have general anesthesia or chemical restraint. They may be trimmed in restraint slings with sedation (Figures 10.17 and 10.18). The roots of the canine teeth are deep and difficult to extract; consequently, the tusks are usually trimmed at the gum line using either Gigli wire or saws. In the adult male, this procedure needs to be performed every 3–6 months. Tusks are slower growing in castrated males and females and may not need to be trimmed. Veterinary advice should be sought to make this determination.

Dental extractions can be performed on the other teeth using standard methods of root elevation followed by extraction (Figure 10.19). Mucoperiosteal flaps may be reflected from the gingiva in the cranial aspects of the oral cavity, using standard techniques of incision and retraction of the gingiva. Use of local anesthetics containing epinephrine as an adjunct to general anesthesia should aid hemostasis by the induction of local vasoconstriction. Oral incisions should be closed with absorbable sutures.

MAXILLOFACIAL AND CRANIOTOMY PROCEDURES

Swine have been used as models for both soft tissue and bone healing, including studies of grafting and implantation of biomaterials. Tissue engineering for bone augmentation and distraction osteogenesis has become an important area of research in maxillofacial surgery (Bradley, 1982; Donovan et al., 1993; Jensen, 2006; Kalkwarf et al., 1983; Ouhayoun et al., 1992; Robinson and Sarnat, 1955; Rosenquist and Rosenquist, 1982; Roth et al., 1984; Sims et al., 1997; Terheyden et al., 1999). Most of the studies have been performed on the mandible, maxilla, and temporomandibular joint. Surgical approaches will be described here.

The body of the mandible can be approached with the pig in dorsal recumbency. An incision made along the ventral aspect of the mandible will provide a relatively bloodless approach to the bone. Dorsal retraction of the skin and platysma muscle will expose the surface of the mandible. The facial vessels at the caudal end of the mandible should be avoided. The masseter muscle may be elevated with the periosteum to expose the lateral surface of the bone and inferior border of the mandible.

The temporomandibular joint may be approached using a lateral incision made dorsal to ventral from an area caudal to the ear and external auditory meatus to the ramus of the mandible following its caudal edge. This area may be readily palpated prior to making the incision. After incising the skin and platysma muscle, the dissection becomes difficult. Branches of the facial nerve and facial, temporal, and auricular arteries and veins should be retracted if possible. The parotid salivary gland should be retracted ventrally. The bodies of the parotidoauricularis muscle will have to be transected. The temporomandibular joint can then be accessed from a caudal direction under

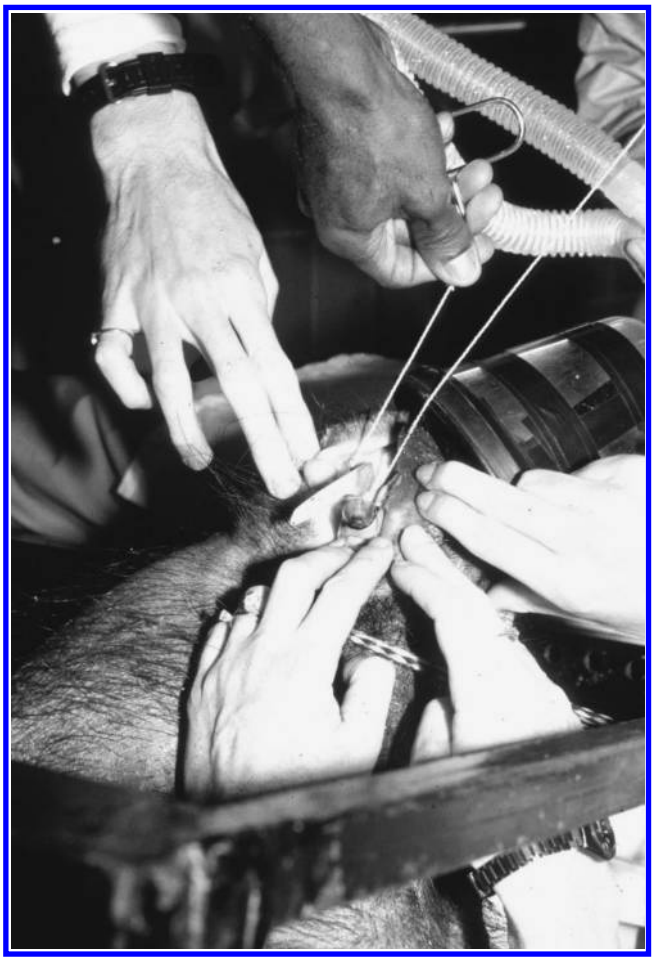

FIGURE 10.17 Cutting the tusks of a sedated boar with Gigli wire.

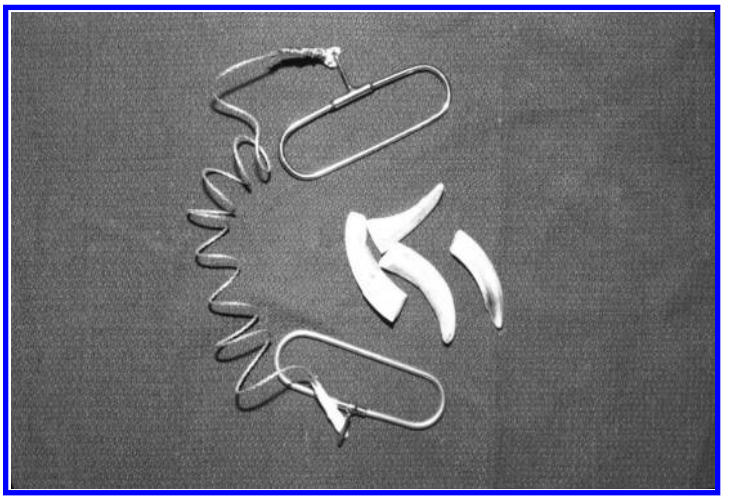

FIGURE 10.18 Trimmed tusks and Gigli wire.

FIGURE 10.19 Elevation of the gum for dental extraction.

the zygomatic arch. If greater exposure is required, the zygomatic arch will have to be transected, taking care not to damage the blood vessels underlying it.

The dorsolateral aspects of the skull can be approached using a midline incision along the crest with the pig in sternal recumbency. The nuchal crest of the pig is quite prominent and is the thickest part of the bone. The superficial muscles along the midline are incised and retracted with the skin. This is followed by incision and retraction of the periosteum laterally from the midline. Using this approach the cranium, frontal sinus, parietal bone, and frontal bone may be approached.

Swine have been developed as a model for endoscopic skull base surgery in the posterior fossa because of the similarities in anatomy with the human (Jarrahy et al., 1999). A curvilinear incision is made caudal to the auricle, and the soft tissue separated from the temporal bone. A burr hole is made down to the level of the intact dura. The endoscope can then be manipulated to visualize the cerebellum, midbrain, and cranial nerves V, VII, VIII, IX, X, and XI as well as the major blood vessels in the region. It is likely that this technique can be extrapolated to other areas of the brain.

The nasal bone, cranial aspects of the frontal sinus, and sinus cavity can be approached using a midline incision along the snout with the pig in sternal recumbency. The nasolabial muscles are incised with the skin and retracted subperiosteally, and then retracted laterally. The nasal bone can be fractured along its suture lines for exposure of the nasal cavity.

The use of Göttingen minipigs as an experimental model for augmentation of the maxillary sinus floor as a treatment for alveolar atrophy (Figures 10.20 through 10.24) (T. Jensen, personal communication) has been described by Terheyden and coworkers (Terheyden et al., 1999). This model is presently used to evaluate a mixture of autogenous bone graft and bone substitutes as graft material (Jensen, 2006). The maxillary sinus is exposed through an extraoral incision below the lower lid. A trap door is made with burrs in the lateral sinus wall and the Schneiderian membrane is elevated. The cavity created between the mucosa and the floor of the maxillary sinus can be packed with a grafting material around the inserted dental implant. The muscles, fascia, and skin are closed in a routine fashion.

These incisions may be closed routinely by repair of the bone with wires, screws, or dental acrylic if defects are created. Bone wax may be used to control bleeding. The muscles, fascia, and skin are closed in a routine fashion.

OCULAR SURGERY

The eyes of the pig are deeply embedded in the sockets, especially in anesthetized animals, and exposure requires the use of ocular retractors. Swine have rarely been used in ophthalmic research

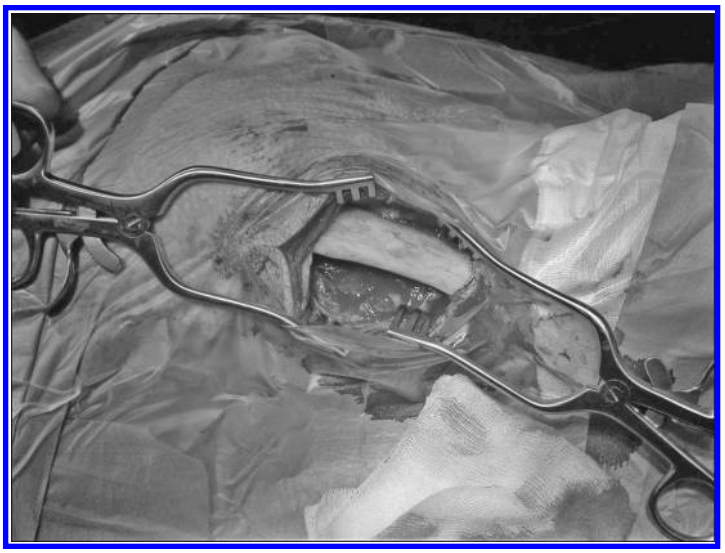

FIGURE 10.20 Exposure of the lateral and inferior border of the mandible. (Courtesy of T. Jensen, Aalborg Hospital, Aarhus University Hospital and Institute of Odontology, Faculty of Health Sciences, University of Copenhagen, Denmark.)

despite some of their anatomic similarities to humans, but they have been used for corneal procedures (Adams, 1988). The surgical procedures and approaches are the same as for other species. Enucleation of the eye will be described in this section.

Allis tissue forceps are used to clamp the margins of the eyelids together. The eyelids are incised in a circumferential fashion beyond their margins, and blunt dissection is initiated at the edge of the orbicularis oculi and into the conjunctiva. After the conjunctiva is dissected to the attachments of the ocular muscles, they are transected. After transection of the muscles, the globe is bluntly

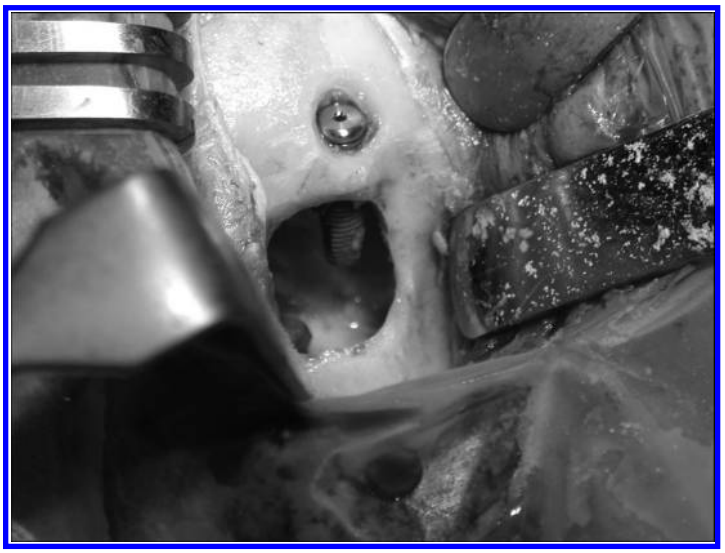

FIGURE 10.21 Insertion of the dental implant into the surgically created cavity. (Courtesy of T. Jensen, Aalborg Hospital, Aarhus University Hospital and Institute of Odontology, Faculty of Health Sciences, University of Copenhagen, Denmark.)

FIGURE 10.22 The entire sinus floor around the implant is packed with grafting material. (Courtesy of T. Jensen, Aalborg Hospital, Aarhus University Hospital and Institute of Odontology, Faculty of Health Sciences, University of Copenhagen, Denmark.)

dissected free of its attachments except for the ocular nerve, artery, and vein. The globe may be drained using needle aspiration at this point to increase exposure. Right-angle forceps are passed behind the globe, and those structures are clamped. The globe is excised on the proximal side of the forceps, and the vessels and nerve are ligated together, followed by removal of the forceps. A biocompatible prosthesis may be inserted or the margins of the incision closed. Care should be taken prior to closure to ensure that all glandular structures have been excised and hemostasis is complete. The conjunctiva and skin are closed in a routine fashion.

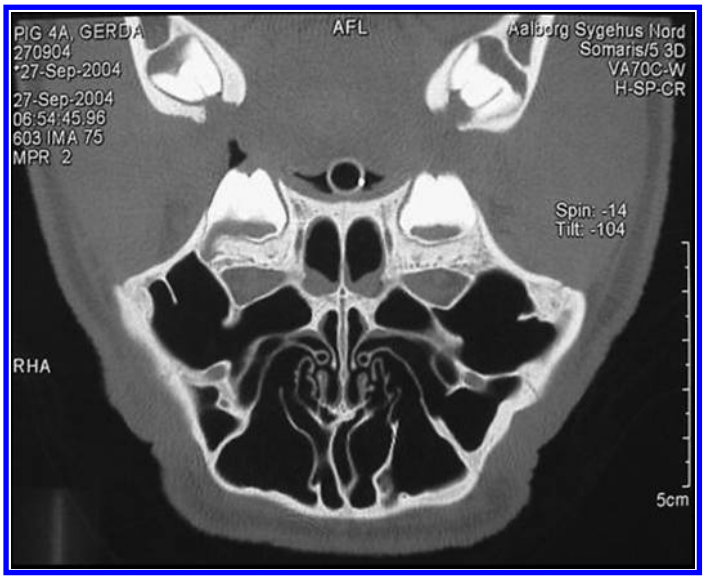

FIGURE 10.23 Preoperative CT scan of the maxillary sinuses. (Courtesy of T. Jensen, Aalborg Hospital, Aarhus University Hospital and Institute of Odontology, Faculty of Health Sciences, University of Copenhagen, Denmark.)

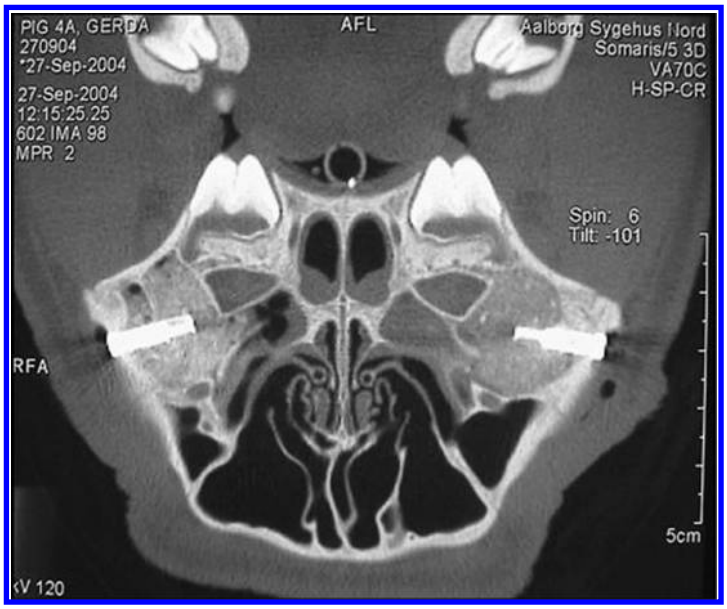

FIGURE 10.24 Postoperative CT scan of the maxillary sinuses with the grafting material packed around the dental implant. (Courtesy of T. Jensen, Aalborg Hospital, Aarhus University Hospital and Institute of Odontology, Faculty of Health Sciences, University of Copenhagen, Denmark.)

Pigs have also been developed as models for retinal transplant (Del Priore et al., 2004; Ghosh et al., 2004; Warfvinge et al., 2005), retinal detachment (Jackson et al., 2003; Jacobs et al., 2002), visual prosthesis development (Sachs et al., 2005), ophthalmic arterial microcatheterization (Requejo et al., 2014) and studies involving vitreous replacement (Rapp et al., 2006; Ravi et al., 2005; Reilly et al., 2008; Swindle et al., 2006a,b; Swindle-Reilly and Ravi, 2010; Quiroz-Mercado et al., 2004). A rhodopsin transgenic pig (Petters et al., 1997; Warfvinge et al., 2005) with a retinitis pigmentosa-like disease has been developed. The mutation Pro347Leu reduces rod photoreceptors significantly by 4 months of age. Cones degenerate more slowly, following sexual maturity. In the cases of these models, the surgical procedures are the same as those performed in humans. In many cases, the procedure to be performed is the goal of the study. For procedures in which the vitreous is removed, it has to be replaced simultaneously with the test substance to prevent retinal detachment.

The predominant number of porcine ophthalmic models in the literature involves the retina because of the anatomic and physiologic characteristics described above. In addition, disease conditions of the retina are likely to cause blindness and thus are of major importance in the research setting (Czajka et al., 2004; Del Priore et al., 2004; Ghosh et al., 2004; Jackson et al., 2003; Jacobs et al., 2002; Kyhn et al., 2008; Landiev et al., 2006; Petters et al., 1997; Ruiz-Edera et al., 2005; Sachs et al., 2005; Shafiee et al., 2008; Warfvinge et al., 2005).

Retinal detachment in humans can develop for a variety of reasons including trauma and metabolic disorders. The condition can be created surgically in swine (Landiev et al., 2006). A lateral canthotomy is created and a circumscript vitrectomy is performed in the region of the detachment. The vitreous is replaced with physiologic saline. A subretinal injection of saline followed by 0.25% sodium hyaluronate is administered using thin glass pipettes. This results in a rhegmatogenous detachment of the retina in the selected area. Variations of this technique can be used to create different types of retinal detachment and damage (Kyhn et al., 2008). A vitrectomy with removal of the posterior hyaloid without creation of a bleb as described above can be performed. Blebs can also be created with injection of Ringers-Lactate following vitrectomy. Diathermia can also be administered on the bleb or a linear retinotomy can be performed in the same area. Retinal holes can be

created with a vitreous cutter made over the bleb (Czajka et al., 2004). This model allows the study of the effects of various surgical insults on the porcine retina, which is essential information in the development of retinal transplants and reparative surgical procedures. Electroretinography and histologic studies of the retina have been performed on these models (Kyhn et al., 2008; Landiev et al., 2006). The retina of the pig reacts in a similar manner as occurs in humans with the condition.

Human vitreous humor is an acellular nonhomogeneous hydrogel composed of 98% water within a network of collagen and interfibrillary hyaluronic acid. The vitreous allows circulation of metabolic solutes and nutrients and acts to hold the retina in place. It is essential to replace vitreous loss as a result of surgery, trauma or deterioration. Porcine vitreous has been demonstrated to be more similar to humans than other common laboratory animals (Swindle and Ravi, 2007). The most promising vitreous substitutes are hydrophilic polymers (hydrogels) with the characteristics of viscoelastic solids. It is anticipated that these would be long-term replacements as opposed to other substances such as silicone oil, which has a variety of complications such as cataracts (Swindle and Ravi, 2007; Swindle et al., 2006a,b).

The pig has been developed as a surgically induced model of glaucoma, which has a similar pathogenesis and deterioration of retinal ganglion cells as the condition in humans (Ruiz-Edera et al., 2005). The technique involves cauterization of the nasal, dorsal and temporal episcleral veins. The intraocular pressure becomes elevated by three weeks postsurgery. Increases of 1.2–1.4-fold were considered to be evidence of glaucoma, which was accompanied by death of retinal ganglion cells. The pig has also been developed as a transgenic model of retinitis pigmentosa with similar deterioration of the retina (Petters et al., 1997).

Porcine corneas and lenses have been utilized to study various surgical repairs and wound healing techniques (Lagali et al., 2007; Liu et al., 2007; Pan et al., 2007; Reilly et al., 2008, 2011; Sugiura et al., 1999; Tong et al., 2006). Laser *in situ* keratomileusis (LASIK) surgical procedures using various devices have been studied in swine (Hernandez-Verdejo et al., 2007). As previously noted, the muscarinic receptors of the cornea are similar to humans which may be a justification for other types of reparative procedures of this type in porcine models (Liu et al., 2007; Tong et al., 2006).

Visual loss due to corneal damage is a significant cause of human sight impairment. Studies have been performed in pigs to study the repair of damaged corneas with tissue engineered implants using collagen-copolymer and cross-linked collagen systems. Long-term studies have demonstrated that the tissue-engineered corneal implants become reinnervated (Lagali et al., 2007). Pig corneas have also been studied as potential xenografts for human corneal transplants (Pan et al., 2007). In pig-to-primate studies, rejection occurred at an early stage for complete penetrating transplantation with immune rejection developing <15 days. However, lamellar corneal xenotransplantation remained clear without signs of rejection for >90 days. In recent years the pig has been developed as a model of blast injury, including injury specific to the eye (Sherwood et al., 2014).

PHARYNGOSTOMY TUBES

A pharyngostomy tube may be surgically implanted when there is a necessity to chronically administer food or medication without mastication. However, it is relatively easy to pass a stomach tube with a mouth gag while the pig is immobilized in a restraint sling. This method of nonsurgical intervention is recommended over the surgical procedure.

The pharynx and proximal esophagus is approached using a ventral midline incision over the larynx. Blunt dissection is continued through the midline, and the esophagus is identified on the dorsum of the trachea by deviating laterally with the dissection when the sternohyoideus muscle is reached. After passing around the esophagus with elastic vessel loops, it is elevated, and a stab incision is made into the lumen. The proximal one-half to two-thirds of the muscle layers of the esophagus are striated muscle, which converts to smooth muscle in its distal length. A premeasured length of soft nasogastric tubing is passed into the stomach. The proper positioning can be determined when stomach gases are noted to be passing through the tube. The tubing is sutured in place

with a purse-string suture. The tubing is tunneled subcutaneously to exit the skin behind the ear. A purse-string suture is placed in the exit site of the skin. The subcutaneous, muscle, and skin layers are closed routinely. The tubing may be taped to the ear for security.

THYROIDECTOMY

The approach to the thyroid gland (see Chapter 1, Figure 1.44) is made with the pig in dorsal recumbency. A ventral midline incision is made, cranial to the manubrium sterni in the last one-third to one-half of the neck (Swindle, 1983). The incision is continued between fascial planes of the sternohyoideus muscle until the thyroid is identified on the ventral surface of the trachea as a dark-colored bilobular structure. The cranial and caudal thyroid artery and vein enter the gland from the dorsal surface and usually cannot be identified in advance. They are bisected and ligated during blunt dissection of the gland from the trachea. The fascial planes of the muscle, the subcutaneous tissues, and the skin are closed in a routine fashion.

PARATHYROIDECTOMY

The single pair of parathyroid glands (see Chapter 1, Figure 1.44) (parathyroid gland III) are difficult to identify (Sack, 1982; Swindle and Bobbie, 1987). They are located on the cranioventral surface of the thymus gland caudolateral to the larynx. A line drawn between the angles of the mandible will provide the surgeon with an approximate location. Because the thymus changes in size with age, the exact location of the dorsal end is variable. These grayish structures are minute (1–4 mm) but usually can be felt to be a definitive structure by rolling them between the thumb and forefinger.

They may either be approached using a ventral midline incision with lateral subcutaneous dissection or a ventral paramedian incision over the sternohyoideus and sternomastoideus muscles. Once the structures are identified, they are removed by blunt dissection. Histopathology is recommended to ensure that they have been removed. Alternatively, the cranial pole of the thymus may be removed if the structures cannot be identified. Hemorrhage is minimal, and vessels usually do not require ligation. The muscle fascia, subcutaneous, and skin layers are closed routinely.

HYPOPHYSECTOMY

The pituitary gland has been surgically removed in both fetal and adult animals (Drisko et al., 1996; Kraeling et al., 1986). The transsphenoidal and parapharyngeal approaches are difficult in swine because of the limitations in exposure through the open mouth and the extensive dissections involved from the ventral midline. The transfrontal, supraorbital approach has been used in the fetus and in gilts; however, the dissection in adult animals is much more difficult. The transorbital approach offers a minimally invasive technique in adult animals (Figure 10.25).

The first step is to perform a right-eye enucleation, including removal of the periosteum, as described previously. A 5-mm burr hole is drilled caudodorsal and parallel to the optic canal. The site is located dorsomedial to the foramen orbitorotundum. The drill is advanced carefully to the level of the dura overlying the juncture between the infundibulum and the infundibular stalk. Care should be taken to avoid the foramen orbitorotundum and the underlying internal carotid artery. If the hole requires enlargement, care should be taken to avoid damage to the underlying blood vessels and the optic chiasm. The dura is pulled away from the pituitary, and a small hole is made with a dura twist hook. Use of suction and microdissection will allow the surgeon to visualize the pituitary. It can be removed, transected, or cannulated at this point. When closing the incision, the burr hole is filled with bone wax to prevent leakage of cerebrospinal fluid (CSF). The socket can be filled with prosthetic material, such as dental acrylic, prior to closing the incision as described earlier for enucleation (Drisko et al., 1996).

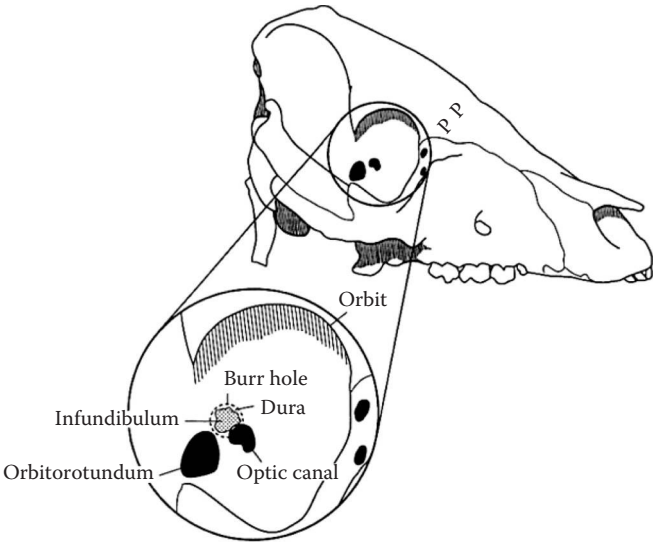

FIGURE 10.25 Hypophysectomy through the transorbital approach. (Reprinted from Drisko, J.E. et al., 1996, *J. Invest. Surg.,* 9(4): 305–311. With permission.)

The supraorbital frontal approach uses the surgical approach to the cranium described previously. A section of the frontal and parietal bone is removed, and care must be taken to avoid damage to the frontal artery and vein. The exposure and craniotomy is performed to expose the left and one-half of the right cerebral hemispheres. The dura mater is incised taking care to avoid damaging the brain. A brain retractor is used to elevate the cerebral hemisphere and expose the region of the hypothalamus. The hypothalamus may be catheterized, transected, or removed as described earlier. The bone fragments are replaced, and the muscular, subcutaneous and skin layers are closed in a routine manner. The authors recommend placement of a drain tube in the dorsum of the cranium for 48 h postoperatively and 50 mg of cortisone pre- and postoperatively. Mineral mix was added to the ration of hypophysectomized gilts as a permanent dietary supplement (Kraeling et al., 1986).

Administration of 100 mL of 10% saline iv intraoperatively may help reduce brain size. Also, an increase in mortality was noted when nitrous oxide was used in the anesthetic protocol (Kraeling et al., 1986). Postoperatively, analgesics are required, and the pig should be monitored closely to maintain homeostasis.

SPINAL CORD ISCHEMIA AND HEMORRHAGE

Spinal cord ischemia can be a complication of aortic surgery (see Chapter 9) or vascular damage. Spinal cord damage usually becomes clinically relevant after 20 min of cross-clamp time in the suprarenal aorta but may be extended in the postrenal aorta. Cross-clamping of the thoracic aorta distal to the branching of the left subclavian aorta for 30 min will consistently produce ischemic damage (Qayumi et al., 1997). Studies of healing of dural substitutes have also been performed following laminectomy (Chapter 11), excision of the dura, and implantation of substitutes such as biomaterials or allografts (Haq et al., 2004). An angiogram of the distal aorta and its branches is illustrated in Figure 10.26.

The model can be surgically produced by performing a left lateral thoracotomy (see Chapter 9) or a left-flank celiotomy (see Chapters 4 and 7) to approach the aorta. The aorta may either be clamped with vascular clamps or encircled with an umbilical tape snare. Alternatively, selected dorsal branches of the aorta may be ligated. Experience from aortic anastomosis (see Chapter 9) demonstrates that this must be more than two contiguous pairs of arteries.

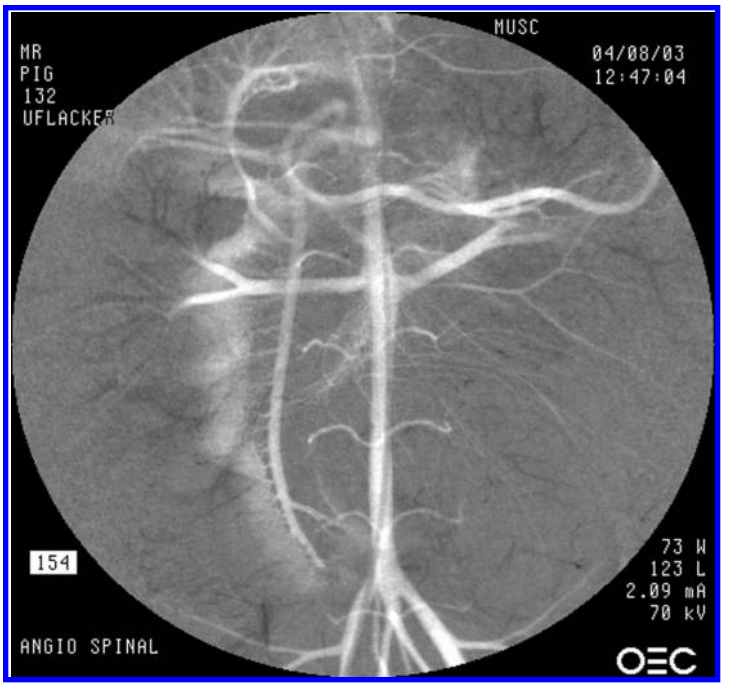

FIGURE 10.26 Angiogram of the distal aorta, renal, spinal and iliac arteries. (Courtesy of Renan Uflacker, Department of Radiology, Medical University of South Carolina.)

Clamping of the prerenal aorta will produce hypertension that must be controlled with blood volume reduction or infusions of Na nitroprusside. Cross-clamping of the aorta will also produce hypoperfusion of all of the distal organs with the potential of dysfunction. Animals may become paraplegic.

If this model is performed as a survival procedure, intensive care of the animal postoperatively is imperative. Housing the animal in deep wood shavings or on padding is necessary. Nutritional support may have to be administered.

Similar types of clinical syndromes occur with spinal cord hemorrhage (Ganz and Zwetnow, 1990) associated with decompression injury models at a simulated dive depth of 200 ft of seawater with decompression after 43 min at 60 ft of seawater per second (Broome et al., 1997; Dick et al., 1997). Approximately two-thirds of the swine will develop clinical neurologic syndromes related to petechia and ecchymoses in the spinal cord. Approximately 20% of the affected swine will die. Additional injuries due to dysbarism may occur and require treatment. This model avoids having to perform surgical procedures.

Experimental allergic encephalomyelitis (EAE) has been induced in minipigs at the National Institutes of Health (NIH) by injection of spinal cord homogenate in Freund's adjuvant with or without pertussis toxin. Animals develop a transient monophasic illness with paresis, which can be graded clinically. The model is proposed as a preclinical animal to study treatments of multiple sclerosis (Singer et al., 2000).

SPINAL CORD INJURY AND NEUROTRAUMA MODELS

A model of pediatric spinal cord injury in domestic pigs 3–5 weeks of age (5–7 kg) was developed (Kuluz et al., 2010). The model was produced by making a dorsal midline incision over T6–8 and removal of the dorsal spinous processes. The ligamentum flavum and epidural fat were removed and the animal allowed to stabilize prior to inducing spinal cord trauma with a 6 mm circular

impactor. Severe complete spinal cord injury without spontaneous recovery of sensorimotor defects was achieved at an impact of 5–8 mm in depth and 60–80 psi. Incomplete injury with spontaneous recovery was achieved with a 3 mm lesion and 30 psi.

When producing a chronic model of injury, the authors indicated that the postoperative care was intensive as expected with models of paralysis. Housing for the animals must include padded floors and probably wood shavings for additional bedding to prevent skin lesions from developing. Antibiotics and analgesics should be included in the immediate perioperative period. Food and water consumption needs to be monitored closely and it is likely that oral gavage of nutrients may have to be given to some animals. Females can be catheterized to empty the bladder but males will have to have the bladder manually expressed or emptied by prepubic needle tap if neurogenic bladder occurs. A neurogenic bowel with constipation may also have to be controlled with enemas or stimulant suppository laxatives if it occurs. The Kuluz group developed a wheelchair apparatus that supported the rear of the body and allowed the pigs to be mobile by using their forelegs.

Isolated cranial neurotrauma is induced both using open and closed cranial techniques (Finnie and Blumbergs, 2002). One method involves use of a modified nail gun with a disc to produce a 46 J shock to produce a subdural hematoma (Dudkiewicz et al., 2008). Other methods involve a circular craniotomy with a fluid percussion of a calculated force or a weight drop or spring-loaded device method (Armisted and Kurth, 1994; Duhaime et al., 2000; Proctor, 2003). In pediatric studies in piglets with postoperative survival for 7 days, the severity of the focal injury following craniotomy and focal trauma with a spring-loaded cylindrical device for 400 ms has been shown to increase progressively with maturation in groups which varied in age (5 days, 1 month, 4 months) (Duhaime et al., 2000, 2006; Missios et al., 2009). An open cranial model of pediatric subdural hematoma was produced in the same age groups by injection of autologous blood into the subdural space. A burr hole was made off midline in a standardized location using suture lines as markers. A 24 g angiocatheter was placed and blood was injected at a rate of 2 mL/m at a predetermined volume of 4.5 mL in 5 days and 5.4 mL in 4 m piglets (approximately 10% of the intracranial volume). The amount and rate are important to prevent mortality due to sudden increases in the intracranial pressure (Durham and Duhaime, 2007). The hole is closed with tissue glue and the scalp sutured. As with the focal trauma there is more resistance to permanent damage in the younger animals. The amount of trauma and location can obviously be varied by location and delivery of traumatic energy. Closed head injury can also be induced by non-impact axial rotations of the head using specialized equipment (Friess et al., 2006). Outcomes vary in severity depending upon the degree of rotation, rotational speed, and number of rotations.

Polytrauma injuries related to traumatic blast injury include CNS, thoracic, abdominal, cardiovascular and/or musculoskeletal trauma and may include all of them in combination (Bauman et al., 2009; Säljö and Hansson, 2008). Military research has utilized actual blast injury and tubes with compressed air discharge. In the university setting, compressed air is the most likely avenue of creating injury that would be used. In most cases, the CNS trauma and debility are likely to be the most prominent and would require intensive aftercare in a chronic model as described above. Included in this intensive protocol would be treatment for shock and/or hemorrhage using standard types of emergency protocols.

In all of these models the use of anesthetics and analgesics would be required, however, selection of the agents should be made after due consideration of the physiologic alterations associated with their use. Selection of the wrong agents may have an undesirable influence on the experimental outcome of the project (Loepke et al., 2002; Rowe et al., 2013).

STROKE MODELS

Pigs are emerging as a stroke model, mainly induced by ligation or occlusion of the middle cerebral artery (Imai et al., 2006; Olsen et al., 2003; Sakoh et al., 2000). All of these methods require a craniotomy either following enucleation or by frontotemporal approaches. In the author's experience, swine are protected against embolic cerebrovascular disease caused by such procedures as chronic

catheterization, because of the anatomy of the rete mirabile. When swine experience embolic disease, it is characterized as transient blindness, which reverses within a few days. Collagen microbeads 380 μm have been utilized to study their embolization effects on the rete mirabile in a chronic model (Turjman et al., 1995). Neurologic effects were not apparent post embolization.

However, a short-term acute model of inducing cerebral ischemia by injection of 0.5 mL of autologous clot into the ascending pharyngeal artery, causing occlusion of the internal carotid artery and rete mirabile, has been developed (Culp et al., 2004). Bilateral snare occlusion of the internal carotid arteries reduces cerebral blood flow, which is rapidly compensated by the basilar artery (Wang et al., 2005). An acute model of pediatric ischemic stroke in 2–4-week-old piglets has been developed using photothrombosis (Kuluz et al., 2007). The surgical procedure involved enucleation of the eye to identify the branches of the middle cerebral artery. After injecting erythrosine B to sensitive, the artery and argon laser was used to create thrombosis which occurred in both the gray and white matter. Consequently, the chronic models will probably have to be produced by direct occlusion of the target artery in the brain. The artery of interest can be approached either via direct craniotomy or a transorbital approach. An acute model of intracranial hemorrhage and hematoma has been produced by direct infusion of blood into the frontal white matter (Wagner et al., 2000). For surgical occlusion models, the artery of interest is identified by angiography, and the area is approached surgically using the surgical techniques identified earlier. See the earlier sections on craniotomy and hypophysectomy for the surgical approaches.

In a chronic model of stroke, postoperative care will require intensive effort to prevent discomfort to the animal. If it has reduced mobility, it is recommended that the animal be housed either with soft padding on the floor and sides of the cage or, preferably, in deep wood shavings to prevent decubitus and scraping injury. Animals may require parenteral nutrition either iv or po. Unless analgesics are contraindicated by the protocol, they should be utilized. In direct surgical ligation models, cerebral edema may have to be controlled.

NEURODEGENERATIVE AND MISCELLANEOUS BRAIN DISORDERS

The use of swine in neuroscience is on the increase because of the translational value of using the pig as a large animal model (Lind et al., 2007; Wakeman et al., 2006). Pigs have been used extensively for study of the serotonin 5-HT system, and the subsets show high homology with humans. Likewise there has been extensive characterization of the dopamine systems. However, other neurotransmitter systems have not been as extensively characterized but appear to be similar to other mammalian species. Transgenic and cloned models are discussed elsewhere in this book.

The model of MPTP-induced Parkinsonism in minipigs has been extensively characterized (Mikkelsen et al., 1999). Following daily sc injections of MPTP 1 mg/kg/day for 6 days, all of the pigs developed typical symptoms of the disease. The pigs exhibited signs of acute toxicity following each injection characterized by spasms and twitches in the muscles and foaming at the mouth which typically resolved within 2 h. The symptoms of the syndrome induced in the pigs included muscle rigidity, hypokinesia, and impaired coordination. In the syndrome, there is a selective loss of dopaminergic somata in the pars compacta of the substantia nigra. There was reduction in the concentrations of DA, DOPAC, and HVA in the putamen and nucleus caudatus. The symptoms in the pigs gradually resolved over the course of months.

Pigs have also been studied for the behavioral effects of diet-induced changes in the brain related to fatty acid intakes and the effects on dopamine metabolism in neonatal pigs (Ng and Innis, 2003). Behavioral effects from many of the areas of study in neurodegenerative diseases and trauma are increasingly important (Dilger and Johnson, 2010; Friess et al., 2006; Lind et al., 2007; Mikkelsen et al., 1999; Ng and Innis, 2003). In general, the various research programs have designed learning and behavioral tasks with equipment specifically designed in house for their studies. In general, it tends to be necessary to have food reward as part of the design of the system since that is the predominate motivator for pigs.

CSF COLLECTION AND EPIDURAL INJECTION

Retrieval of CSF from the spinal cord and epidural administration of pharmacological agents may be necessary for some experimental procedures. Epidural administration of analgesics, such as morphine, may be desirable for preemptive analgesia for surgical procedures caudal to the thorax. The epidural cavity or space is the site of injection of substances such as analgesics. The subarachnoid cavity is the site of interest for obtaining CSF. CSF formation rates for pigs may be estimated at 0.9 mL/min although there will be variations based upon breed and size (Stromholm et al., 1994).

The vertebral bone structure in the pig is massive compared to other large animal species used in research. In addition, the intervertebral spaces are narrow and the dorsal processes of the vertebra tend to interfere with access to the vertebral spaces because of their size and caudal orientation. The pig does not normally have the ability to flex the spine as much as other species, giving them a more stiff posture. The vertebral formula is C7, T14–T15, L6–L7, S4, Cy20–Cy23. The sacral vertebrae are partially fused. The spinal cord terminates with the conus medullaris at S2–S3 and follows down the remaining vertebra with the cauda equina and the filum terminale. This is unlike the human in which the conus medullaris is located at L1–L2. The epidural space tends to contain fatty deposits. The cross section of the vertebral canal from outside to inside is as follows: dorsal longitudinal ligament, epidural cavity, dura mater, subdural cavity, arachnoid membrane, subarachnoid cavity, pia mater, and spinal cord.

Epidural Injection

Swine must be anesthetized for these procedures (Boogerd and Peters, 1986; Punto, 1980). Complete aseptic technique should be utilized when invading the spinal canal. This includes shaving, surgical skin preparation, and wearing sterile gloves. Most porcine spinal canals can be accessed using 20–22 g, 1.5–3.0 in. (3.8–7.6 cm) spinal needles (Figure 10.27).

Epidural administration of analgesics for preemptive analgesia is the most common procedure for which this technique is used. However, administration of test substances or radiopaque solutions may require this technique. Epidural administration is usually performed in the lumbar region because the regional analgesia is only effective for procedures caudal to the thorax. It is necessary to flex the spine to separate the intervertebral spaces. This is performed either by hanging the pig's

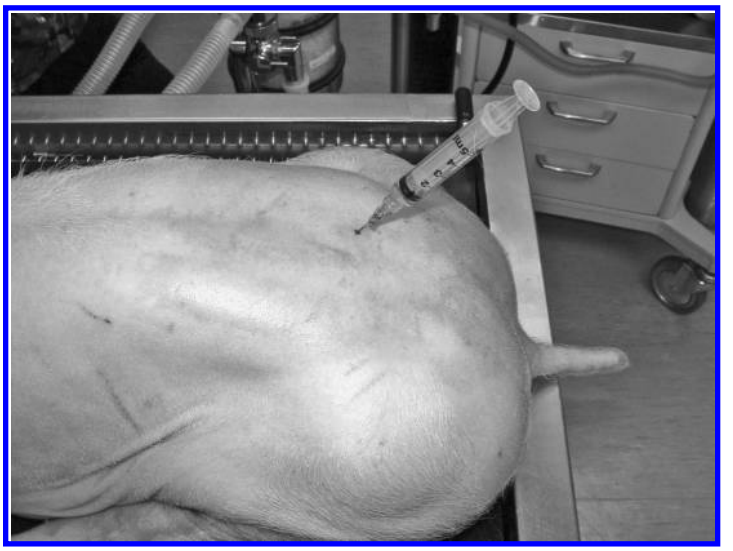

FIGURE 10.27 Epidural injection in the lumbar region.

rear legs off the end of a table while it is in sternal recumbency or by bending the rear legs forward under the abdomen in the same position.

An imaginary line is drawn between the most cranial aspects of the bilateral tuber coxae (wings of ileum), which are readily palpable. The intervertebral space cranial to this line will be L5–L6 or L6–L7. The needle is placed between the palpable dorsal spinous processes and advanced slowly through the intervertebral space until a popping sensation is felt and there is a lack of resistance. If the vertebral body is hit, the needle will not advance, and it should be withdrawn to try again. After entry into the site, the stylet is removed from the needle to ensure that blood or other fluid does not appear in the needle hub. The syringe is then attached, and the injection is given. There should not be any resistance to the injection if it is in the epidural space. Catheters can be passed into the epidural space by the same technique. Larger catheters may require a partial dorsal laminectomy to place them in this position.

CSF Collection

The area is prepped as per the directions above. CSF can be obtained from the lumbar region by use of the same technique and landmarks as described earlier. A greater volume can be obtained from the cisterna magna accessed through the foramen magnum (Figure 10.28). If this area is used, the pig's head is leaned off the end of a table to flex the neck. Alternatively, the pig may be placed in lateral recumbency and the neck flexed by an assistant. The caudal end of the occipital bone and the nuchal tubercles are palpated. The needle is passed slightly caudal to this area at an angle (approximately 60°) toward the oral cavity to enter the foramen magnum cranial to the body of the axis.

For CSF collection, the subarachnoid cavity is the site of interest. The spinal needle is passed into the epidural space as described earlier. Then a slight resistance is felt as the arachnoid membrane is penetrated. The stylet is removed from the needle and clear CSF fluid will drip from the needle if the location is correct. Passing the needle too deep will penetrate the spinal cord, and a reflex jerk will be observed. If blood comes from the needle, then either the venous plexus or a small artery has been hit. These problems should not occur if the needle is passed on the midline in the fashion described earlier. CSF catheters can be implanted chronically into the brain, using a burr hole in the cranium over the site of the central system in the brain (Figure 10.29). CSF catheters must be skillfully implanted to prevent infection and migration out of the burr hole. A technique of

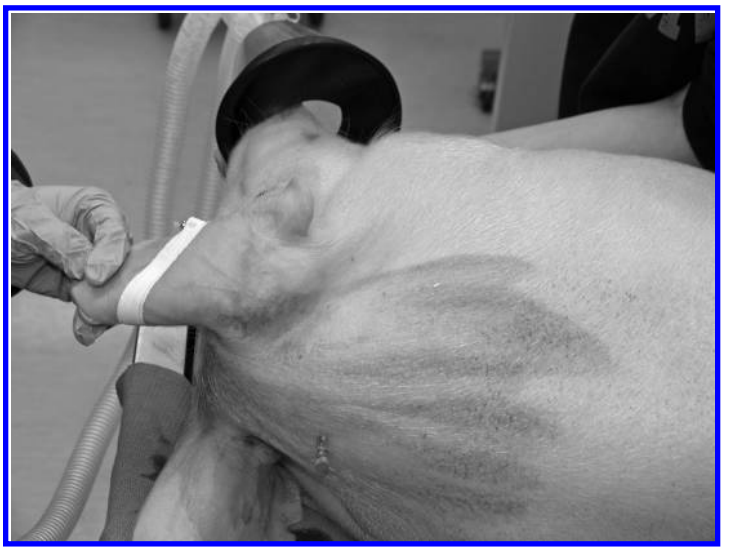

FIGURE 10.28 Spinal tap of the cisterna magna.

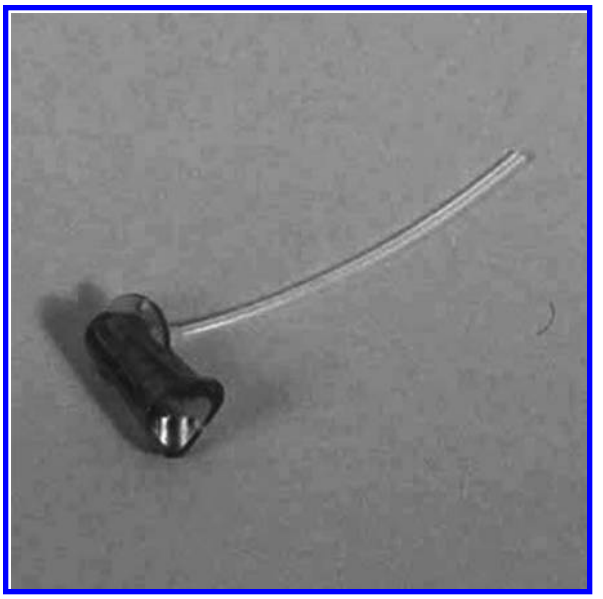

FIGURE 10.29 Cerebrospinal fluid catheter for chronic implantation. (Courtesy of Da Vinci Biomedical.)

short-term implantation of commercially available CSF catheters into the ventricular system of the brain has been described (Kaiser and Fruhauf, 2006). The authors implanted 40 kg farm pigs for a few days by implanting a catheter 1 cm paramedian from the sagittal suture caudal to the os frontale. A burr hole was made through the cleared tissues and the catheter inserted slowly until CSF was encountered. Bone cement was used to fix the catheter in place. Mean values of intracranial pressure (8 mmHg) and cerebral perfusion pressure (61 mmHg) were determined. The author used a similar technique to implant a CSF catheter chronically into the ventricle of immature minipigs for 6 months. During that time the frontal sinus extended caudally and completely enveloped the implanted catheter. Thus a modification of the technique which includes running a catheter caudally to avoid this issue needs to be made.

An exact calculation is not available but, generally, administration of <5 mL of solution or collection of 5–10 mL of CSF twice a week is not harmful to 25–50 kg swine.

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11 Musculoskeletal System and Orthopedic Procedures

Jan H. Duedal Rölfing and M. Michael Swindle

CONTENTS

ANATOMY AND TERMINOLOGY

While literature describing swine anatomy is comprehensive and amply illustrated, scientific evidence concerning the skeletal maturity of swine is sparse. An excellent atlas of the topographical anatomy as well as an extensive dissection atlas of the musculoskeletal system of the domestic pig has been published (Popesko, 2012; Sack, 1982; Sisson, 1975). Laboratory dissection manuals for the fetal pig (Chiasson et al., 1997; Donnelly and Wistreich, 1996) and a complete description of the anatomy and physiology of the musculoskeletal system in the domestic pig are also available (Novakofski and McCusker, 2001).

The term "bone" is ambiguous and may convey one of three different meanings. The first meaning, defined by the American Society of Bone and Mineral Research, is: *bone matrix including mineralized and not yet mineralized matrix* (*osteoid*). The second is: *mineralized bone matrix excluding osteoid*, and the third meaning is bone as a *tissue including bone matrix, bone marrow and soft tissue*; in the interest of terminological uniformity, the third meaning should be referred to as *bone tissue* (Dempster et al., 2013).

Bone can be subdivided into two structural types: *cancellous*, also known as trabecular, spongy or porous bone, and *cortical*, also known as compact bone. This classification is based on porosity and unit microstructure. In humans, *cancellous bone* is found at the end of long bones, in vertebrae

and in flat bones such as the bones in the pelvis. *Cortical bone* is found primarily in the shaft of long bones and forms the outer shell around cancellous bone at the end of joints and vertebrae.

Osteoid can be qualified as either *woven bone* with a random, disorganized orientation of collagen fibers resulting in weak mechanical properties, or *lamellar bone*, with aligned collagen fibers and strong mechanical characteristics. Woven bone is found in fetal bones and at the initial stages of fracture healing before remodeling into lamellar bone has taken place.

Unlike humans, swine and most other large animals have *plexiform bone* which is more rigid than human bone; its structure is a scaffolding of woven bone sandwiched with blocks of lamellar bone (Hillier and Bell, 2007). Nevertheless, bone regeneration, repair, and remodeling in pigs compare favorably with humans (Pearce et al., 2007; Turner, 2001). For in-depth information about bone structure, nomenclature, terminology, and abbreviations, refer to Dempster et al. (2013), Hillier and Bell (2007), and standard histologic textbooks.

The vertebral formula of swine is C7, T14-15, L6-7, S4, Cy20-23. Some miniature breeds may have one less thoracic and/or lumbar vertebra. There are seven sternal and seven asternal ribs. If a 15th rib is present, it is usually floating rather than being attached to the cartilage of the costal arch. The clavicle is absent. In the forelimb, there are eight carpal bones, four metacarpal bones (2–5) present. The third and fourth metacarpal bones carry the weight-bearing chief digits, while the second and fifth bear accessory digits. The chief digits comprise of three phalanges and three sesamoid bones. In the hind limb, there are seven tarsal bones, four metatarsal bones, and phalanges with sesamoid bones present (Sack, 1982; Sisson, 1975).

Human bone consists of 70% inorganic, 25% organic, and 5% water components. The inorganic component is predominately calcium phosphate hydroxyapatite. Type 1 collagen is 90% of the organic portion and the remaining 10% is a mixture of protein, proteoglycans, and phospholipids.

Cartilage is nonvascular and composed of chondroblasts synthesizing extracellular matrix, which is comprised of collagen fibers, elastin fibers and a ground substance rich in proteoglycans. Depending on the type and amount of fibers in the extracellular matrix, three types of cartilage are described. *Hyaline cartilage* is most abundant and covers bone and joint surfaces. *Fibrocartilage* is the strongest type of cartilage and provides support and rigidity. It has a limited distribution and is found in intervertebral discs, symphysis pubis, and certain joints. The third type, *elastic cartilage,* is of no particular interest in orthopedics, due to its location in the auditory tubes, external ear, epiglottis, and larynx.

All quadrupeds deviate from the bipedal human in terms of size, geometry and function of the cartilage, long bones and vertebrae (An and Friedman, 1999). Swine muscles have a predominance of Type II B muscle fibers (Figure 11.1) with a lesser extent of Type IIA, IIC, and IIX fibers. Locomotion characteristics of swine, as well as most other quadrupeds, are also dissimilar from those of humans (Adams, 1988; MacEwen, 1973; Salter, 1968). Colored images of muscle are located in the DVD attached to this textbook.

However, as the abundance of orthopedic porcine models indicates, many anatomical structures compare favorably to that of the human. For example, the porcine size and geometry of the knee (stifle joint), as well as cartilage thickness, more closely imitate the human analog than small animal models and canine, cattle or sheep (Ahern et al., 2009; Chu et al., 2010; McLure et al., 2012). Hence, the tibiofemoral cartilage is a common area of comparative modeling for studies involving cartilage healing and metabolism.

SKELETAL MATURITY

Original scientific publications regarding skeletal maturity of pigs are scarce and extremely difficult to obtain. As a consequence, literature abounds with secondary and circular references, and even "private communication with experts" without any coupling to the original publications. This practice should be avoided under any circumstances; instead, referencing original sources is imperative. The following critical review, while likely incomplete, strives to fulfill this prerequisite and clearly states when this goal is not achieved.

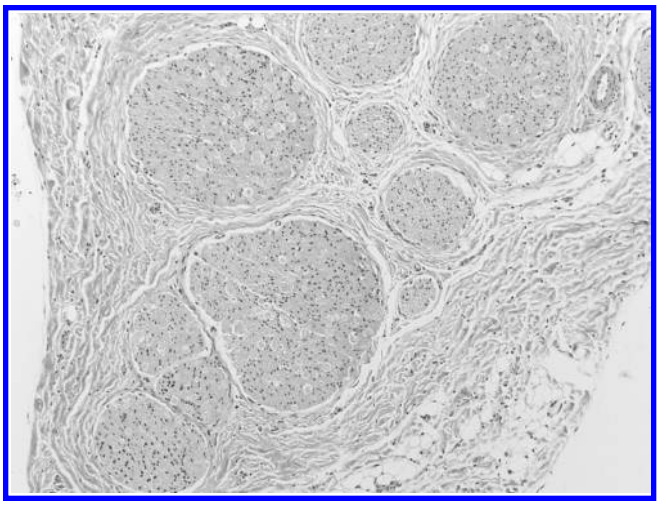

FIGURE 11.1 Histologic section of skeletal muscle and peripheral nerve. H&E, ×100.

Skeletal maturity defined as closure of the epiphyses of the long bones varies among breeds of swine. Dates of epiphyseal closure for some of the breeds of miniature pigs are: Hanford minipigs, 2–4 years; Yucatan minipig, 2–3 years and Yucatan micropig 1.5–2 years (Swindle, 2008: no original publications cited).

In 1975, Sisson published tables detailing the timing of epiphyseal closure in *the* pig. Note that "pig" is used generically and no subspecies named. While this work is not original either, the author refers to French and Italian studies dated 1951 and 1897, respectively (Bruni and Zimmerl, 1951; Lesbre, 1897). According to Sisson, these studies report the following approximate time points of epiphyseal closure: 1 year for the scapula, distal humerus, proximal radius, middle phalanx, and pelvis; 2 years for the proximal phalanx, distal metacarpal III, and distal tibia; 2.5 years for the distal fibula and calcaneus; and 3–3.5 years for the proximal humerus, distal radius, proximal and distal ulna, proximal and distal femur, proximal tibia and proximal fibula. Other bones, for example, the spine, were not described. The high correlation between the referenced studies indicates either high accuracy or citing of the same source. Because the publications could not be retrieved or accessed, originality of these studies could not be verified.

For Göttingen minipigs, information about closure of growth plates and skeletal maturity is readily accessible. However, these reports are contradictory as evidenced by the fact that age at closure of growth plates varies from 16–24 months up to 42 months (Minipigs.dk, 2010; Tsutsumi et al., 2004; Tsutsumi, 2012). After enquiry, the company admitted lack of scientific substance of the first publication and promised either to remove the illustrated graph from their homepage or alter it in accordance with the latter two publications. These publications report that closure of the growth plates of vertebral body L2 and femur begins at 21 and 25 months of age, respectively, and is completed at 42 months of age. Bone length increases until 21 months in the vertebra and until 28 months in the femur. Tsutsumi stresses the fact that epiphyseal closure in this strain of minipigs occurs at 14%–23% of life expectancy. This timing correlates closer to the human time point of growth plate closure and thus better mimics bone growth velocity than competitive large animal models, for example, dogs, sheep, or cynomolgus monkeys (Sisson, 1975). Tsutsumi concludes that epiphyseal closure in female Göttingen minipigs occurs later than in other miniature swine, but this conclusion was drawn from yet another circular reference and therefore probably incorrect (Tsutsumi et al., 2004).

In the absence of more compelling evidence for determination of skeletal maturity in the different strains of swine, surrogate markers other than epiphyseal closure may be considered. Peak bone mass defined as the amount of bone present at skeletal maturity is less precise. In humans,

the increase in peak bone mass after epiphyseal closure continues for almost a decade, albeit at a slow pace (Matkovic et al., 1994). Peak bone mass is reached at 6.3 years in Göttingen minipigs (Tsutsumi et al., 2004). In Sinclair minipigs, growth plates of lumbar vertebrae L2-L4 were open at a mean age of 1.2 years, and found closed when examining pigs at 4.8 years of age (Borah et al., 2000). Because investigation took place only at these two time points, closure timing cannot be exactly determined from this study. Also in Sinclair (S-1) miniature swine, bone mineral content and bone mineral density of lumbar vertebrae L1–L4 increased until 3–4 years of age. Based upon these findings, Bouchard et al. (1996) concluded that adulthood is reached between 2.5 and 3 years of age. The degree of mineralization of cancellous and cortical bone in the mandibular condyle of domestic pigs (*Sus scrofa domestica*) reaches a plateau at 40–60 weeks (Willems et al., 2010).

In conclusion, the scientific evidence regarding skeletal maturity is not persuasive. Yet, the essential significance of skeletal maturity is highlighted in several papers, for instance Murray et al. (2010) showed that the intrinsic healing potential after anterior cruciate ligament decreases with age. Therefore, unless studying developmental or deformity models, the studied animals should be as old as possible, and the condition of adjacent growth plates should be reported.

ORTHOPEDIC RESEARCH MODELS IN SWINE

A carefully chosen, reliably reproducible and appropriate large animal model for the study of a given interest is the final step before conducting clinical trials. The requirements for an ideal animal model are "convenience, relevance (comparability to the human condition), and appropriateness: a complex of other factors that make a given species the best for studying a particular phenomenon" (Turner, 2001). Hence, the following considerations should influence the choice of animal model: "(1) appropriateness as an analog, (2) transferability of information, (3) genetic uniformity of organisms where applicable, (4) background knowledge of biological properties, (5) cost and availability, (6) generalizability of the results, (7) ease and adaptability to experimental manipulation, (8) ecological considerations, and (9) ethical and societal implications" (Turner, 2001).

In comparison to other large animals, swine possess several advantages in relation to testing novel orthopedic implants and treatment strategies on the road to clinical translation. Swine are readily available and inexpensive; their bone remodeling and bone regeneration rate of 1.2–1.5 μm/day is comparable to the human bone regeneration rate of 1.0–1.5 μm/day (Ahlmann et al., 2002; Laiblin and Jaeschke, 1979; Pearce et al., 2007; Schmitt et al., 2013). Limitations include differences in mechanical loading due to the skeletal axis of quadrupeds compared with humans and nonhuman primates, as well as late skeletal maturation. The drawbacks of late skeletal maturity and massive size of the domestic pig have led researchers to use miniature swine for orthopedic research (Bendtsen et al., 2011; Ebihara et al., 2012; Murray et al., 2010). Because they are bred and stabled under highly controlled conditions, these breeds are more expensive than their domestic counterparts. Hence, health information and specific-pathogen-free animals are available. Furthermore, a more genetically homogeneous study population is less likely to mask minor but possibly relevant differences between study groups. Limiting the study population to either female or male pigs presumably diminishes biological variability. In Göttingen minipigs, the female is associated with significantly higher bone volume and bone density (Bollen et al., 2006).

For chronic longitudinal studies, miniature breeds are preferable to large domestic breeds; the domestic farm pig's rapid growth and increased body weight are detrimental to performing chronic procedures. "Relative Body Weights and Growth of Domestic and Miniature Pigs" are provided in Table 1.1. Selection of the appropriate breed of swine should depend upon the goals of the study and local availability.

Further information about benefits and shortcomings of swine in orthopedic research can be found in two valuable reviews (Pearce et al., 2007; Turner, 2001). In conclusion, recent advances in skeletal tissue engineering and tissue regeneration make the need for reliable, reproducible large

animal models for preclinical testing more and more imperative. Swine, and miniature swine in particular, should be considered when choosing large animal models for validation of encouraging *in vitro* or small animal data.

Scientific reproducibility and use of unambiguous terminology is a cornerstone of research. Therefore, adherence to well-defined and recognized guidelines is of paramount importance both when planning evaluation and methodology and when reporting findings. *The Journal of Bone and Mineral Research* provides authors with compulsory guidelines regarding bone histomorphometry and micro-computed tomography (μCT) (Bouxsein et al., 2010; Dempster et al., 2013). The Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guideline includes a checklist for authors to consult before submitting manuscripts, but the guideline should also be considered—and its criteria met—throughout the entire research process (Kilkenny et al., 2010).

Analgesia and Veterinary Care

All invasive orthopedic procedures should be considered capable of causing significant pain. Therefore, preemptive analgesia is required for all procedures. This could include administration of systemic opioids or NSAIDs, local anesthetic administration, and/or epidural analgesic administration.

For procedures involving major muscular manipulations, the use of NSAIDs in combination with buprenorphine has been found to be superior to the use of either agent on its own. Although NSAIDs have been implicated in delay of cartilage and bone healing, it is unlikely that this would be significant with short-term use as preemptive and postoperative administration for a few days (Dodwell et al., 2010; Kurmis et al., 2012). However, the use of opioids and local anesthetics should be planned for chronic studies. Infusion pumps may be utilized to provide targeted infusion of surgical sites with local anesthetics.

Swine are relatively inflexible and may have difficulty with locomotion following some procedures. In this case, padded cages may be necessary for providing relief. The veterinary staff should be consulted in advance of these types of procedures in order to plan for alleviation of potential postoperative complications.

Guidelines for veterinary care and local regulations must be followed in order to fulfill the obligation to plan and conduct animal experiments in accordance with the highest scientific, humane, and ethical principles. For U.S.-based readers "Guide for the Care and Use of Laboratory Animals," 2011 and for European readers "Guidelines for the veterinary care of laboratory animals: report of the FELASA/ECLAM/ESLAV Joint Working Group on Veterinary Care," 2008 provide an overview.

Several standardized porcine models for testing of novel treatment strategies and implants exist. A brief introduction to the available models and key references is given below.

Developmental and Deformity Models

The pig's already-described rapid growth from birth to sexual maturity may be conducive to the study of musculoskeletal development. The proportion and distribution of muscles and bones is similar to humans. Also, the closure of the epiphyseal plates provides a mechanism for studying effects of treatments on epiphyseal development in a relatively short period of time in contrast to humans, where closure does not occur for several decades (An and Friedman, 1999). Epiphysiodesis as a means for correction of limb deformities in children can also be studied in swine; hemiepiphysiodesis is achieved either with the Blount stapling technique, wire loops, or tension-band plating with similar results (Burghardt et al., 2011; Gottliebsen et al., 2013).

Femoral cortical bone has been studied extensively for its structural characteristics and their changes with age in swine at 6, 12, and 42 months of age (Feng and Jasluk, 2011). Lamellar bone was most predominant at 6 and 12 months. Resorption sites as an indicator of bone remodeling

were more prominent in younger animals. Secondary osteons were more prevalent in the older group and both mineral content and mineral–organic ratio increased with age. The changes during aging corresponded to increased elastic modulus and tensile strength. In another study, the majority of changes in the number of mature collagen cross-links and mineralization take place prior to 40 weeks of age (Willems et al., 2010). Injury biomechanics in parietal bones and coronal sutures were studied in 3- to 21-day-old piglets and it was found that days of bone maturity in pigs correlated to months in infants <18 months of age (Baumer et al., 2009). Porosity and bending stiffness increased with age.

Scoliosis models in swine exist. Despite differences in the vertebral axis of swine compared with humans, a three-dimensional deformity mimicking the human condition can be achieved. Both non-invasive and operative techniques have been described, usually including unilateral tethering. Sagittal deformity estimated with Cobb angles ranged from 30° to 60° after 3 months in these models. Lordosis and rotation were also affected, hence allowing the study of new instrumentation and treatment options in a setting simulating the three-dimensional deformity in humans (Accadbled et al., 2011; Chay et al., 2012; Odent et al., 2011; Schwab et al., 2009).

Orthopedic Tissue Engineering Models

Healing of long bones and craniofacial bones has been studied in swine and in recent years there has been increased interest in this area. (Aparicio et al., 2011; Doll et al., 2001; Endres et al., 2008; Lethaus et al., 2010; Spies et al., 2010). Many of these studies address issues of healing in the presence of implants and grafts, for example, biocompatibility and ingrowth abilities. Inherent in most studies of tissue defects is the size of the lesion. Critical-sized lesions are those that would not heal on their own without such interventions as grafts. Critical size defects vary with anatomical location and physical characteristics of the pig. For example, wound healing would be expected to occur more rapidly in immature animals. Standardized critical size defect models for the calvaria and long bones have been described in detail (Figure 11.2; Jensen et al., 2014; Rölfing et al., 2014; Schmitt et al., 2013; Spies et al., 2010).

Cartilage regeneration can also be studied in swine. The International Cartilage Repair Society has published recommendations regarding preclinical studies and advocates the use of pigs for pivotal studies once proof of principle has been established in small animal models (Hurtig et al.,

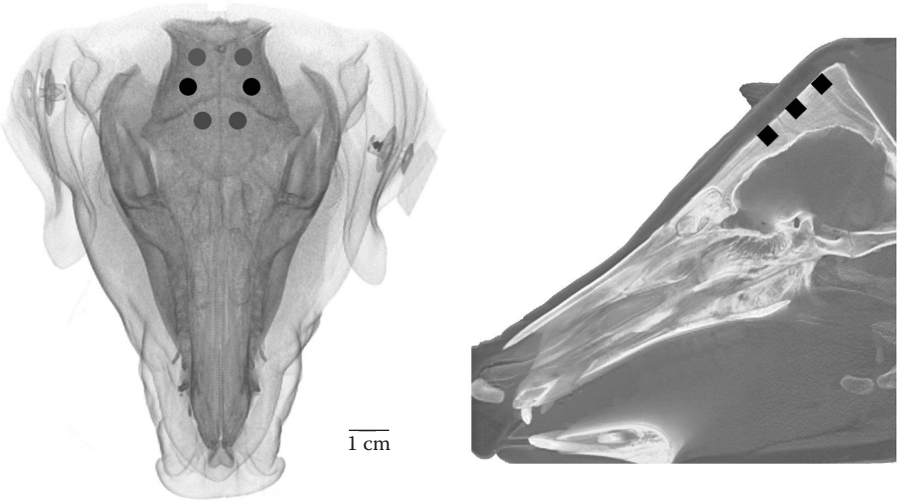

FIGURE 11.2 Position of six bone defects (Ø 10 mm, $h = 1$ cm) in the frontal and parietal bone of a pig ((Danish Landrace × Yorkshire) × Duroc).

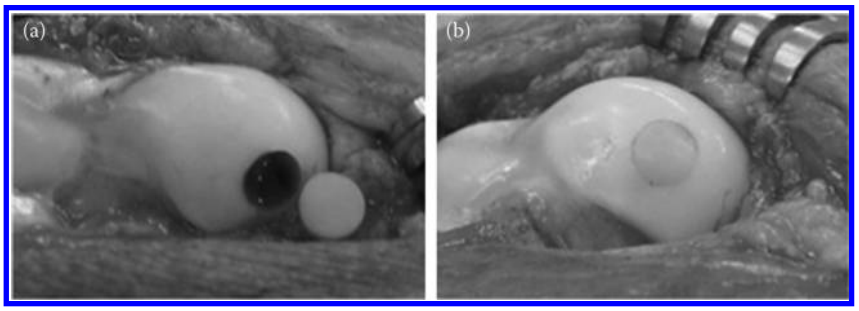

FIGURE 11.3 Weight-bearing implants for cartilage repair in femoral condyles. (a) Excised cartilage, (b) implant after insertion.

2011). Because of the similarities between human and porcine cartilage as described above, osteochondral defects are a major area of study (Cui et al., 2011; Gotterbarm et al., 2006; Peretti et al., 2006; Petersen et al., 2008; Vasara et al., 2006). The cartilage within the femoral–tibial joint is the predominant area in which procedures are performed (Figure 11.3). Many of the studies involve creating circular punch defects on the articular surface after surgical approaches through the cranial-lateral aspect of the joint. Graft and lesion sizes vary considerably in the literature, however, as a general rule, the larger the defect and graft the higher the failure rate. In mature animals, osteochondral lesions of 5–7 mm in diameter are probably the critical size (Gotterbarm et al., 2006). Non-weight bearing cartilage lesions can be created in the patellar cartilage. Uniform, standardized positioning of the joint is important when acquiring MRI scans, because flexion, alignment and loading of the stifle joint (knee) have significant impact on T_2, T_2^* measurements (Shiomi et al., 2010a,b). The porcine knee has also been utilized for studies of ligament repair and replacement (Black et al., 2000; Yamanaka et al., 1999).

Osteoporosis and osteopenia have also been studied in swine. (Ahn et al., 1997; Mosekilde et al., 1993; Seiler et al., 1996; SinclairResearch.com, 2013; Swiontkowski et al., 1993; Tatara et al., 2008). These models utilize the spinal vertebrae and the femoral head and neck. Osteopenia of the axial skeleton and long bones was created by means of gastric fundectomy after 8 months (Tatara et al., 2008). In Sinclair minipigs, a model of osteopenia has been produced by restricting dietary calcium <0.37% with ovariectomy (Mosekilde et al., 1993; SinclairResearch.com, 2013). Bone loss occurs rapidly—within 2 months. Bone weight, bone mineral density, and bone mineral content of the vertebrae and long bones were significantly decreased during the postoperative period in the fundectomized pigs.

Trauma, Infection, and Transplantation Models

Osteonecrosis of the femoral head can be induced via femoral neck injury with an osteotome and wire-banding simulating a fracture with reduced blood flow (Swiontkowski et al., 1993).

Healing of traumatic soft tissue lesions including burn and blast injuries, and the feasibility of porcine models for the study thereof were highlighted in several studies (Christensen et al., 2012; Dai et al., 2011; Gaines et al., 2013; Li et al., 2012; Singer et al., 2000; Sullivan et al., 2001; Wang et al., 2010).

Tendons and their biomechanics, optimal suturing technique, and surgical reconstruction can also be studied in pigs. Notably, reports about ligaments of the knee and tendon repair have been published (Duenwald-Kuehl et al., 2012; Sakaguchi et al., 2012; Viinikainen et al., 2008).

Osteomyelitis can be studied by injecting *Staphylococcus aureus* into the blood stream (Jensen et al., 2010). Localized infections can be produced by injection of the same or similar organisms into surgical lesions or the arterial supply to the bone of interest (Johansen et al., 2011).

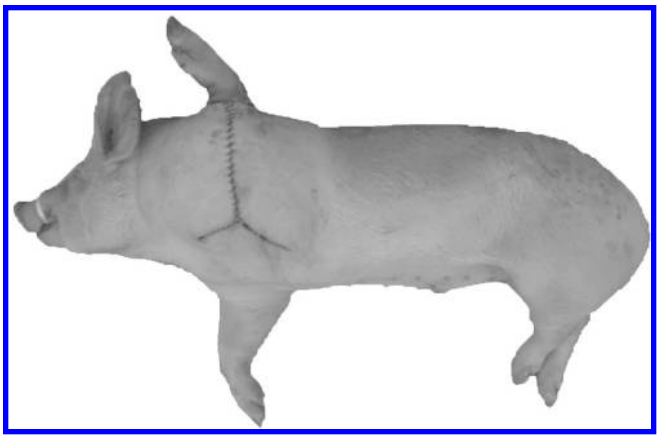

FIGURE 11.4 Heterotopic limb transplantation into a subcutaneous pocket with vascular anastomoses to the axillary vessels. (From Kiermeir, D.M. et al. 2013. *Microsurgery.* 141–147. 2012. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)

Exploration of traumatic amputation and treatment regimens has been conducted in porcine models (Constantinescu et al., 2010). Transplantation of vascularized allografts such as a gracilis myocutaneous flap or entire limbs are possible (Figure 11.4; Kiermeir et al., 2013; Leto Barone et al., 2013; Mathes et al., 2003).

Xenotransplantation, for example, the transplantation of porcine cells, tissues or organs into humans for the treatment of tendon, cartilage, muscular and bone lesions, is another area of increased interest (Laurencin and El-Amin, 2008). Porcine-derived bone and cartilage scaffolds have been employed for tissue engineering, isolation of bone marrow stem cells, and osteochondral composites. Furthermore, Food and Drug Administration-approved tendon augmentation grafts are commercially available. It is likely that swine will continue to be a target animal for development of xenotransplantation into humans in these fields.

Spinal Models

The appropriateness and limitations of porcine spinal models are discussed above. Similarities and differences between the spine of large animals and humans were made available (Sheng et al., 2010; Yingling et al., 1999).

Spinal fusion models via anterior and posterolateral approaches are highly utilized and have been validated (Alitalo, 1979; Christensen, 2004; Foldager et al., 2008; Li et al., 2002, 2004; Zou et al., 2004).

Biomechanics and kinematics, including the necessity of standardized measurements hereof, are described in detail (Beckstein et al., 2008; Kaigle et al., 1997; Keller et al., 1990; Showalter et al., 2012). The porcine spine has been used to test pedicle screw placement, fixation, and its effects if the neural elements of the spine are contacted by the screws (Lewis et al., 2001; Xue et al., 2010).

Models mimicking intervertebral disc degeneration (IDD) are numerous. IDD can be induced either by a stab wound of the annular fibrosus, nucleotomy, or chemically via injection of chondroitinase ABC and chymopapain (Bendtsen et al., 2011; Leckie et al., 2012; Omlor et al., 2009; Pfeiffer et al., 1994; Yoon et al., 2008).

Minimally invasive approaches, and recently robot-assisted techniques as well, can be learned and their safety determined (Olinger et al., 1999; Yang et al., 2011). For scoliosis models, please refer to the paragraph above: Developmental and Deformity models. For animal models concerning spinal cord and nerve root, see Chapter 10.

Oral and Facial Models

Orofacial and dental researchers have also utilized swine and, in particular, the miniature pig for large animal studies (Wang et al., 2007). For example, it was shown in a comparative macroscopic study that the temporomandibular joint (Chapter 10, Figures 10.8 and 10.10) of swine is more similar to humans than many other species (Bermejo et al., 1993). The authors concluded that the pig was an appropriate animal model for the study of temporomandibular joint abnormalities. The pig has a reciprocally fitting meniscotemporal joint and a condylomeniscal joint of the condylar type. The size of the articular structures, the shape of the meniscus, and the omnivorous chewing characteristics of swine provide additional justification for the use of this model over that of the rodents, rabbits, carnivores, and herbivores that were examined. Please refer to Chapter 10 for further information on head and neck surgery.

SURGICAL APPROACHES TO BONES AND JOINTS

Surgeons can determine their surgical approaches (Figures 11.5 and 11.6) to bones and joints of the pig by means of their knowledge of other species, anatomic inspection and palpation, and radiographs. Surgical approaches that avoid major muscle dissection and vascular ligation to the major bones and joints are described here. Variations of these procedures may be dictated by the experimental protocol. Consulting an anatomical atlas while reading the specific surgical approach is advised (Novakofski and McCusker, 2001; Popesko, 2012; Sack, 1982).

In general, muscles may be transected, dissected between muscular fibers, or preferably divided in the fascial plane between muscle bellies. The fascial sheath surrounding the muscle provides the predominant strength for holding sutures and must be included when suturing surgical incisions. Sutures may pass all the way through a muscle body, and the sheath on both sides of the muscle or the muscular sheath can be sutured by itself (Swindle, 1983).

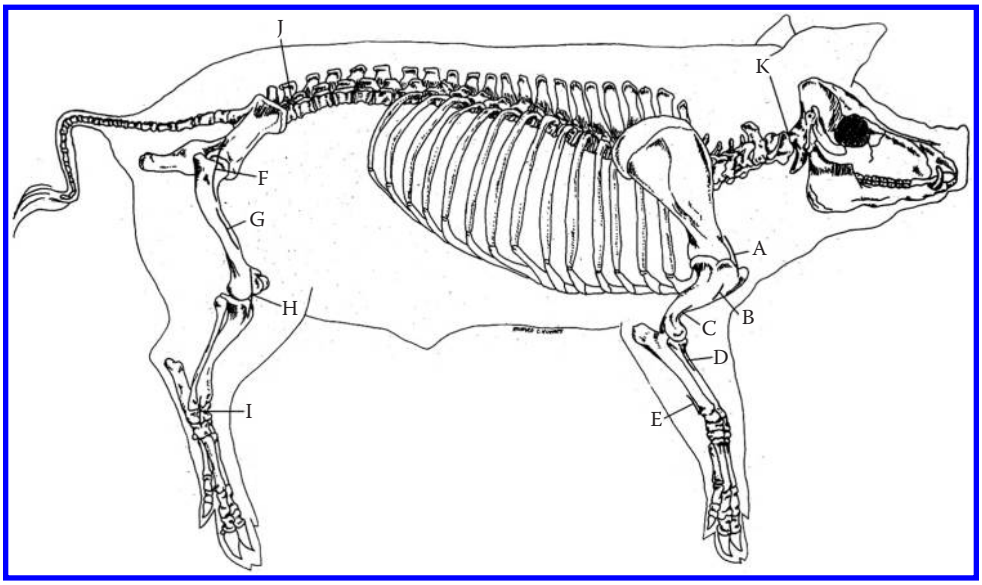

FIGURE 11.5 Surgical approaches to the bones and joints. A—shoulder joint, B—proximal humerus, C—distal humerus, D—proximal radius, E—ulna, F—hip joint, G—femur, H—stifle joint (knee), I—tarsocrural (hock) joint, J—subarachnoid space injection site, K—cisterna magna injection site.

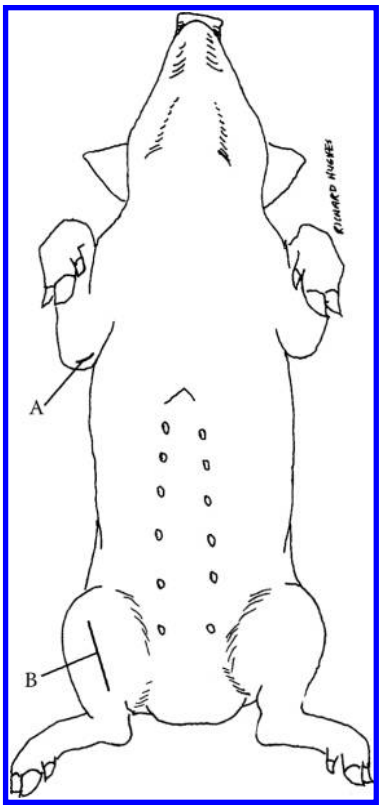

FIGURE 11.6 Surgical approaches to the bones and joints. A—medial aspect of the elbow. B—medial approach to the tibia.

Foreleg

The shoulder joint is approached in the craniolateral aspect after flexion. The greater tubercle of the humerus is prominent, and the caudal part is readily palpated. The incision is made between the cranial and caudal portions of the tubercle alongside the infraspinatus tendon. The infraspinatus tendon may have to be transected at its attachment in order to be retracted caudally. The infraspinatus nerve should be avoided.

Because of the massiveness of the musculature, the humerus is not readily approached surgically. Surgical approaches from the lateral aspect will probably involve transecting musculature. The proximal shaft can be approached between the brachiocephalic muscle and the distal portion of the deltoid muscle. The distal portion of the shaft can be approached between the brachiocephalic and biceps brachii muscles cranially, and the lateral head of the triceps caudally. The radial nerve and deep branches of the brachial vessels should be avoided. The medial aspect is even more difficult to approach because of the close attachment of the leg to the trunk at that level. The distal shaft can be approached between the biceps brachii and the medial head of the triceps. The brachial artery and vein, and the ulnar nerve should be avoided.

The elbow joint is approached from a medial aspect between the medial epicondyle of the humerus and the tuber olecrani of the ulna. The tensor fasciae antebrachii muscle will have to be partially transected, and the medial head of the triceps reflected. Laterally, a surgical approach can be made by reflecting the lateral head of the triceps proximally, and subsequent incision of the anconeus muscle.

The radius can be approached from a lateral incision between the common digital extensor and the ulnaris lateralis. The distal shaft of the radius can be approached medially between the extensor carpi radialis and the pronater teres with caudal reflection of the digital flexors. The radial artery and vein, and the median nerve should be avoided.

The ulna is more accessible from the caudal aspect between the deep digital flexor and the ulnaris lateralis. Even in large swine, the bones and joints of the distal limb can be readily palpated and are easily approached from the cranial aspect.

Hind Limb

The approach to the femoral head and neck and acetabulum is difficult even in small pigs. The trochanter major can be palpated with difficulty below a line drawn from the tuber coxae and the tuber ischiadicum. A curved incision starting above this area and following the line of the femur is made to expose the juncture of the gluteus medius, the gluteus superficialis, and the tensor fasciae latae. Dissection through this juncture of fascia and tendinous attachments with retraction of the vastus lateralis and biceps femoris caudally will expose the greater trochanter and cranial aspect of the hip joint. Depending upon the experimental procedure, full exposure of the femoral head and neck and the joint will require cutting the tendinous attachments of these muscles and further deep dissection.

The femoral shaft can be exposed by an incision following the lateral aspect of the bone and dissecting between the fascia of the tensor fascia latae and the biceps femoris.

The stifle joint (knee) is approached from a craniolateral incision after flexion. The incision is made lateral to the patella and the patellar ligament through the infrapatellar fat pad. The patella can be displaced laterally if required. The tibia is subcutaneous on the medial aspect and can be surgically approached at any level with minimal difficulty. The medial saphenous artery, vein, and nerve should be avoided.

The tarsocrural joint (hock) can be approached in either the lateral or medial aspect of the cranial border of the calcaneus. The malleolus of the fibula and the tuber olecrani can be palpated as landmarks, and the incision line should be between them. On the lateral aspect, the saphenous vein should be avoided. On the medial aspect, the collateral ligaments and tendons are more prominent. The digits may be approached as described for the foreleg and the amputation previously described.

Vertebral Column

The bones of the vertebral column of the pig are massive compared to many other species, and the intervertebral spaces are relatively narrow. With the pig in sternal recumbency, the vertebra can be approached from a dorsal midline incision as in other species (Haq et al., 2005; Li et al., 2002, 2004; Zou et al., 2004). In general, this involves stripping the paravertebral muscles and retracting them laterally. A paramedian abdominal incision with a retroperitoneal approach has also been used (Zou et al., 2004). A laminectomy can then be performed to expose the spinal cord. Detailed studies of spinal cord fusion have been performed (Christensen, 2004). The Göttingen minipig was used for chronic studies because of its well-defined vertebral pedicles, which make screw-type techniques feasible, and because of its early growth plate closure. Using posterior-lateral techniques, fusion can be achieved in 3 months with this model.

A ventral midline approach can be made to the cervical and lumbar vertebrae. For the cervical vertebrae, the trachea and esophagus must be directed laterally and care must be taken not to damage the vagal sheath. The lumbar vertebrae can be approached from a ventral midline incision (Alitalo, 1979). The viscera must be retracted and packed off with laparotomy sponges within the abdomen. The aorta, vena cava, and branching vessels must be retracted laterally to expose the

vertebrae underlying the ventral longitudinal ligament. Care should be taken to not damage the lymphatics in the region or to ligate them if they are transected.

Injections into the subarachnoid space can be made between the last two lumbar vertebrae (see Chapter 10 for a complete description of the techniques). The lateral wings of L5 are at the level of the tuber coxae. More cranial positions are difficult because of the conformation of the spinous processes of the vertebrae and the difficulty in flexing the vertebral column. The pig is positioned with the hindquarters hanging over the end of a table to flex the vertebrae. A spinal tap needle is slowly inserted until either cerebrospinal fluid drips from the needle or the position is confirmed radiographically (Boogerd and Peters, 1986; Punto, 1980). The cisterna magna can be accessed by flexing the head off the table or over a sandbag. The space is narrow, and the needle should be directed cranioventrally until access is confirmed by dripping of cerebrospinal fluid.

TENDON AND LIGAMENT REPAIR

The major tendons that are substantial in size and accessible through superficial surgical dissection are the common calcaneus and the popliteus. The digital extensor and flexor tendons are also readily accessible on the distal portion of the metacarpal and metatarsal bones, and, if small to midsized tendons are preferred, on the cranial and palmar surfaces of the digits. The patellar ligaments are the main superficial ligaments. These tendons and ligaments may be used in wound-healing studies and may also require clinical repair owing to trauma, especially of the digital extensor and flexor ligaments.

Methodology for suturing the ligaments is the same as for other species and has been described in the pig (Swindle, 1983). The common calcaneal tendon includes the tendons of the superficial flexor and the larger oval gastrocnemius tendon. It is approached with a caudal vertical incision along the distal end of the tibia ending at the tuber calcis. The popliteal tendon is approached using a similar incision along the caudal aspect of the humerus ending at the olecranon. The digital tendons can be palpated on the distal aspect of the legs and direct incisions made over them. The patellar ligaments can be approached using a vertical incision along the cranial aspect of the stifle joint (knee), with the ventral patellar ligament being the most accessible.

After transecting the ligament or tendon, the edge is grasped with forceps and repaired using the Bunnell-Mayer technique (Figure 11.7). Starting well back from the edge of the incised tendon, a transection suture is placed through the tendon from side to side, using a double-armed suture with nonabsorbable material. The ends of the suture are left equidistant from the tendon. In an alternating

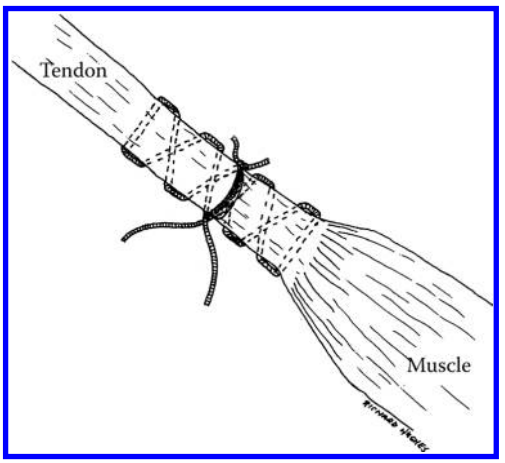

FIGURE 11.7 Bunell-Mayer's suturing technique for repair of a tendon.

pattern, the needles reenter the tendon at a 45° angle aimed distally. Sutures are continued distally in this crossing pattern until the outer edges of the severed tendon are exited. The same suture pattern is then placed in the distal end of the severed tendon. The ends of the sutures are tied along the lateral and medial edges of the tendon at the incised edge. As an alternative to Bunnell-Mayer, Kessler's locking loop, Yotsumoto-Dona or Krackow technique may be applied (Viinikainen et al., 2008). For an extensive review and additional suture patterns, the reader is referred to Viinikainen.

The subcutaneous tissues and skin are closed in a routine fashion. Postoperatively, the limb may require immobilization in a plaster cast or require some other form of support for a short time.

BONE MARROW ASPIRATION

Bone marrow aspiration to collect samples may be performed with standard bone marrow aspiration needles. The medial aspect of the proximal tibia, approximately at the level of the tibial crest, is a suitable site for obtaining bone marrow samples in all ages of swine (Figure 11.8). There is a concave depression just distal to the tibial crest which is the preferred site for collection because it has thin cortical bone which is easily penetrated. Bone marrow in the long bones tends to become fatty in most breeds at sexual maturity. Bone marrow may also be aspirated from the dorsal aspect of the tuber coxae (Figure 11.9) and from the midsternum (Figure 11.10). Sternal samples are best obtained from neonatal and juvenile animals. After aseptic preparation of the skin, the bone marrow aspiration needle with a stylet is inserted through the skin and muscle to the bone. It is then rotated back and forth while exerting forward pressure. A popping sensation will be felt when the needle penetrates the cortex. The stylet is removed and the sample is collected with a syringe containing saline or preservative. The needle is removed when the sample is obtained. The same technique can be used to inject substances into the bone marrow. Bone marrow injections are absorbed in a similar manner to i.v. injections (Laber-Laird and Swindle, 1996).

AMPUTATION OF A DIGIT AND HOOF TRIMMING

The principal digits of the pig are numbers III and IV. Vestigial digits, numbers II and V, form the dewclaws, which are positioned caudal to the principal digits. All terminate in hooves that may periodically have to be trimmed with hoof nippers on swine that are not housed on concrete floors or

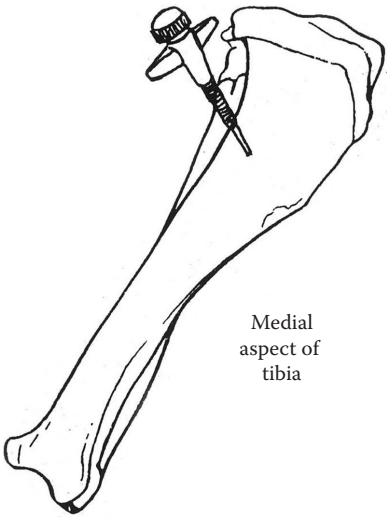

FIGURE 11.8 Bone marrow aspiration site in the medial aspect of the tibia.

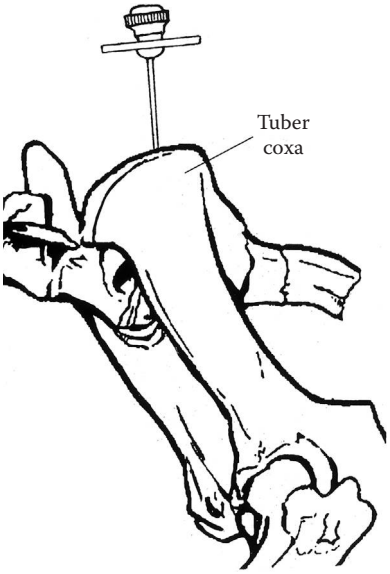

FIGURE 11.9 Bone marrow aspiration site in the tuber coxae (iliac crest).

rough material that allows constant wear of the hoof (Figure 11.11). As the dewclaws are non-weight bearing, their amputation does not result in any consequential debility to the pig. If the principal digits become injured or infected, they may require amputation. The pig will retain its locomotive ability as long as one of the pair is retained intact.

The digit is amputated in the joint either between the metacarpal bone and the proximal phalanx or between the proximal and middle phalanx, depending upon the extent of the lesion. A horizontal incision is made across the dorsal aspect of the digit distal to the joint in which the amputation is to be performed. Two vertical incisions are made up the sides of the digit extending to the ventral surface of the joint in which the amputation is being performed. The dorsal flap is then bluntly dissected to the surface of the joint. The branches of the digital veins on the dorsal surface are ligated and the tendons severed at their insertions on the bones. A flap is then dissected on the palmar surface of the digit and the vessels and tendons are similarly transected. The skin on the palmar surface is transected horizontally cranial to the joint. The joint is then disarticulated and amputated. The tendons and muscles are trimmed and tacked down across the ventral surface of the joint with stay sutures. The ventral aspect of the dorsal flap is sutured with simple interrupted sutures to the skin on the palmar surface of the joint. This will leave a skin flap that covers the joint from the dorsal to the palmar aspect of the joint.

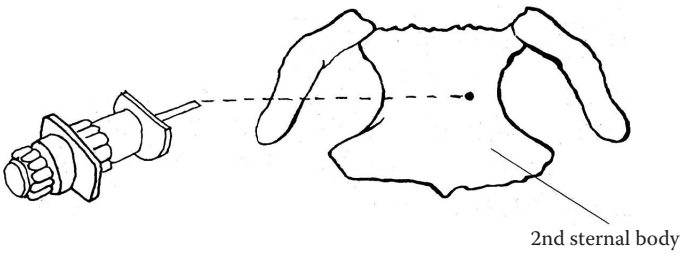

FIGURE 11.10 Bone marrow aspiration site in the second sternal body.

FIGURE 11.11 Trimming a hoof with hoof nippers.

The foot is bandaged leaving the intact digit uncovered. The animal should be housed in clean dry bedding, and the bandage changed daily for the first few days following surgery.

TAIL AMPUTATION

Swine routinely traumatize the tails of less dominant animals when housed in groups. This may require corrective surgery. The tail is amputated between the bodies of the coccygeal vertebrae in the joint. It is best to amputate the entire length of the tail to prevent further trauma.

A V-shaped flap is made with the long ends of the flap on the dorsal and ventral aspects of the tail. The blood vessels along the ventral and lateral surfaces of the tail are identified and ligated. The tail is disarticulated in the joint, and the flap is sutured using a simple interrupted pattern.

HERNIA REPAIR

Both umbilical and inguinal hernias occur spontaneously in swine. Inguinal hernias and retained testicles are more common on the left side (St-Jean and Anderson, 1999). Both conditions have a genetic predisposition.

For inguinal hernias, the pig is placed in dorsal recumbency. A skin incision is made directly over the inguinal canal, which can be readily palpated on a line drawn between the mid to cranial aspect of the cranial surface of the thigh and the midline. After the skin incision is made, the edges of the femoral ring are bluntly dissected away from the herniated tissues, which typically include omentum and fat without the intestines. The spermatic cord should not be damaged in this process. After the herniated tissue is free from attachments, it is bluntly replaced into the abdominal cavity. It may have to be held in place with a blunt instrument, or the pig's hindquarters directed upward, to move the herniated tissues and viscera away from the surgical site. The muscles surrounding the inguinal ring are sutured together with simple interrupted sutures using 2/0 or 3/0 nonabsorbable synthetic suture material. The inguinal canal should not be sutured so tightly as to constrict the blood flow to the blood vessels in the spermatic cord. The skin and subcutaneous tissues are closed in a routine manner.

An inguinal hernia may be created experimentally in small swine by making an incision over the inguinal ring and incising the muscles on the cranial edge of the ring (Garcia-Ruiz et al., 1998). This relaxation of the ring should lead to herniation postsurgically and can be tested at the time of surgery by applying pressure to the abdomen to cause the viscera to herniate.

Umbilical hernias are usually closed around herniated omentum and fat, and are rarely clinically relevant. The umbilical hernia may be closed after making a skin incision directly over the herniation and by either replacing or excising the eviscerated material. The umbilicus is closed with nonabsorbable sutures as described previously.

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12 Cardiovascular Catheterization, Electrophysiology, and Imaging Laboratory Procedures

Daniel D. Myers Jr., Patrick Lester, Marisa L. Conte, and M. Michael Swindle

CONTENTS

INTRODUCTION

Swine have been used extensively in procedures involving the use of cardiovascular catheterization laboratories, fluoroscopic imaging, and interventional radiology techniques (Gaymes et al., 1995; Smith et al., 1989; Swindle et al., 1992, 1994). The anatomy and surgical procedures involving the cardiovascular system have been discussed in Chapter 9. The relationship of the vessels entering and exiting the heart is illustrated here (Figure 12.1). Recommended anesthetics for cardiovascular protocols and methods of prevention of cardiac arrhythmias are discussed in Chapter 2. The purpose of this chapter is to provide practical guidance to the use of swine in catheterization and fluoroscopy laboratories. Atherosclerosis is also discussed in this chapter because of the close relationship of angioplasty techniques to the study of that technique. Additional images and videos of some of the techniques described in this chapter are included on the DVD attached to this book.

PERIPHERAL VASCULAR ACCESS FOR CARDIOVASCULAR CATHETERS

Access to the femoral and neck vessels (see Chapter 9, Figures 9.39 through 9.51) via surgical cut down procedures has been discussed in detail in Chapter 9. The technique of catheterization of these vessels using percutaneous techniques (Seldinger technique) (Figures 12.2 through 12.8) for cardiovascular research has been published (Gaymes et al., 1995; Goldman and Shuros, 2000; Smith et al., 1989). Advantages of percutaneous techniques include minimal damage to the catheterized vessels,

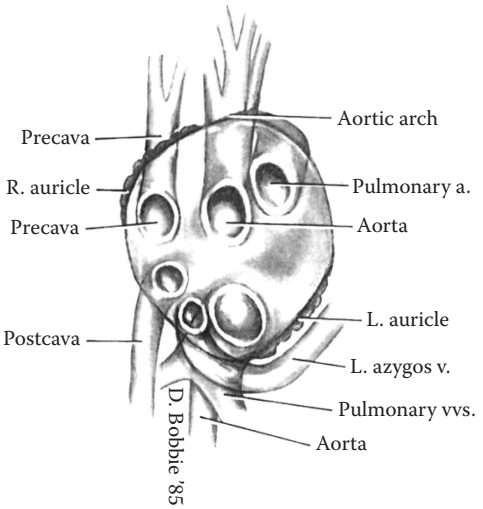

FIGURE 12.1 Relationship of the blood vessels entering and exiting the heart cap. (Reprinted from Swindle et al., 1986, *Lab. Anim. Sci.*, 36(4): 357–361. With permission.)

a shorter healing time than surgical procedures, and an increased likelihood that serial catheterizations can be performed in the same animal. The technique of locating and accessing blood vessels for percutaneous catheterization is discussed here. The technique can be guided with fluoroscopy or sonography (Goldman and Shuros, 2000).

The femoral vessels are located with the pig in dorsal recumbency and the legs restrained caudolaterally. If the legs are stretched too tightly, it will be more difficult to determine the location of the vessels than if the hip joint remains slightly flexed. The medial saphenous artery may be routinely palpated as it crosses the medial aspect of the stifle (knee) joint. The arterial pulse may be palpated until it enters the deep muscles of the leg to join the femoral artery between the cranial

FIGURE 12.2 Seldinger technique: An introducer needle is guided into the femoral artery.

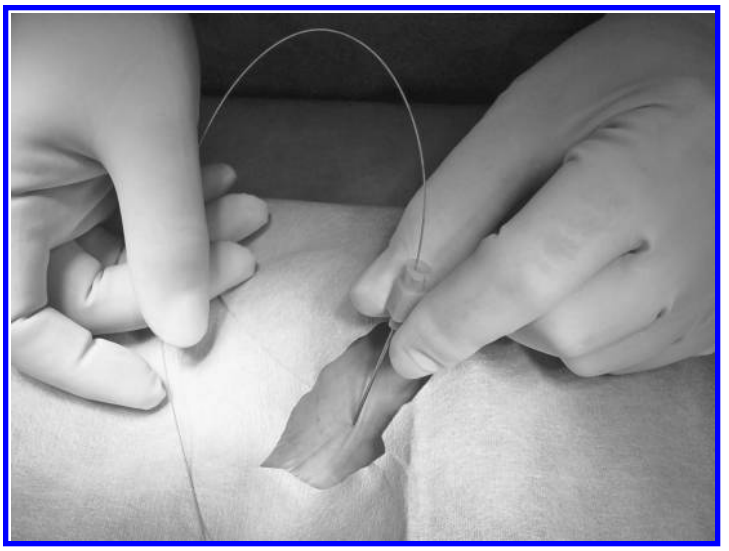

FIGURE 12.3 Seldinger technique: After arterial blood is noticed dripping from the needle, a guidewire is passed through the needle into the artery with the soft bent tip first.

sartorius and caudal gracilis muscles in the femoral canal (see Chapter 9, Figure 9.44). The femoral canal is palpated and the vessels are located lateral to the edge of the sartorius muscle. The femoral pulse is difficult to palpate, even in smaller animals, and the anatomic guide to location is usually more reliable.

A saline filled 19-gauge (ga) 1.5-in. percutaneous needle is inserted through the caudal edge of the sartorius muscle approximately one-third to one-half of the distance from the entrance of the median saphenous artery into the femoral canal and the hip joint (Figure 12.2). The needle should enter the skin at a 45° angle in a craniolateral direction. The needle is advanced slowly until blood

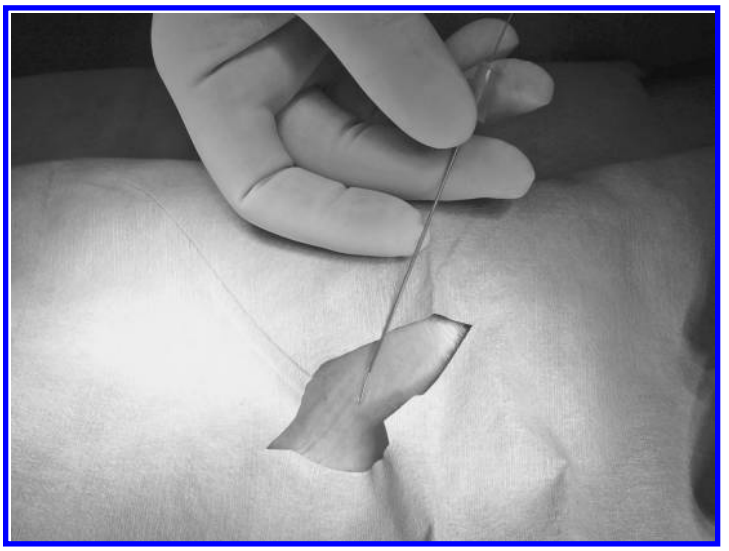

FIGURE 12.4 Seldinger technique: After the guidewire is passed into the artery, the needle is withdrawn.

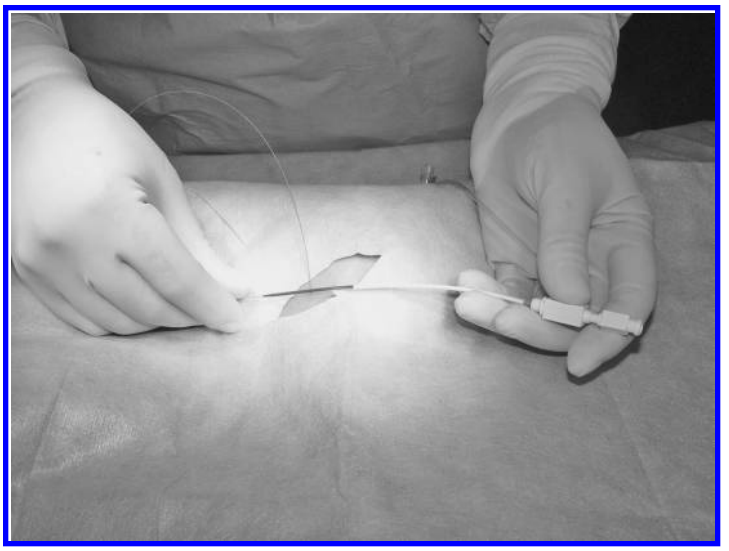

FIGURE 12.5 Seldinger technique: The dilator and sheath are passed over the guidewire into the artery. A small skin incision will facilitate passage of the device through the skin.

appears in the needle or the passage through the vessel is felt by a popping sensation. The tip of the needle is manipulated until a free flow of blood is obtained. Venous and arterial blood may be distinguished by pressure and color. The vein is usually encountered first, is caudal, and slightly overlaps the artery. The nerve is cranial to the artery. If the vessel cannot be catheterized percutaneously after three tries with the needle, a surgical cut down should be performed because of the high probability of vasospasm.

When the vessel is accessed, as indicated by bleeding, a 0.021 guidewire is inserted into the needle with the soft tip first (Figure 12.3). It is advanced cranially to the site of interest using

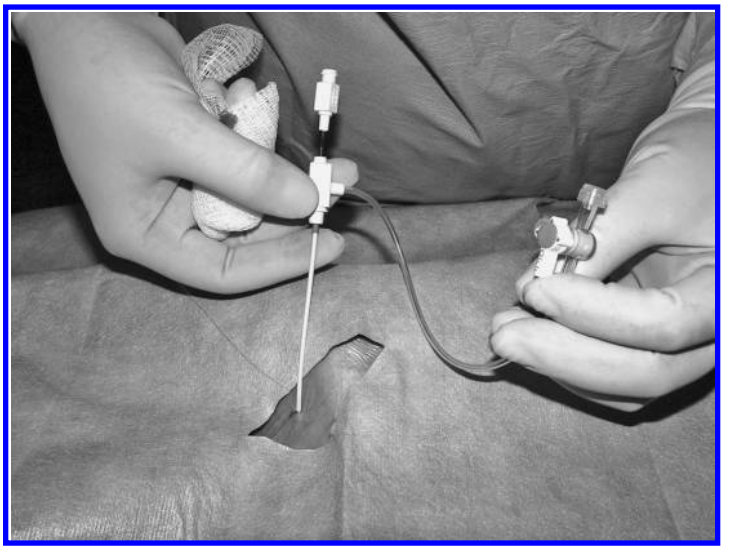

FIGURE 12.6 Seldinger technique: After the dilator and sheath are passed into the blood vessel, the guidewire is withdrawn.

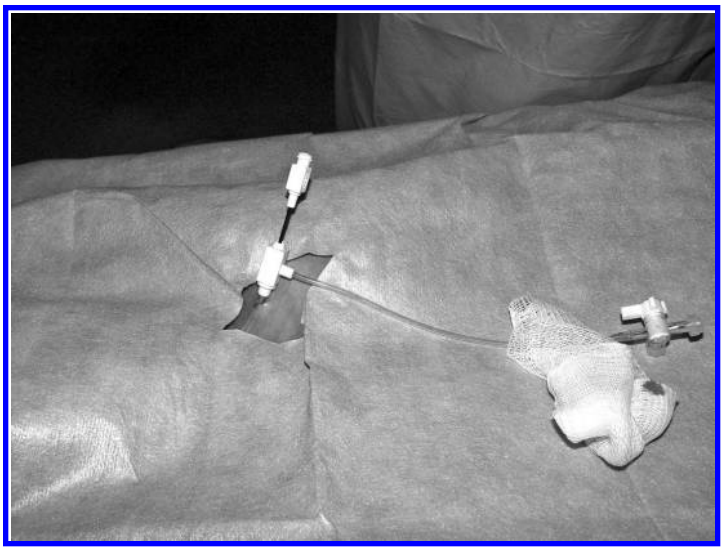

FIGURE 12.7 Seldinger technique: After the dilator and sheath are passed fully into the artery, the dilator is withdrawn.

fluoroscopy for guidance. A skin nick is made at the site of needle entry, and the needle is removed (Figure 12.4), leaving the wire in place. A dilator inside a 5- to 7-French (Fr) sheath with a side arm is passed over the guidewire (Figure 12.5). It is advanced into the vessel using a gentle back-and-forth rotational movement. The dilator and sheath should be wetted and filled with saline prior to their introduction (Figure 12.6). After full advancement and demonstration of blood reflux, the dilator and guidewire are removed (Figure 12.7), and the sheath is flushed with heparinized saline (Figure 12.8). Two sheaths may be placed in the same vessel by passing a second guidewire into

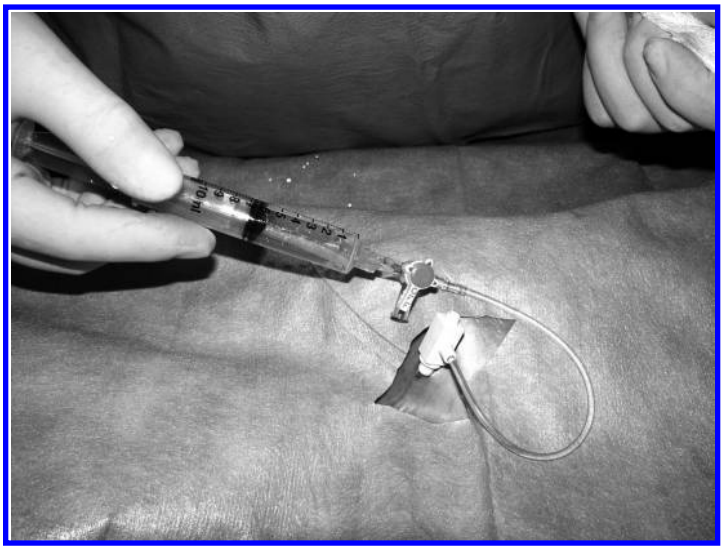

FIGURE 12.8 Seldinger technique: The sheath is now filled with heparinized saline and is ready for catheter passage into the circulatory system.

the first sheath, removing the sheath, and passing two smaller dilators and sheaths over the individual wires. It is also possible to catheterize both the artery and vein on the same side. The medial saphenous artery, but not the vein, can be catheterized with a smaller needle and guidewire and can accommodate up to a 5-Fr catheter in 25- to 30-kg swine. It is possible to insert a small guidewire and cannulate the central cardiovascular system from this vessel. However, digital pressure will be necessary to direct the tip of the catheter more deeply at the juncture of the saphenous and femoral arteries to facilitate passage at the angle between these vessels.

It is possible to pass up to an 11-Fr sheath in 15-kg swine. However, use of the smallest possible size is advisable, because larger catheters are more likely to cause permanent damage to the vessels. For most procedures, 5- to 9-Fr sheaths suffice. When the sheath and catheters are removed, digital pressure should be applied for approximately 5 min to prevent the formation of a hematoma. If the animal has been systemically heparinized, the activated clotting time (ACT) should be allowed to return to baseline before removal of the sheath. The skin can usually be repaired with a single suture.

The external jugular vein, internal jugular vein, and carotid artery can be catheterized using a similar technique (see Chapter 9, Figures 9.39 through 9.42). Larger sheath sizes can be used, especially in the external jugular vein. The pig is placed in dorsal recumbency, and the forelegs are restrained caudally. The anatomic locations for percutaneous needle insertion can be identified. A triangle is identified with the cranial surface of the first rib as the base, the apex represented by the jugular furrow. At the middle of this triangle along the lateral aspect of the jugular furrow, the needle is inserted through the juncture of the sternohyoideus and sternomastoideus muscles. This location is approximately one-quarter of the distance between the manubrium sterni and the ramus of the mandible. The carotid arterial pulse and the wings of the cervical vertebrae can usually be palpated along the lateral aspect of the trachea even in large swine. The internal jugular vein, carotid artery, and vagus nerve are located in a sheath on the floor of the cervical vertebrae. Depending upon whether the catheterization direction is cranial or caudal, the needle is inserted at an angle in the appropriate direction.

Other vessels (Chapter 1, Figures 1.21 through 1.33), such as the auricular and cranial abdominal vessels, can be cannulated to gain access into the central circulation in large swine, depending upon the indications for catheterization. These techniques, as well as the chronic catheterization techniques previously described, can be modified for a pig of any size.

ANESTHETICS AND ADJUNCT DRUGS

General anesthetic and analgesic techniques have been described in detail in Chapter 2 with additional dosages in Chapter 9; however, some specific recommendations for cardiac catheterization procedures can be made. As a general rule, isoflurane +/– nitrous oxide should be the default agent unless it is contraindicated by the protocol. When procedures involve the heart and great vessels, caution should be taken to avoid anesthetic agents that are proarrhythmic or cardiodepressant. Agents that have been associated with adverse cardiac events in these procedures include alpha-2 agonists (e.g., xylazine or dexmedetomidine), tiletamine–zolazepam, and propofol (hypotension and apnea with rapid bolus administration) (Ko et al., 1997; Lefkov and Müssig, 2007; Swindle, 2008).

When performing intracardiac procedures or catheterization of the coronary arteries, prophylactic treatment for prevention of arrhythmias and vasospasm should be utilized (Table 12.1). A complete listing of cardiac emergency drugs and procedures is in Chapter 2.

Swine are prone to development of ventricular fibrillation when the myocardium is irritated or coronary blood flow is restricted. Amiodarone or lidocaine can be administered i.v. prior to cardiac manipulation until the procedure is completed. Amiodarone is indicated for atrial fibrillation, ventricular tachycardia, and ventricular fibrillation (in conjunction with DC countershock therapy). Due to its potential to cause hypotension, intravenous boluses should be administered

TABLE 12.1
Adjunct Agents for Interventional Procedures

Drug	Dosage	Indication
Amiodarone	5–10 mg/kg i.v. as a bolus followed by 0.5–3.5 mg/kg/h infusion	Antiarrhythmic, class I and class III
Aspirin	80–325 mg p.o., sid	Platelet inhibition
Clopidogrel	150–300 mg loading dose p.o. one day prior to procedure 75 mg/day (25–50 kg pig) p.o., sid	Platelet aggregation inhibition
Dalteparin	50–75 μg/kg s.c., sid or bid	Low-molecular weight heparin anticoagulant
Diltiazem	2–4 mg/kg p.o., tid	Antiarrhythmic, class IV, Ca^{+} channel blocker
Enoxaparin	2 mg/kg SQ q12 h	Low-molecular weight heparin anticoagulant
Heparin	150–300 IU/kg, i.v. q 1–2 h	Anticoagulant, increase ACT >300 s
Indomethacin	50 mg rectal suppository the day prior to the procedure	Antiprostaglandin effect
Lidocaine	2–4 mg/kg i.v. bolus followed by 50 μg/kg/min i.v. infusion	Vessel antispasmotic, antiectopic
Magnesium sulfate	500–1 g i.v. immediately prior to stenting	Electrolyte replacement—antiarrhythmic
Nitroglycerine	200 μg diluted into 2 mL saline i.v. slow infusion to effect	Vessel dilation

slowly over several minutes. Lidocaine infusions are antiectopic and help prevent vasospasm when the blood vessels are invaded by catheters. It should be noted that the pharmacological response to antiarrhythmic therapy in swine has variable success and in some cases may be proarrhythmic (Williams et al., 2012). In addition, DC countershock is less successful due to porcine thoracic muscular impedance. As a result, it is most important to focus on maintaining proper circulatory, respiratory, acid–base, fluid, and electrolyte balance during anesthesia for coronary angiography procedures.

Diltiazem (class IV antiarrhythmic Ca channel blocker) is useful as prophylaxis prior to performing intracoronary procedures in order to achieve coronary dilation and to control supraventricular tachycardia. It is generally administered orally the day before the surgery, but can also be administered intravenously during surgery. Nitroglycerin should be administered slowly as an i.v. infusion at the aortic root to provide short-term vasodilation for catheterization of the coronaries.

Anticoagulation is indicated during some procedures and in the postoperative period when some intravascular devices or grafts are implanted. Aspirin is relatively safe but may be insufficient for some protocols. If the pig shows signs of gastric upset, low-dose aspirin (80 mg) may be administered with H-2 antagonists, proton pump inhibitors, or antacids in efforts to reduce gastritis. In order to mimic human percutaneous coronary stenting procedures, additional antiplatelet therapy may be warranted by administering clopidogrel in conjunction with aspirin. Antiplatelet therapy is generally started the day before surgery and continued daily post-operatively (Carpenter et al., 2011; Williams et al., 2012). Heparin is generally administered intraoperatively for short-term anticoagulation by increasing the ACT. It is generally not necessary to administer protamine to reverse it because it

has a short-term effect of approximately 45 min in swine. Low-molecular weight heparin injections may be used as an alternative to heparin, especially for chronic dosing in long-term studies involving implanted devices or grafts (McKellar et al., 2011). Pigs show a wide individual variation in their sensitivity to these agents. If a pig is sensitive to the injectable preparations, bleeding from the injection site is a frequent occurrence. Changes in the feces indicative of intraintestinal bleeding should be monitored for oral preparations. The exact dosages of these agents in swine have not been determined. If a problem with hemorrhage occurs, administration of the agent should be halted or the dosage reduced.

Indomethacin, a non-steroidal anti-inflammatory drug, helps prevent the prostaglandin-stimulating effects of some procedures and alleviates pulmonary platelet clotting which can result in pulmonary hypertension. There can be severe toxic effects with this agent if it is administered orally or as an injection. Inserting a rectal suppository the night before a procedure has been demonstrated to be safe and effective.

HEMODYNAMICS

Hemodynamic measurements may be taken employing the cardiovascular catheterization techniques described in the preceding text, using fluoroscopic guidance to see the catheter placement, and noting the morphology of the pressure waves (Figure 12.9). Standard-sized catheters used for measurements in humans, such as Swan-Ganz catheters in the 5- to 7-Fr range of sizes, work for most laboratory swine. The length of the catheter to be used should be determined in advance.

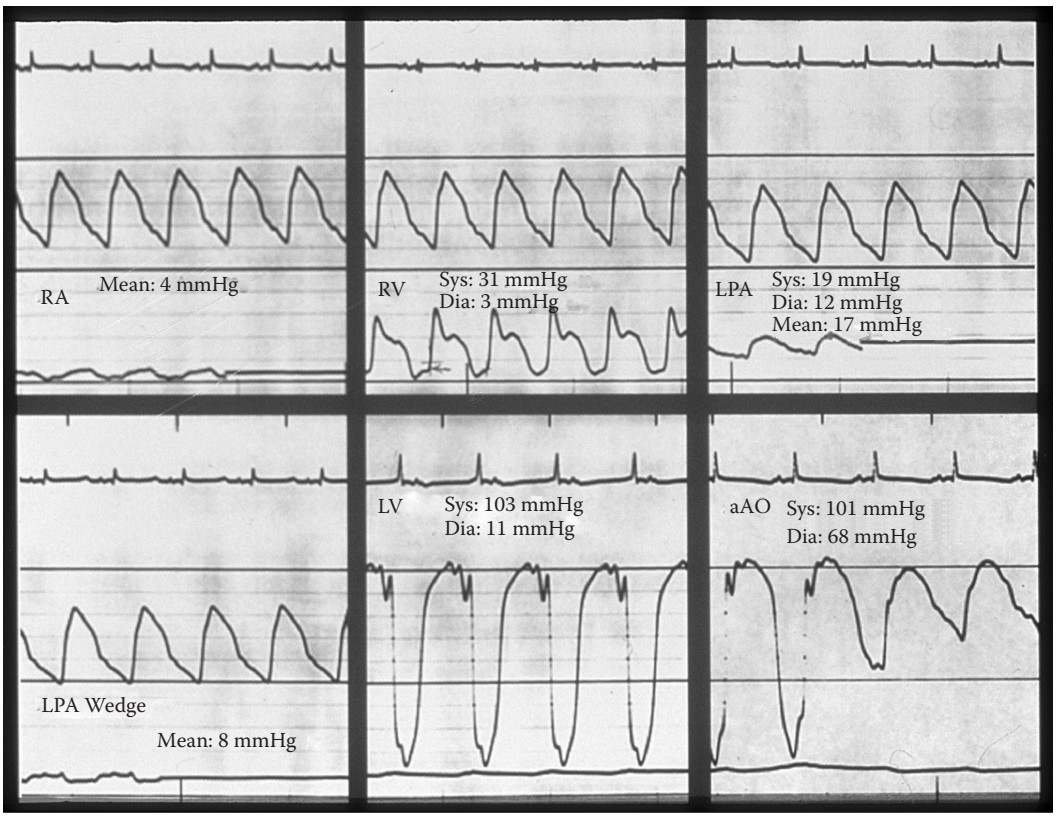

FIGURE 12.9 Pressure wave recording. RA—right atrium, RV—right ventricle, LPA—left pulmonary artery, LPA wedge—left pulmonary artery wedge, LV—left ventricle, AO—aorta.

As a general rule, pediatric lengths work best in swine. Echocardiography can also be used as a noninvasive technique for obtaining some measurements. Chronic cardiovascular catheterization, as described in Chapter 9, may be performed if it is desirable to take hemodynamic measurements without sedation. Peripheral cuff measurements from the tail or the medial saphenous artery can also be used for limited measurements of systemic pressure. Physiological and monitoring equipment used for other species is appropriate for swine.

Pulse oximetry is a useful technique for monitoring oxygenation (Chapter 2, Figures 2.14 through 2.17). Human finger cuffs may be used on the dewclaws, tail, ear, or tongue. If the soft tissues are chosen, the cuff will periodically have to be repositioned because of vascular compression. Arterial blood gases may be measured using the same techniques that are applicable to humans and other species.

Hemodynamic values are affected by age, weight, breed, measurement technique, body temperature, ventilation, anesthetic administration, and pathological conditions. Consequently, comparison to normal values in the literature should be made with caution. In cases of measurements taken under anesthesia, it is not always possible to reproduce the exact anesthetic level from the description in the literature. If possible, each animal should be used as its own control and experimental measurements compared to the baseline. As a general rule, sexually mature miniature swine have hemodynamics that closely resemble those of adult humans.

The animals should be matched for age, body weight (BW), and breed, if possible. Hemodynamic values should be indexed to body surface area (BSA). Formulas are based on dissection and measurement of the skin of farm pigs. One formula, $BSA = 0.097 \times BW \text{ in } kg^{0.633}$ (Brody and Kibler, 1944), should be used with caution because the conformation of farm pigs has changed substantially since the time of that publication. The formulas were recalculated more recently to reflect those differences. Kelley et al. (1973) provided two formulas: $BSA\ (cm^2) = 734\ (BW_{kg})^{0.656}$ and $BSA\ (cm^2) = 3996 + 110\ (BW_{kg})$. The first is a geometric formula and correlates better with other formulas than the second linear formula. Wachtel et al. (1972) used a different formula to calculate BSA in miniature swine, which were determined to be different from farm pigs. That formula is as follows: $BSA\ (m^2) = 0.121\ BW_{kg}{}^{0.575}$. Another formula based upon metabolism has been described in Göttingen minipigs (Bollen et al., 2000), $BSA\ (m^2) = (70 \times BW^{0.75})/1000$. The author has used the formulas in comparison to farm pigs of various sizes and the final results are reasonably close to each other regardless of the formula used. Another formula was determined for BSA in neonatal swine less than 2 kg (DeRoth and Bisaillon, 1979). That formula is as follows: $BSA\ (cm^2) = 337.2 + 0.553$ BW (g). The formulas that are most applicable to current research are probably the Kelley formula: $BSA\ (cm^2) = 734\ BW_{kg}{}^{0.656}$ or $BSA\ (m^2) = 0.0734\ BW_{kg}$ for farm pigs and the Wachtel formula $BSA\ (m^2) = 0.121\ BW_{kg}{}^{0.575}$ for miniature breeds. The neonatal and Göttingen formulas may be more applicable for some studies.

All anesthetic protocols have some effects on hemodynamics; however, they can be minimized as discussed in Chapter 2. A complete review of the effects of hemorrhagic shock on the hemodynamics of domestic farm swine as well as hemodynamic methods has been published (Hannon, 1992; McKirnan et al., 1986). In addition, the effects of age on hemodynamics in domestic farm swine under pentobarbital anesthesia (Buckley et al., 1979) and comparison to different breeds of swine, dogs, and humans (Benharkate et al., 1993; Konrad et al., 2000; McKenzie, 1996; Vogl et al., 1997) have been published. In general, blood pressure increases and heart rate decreases with age. Some examples of hemodynamic values and cardiac sizes from the literature are listed in Tables 12.2 through 12.5. Hemodynamics associated with particular systems are also discussed in Chapters 5 and 9, and in the Appendix. The DVD contains additional data including the farm pig hemodynamics charts from Hannon (1992).

Comparisons were made among 4-month-old Hanford miniature swine, Yucatan miniature swine, and Yucatan microswine under a surgical plane of isoflurane anesthesia (Smith et al., 1990). Table 12.5 compares hemodynamic values and Table 12.2 compares cardiac morphometrics from this study. Table 12.3 contains measurements that were made in 20- to 22-week-old Yucatan

TABLE 12.2
Cardiac Morphometrics for Three Groups of Miniature Swine

	Hanford	Minipig	Micropig
Heart weight (g)			
Total	117 ± 4[a,b]	61 ± 2	53 ± 2
Right-ventricular (RV) free wall	23 ± 1[a,b]	13 ± 1	11 ± 1
Left-ventricular (LV) free wall	39 ± 1[a,b]	23 ± 1	19 ± 1[c]
Wall thickness (cm)			
Right ventricular	4.1 ± 0.1	4.4 ± 0.2	4.5 ± 0.2
Left ventricular	10.4 ± 0.3	11.2 ± 0.3	10.2 ± 0.3
Heart weight/body weight	4.6 ± 0.1[a,b]	5.7 ± 0.1	5.5 ± 0.1
LV weight/body weight	1.5 ± 0.1[a,b]	2.2 ± 0.1	2.0 ± 0.1
RV weight/body weight	0.9 ± 0.1[a,b]	1.2 ± 0.1	1.2 ± 0.1

Source: Reprinted from Smith, A.C. et al., 1994, *Lab. Anim. Sci.,* 40(1): 47–50. With permission.

Note: All values are reported as mean ± standard error of the mean.

[a] Significant ($p < 0.05$) difference between minipig versus Hanford.

[b] Significant ($p < 0.05$) difference between micropig versus Hanford.

[c] Significant ($p < 0.05$) difference between minipig versus micropig.

micropigs under isoflurane anesthesia (Corin et al., 1988). Table 12.4 contains measurements that were made in domestic farm swine, 10–15 kg, without anesthesia, previously implanted with chronic atrial lines (Smith et al., 1989). Serial hemodynamic values from unanesthetized farm pigs can be found in Table 5.3.

ANGIOPLASTY BALLOON TECHNIQUES, INTRAVASCULAR DEVICE IMPLANTATIONS, AND RESTENOSIS

Swine have been used for testing angioplasty balloon techniques, implantation of intravascular devices, and interventional radiology procedures (Amin et al., 1999; Anfinsen et al., 1999; Derdeyn et al., 1997; Dondelinger et al., 1998; Gal and Isner, 1992; Gepstein et al., 1999; Grifka et al., 1993; Jumrussirikul et al., 1998; Lock et al., 1982, 1985; Lund et al., 1984; Magee et al., 1998; Massoud et al., 1997; Mitchell et al., 1994; Morrow et al., 1994; Mukherjee et al., 2003; Murphy et al., 1992; Pawelec-Wojtalik et al., 2005; Randsbaek et al., 1996; Rashkind et al., 1987; Rodgers et al., 1988; Schalla et al., 2005; Schwartzman et al., 2001; Sideris et al., 2002; Solomon et al., 1999; Swindle et al., 1992; Uflacker and Brothers, 2006; White et al., 1992a; Windhagen-Mahnert et al., 1998; Wood et al., 2005) (Figure 12.10). The techniques of using these devices involve the procedures described previously for vascular access, as well as the procedures for surgical approaches to peripheral vessels and cannulations described in Chapter 9. Models of aneurysm for endovascular device closure are also discussed in the same chapter. The anatomic depictions of the vessels in a subsequent section of this chapter, Angiography, and the additional images in the DVD attached to this book may be of use in designing protocols and approaches for these procedures. Sizes of the heart and blood vessels may be estimated from these images. The manufacturer's directions for use of these devices should be followed.

Angioplasty balloon techniques (Figure 12.11) have been utilized to reopen intracardiac and intravascular shunts in neonates, to provide models of shunt patency for device closure, to produce left-to-right shunts for volume overload cardiac hypertrophy, and to study the effects of angioplasty

TABLE 12.3
Yucatan Swine Hemodynamic Data

QP/QS	Controls (Mean ± SEM)
Age (weeks)	21 ± 1
Wt (kg)	21 ± 2
HR (beats per minute)	116 ± 13
LVMI (g/kg)	2.4 ± 0.4
EDVI (mL/kg)	1.9 ± 0.5
ESVI (mL/kg)	0.8 ± 0.3
EF (%)	0.58 ± 0.08
IP (mmHg)	81 ± 8
PAP (mmHg)	21 ± 10
PCW (mmHg)	4 ± 4
V_2 (mL/min/kg)	2.6 ± 0.3
FSVI (mL/kg)	1.1 ± 0.26
CI (L/min/kg)	0.12 ± 0.03
SVRI (dyn s/cm^5 kg)	152 ± 71
PVRI (dyn s/cm^5 kg)	37 ± 17
EDS (dyn/cm^2)	9 ± 5
ESS (dyn/cm^2)	111 ± 28
E_{max}	2.2 ± 0.7
E_{maxc}	84 ± 27
V_{cf50} (circ/s)	1.04 ± 0.14
V_{cf100} (circ/s)	0.68 ± 0.14

Source: Reprinted from Corin, W.J. et al., 1988, *J. Clin. Invest.*, 82(2): 544–551. With permission.

Note: QP/QS = ratio of pulmonary to systemic blood flow, Wt = body weight, HR = heart rate, LVMI = left-ventricular muscle mass index, EDVI = end-diastolic volume index, ESVI = end-systolic volume index, EF = ejection fraction, IP = incisural pressure, PAP = mean pulmonary artery pressure, PCW = pulmonary capillary wedge pressure, V_2 = oxygen consumption, FSVI = forward stroke volume index, CI = systemic cardiac index, SVRI = systemic vascular resistance index, PVRI = pulmonary vascular resistance index, EDS = end-diastolic stress, ESS = end-systolic stress, E_{max} = maximum systolic elastance, E_{maxc} = E_{max} corrected for end-diastolic volume, V_{cf50} and V_{cf100} = mean velocity of circumferential fiber shortening at a common end-systolic stress of 50 and 100 kdyn/cm^2.

on stenosed valvular structures and atherosclerotic lesions (Lock et al., 1985; Lund et al., 1984; Mitchell et al., 1994; Rashkind et al., 1987; Sideris et al., 2002; Smith et al., 1997; Bloch Thomsen et al., 1998). Transient decreases in systemic blood pressure will probably be noted with the inflation of angioplasty balloons. The angioplasty balloon should be filled with contrast material to observe inflation better and to observe for leaks. Catheters filled with air may cause air embolism if the balloon ruptures.

Atrial septostomy has been performed in 18- to 27-kg domestic swine to produce a patent foramen ovale (Mitchell et al., 1994) (Figure 12.11). A transseptal catheter was advanced from the femoral vein to cross the closed fossa ovalis. After exchanging the catheter with an angioplasty balloon catheter, the balloon was inflated three times with 3–4 atm of pressure for 10–15 s. No reclosure of the septum was noted with balloon sizes greater than 12 mm during long-term studies. Modifications of this technique have been used in larger pigs, 28–33 kg (Bloch Thomsen et al., 1998). Smaller pigs

TABLE 12.4
Weekly Catheterization Results of Chronic Atrial Line Implantation: Control of Animals

	Week 1
Left ventricle	
Ejection fraction (%)	59 ± 1
End-diastolic volume (cc)	54 ± 4
Peak systolic pressure (mmHg)	104 ± 4
End-diastolic pressure (mmHg)	2 ± 1
Right ventricle	
Ejection fraction (%)	53 ± 3
End-diastolic volume (cc)	56 ± 4
Peak systolic pressure (mmHg)	24 ± 1
End-diastolic pressure (mmHg)	2 ± 1

Source: Reprinted from Smith, A.C. et al., 1989, *J. Invest. Surg.*, 2(2): 187–194. With permission.

Note: Data presented as mean ± standard error of the mean. There was no significant difference in weekly values. ($p < 0.75$, $n = 4$).

(10–13 kg) have also been used to produce this model for study of transcatheter patch occlusion (Sideris et al., 2002). Histologically, the septums healed with collagenous scar tissue and reendothelization (Mitchell et al., 1994; Sideris et al., 2002). Acute studies have been performed with larger angioplasty balloons (18 mm) in swine 20–40 kg, which produced similar-sized defects, measured postmortem or by fluoroscopy or magnetic resonance imaging (MRI) (Schalla et al., 2005).

TABLE 12.5
Hemodynamic Parameters for Three Groups of Miniature Swine

	Hanford	Minipig	Micropig
Heart rate [beats per minute (bpm)]	105 ± 7	112 ± 3	106 ± 5
Left ventricle			
Systolic pressure (mmHg)	116 ± 4[a,b]	58 ± 2	59 ± 3
Diastolic pressure (mmHg)	4 ± 1	3 ± 1	6 ± 2
Right ventricle			
Peak pressure (mmHg)	30 ± 1[a]	24 ± 2	27 ± 2
Diastolic pressure (mmHg)	4 ± 1[a]	2 ± 1	5 ± 1[c]
Mean arterial pressure (mmHg)	89[a,b]	48 ± 3	53 ± 2
Mean pulmonary artery pressure (mmHg)	19 ± 1[a]	15 ± 1[c]	20 ± 2
Right atrial pressure (mmHg)	9 ± 1 [a,b]	3 ± 1[c]	6 ± 1
Pulmonary capillary wedge pressure (mmHg)	12 ± 1	12 ± 2	11 ± 1

Source: Reprinted from Smith, A.C. et al., 1994, *Lab. Anim. Sci.,* 40(1): 47–50. With permission.

Note: Measurements taken under isoflurane anesthesia. All values are reported as mean ± standard error of the mean.

[a] Significant ($p < 0.05$) difference between minipig versus Hanford.

[b] Significant ($p < 0.05$) difference between micropig versus Hanford.

[c] Significant ($p < 0.05$) difference between minipig versus micropig.

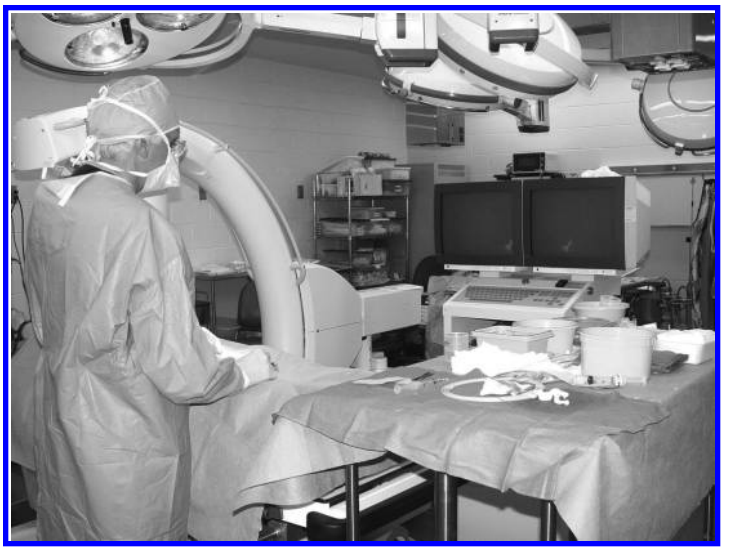

FIGURE 12.10 Catheterization laboratory using C-arm fluoroscopy.

Domestic swine weighing 5 kg were used to create a model of patent ductus arteriosus (PDA). A 0.035-in. guidewire was passed distal to the aortic arch from the right femoral artery. A 5-Fr curved catheter was advanced over the guidewire, and the guidewire was advanced across the PDA into the right ventricle. The catheter was exchanged for a 6-mm diameter angioplasty balloon. The balloon was inflated to 8–10 atm of pressure for 2–5 min. No reclosures of the PDA were noted in long-term studies (Lund et al., 1984). This method offers an alternative to keeping the PDA open with multiple injections and long-term infusions of prostaglandins (Starling et al., 1978).

A genetic model of subaortic ventricular septal defect (VSD) (Figure 12.12), which develops clinical syndromes similar to humans, exists in Yucatan minipigs (Amin et al., 1999; Corin et al., 1988; Fu et al., 2006; Swindle et al., 1990, 1992). This perimembranous defect has been used to test interventional closure devices. Swine have also been used as models to refine techniques of

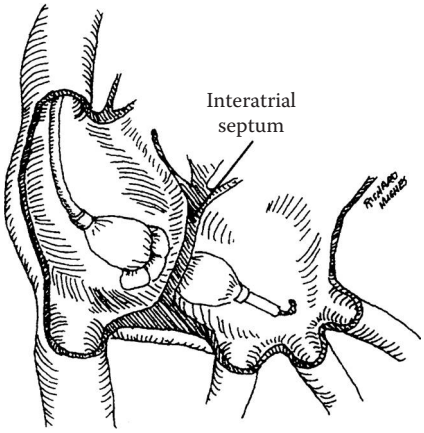

FIGURE 12.11 Method of using angioplasty balloon catheter inflation to create a shunt.

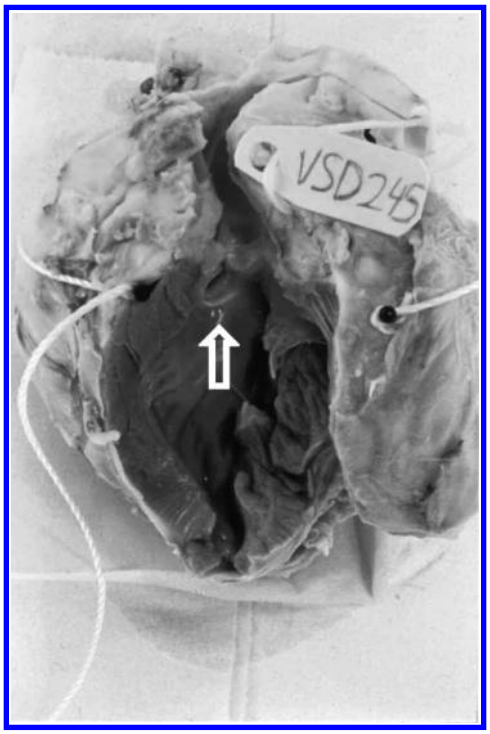

FIGURE 12.12 Subaortic ventricular septal defect (arrow). (Courtesy of R.P. Thompson, PhD, Medical University of South Carolina.)

closure of ventricular defects using larger catheter devices (Figures 12.13 and 12.14). A hybrid procedure of performing a substernal incision, opening the pericardium, and inserting the device through the left-ventricular apex (Pawelec-Wojtalik et al., 2005) as well as a technique for passing the devices through the free wall of either ventricle (Amin et al., 1999) have been described.

A model of restenosis of the coronary artery following balloon angioplasty or stent implantation (Figures 12.15 and 12.16) has been developed in swine (Murphy et al., 1992; Willette et al., 1996). The model may be produced by overinflation with a balloon angioplasty catheter by 25%–75% (20 s, 10 atm of pressure) repeated three times. Neointimal formation occurs in approximately 50% of the miniature or domestic swine subjected to this procedure. The implantation of various stents reliably produces 100% neointimal formation. The stent needs to be oversized (>30%) in the coronary artery (Willette et al., 1996). Restenosis following the procedure may be noted in as short a period as 2 weeks but consistently occurs in 4–6 weeks with some stents.

In one of the investigations, it was noted that pigs developed postinfarction ventricular aneurysm similar to the clinical syndrome in humans. The infarctions and aneurysms occurred in all animals that developed occlusion following implantation of the stent. This model provides distinct advantages to surgical implantation of graft material into windows created in the ventricular wall. It can be performed without a thoracotomy and provides a more similar pathogenesis to the human situation (Murphy et al., 1992).

Interventional catheter techniques are also utilized for embolotherapy (Derdeyn et al., 1997), closure of arteriovenous malformations (Massoud et al., 1997), and tracking techniques during intervention (Solomon et al., 1999; Wood et al., 2005). They have also been used for development of stent devices for closure of aneurysms (Figures 12.17 and 12.18). Creation of these lesions is discussed in Chapter 9. Postoperative and intraoperative antithrombotic therapy may be necessary in the models of stent implantation.

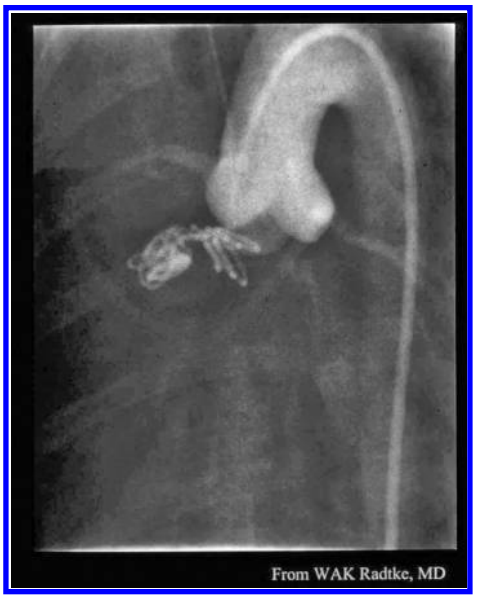

FIGURE 12.13 Fluoroscopic view of coil after placement into a VSD. (Courtesy of W.A.K. Radtke, MD, Nemours Cardiac Center, Wilmington, DE.)

As a general rule, vascular growth in a young domestic pig can be expected to increase in diameter by 35%–40% and in length by 25%–30% over a 6-month time period. Growth in miniature pigs is substantially less. Heart and vessel sizes are discussed in Chapter 9, and data are available in Tables 9.1 through 9.3. As a guideline for sizing of devices, sample measurements are provided in the following text and in the Appendix:

FIGURE 12.14 VSD has been closed by a fibrous membrane, which has formed over the coil. (Courtesy of W.A.K. Radtke, Nemours Cardiac Center, Wilmington, DE.)

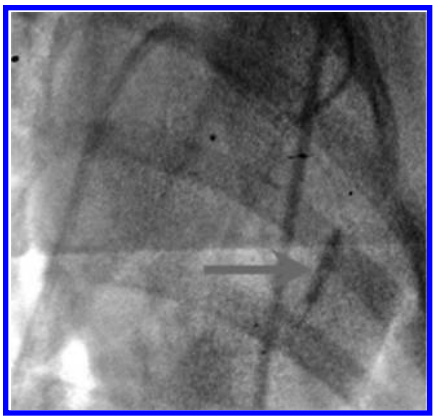

FIGURE 12.15 Fluoroscopic view of a stent (arrow) being placed in the circumflex coronary artery. (Courtesy of Michael Sturek, PhD, Indiana University.)

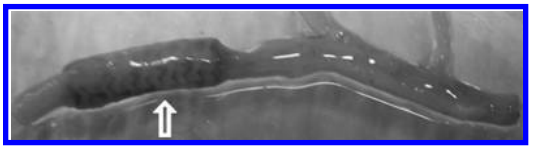

FIGURE 12.16 Necropsy specimen of a coronary artery with an implanted stent (arrow). (Courtesy of Michael Sturek, PhD, Indiana University.)

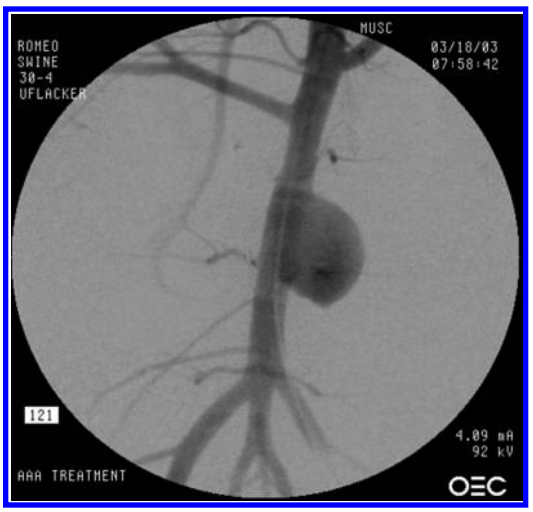

FIGURE 12.17 Fluoroscopic image of an artificially created aneurysm on the distal aorta (Chapter 9 for technique). (Courtesy of Renan Uflacker, MD, Department of Radiology, Medical University of South Carolina.)

1. Göttingen miniature swine weighing 20–25 kg had the following diameter measurements *in vivo*: left anterior descending coronary artery, 1.4 mm ID (inside diameter) and 1.9 mm OD (outside diameter) (N. Grand, personal communication).
2. Yucatan microswine weighing 14–33 kg had the following diameter measurements *in vivo*: iliac arteries, 5 mm; femoral arteries, 3.5 mm; popliteal arteries, 2.0 mm (Gal and Isner, 1992).

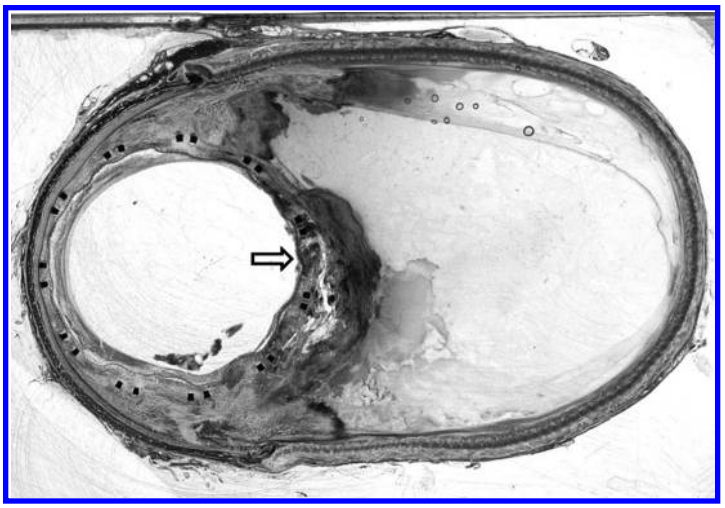

FIGURE 12.18 Histologic section of a stent (arrow) implanted into the lumen of the aortic aneurysm in Figure 12.17. (Courtesy of Renan Uflacker, MD, Department of Radiology, Medical University of South Carolina.)

3. Yucatan miniature swine weighing 10–20 kg had the following measurements *in vivo*: coronary arteries, 2.0–3.5 mm; iliac artery, 2.5–4.0 mm (White et al., 1992a).
4. Hanford miniature swine weighing 15 kg had coronary artery diameters of 2.0–2.5 mm (Rodgers et al., 1988).

Necropsy measurements of the diameters of various arteries and veins were collected by the author. *In vivo*, it can be expected that these vessel sizes can be expanded. The measurements recorded were the following:

1. Yucatan micropig (29 kg): aortic arch—1.2 cm, abdominal aorta—6 mm, external iliac artery—2 mm, internal iliac artery—1 mm, portal vein—1.0 cm, postcava—2 cm.
2. Yucatan miniature pig (47.6 kg): carotid—4 mm, external jugular—6 mm, internal jugular—4 mm, aortic arch—1.1 cm, pulmonary trunk—1.1 cm, renal artery—4 mm, heart weight—0.2 kg, heart length—95 mm, heart circumference—230 mm, LV chamber length—48 mm, RV chamber length—75 mm, coronary sinus—10 mm, mitral valve diameter—63 mm, tricuspid valve diameter—65 mm.
3. Yucatan miniature pig (109 kg): aortic arch—1.1 cm, abdominal aorta—6 mm, external iliac artery—4 mm, internal iliac artery—3 mm, renal artery—2 mm.
4. Hanford miniature pig (43.6 kg): aortic arch—1.3 cm, abdominal aorta—4 mm, external iliac artery—3 mm, internal iliac artery—2 mm, postcava—4 mm.
5. Landrace domestic pig (70 kg): aortic arch—1.2 cm, abdominal aorta post renal—5 mm, external iliac artery—4 mm, internal iliac artery—3 mm, renal artery—2 mm, carotid artery—3.5 mm, coronary artery—3.0 mm.
6. Yorkshire domestic pig (25 kg): aortic arch—2.1 cm, abdominal aorta pre renal—11 mm, abdominal aorta post renal—10 mm, external iliac—6 mm, internal iliac—3 mm, carotid artery—5 mm, pulmonary artery—2.1 cm, precava—12 mm, postcava—12 mm, portal vein—16 mm, renal artery—4 mm, vena cava—11 mm.
7. Yorkshire domestic pig (47.5 kg): aortic arch—2.2 cm, abdominal aorta prerenal—15 mm, abdominal aorta post renal—8 mm, external iliac—6 mm, internal iliac—4 mm, renal artery—5 mm, coronary—3 mm.

ATHEROSCLEROSIS

Atherosclerosis in swine can occur spontaneously over time on a normal diet (Casani et al., 2005; Fuster et al., 1991; Skold et al., 1966). Pigs spontaneously develop atherosclerosis which can be accelerated by feeding an atherogenic diet (Davies, 2009; Koskinas et al., 2010; Reiser et al., 1959; Skold et al., 1966). The pig is large enough to allow for noninvasive measurements of arteries and for harvesting sufficient arterial tissue for analysis, has a human-like lipoprotein profile, and develops lesions in the coronary arteries. Due to its size and vascular similarities of swine to humans, it is a good translational model for evaluating the effects of atherosclerosis on the arterial endothelial layer. Atherosclerotic lesions generally occur at arterial bends, characterized by flow separation and low shear stress (Davies, 2009). If allowed to develop over time, mild atherosclerotic lesions first appear in coronary arteries, and both atherosclerotic plaque distribution and composition (lipid, fibrinogen, smooth muscle cells, and macrophage content) follow a pattern comparable to that in humans (Casani et al., 2005; Gal et al., 1992; White and Bloor, 1992; White et al., 1992b). Swine may also develop hypercholesterolemia and atherosclerotic lesions by high-fat diet induction, reaching plasma cholesterol levels similar to those found in humans (Getz and Reardon, 2012). Feeding pigs a 1.5% cholesterol, 19.5% lard diet for up to 30 weeks induced a hypercholesterolemia and generated predominantly lipid-laden foam cell atherosclerotic lesions in atherosusceptible areas of the aorta (Gerrity, 1981a,b). Human-like pig lipoprotein metabolism may determine the similar response that swine have to atherosclerosis (Marzetta and Rudel, 1986). As conventional swine age and grow, the degree of atherosclerosis becomes increasing similar to humans. To overcome size-related problems, minipigs are increasingly being used as animal models in chronic long-term studies because of their small size and low-growth rate, which allows them to maintain weight and size throughout adulthood (Badimon et al., 2013). Swine, unlike other animal models, develop coronary restenosis after gradual occlusion of the coronary vessels induced by both balloon injury and atherogenic diet as in humans (Rodgers et al., 1990; Stanton and Mersmann, 1986; White and Bloor, 1992; White et al., 1992b).

Models of atherosclerosis (Figure 12.19) can be produced by inflation of angioplasty balloons in the artery and denudation of the endothelium by pulling back on the inflated balloon in swine fed an atherogenic diet (Attie et al., 1992; Gal and Isner, 1992; Lee et al., 1986; Rodgers et al., 1988; White et al., 1992a). The denudation procedure should be repeated two to three times. Fogarty embolectomy catheters (5–6 Fr) may be used for this technique after being inflated to 6–8 atm of

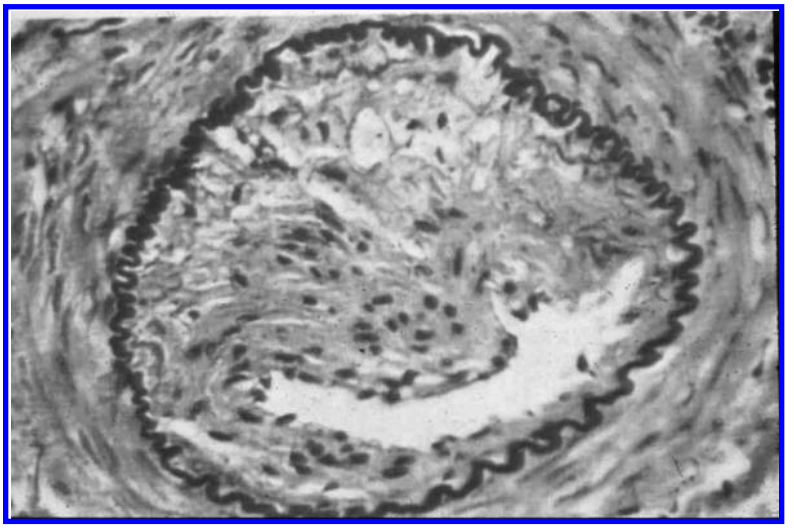

FIGURE 12.19 Coronary arterial atherosclerotic plaque. H&E, ×100.

pressure. Other angioplasty catheters can also be used. Animals must be fed an atherogenic diet of 0.5%–4.0% cholesterol. The cholesterol is added as a supplement in lard, or atherogenic food oils and fats are mixed in standard diets. Alternatively, diets may be commercially prepared by food manufacturers. No universal standard for the amount of cholesterol to be included in the diet has been developed, but it is likely that 2% cholesterol is adequate for most studies. The diet should be fed to the animals starting 2 weeks prior to the procedure and must be continued for up to 12 weeks following the procedure to have radiographically visible occlusal lesions. Swine fed atherogenic diets alone may not develop significant atherogenic lesions within a year. The arteries most utilized in these techniques are the coronaries and the iliacs. The iliacs are approached from catheters introduced into the carotid artery and the coronaries from the femoral artery. Anesthetic and ancillary drug administration for coronary artery catheterization is discussed in Chapter 2.

Gal and Isner (1992) concluded that the Yucatan micropig may be a more favorable model for the development of these lesions than other miniature and domestic swine after comparing their results with the literature. The model can then be tested for balloon angioplasty of the occlusal lesions, atherectomy techniques, stent implantation, and local and systemic drug therapies.

Goodrich et al. (2003) developed a Yucatan minipig model of menopause and atherosclerosis. Sexually mature Yucatan micropigs were ovariectomized and fed a 4% cholesterol diet free of plant phytoestrogens with 40% of the calories from fat for 6 months. This model results in generalized atherosclerotic plaques including significant lesions in the coronary arteries. Swine had changes in serum lipid and C-reactive protein levels that were similar to humans in this study.

Expandable stents can be placed in atherogenic and normal arteries to provide patency and for local drug delivery. The technique involves placing an expandable stent over an inflatable balloon and then inflating the balloon after the stent is in the proper location (Figures 12.15 and 12.16). The balloon is then deflated and the catheter removed. Stents are most commonly employed to study percutaneous transluminal coronary angioplasty (PCTA) but have also been used for other vascular models such as surgically produced coarctation of the aorta or iliac artery stenosis (Gal and Isner, 1992; Grifka et al., 1993; Lock et al., 1982; Morrow et al., 1994; Murphy et al., 1992; White et al., 1992a).

SWINE AND METABOLIC SYNDROME

Metabolic syndrome is a disorder of energy utilization and storage. It is characterized and diagnosed by a cluster of risk factors for non-insulin-dependent diabetes mellitus (NIDDM; type II diabetes) and cardiovascular disease which are generally classified as a combination of insulin resistance (DeFronzo and Ferrannini, 1991), central obesity, raised plasma triacylglycerol concentrations, reduced high-density lipoprotein cholesterol, increased low-density lipoprotein cholesterol and hypertension (Eckel et al., 2005; Pi-Sunyer, 2007). In humans, metabolic syndrome leads to high morbidity and mortality (Ford et al., 2002; Litten-Brown et al., 2010; Poole and Byrne, 2005). Presently, the porcine model, which exhibits three or more of the clinical signs, is now generally considered the optimum non-primate model for investigating metabolic syndrome (Spurlock and Gabler, 2008). Of interest, different swine breeds have different responses to metabolic syndrome diseases. For instance, the Ossabaw minipig provides a better model than Yucatan for the metabolic syndrome as they exhibit obesity, insulin resistance, and hypertension, all of which are absent in the Yucatan (Litten-Brown et al., 2010). The Göttingen minipig breed is an excellent model for studying the effect of dietary high-fat intake on obesity, glucose homeostasis, and susceptibility to diabetes (Cirera et al., 2010; Johansen et al., 2001; Larsen et al., 2002; Spurlock and Gabler, 2008).

ELECTROPHYSIOLOGY AND ELECTROCARDIOGRAPHY AND TELEMETRY

Swine have been used as models for study of cardiac electrophysiology and cardiac arrhythmias (Gillette et al., 1991; Huang et al., 2001; Hughes, 1986; Khan et al., 2001; Lin et al., 1999; Nahas et al., 2002; Schumann et al., 1993, 1994; Smith et al., 1997; Verdouw and Hartog, 1986).

FIGURE 12.20 Pig restrained in a sling with adhesive ECG leads attached.

The position of the heart in the pig as well as other quadrupedal animals is different from the human, and the electrocardiography (ECG) monitoring is slightly different. In the Göttingen, minipig triangular leads (Nehb-Spöri leads) have been shown to be better than bipolar or unipolar leads for monitoring QRS patterns while being restrained in a sling (Nahas et al., 2002). The cubital and stifle joint areas are the preferred location for standard limb leads. The dorsolateral neck, sacrum, and xiphoid process are the preferred positions for triangular leads. The authors recommended these positions based on a detailed study of the minipig and the topographical relationship of the heart to the sternum. The heart projection is from the second to the fifth intercostal space, and the right ventricle forms a sharp angle to the sternum. Adhesive leads typically need to be taped in position although cleaning the skin with alcohol helps with their security. Various software programs used in humans can be utilized to process and analyze continuous ECG recordings and look for abnormalities. Illustrations of lead placement techniques and recordings are illustrated in Figures 12.20 through 12.23. Tables 12.6 through 12.8 contain selected ECG parameters for minipigs.

The anatomy of the conduction system of the porcine heart has been compared to other species, including humans (Bharati et al., 1991; Truex and Smythe, 1965). The principal difference between the porcine conduction system and that of humans is the presence of large numbers of

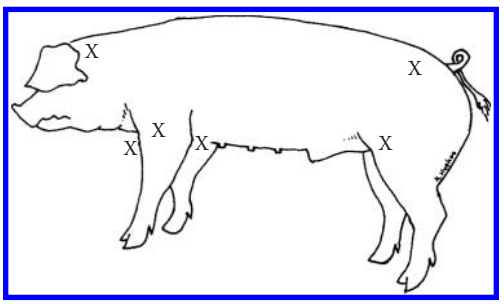

FIGURE 12.21 ECG lead placement is marked by an X on each location (limbs, sternum, manubrium, neck, sacrum). The limb leads are bilateral.

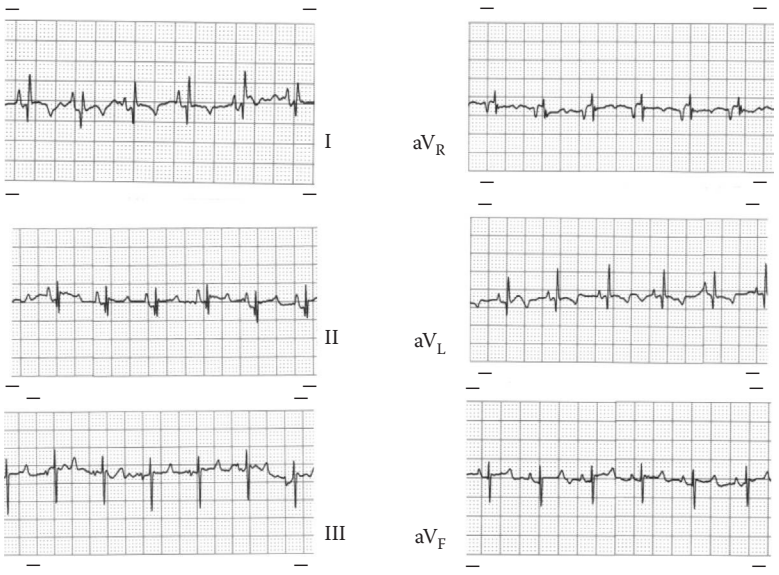

FIGURE 12.22 Surface ECG tracings of leads I, II, III, aV$_R$, aV$_L$, and aV$_F$ recorded at 25 mm/s. The leads were placed in the cubital position over the elbow joint of the forelegs and the knee joint of the hind limbs while the pig was restrained in the sternal position in a sling. The 22-kg domestic pig was unsedated.

adrenergic and cholinergic nerve fibers in the atrioventricular (AV) node and bundle branches. Nerve cells are lacking in the fibers. The Purkinje fibers are large, well differentiated, and easily identifiable in the endocardium (Chapter 9, Figures 9.12 and 9.13). Humans have some nerve fibers, no ganglia, and smaller Purkinje fibers. Swine may have more of a neuromyogenic rather than a predominantly myogenic component to the conduction system because of these differences.

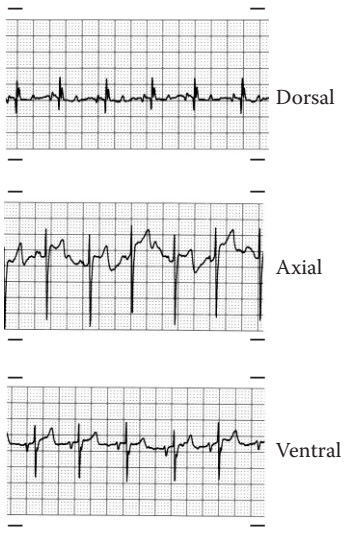

FIGURE 12.23 Triangular ECG tracings with the electrodes placed behind the ear, on the sacrum, and on the sternum. This is the same pig as in Figure 12.22.

TABLE 12.6
Selected ECG Parameters for Göttingen Miniature Swine

	Male (*N* = 24)			Female (*N* = 24)		
	Average	**SD (+/–)**	**# of Measurements**	**Average**	**SD (+/–)**	**# of Measurements**
Age (months)	4.0	0.3	24	3.8	0.3	24
Heart rate (bpm)	119	29	47	126	23	48
P (ms)	34	5	46	36	5	48
PR (ms)	78	11	47	75	8	48
QRS (ms)	36	6	47	39	4	48
QRT (ms)	240	22	47	235	17	48

Source: Courtesy of Ellegard Göttingen Minipigs ApS, Dalmose, Denmark.

TABLE 12.7
Selected ECG Results for Juvenile and Young Adult Hanford Miniature Swine

	Male				Female			
	N	**Average**	**SD**	**Range**	***N***	**Average**	**SD**	**Range**
HR (bpm)	24	115	17.7	84–144	24	118	18.4	84–144
RR (ms)	24	536	83.3	417–714	24	523	84.5	417–714
PR (ms)	24	106	15.6	80–140	24	115	15.3	80–140
QRS (ms)	24	35	5.1	30–40	24	35	5.1	30–40
QT (ms)	24	261	23.3	220–300	24	258	25.8	210–300
QTc (Fridericia's formula) (ms)	24	322	21.0	277–365	24	321	24.5	273–361

Source: Courtesy of Sinclair Research Center, Auxvasse, MO.
Note: Age: ~ 4–8 months.

TABLE 12.8
Selected ECG Parameters for Juvenile Sinclair Miniature Swine

	Male			Female		
	Average	**SD**	**Range**	**Average**	**SD**	**Range**
RR (s)	0.36	0.08	0.24–0.56	0.37	0.07	0.28–0.50
HR (bpm)	172	36	108–252	168	30	120–216
PR (mm)	4.16	0.46	3.39–5.52	4.10	0.57	3.20–5.61
QT (mm)	10.79	1.45	8.27–13.78	10.79	1.04	8.46–12.72
QT (s)	0.22	0.03	0.17–0.28	0.22	0.02	0.17–0.25
QTc (Bazett's) (s)	0.36	0.02	0.33–0.40	0.36	0.02	0.32–0.40

Source: Courtesy of Sinclair Research Center, Auxvasse, MO.
Note: *N* = 32, age = ~3–4 months.

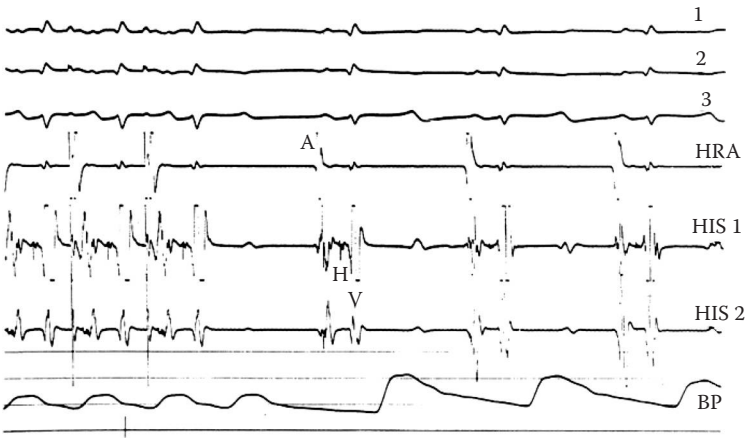

FIGURE 12.24 Surface and intracardiac electrocardial recordings. 1,2,3—surface leads, HRA—high right atrium, HIS 1 and HIS 2—distal and proximal bundle of His catheter recordings, BP—blood pressure, A—atrium, H—bundle of His spike, V—ventricle. (Reprinted from Gaymes, C.H. et al., 1995, *J. Invest. Surg.*, 8(2): 123–128. With permission.)

The sinus rhythm rate and conduction system velocity rates vary depending upon the age and weight of the pig; in smaller pigs, they are more rapid (Huang et al., 2001; Lin et al., 1999). Cardiac maturity in minipigs is achieved at approximately 60 days of age (Lin et al., 1999). In general, heart rates are more rapid than for humans of equivalent maturity. Heart conduction system rates are also affected by the anesthetic protocol. Long QT segments, with the T wave sometimes becoming superimposed with the following P wave, may be observed in deep surgical anesthesia. Ketamine-midazolam or sufentanil infusions (see Chapter 2) may be used to shorten the QT interval if required (Schumann et al., 1993, 1994). Swine have been demonstrated to have similar enough conduction system parameters (Figures 12.24 and 12.25) to be used for pacemaker testing (Brownlee et al., 1997;

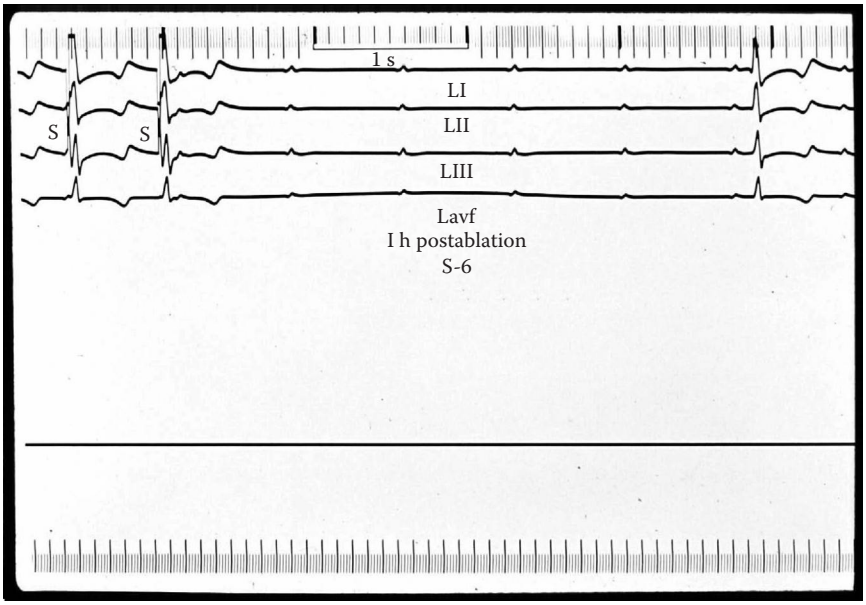

FIGURE 12.25 Surface leads demonstrating complete heart block following cryoablation of the bundle of His. The heart block is illustrated when the pacemaker has been turned off.

TABLE 12.9
Threshold Voltage (Volts) Requirements: Domestic Swine 25–30 kg

Electrode Location	Mean Pulse Duration (ms)	Pig (Range)
SA node	1.0	0.36–0.58
	0.5	0.58–0.96
Atrial appendage	1.0	0.26–0.60
	0.5	0.27–1.21
Right ventricle	1.0	0.15–0.35
	0.5	0.23–0.51
Left ventricle	1.0	0.17–0.43
	0.5	0.31–0.69

Source: Reprinted from Hughes, H.C., 1986, *Lab. Anim. Sci.*, 36(4): 348–350. With permission.

Hughes, 1986; Smith et al., 1997) and for study of ventricular arrhythmias (Verdouw and Hartog, 1986). Arrhythmias following myocardial infarction are discussed in Chapters 2 and 9. Right cardiac vagal denervation created by surgery in neonatal pigs leads to decreased QT and RR intervals as well as and sudden pauses in sinus rhythm 6 weeks following surgery (Khan et al., 2001). The electrophysiological (EP) testing techniques of Gillette and Garson (1981) and Gillette and Griffin (1986) have been modified for use in swine (Smith et al., 1997). Noninvasive programmable stimulation (NIPS) that performs EP using a telemetry system has also been successfully used in Hanford miniature swine (Smith et al., 1997). Examples of intracardiac electrophysiological parameters in anesthetized domestic swine used for pacemaker testing are listed in Tables 12.9 through 12.11.

The NIPS program is summarized below as follows:

1. Verify programming parameters of the pacemaker. Surface electrocardiogram should be recorded simultaneously.
2. Retrieve diagnostic data collected by the NIPS.
3. Intracardiac electrogram is checked for proper lead placement.
4. The stimulation threshold is determined for atrial and ventricular leads by pacing at 50 ms shorter than the shortest sinus rhythm cycle.
5. AAI and VVI modes are used to perform pacing stimulation protocols for the atrium and ventricle separately.

TABLE 12.10
Threshold Current (mA) Requirements: Domestic Swine 25–30 kg

Electrode Location	Mean Pulse Duration (ms)	Pig (Range)
SA node	1.0	0.81–1.25
	0.5	1.54–2.70
Atrial appendage	1.0	0.37–1.55
	0.5	0.47–2.87
Right ventricle	1.0	0.24–0.74
	0.5	0.43–1.21
Left ventricle	1.0	0.38–0.80
	0.5	0.72–1.44

Source: Reprinted from Hughes, H.C., 1986, *Lab. Anim. Sci.*, 36(4): 348–350. With permission.

TABLE 12.11
Noninvasive Programmable Stimulation Values for Hanford Miniature Swine

	VTHRESH	VBURST	VERP	DPPRV S2	S3	ATHRESH	ABURST	AVNERP	AERP	DPPRA S2	S3
Mean	0.07	220.98	228.51	226.45	163.01	0.25	233.55	249.88	210.25	221.15	166.73
Standard deviation	0.07	29.01	20.54	19.54	27.22	0.20	35.09	27.91	30.51	34.53	33.77
Range, minimum	0.03	140.00	180.00	170.00	110.00	0.03	160.00	210.00	150.00	140.00	100.00
Range, maximum	0.45	290.00	320.00	330.00	260.00	1.00	370.00	340.00	320.00	310.00	270.00

Source: Reprinted from Smith, A.C. et al., 1997, *J. Invest. Surg.*, 10(1–2): 25–30. With permission.

Note: Refer to the description of electrophysiological testing in text for explanation of pacing protocols. VTHRESH = ventricular threshold, VBURST = ventricular burst pacing, VERP = ventricular effective refractory period, DPPRV = double premature beats into paced right ventricle, S2 = first premature beat, S3 = second premature beat, ATHRESH = atrial threshold, ABURST = atrial burst pacing, AVNERP = AV node effective refractory period, AERP = atrial effective refractory period, DPPRA = double premature beats into paced right atrium.

6. Pacing procedures:
   a. Eight beats of burst pacing (300 ms) and determination of the rate at which 2:1 block occurs (decrease cycle by 10 ms each time).
   b. Deliver a single premature beat into the paced chamber (SPRA/V). Start at 400 ms for eight beats and deliver a premature beat at 380 ms. Decrease the interval of premature beat delivery by 20 ms until 300 ms. The interval at which the premature beat was introduced is decreased by 10 ms until capture discontinues. The shortest interval without capture of either chamber is the atrial or ventricular effective refractory period (AERP or VERP).
   c. Deliver two premature beats into the paced chamber (DPPRA/V) after eight beats of 400 ms. The two premature beats are introduced at a cycle length 50 ms greater than AERP or VERP. The introduction of each premature beat is decreased by 10-ms intervals until it is no longer captured. The protocol is concluded when AERP or VERP is reached.
7. Reprogram the pacemaker to DDD mode for previous pacing parameters.

Table 12.11 contains values recorded from this technique in Hanford miniature swine (Smith et al., 1997). Other telemetry devices have been developed for swine. The telemetry units illustrated in Figure 12.26 may be used for blood pressure, temperature, and a variety of biopotential measurements. Devices may be customized for studies in swine.

When telemetry systems are implanted for the cardiovascular system, they should be implanted in the jugular furrow as described for pacemaker units in the section that follows. Instead of using the carotid and jugular veins, the superficial cervical vessels can be used to accommodate the smaller telemetry leads. The superficial cervical vessels are located near the level of the thoracic inlet dorsal to the external jugular vein. When implanting telemetry devices caudal to the diaphragm, such as ones using the femoral vasculature, the device should either be implanted in the dorsal flank or in the retroperitoneal region (Willens et al., 2014). Implanting the devices in the subcutaneous tissues along the ventrum will have a high likelihood of eroding through the skin. The subcutaneous pocket surgical technique is described for vascular access ports in Chapter 9 and techniques are included in videos on the DVD attached to this textbook.

The LifeShirt System® for data collection in animals is being modified for swine from an established human ambulatory monitoring technology (A. Derchak, 2006, personal communication,

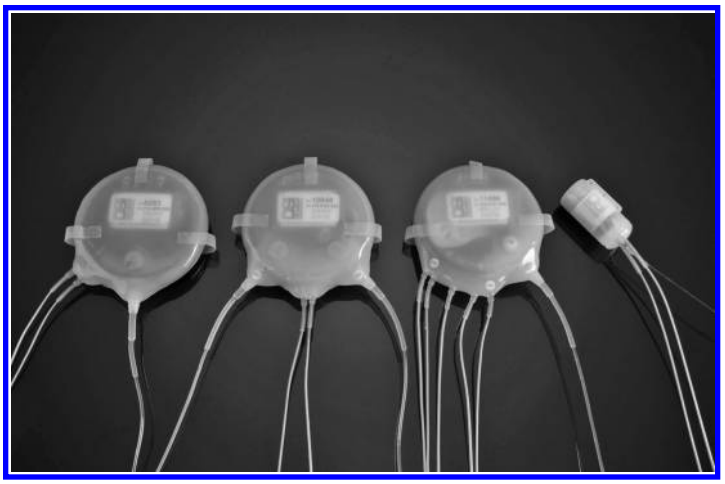

FIGURE 12.26 Data Sciences telemetry transmitters. The unit on the left measures blood pressure, temperature, and one biopotential channel. The second unit measures two pressure channels and one biopotential channel. The third unit measures one pressure channel and two biopotential channels. The unit on the right is for rats and provides a size comparison. These units are for pigs >2.5 kg. Biopotential channels can be used for ECG, EEG, or EMG. (Courtesy of Data Sciences International.)

VivoMetrics, Inc.) (Figures 12.27 and 12.28). This noninvasive equipment is an alternative to implanted telemetry units and chronic catheterization for the continuous long-term collection of respiratory and cardiovascular data in unanesthetized animals. Respiratory data are collected with two respiratory inductance plethysmography bands, one circling the thoracic cavity and the other circling the abdomen. The respiratory signals provide breathing frequency and all timing aspects of the respiratory cycle (e.g., inspiratory time, expiratory time, total time, duty cycle, etc.). Potentially other parameters can be collected if there is calibration using a spirometer (tidal volume, minute ventilation, inspiratory flow, and expiratory flow). ECG is captured with a single-lead

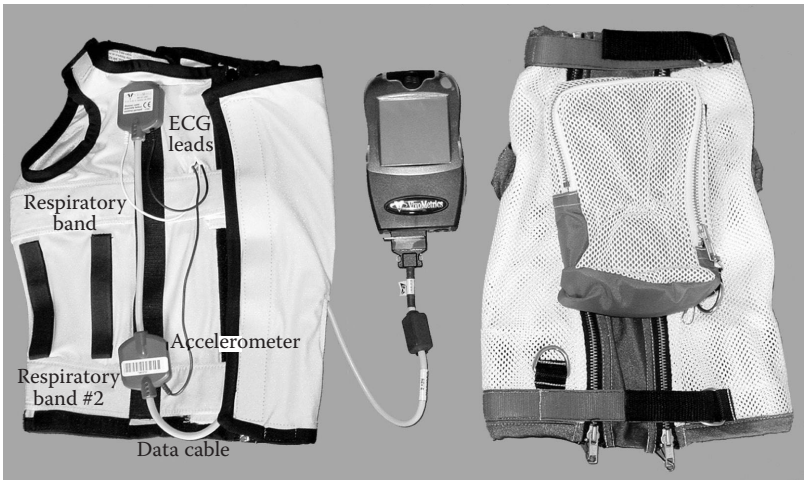

FIGURE 12.27 Complete LifeShirt System—the LifeShirt garment is on the left. The data recorder contains a removable flash memory card, the battery, and an interactive touch screen that can be used to mark study events (treatment, dosing, etc.), as well as to display waveforms for the evaluation of signal quality. The jacket on the back provides protection for the equipment and contains a pouch in which the data recorder is stored during recording periods. (Courtesy of A. Derchak, VivoMetrics, Inc.)

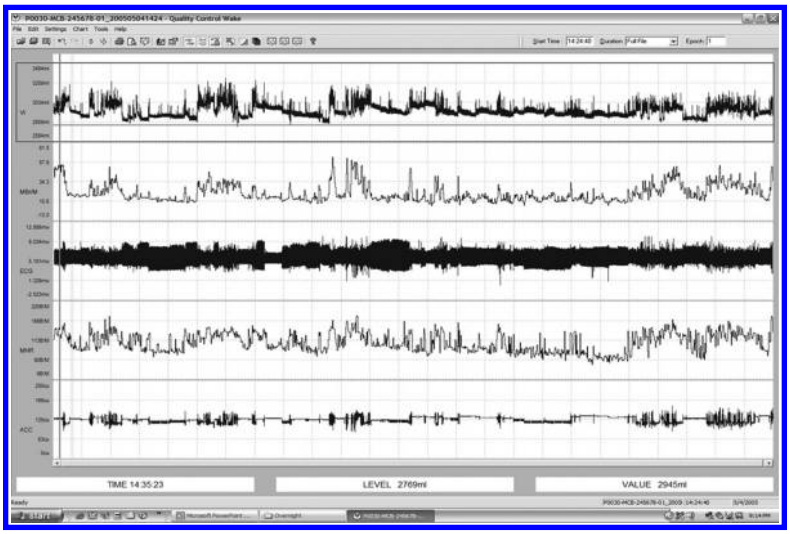

FIGURE 12.28 Example of LifeShirt data—19 h and 25 min of data. VT—tidal volume, MBr/M—minute median breathing frequency, ECG—electrocardiogram, MHR—minute median heart rate. Activity and postural changes can be noted in the accelerometer trace (ACC). The multiple signals provide researchers with the ability to evaluate unrestrained data and make comparisons between periods of similar activity and/or body orientation. (Courtesy of A. Derchak, VivoMetrics, Inc.)

arrangement, which can be applied in a species-specific manner, and activity and postural changes are continuously monitored with a triaxial accelerometer. Additional data streams, including SpO_2, skin temperature, and other measures, can be collected on a protocol-specific basis. Currently, all data are recorded to removable flash memory for subsequent analysis (Figure 12.28). However, addition of radio transmission of high-resolution waveforms to a computer for real-time evaluation is under development.

PACEMAKER IMPLANTATION AND ENDOCARDIAL PACEMAKER LEADS

The technique for epicardial pacemaker implantation is described in Chapter 9. In this section, the implantation of endocardial pacemaker leads and the implantation of the pulse generator in the neck are described.

Hanford miniature swine weighing >45 kg have similar intracardiac measurements to the human heart, and single-pass 11- to 13-cm pacemaker leads designed for adult humans can be inserted for testing (Figures 12.29 and 12.30) (Brownlee et al., 1997). Smaller swine of the same breed have been used for pacemaker implantation after conduction system ablation (Gillette et al., 1991; Rabkin et al., 2004; Smith et al., 1997). Both single-pass leads and separate atrial and ventricular leads have been used for dual chamber pacing. The angle of entry into the heart is different in swine, as it is in other quadrupedal animals, and adjustments in the technique of passing a lead into the heart using fluoroscopic guidance must be made.

A left external jugular cut down surgery is performed as described in Chapter 9. The pacemaker generator will be implanted in the pocket formed by this surgery (Figures 12.31 and 12.32); consequently, dorsal dissection between the muscle planes along the path of the external jugular vein to accommodate the device will be required. Elastic vessel loops are applied to the external jugular vein, and a venotomy is made with iris scissors. The pacemaker leads are passed into the heart through the venotomy. The ventricular lead is implanted first if two leads are required. Either screw-in or tined lead tips will work in the ventricle; however, screw-in leads work best in the atrium.

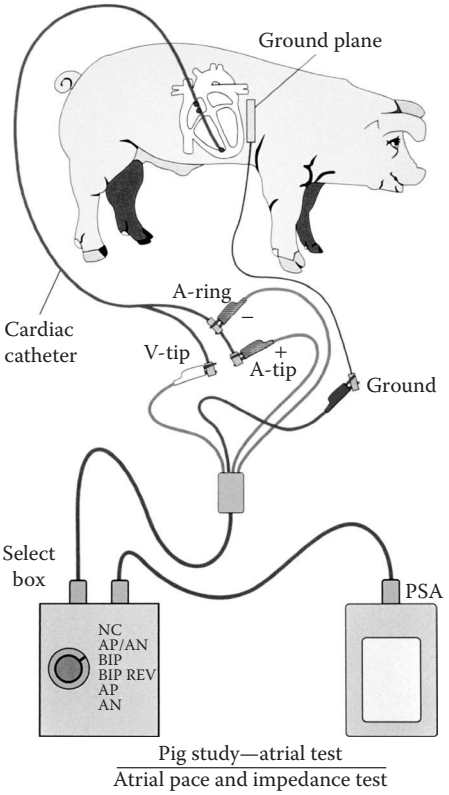

FIGURE 12.29 Atrial lead testing. (Courtesy of Cardiac Control Systems.)

When the ventricular lead tip is observed at the tricuspid valve, the insertion wire is retracted 1–5 cm so that the tip of the lead is floppy and can be manipulated through the valve. The internal guidewire can be slightly curved along the distal end to facilitate the manipulation. Depending upon the location of the lead tip, either a counterclockwise or a clockwise twist is performed on the lead while inserting it through the valve. The tendency of the lead will be to pass into the postcava, and multiple insertion attempts to correct the angle usually have to be made. Once the lead tip is in the ventricle, the tip will pass into the pulmonary outflow tract if it is passed between tendinous chordae. The goal of the insertion is to implant it into the apex. This can be accomplished by inserting the internal guidewire fully into the pacemaker lead after it has been passed into the ventricle.

For single-pass leads (Figure 12.33) after the ventricular tip is secured, the electrodes are placed close to the entrance of the precava into the atrium. For atrial screw-in leads, the implantation site should be the high right atrium. Care must be taken when inserting this lead to avoid overmanipulation that will dislodge the ventricular lead. The internal guidewire is retracted slightly to place the lead in the proper location using a counterclockwise twist. The tip is then screwed into the atrial wall.

After the electrode tips are implanted, they are tested to ensure that appropriate impedance and threshold values are obtained. The manufacturer's instructions for the electrodes should be followed, and similar electrophysiology values to humans can be expected with implantation of these devices in swine (Brownlee et al., 1997; Hughes, 1986; Smith et al., 1997). The phrenic nerve runs along the course of the pre- and postcava, and diaphragmatic stimulation may occur with improper positioning of the lead electrodes or too high a threshold value. The pacemaker leads are secured in place with nonabsorbable suture ligatures around the proximal

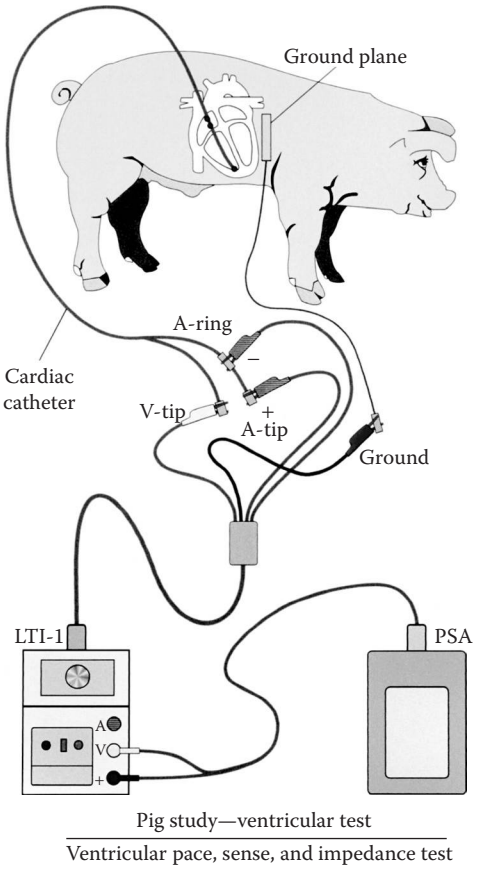

FIGURE 12.30 Ventricular lead testing. (Courtesy of Cardiac Control Systems.)

and distal venotomy sites and around the suture sleeves on the leads. The pacemaker generator is attached, and excess lead material is coiled around the device. The device is implanted into the pocket, and its placement is checked to ensure that it is not stimulating the skeletal muscle in the region. The pocket is enclosed by suturing the muscles over the pacemaker generator. The closure between the sternohyoideus and the sternomastoideus muscles should completely cover the device. Leaving dead space in the pocket will lead to seroma formation. The fascial layers are closed using synthetic absorbable sutures, and a subcuticular suture pattern is used to close the skin.

Pacemaker wand telemetry systems should be able to communicate with the device through the skin and muscle if it is held next to the skin. Some manipulation of the wand may be necessary to achieve the desired communication. The wand should be checked for its ability to communicate with the pacemaker generator prior to closing the muscle layers.

CONDUCTION SYSTEM ABLATION

Conduction system ablation is used to study electrophysiology of the heart, techniques for ablation of aberrant conduction system pathways, and the function of cardiac pacemakers. Heart block and regional ablation of the conduction system of the heart have been produced in animals by traumatic damage to the area of interest using surgical techniques. These have included cutting, thermal damage, tight sutures, and injection of formalin into the SA node, AV node, bundle

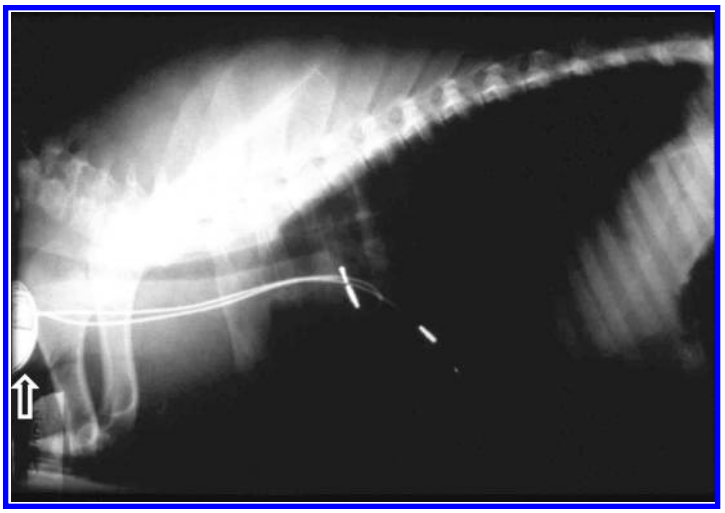

FIGURE 12.31 Lateral radiographs of placement of atrial and ventricular leads. The pacemaker generator has been implanted in the neck (arrow).

of His, and bundle branches. Some of these techniques involve the use of a right atriotomy, and most have a high failure rate (Gardner and Johnson, 1988). Transvenous ablation of the conduction system has been developed using catheter delivery of cryothermia (Figures 12.25, 12.34, and 12.35), radiofrequency, direct current, microwave, ultrasonic, and laser trauma to the conduction system. These methods have been utilized in swine (Anfinsen et al., 1999; Fujino et al., 1991, 1993; Gepstein et al., 1999; Gillette et al., 1991; Jumrussirikul et al., 1998; Mukherjee et al., 2003; Ohkubo et al., 1998; Schwartzman et al., 2001; Smith et al., 1997; Windhagen-Mahnert et al., 1998).

The technique of transvenous ablation involves the positioning of intracardiac tripolar electrode catheters into the heart to record electrical signals of the heart. The area of interest is positioned between two of the electrodes. An ablation catheter is guided fluoroscopically until the tip of the

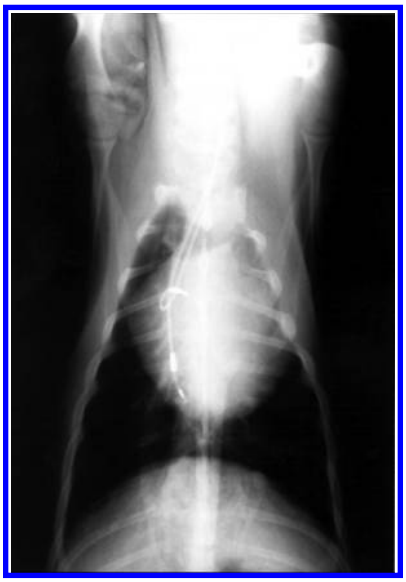

FIGURE 12.32 Ventrodorsal radiograph of placement of atrial and ventricular leads.

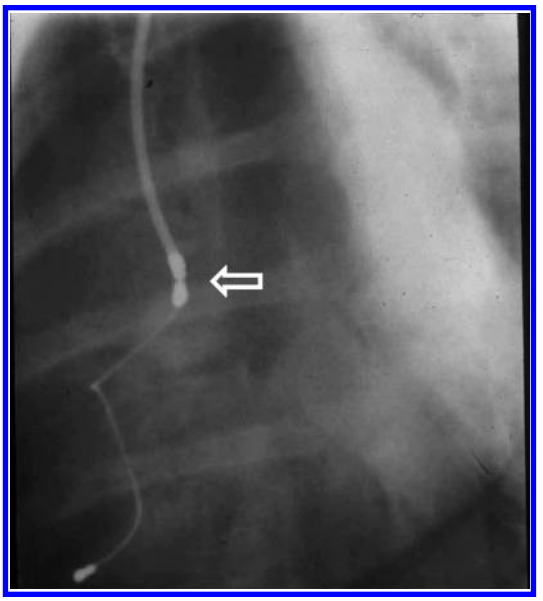

FIGURE 12.33 Single-pass pacemaker lead implantation. The atrial lead rolls in the atrium (arrow), and the ventricular lead is implanted in the apex. The indentation in the lead half way between them is the lead passing through the tricuspid valve.

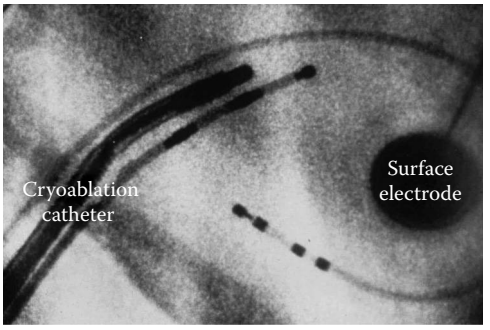

FIGURE 12.34 Cryoablation catheter at the bundle of His (large catheter). The other catheters are electrodes for recording the intracardiac electrogram (small catheters). The round object is a surface ECG lead.

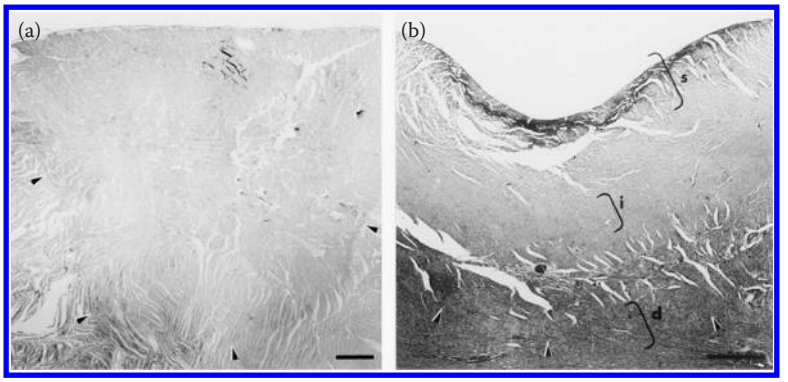

FIGURE 12.35 Histologic section comparing cryoablation (a) and radiofrequency (b) lesions in the heart. The cryoablation lesion is a homogeneous scar and the radiofrequency lesion is a cratered char-type lesion. (Courtesy of R.P. Thompson, PhD, Medical University of South Carolina.)

catheter can be seen at the area of interest, and the energy to ablate the conduction system is delivered using the applicable generator. Recordings of the electrical signals of the heart will indicate when a lesion has been delivered. When producing heart block, it is necessary to provide ventricular pacing. Currently, the two most common types of energy delivery systems in use are radiofrequency and cryothermia. The catheters are usually introduced into the heart from the femoral vessels, as described earlier.

The catheter size, amount of energy delivered, length of delivery of energy, and necessity to repeat the procedure vary depending upon the type of ablation selected and the design characteristics of the catheter and equipment. From our experience, the following examples are given for cryoablation and radiofrequency ablation in 14- to 22-kg Hanford pigs. Cryoablation of the bundle of His: 11-Fr catheter, 60°C, 180 s, three freeze-and-thaw cycles. Radiofrequency ablation of the bundle of His: 5-Fr catheter, 50-W energy output, 70–90°C, 30 s, repeated once.

ANGIOGRAPHY

Angiography may be performed in swine in a similar fashion to other species. A review of coronary angiography and percutaneous coronary intervention procedures in the porcine model has been published (Williams et al., 2012). A complete anatomic study of the vascular system using angiography has been published as a guide for using farm pigs in training in interventional radiology (Dondelinger et al., 1998). The angiographic images included in this section are provided to give anatomic guidance of structures of major importance in research in swine (Figures 12.36 through 12.50). They were taken in 16-kg Yorkshire swine with iodine contrast solution (iohexol 350 mg/mL). An 8-Fr 110-cm Cordis catheter (inside dimension, 1.2 mm) with side holes was used. A Cordis-automated injector was set at a volume of 14 mL, a delay of 0.15 s, a pressure of 490 psi, and a rate of 14 mL/s. The coronary arteriograms were taken using 7-Fr Judkins left and right coronary artery catheters with the same contrast material in a 43-kg Hanford pig. Instead of an automated injector, 10 mL of contrast solution was injected manually during cinefluorography. The size marker in the films is an 18-ga 1.5-in. hypodermic needle. Additional images can be found in the DVD attached to this textbook.

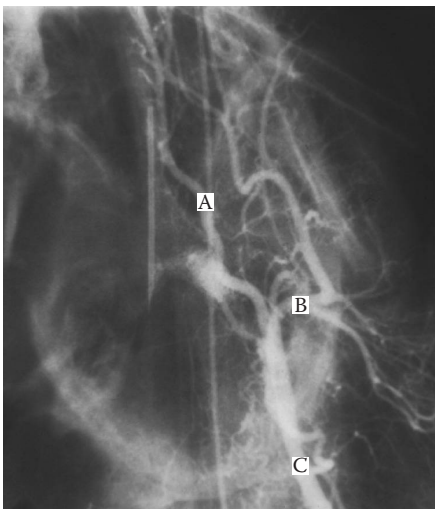

FIGURE 12.36 Ventrodorsal view of a cerebral angiogram with the catheter placed in the carotid artery. A—rostral cerebral artery, B—external carotid artery and branches, C—internal carotid artery.

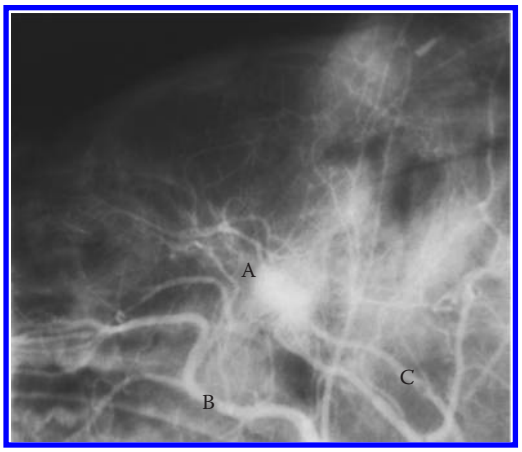

FIGURE 12.37 Lateral view of a cerebral angiogram with the catheter placed in the carotid artery. A—rostral cerebral artery, B—facial artery, C—internal carotid artery.

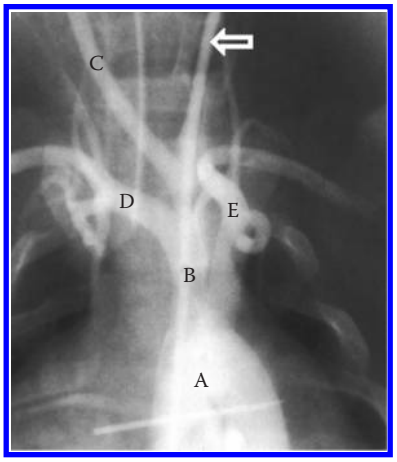

FIGURE 12.38 Ventrodorsal view of the arteries of the cranial thorax and neck with the catheter (arrow) placed at the aortic root. A—aorta, B—brachiocephalic trunk, C—right carotid artery, D—right subclavian artery, E—left subclavian artery.

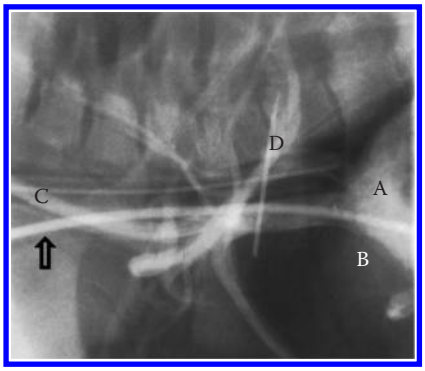

FIGURE 12.39 Lateral view of the arteries of the cranial thorax and neck with the catheter (arrow) placed at the aortic root from the left carotid artery. A—aorta, B—brachiocephalic trunk, C—right carotid artery, D—costocervical trunk.

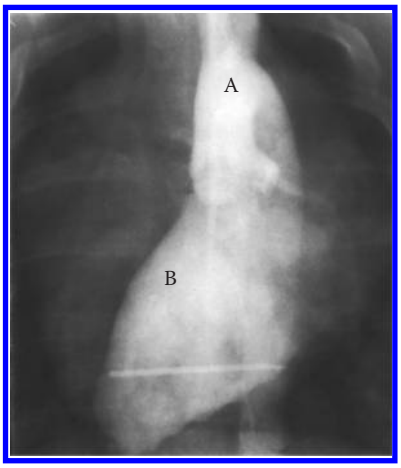

FIGURE 12.40 Ventrodorsal view of the left-ventricular chamber. A—aorta, B—left ventricle.

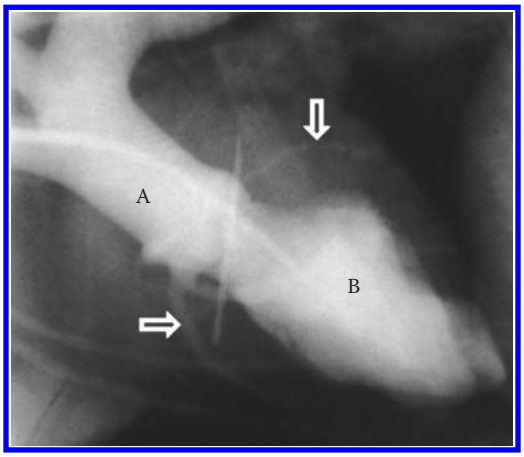

FIGURE 12.41 Lateral view of the left-ventricular chamber. The coronary arteries are apparent (arrows). A—aorta, B—left ventricle.

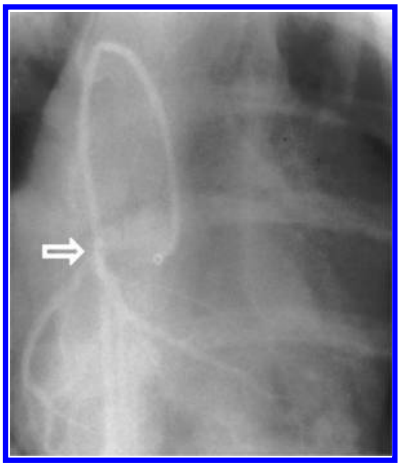

FIGURE 12.42 Left coronary (arrow) arteriogram, ventrodorsal view.

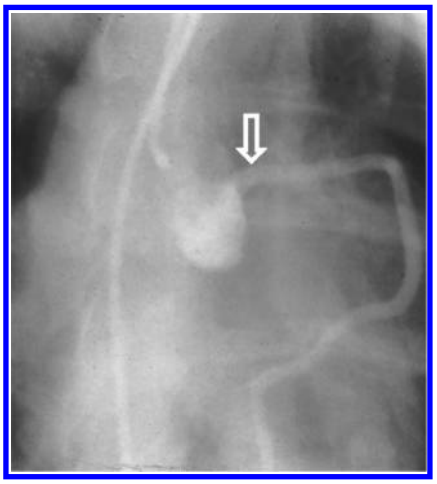

FIGURE 12.43 Right coronary (arrow) arteriogram, ventrodorsal view.

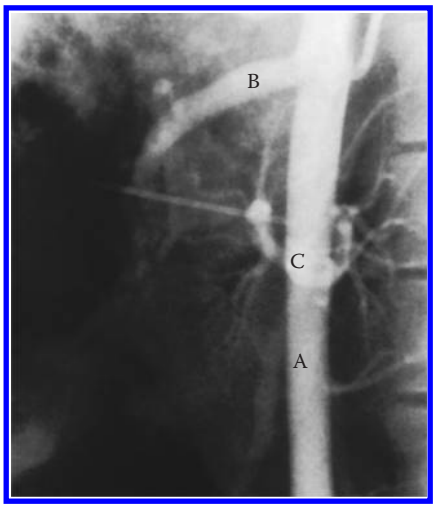

FIGURE 12.44 Ventrodorsal view of the root of the celiac and cranial mesenteric arteries. A—aorta, B—celiac artery, C—cranial mesenteric artery.

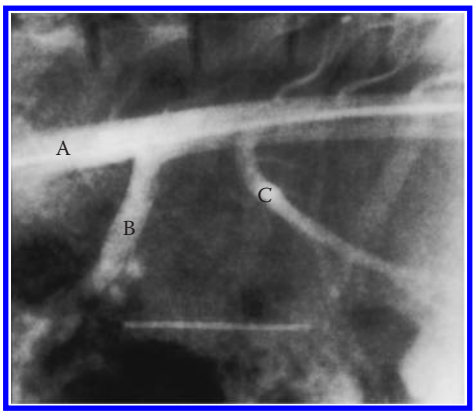

FIGURE 12.45 Lateral view of the root of the celiac and cranial mesenteric arteries. A—aorta, B—celiac artery, C—cranial mesenteric artery.

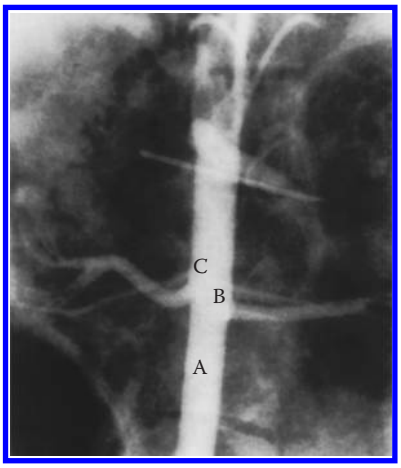

FIGURE 12.46 Ventrodorsal view of the renal arteries. A—aorta, B—right renal artery, C—left renal artery.

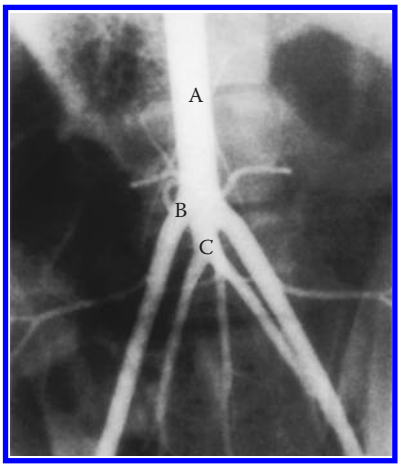

FIGURE 12.47 Ventrodorsal view of the iliac arteries. A—aorta, B—external iliac artery, C—internal iliac artery.

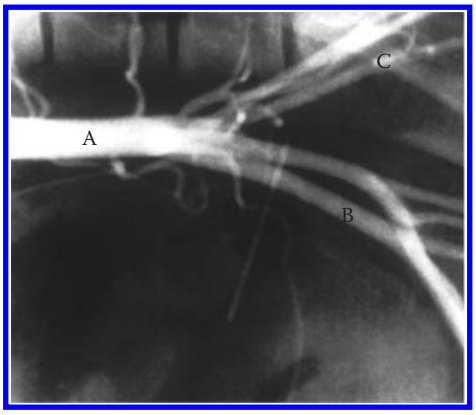

FIGURE 12.48 Lateral view of the iliac arteries. A—aorta, B—external iliac artery, C—internal iliac artery.

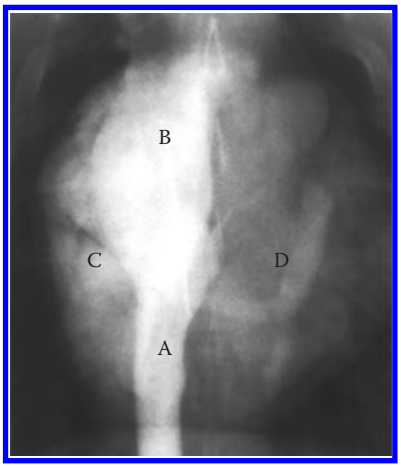

FIGURE 12.49 Ventrodorsal views of the right side of the heart. A—caudal vena cava, B—right atrium, C—right ventricle, D—reflux of contrast into the coronary sinus and left azygous (hemiazygous) vein.

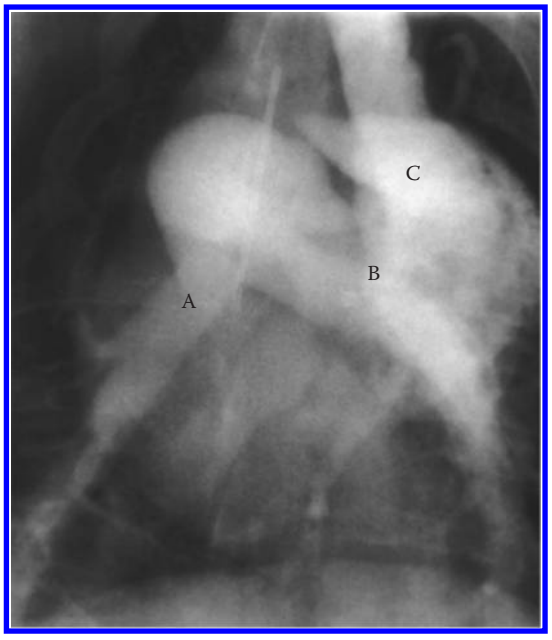

FIGURE 12.50 Ventrodorsal view of the pulmonary veins. A—right pulmonary artery, B—left pulmonary artery, C—left atrium.

ECHOCARDIOGRAPHY

Echocardiography can be challenging in swine because of the narrow intercostal spaces and wide ribs. Standard equipment used in humans can be utilized for porcine studies. Echocardiographic images of domestic, miniature, and fetal swine have been published (Konrad et al., 2000). The figures in this chapter (Figures 12.51 through 12.58) are examples of images of the heart in an 8-month-old female Hanford miniature pig weighing 36.5 kg. Comparisons of normal hearts with hypertrophic hearts are made in Table 12.12. Normal echocardiographic values for Göttingen minipigs are located in Tables 12.13 through 12.15. Additional images are on the companion DVD.

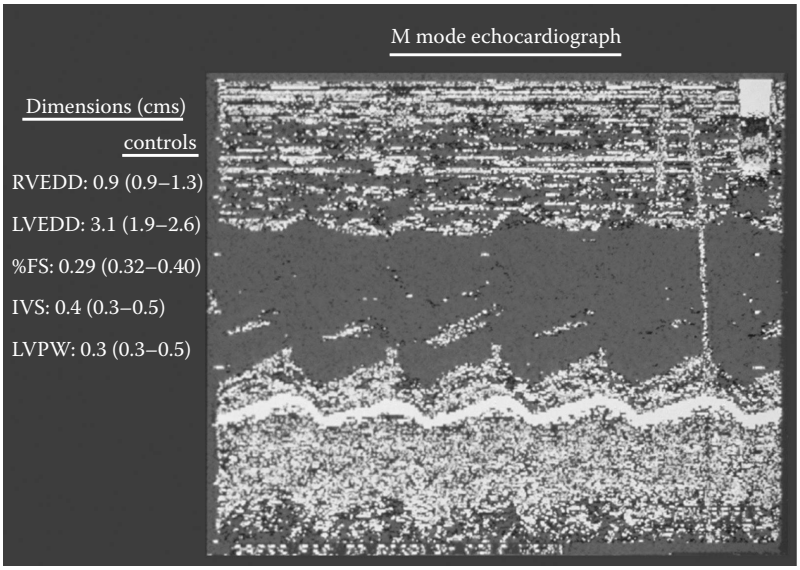

FIGURE 12.51 M mode echocardiogram. RVEDD—right-ventricular end diastolic dimension. LVEDD—left-ventricular end diastolic dimension, %FS—fractional shortening, IVS—interventricular septum, LVPW—left-ventricular posterior wall.

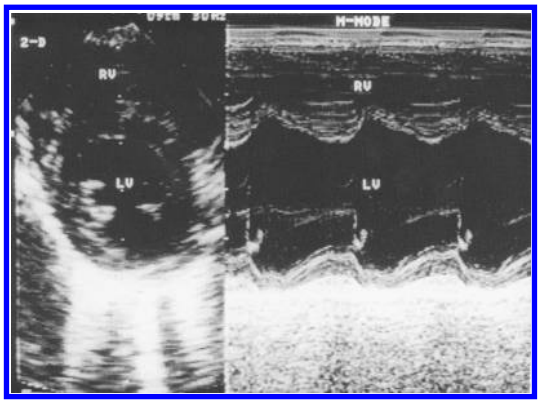

FIGURE 12.52 2D directed M mode electrocardiogram short axis view. LV—left ventricle, RV—right ventricle.

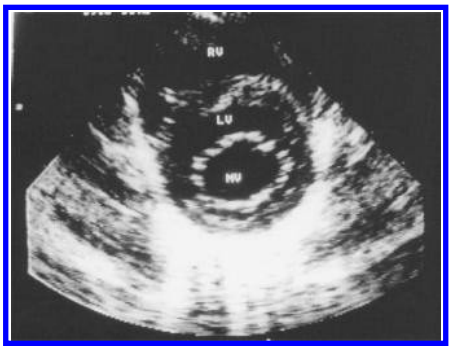

FIGURE 12.53 2D short axis view electrocardiogram. RV—right ventricle, LV—left ventricle, MV—mitral valve.

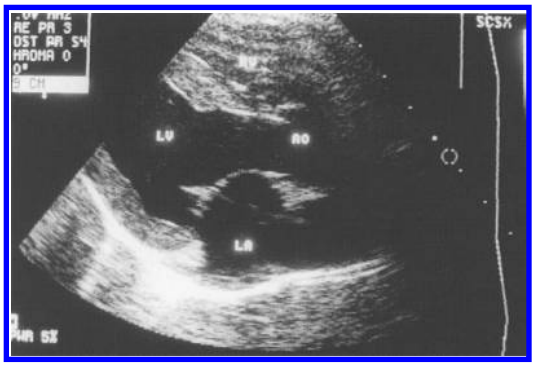

FIGURE 12.54 2D long axis view (parasternal) echocardiogram. LV—left ventricle, AO—aorta, LA—left atrium.

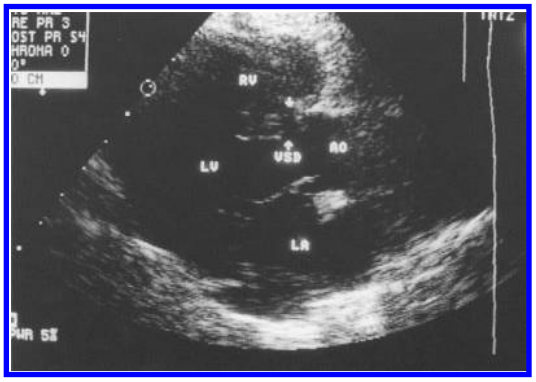

FIGURE 12.55 2D long axis view echocardiogram of a pig with a ventricular septal defect (VSD). RV—right ventricle, LV—left ventricle, AO—aorta, LA—left atrium.

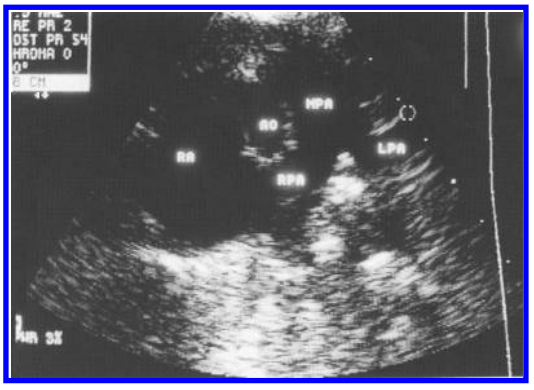

FIGURE 12.56 2D short axis view (great vessels). AO—aorta, MPA—main pulmonary artery, LPA—left pulmonary artery, RPA—right pulmonary artery, RA—right atrium.

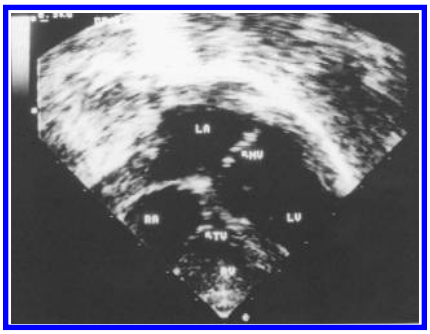

FIGURE 12.57 2D apical four chamber view echocardiogram. LA—left atrium, MV—mitral valve, RA—right atrium, LV—left ventricle, RV—right ventricle, TV—tricuspid valve.

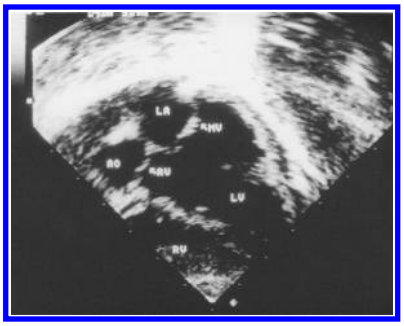

FIGURE 12.58 2D four chamber long axis view. LA—left atrium, AO—aorta, MV—mitral valve, AV—aortic valve, LV—left ventricle, RV—right ventricle.

TABLE 12.12
Demographic and Morphologic Characteristics of Pigs with Hypertrophic Cardiomyopathy (HCM) and of Clinically Normal Control Pigs

Parameters	Pigs with HCM	Control Pigs	*P* Value
No. of pigs	8	25	NA
Age (months)	7.3 ± 1.3	7.2 ± 0.8	NS
Male (%)	75	44	NS
Body weight (kg)	114.0 ± 32.0	112.0 ± 16.0	NS
IVS (mm)			
End diastolic	16.2 ± 1.0	13.1 ± 7.5	<0.01
End systolic	22.4 ± 1.6	17.1 ± 1.0	<0.01
LV caudal free wall (mm)			
End diastolic	14.5 ± 2.5	13.1 ± 1.4	NS
End systolic	23.4 ± 1.6	20.2 ± 0.9	NS
LV cavity dimension (mm)			
End diastolic	44.0 ± 12.9	47.3 ± 6.3	NS
End systolic	26.0 ± 7.6	28.4 ± 6.1	NS
Fraction shortening (%)	36.7 ± 13.9	39.1 ± 10.8	NS
Left arterial dimension (mm)	29.0 ± 10.1	28.0 ± 4.3	NS
Aortic root dimension (mm)	27.3 ± 10.0	28.6 ± 3.8	NS

Source: Reprinted from Lin, J.H. et al., 2002, *Comp. Med.* 52(3): 238–242. With permission.

Note: Data are expressed as mean ± SEM. Echo measurements of Landrace pigs under anesthesia. LV = left ventricular, IVS = intraventricular septum, NS = not significant, NA = not applicable.

TABLE 12.13
Echocardiographic Values in Female Göttingen Minipigs

Parameter	Mean	SD	Minimum	Maximum
Body weight (kg)	13.15	3.25	8.1	21.9
Motor activity[a]	1.77	0.65	1	3
Heart rate (bpm)	115.10	20.65	84	216
Rhythm[b]	0.96	0.22	0	1
IVSD (cm)	0.59	0.13	0.41	1.01
IVSS (cm)	1.07	0.24	0.62	1.99
LVCWD (cm)	0.61	0.14	0.38	1.09
LVCWS (cm)	1.1	0.21	0.69	1.59
LVDD (cm)	2.96	0.33	2.02	4.08
LVDS (cm)	1.53	0.33	0.96	2.56
FS (%)	48.63	8.03	27.65	67.06
EF	0.86	0.07	0.62	0.96
PIVST (%)	0.81	0.30	0.194	1.43
PLVCWT (%)	0.84	0.34	0.18	1.67
EDV (mL)	35.30	12.63	13.05	73.36
ESV (mL)	6.96	4.32	1.84	23.67
SV (mL)	28.34	10.38	5.58	60.16
CO (mL/min)	3,254.78	1,265.57	624.96	6,256.64
CI (mL/min/kg)	248.43	86.47	77.15	509

Source: Reprinted from Konrad, D. et al., 2000, *Comp. Med.*, 50(4): 405–409. With permission.

Note: $N = 58$, bpm = beats per minute, IVSD = diastolic diameter of interventricular septum, IVSS = systolic diameter of IVS, LVCWD = diastolic diameter of left-ventricular caudal wall, LVCWS = systolic diameter of LV caudal wall, LVDD = LV diastolic diameter, LVDS = LV systolic diameter, FS = fractional shortening, EF = ejection fraction, PIVST = percentage of thickening of IVS, PLVCWT = percentage of thickening of LV caudal wall, EDV = end diastolic volume, ESV = end systolic volume, SV = stroke volume, CO = cardiac output, and CI = cardiac index.

[a] 0 = Calm and cooperative animal, 3 = maximal excitation, no cooperation.

[b] Binary—0 = no arrhythmia, 1 = arrhythmia present.

TABLE 12.14
Sonographic Values for Aortic Parameters in Göttingen Minipigs

Parameter	Mean	SD	Minimum	Maximum
AD prox ARS (cm) (ADP)	0.75	0.09	0.61	1.18
AD dist ARS (cm) (ADDi)	0.75	0.09	0.61	1.11
ADD (cm)	0.82	0.12	0.62	1.21
ADS (cm)	0.88	0.13	0.69	1.29
ADD:ADS	0.93	0.03	0.85	0.98
V_{max} (m/s)	1.94	0.45	1.09	2.95
Aortic morphology	0.03	0.18	0.00	1.00
Systolic window[a]	1	0	1	1
Doppler color[b] variegation	0	0	0	0

Source: Reprinted from Konrad, D. et al., 2000, *Comp. Med.*, 50(4): 405–409. With permission.

Note: $N = 58$ female pigs, AD prox ARS = aortic diameter proximal to the right renal artery, AD dist. ARS = aortic diameter distal to the right renal artery, ADD = aortic diastolic diameter, ADS = aortic systolic diameter, ADD:ADS = ratio of ADD to ADS, and V_{max} = maximum flow speed.

[a] Systolic window—1 = present, 0 = not present.

[b] Doppler color variegation—0 = not present.

TABLE 12.15
Effect of Body Weight on Echocardiographic Parameters

Parameter	Equation for Regression	Coefficient of Correlation
LVDD	1.739 + 0.093 W	0.69*
LVDS	0.821 + 0.054 W	0.53*
EDV	0.885 + 2.618 W	0.67*
ESV	–2.764 + 0.740 W	0.56*
SV	3.644 + 1.881 W	0.59*
CO	344.63 + 221.39 W	0.57*

Source: Reprinted from Konrad, D. et al., 2000, *Comp. Med.*, 50(4): 405–409. With permission.

Note: $N = 58$ female minipigs, W = body weight (kg), LVDD = LV diastolic diameter, LVDS = LV systolic diameter, EDV = end diastolic volume, ESV = end systolic volume, SV = stroke volume, CO = cardiac output.

*$p < 0.001$.

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13 Endoscopy and Minimally Invasive Surgery

M. Michael Swindle and Katherine A. Morgan

CONTENTS

INTRODUCTION

In the current healthcare climate which highly values patient safety, quality, and cost efficiency, the simulated surgical experience, outside the clinical arena, is essential to surgical training. Great attention has been paid to the development of virtual reality (VR) simulators, which allow for basic surgical tasks to be practiced and mastered, and then competency to be documented in a consistent fashion. VR technology has also evolved to accommodate this role, including improved visual and haptic experience as well as the development of a robust associated curriculum. However, the animal model, and in particular the swine model, maintains an important role in simulated surgical training. The animals represent a unique medium for teaching tissue handling and dissection techniques and mastery of operative device technology. They are a cardinal mechanism for

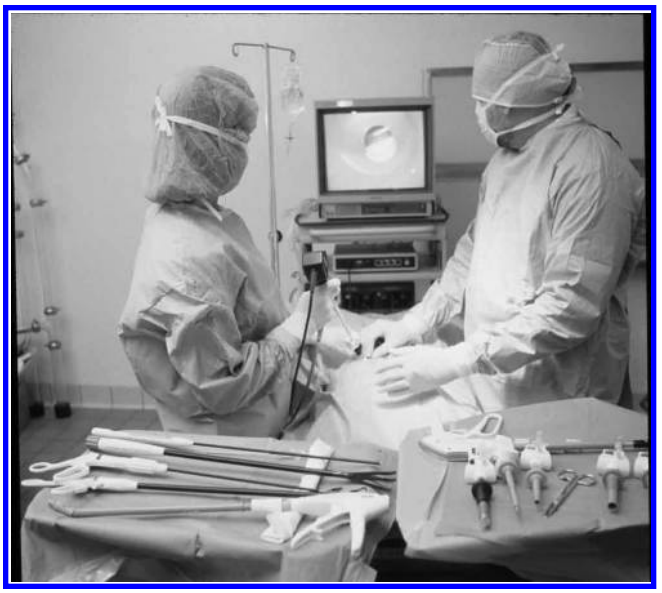

FIGURE 13.1 Operating room for laparoscopic surgery.

learning complex tasks and refining techniques to improve patient care. In addition, given the rapid pace of innovation in minimally invasive surgery, including advanced techniques in therapeutic endoscopy, complex laparoscopy, and robotic operations, the swine model is an invaluable resource in procedure development and training. The DVD attached with this textbook contains demonstration videos of both laparoscopic and natural orifice transluminal endoscopic surgical procedures performed as nonsurvival procedures in a pig (Figure 13.1).

GENERAL CONSIDERATIONS

Methodologies of prepping and anesthetizing the animals for these procedures, as well as the anatomy, are discussed in the various system chapters and in Chapter 2. Fasting instructions and methods of bowel evacuation for gastrointestinal (GI) procedures are discussed in Chapter 4 and our laboratory method is given in Table 13.1. Fasting for 48 h in a cage without contact bedding is essential for these procedures. It is not desirable to eliminate water unless it is of importance to a particular gastric procedure. Electrolyte or glucose and protein supplements, as described in Chapter 4, may be given as liquid diets during the 48-h fast without producing significant residue. This also provides the animals with a form of nutrition that will prevent them from being compromised physiologically for these procedures. Animals should be fully anesthetized and intubated when performing laparoscopic or endoscopic procedures (Chapter 2).

ANATOMIC CONSIDERATIONS FOR FLEXIBLE ENDOSCOPIC RESEARCH

Porcine models in the 20–34-kg weight range are acceptable as models for most endoscopic procedures in clinical practice (Freys et al., 1995; Ma and Fang, 1994; Noar, 1995; Pasricha et al., 1995; Rey and Romanczyk, 1995; Santos Garcia-Vaquero and Uson Gargallo, 2002). The porcine model has both advantages and disadvantages as a model for these techniques (Figures 13.2 through 13.6). The porcine esophagus is on average 10 cm longer than that of the human, and the swine stomach has a tighter J-shaped curve. These two features make it difficult to intubate the duodenum. The swine stomach has a muscular outpouching, the torus pyloricus, which is located adjacent to the pylorus (Freys et al., 1995; Rey and Romanczyk, 1995). This structure can serve as an excellent

TABLE 13.1
Endoscopy Fasting Protocol (30–40 kg Pig)

TWO DAYS BEFORE PROCEDURE

Pig eats normal morning feed.	NPO sign placed at 1 p.m.	Pig receives 12 g VISICOL™ (8 Tablets) with 3–8 oz cans ENSURE™ w/equal parts of water.	Never restrict pigs from water unless it is a stomach surgery and then withhold water for only 4 hours prior to surgery.

ONE DAY BEFORE PROCEDURE

Morning: 4 cans of ENSURE w/water and 30 g (20 tablets) VISICOL™	Afternoon: 4 cans ENSURE w/water and 30 g (20 tablets) VISICOL™

VISICOL™ (sodium phosphate monobasic monohydrate, USP, and sodium phosphate dibasic anhydrous)
ENSURE™ (Ross Nutrition/Abbott Labs)
30 g dose = 20 tablets
1 tablet = 1.5 gm
Tablets are crushed and dissolved in liquid diet. A blender can be used.

4 cans ENSURE™ with water the night of the procedure.	Regular feeding starts the next morning.

ALTERNATIVE

Two to three days of fasting on a liquid diet provide a satisfactory cleansing for many procedures, especially in the upper GI tract. In that case, ENSURE™ (24 oz) and Gatorade™ (24 oz) BID are given in separate pans for the days prior to surgery. When giving liquid diets it is essential that the bowls be tightly attached to the cage otherwise they will spill and spoil the liquids. Gatorade is sticky and may attract flies but it does supply electrolytes. Data has shown that blood chemistry and electrolyte values are not affected until after 48 hours of total fast.

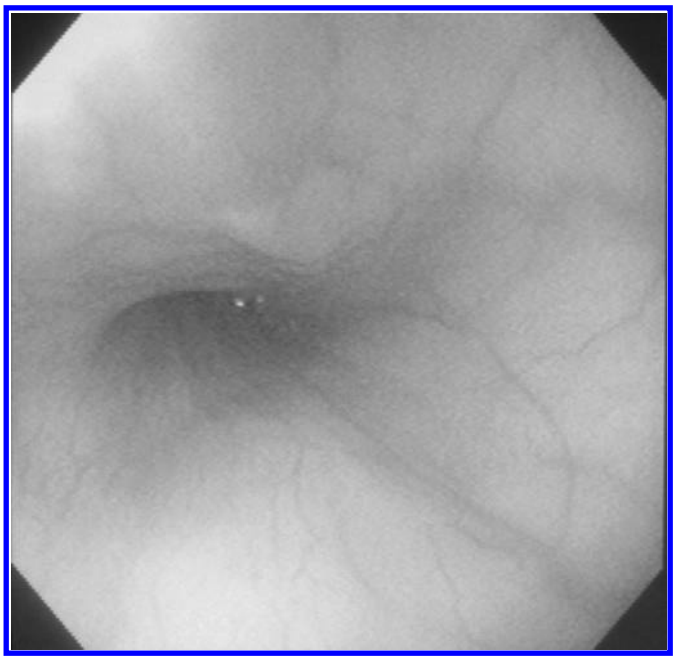

FIGURE 13.2 Endoscopic view of the esophagus.

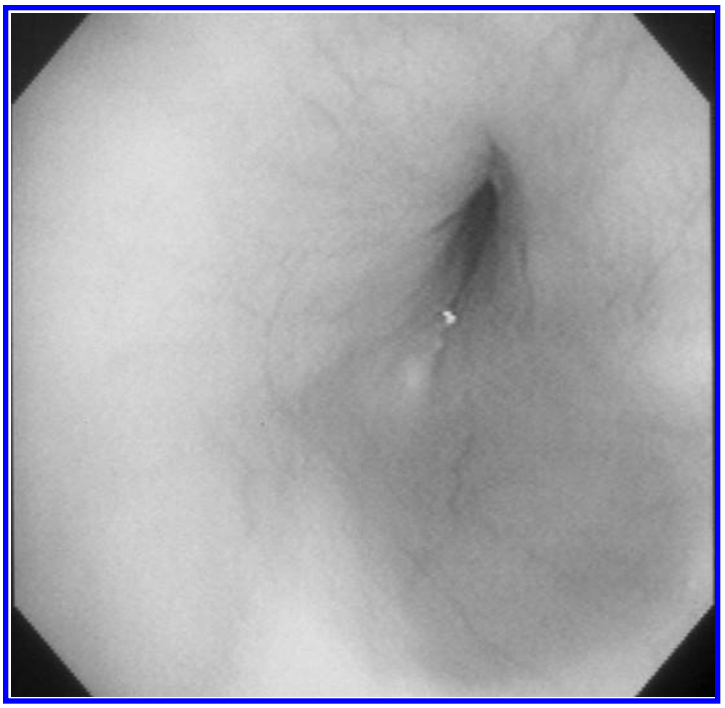

FIGURE 13.3 Endoscopic view of the lower esophageal sphincter.

tool to teach the use of various endoscopic cutting devices, where precision in movement is mandatory. The biliary papilla is located 1–2 cm distal to the pylorus, just behind the torus pyloricus and is separate from the pancreatic papilla. The orifice of the pancreatic duct is located 7–12 cm distal to the biliary papilla and is extremely difficult to find endoscopically but is readily identifiable in open surgery. The biliary sphincter serves as an excellent model for the study of the human sphincter of Oddi (see Chapter 5). Sphincter of Oddi dysfunction is a disorder in humans characterized by episodes of epigastric or right upper quadrant (RUQ) pain with or without abnormal liver tests during episodes of pain, or a dilated bile duct. Detection of this disorder is best accomplished with

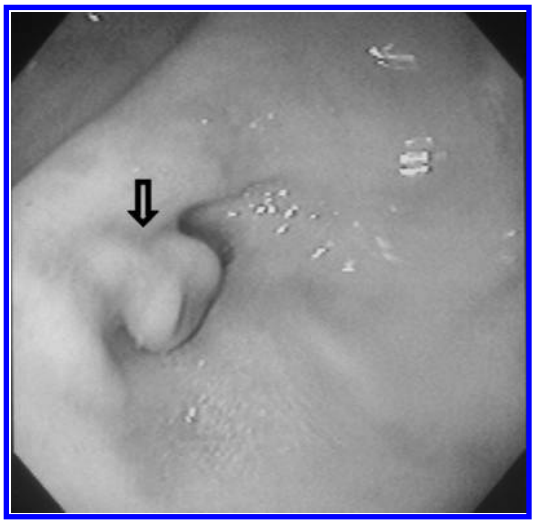

FIGURE 13.4 Endoscopic view of the gastric antrum and the torus pyloricus (arrow).

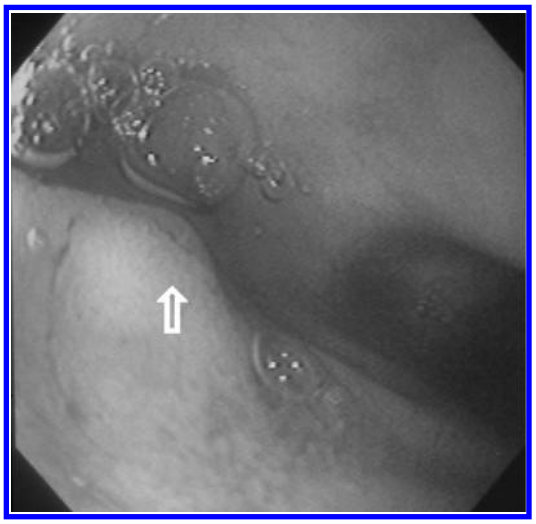

FIGURE 13.5 Endoscopic view of the biliary papilla (arrow).

an endoscopic test called *sphincter of Oddi manometry*. Our understanding of the human condition has been greatly enhanced by studying the swine sphincter using sphincter manometry (Noar, 1995; Pasricha et al., 1995). The pig demonstrates basal sphincter pressures as well as phasic waves, in which the rate and amplitude can be recorded and studied, including pharmacological manipulation (Bak et al., 2000). The functions of the pancreas and pancreatic duct (see Chapter 6) are similar to those of humans, even though the gross anatomy is different (Mullen et al., 1992; Pasricha et al., 1995; Stump et al., 1988).

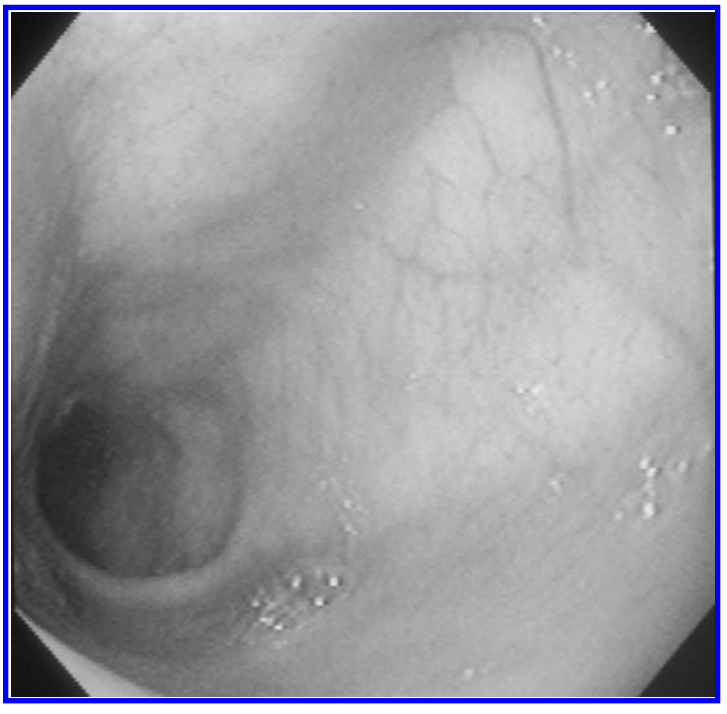

FIGURE 13.6 Endoscopic view of the duodenum.

The conformation of the swine colon is very different from that of the human (Chapter 4). The colon forms a spiral, which does not exactly simulate the human colon when studying colonoscopy. Techniques have been described in which the swine colon is "unspiraled" in open surgery and tacked down so as to simulate the conformation of the human colon. In experiments that require colon cleansing, we have found that use of sodium phosphate (NaP) in a pill-based colon cleansing prep works very well in swine (VISICOL™, InKine Pharmaceuticals, Inc., Blue Bell, PA). The pills are included in the feedings and are well tolerated by the pigs.

PERFORMING ENDOSCOPY

The basic technique of GI endoscopy involves careful passage of the lubricated endoscope to the area of interest. Frequently, overtubes are passed over the endoscope once the scope is in the distal esophagus. The overtubes are designed with spiral coils in the wall to prevent kinking and have a bite block fixed to the end, which fits securely in the pig's mouth. The overtubes are usually 45–54 French (15–18 mm diameter) and facilitate repeated intubation of the pig, which is often required when performing therapeutic procedures. The bite block protects the scope from the pig's teeth (which can easily tear the plastic covering of the scope shaft) and has a soft valve covering the end, which allows easy movement of the scope in and out but prevents air from escaping. Air insufflation is a routine and important part of endoscopic procedures. It is also essential in endoscopic procedures to avoid overinsufflation (>8 mmHg) of the gut lumen. This can produce a vagal response, resulting in cardiac arrest. Insufflation should be performed slowly and judiciously as required for observation rather than continually administering air.

Intubation can be a particularly difficult aspect of performing endoscopy in the swine. The pharyngeal anatomy is different from that of the human, and care should be taken to avoid passing the device into the pharyngeal diverticulum when entering the esophagus. If this occurs and too much pressure is exerted, one can easily cause a pharyngeal perforation.

Gut motility can be a problem, especially when working in the small bowel, and when needed, Glucagon® (Eli Lilly, Indianapolis, IN) can be administered intravenous (IV) to slow peristalsis. It is administered in 0.25–0.5-mg dosages by slow injection, as required.

Once the proximal esophagus is entered, it is relatively easy to pass the scope into the stomach. There is inherent angulation between the distal esophagus and the stomach, and this must be carefully negotiated. Within the stomach, there is invariably some bile-stained fluid or residual food, which can be aspirated through the scope. The scope must be advanced quite far along the greater curvature of the stomach before one approaches the pylorus. Intubation of the pylorus can be difficult because of the extreme J shape of the stomach (Figures 13.3 through 13.5). Once past the pylorus, the opening of the bile duct can be identified in a location approximately 1 cm distal to the torus pyloricus. One needs a side viewing endoscope to locate the opening, and the instrument must have an elevator function to be successful in passing an endoscopic accessory into the bile duct. The pancreatic orifice is indistinct but can be located in most animals, with experience (Noar, 1995; Pasricha et al., 1995). The small intestine may be observed using the same methodologies as for other species. Air or saline tonometry can be used to estimate GI mucosal carbon dioxide tension in swine (Salzman et al., 1994).

ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY

Endoscopic Retrograde Cholangiopancreatography (ERCP) is a technique (Figure 13.7) for which the pig may have particular usage, even with the differences in anatomy noted in the preceding text (Gholson et al., 1990; Noar, 1995). The pig is the primary model for biliary sphincterotomy and biliary stenting. Nonhuman primates and cats have a common opening for the biliary and pancreatic duct; however, cats are too small for most equipment, and appropriately sized primates, such as the baboon (Siegel and Korsten, 1989), are expensive and difficult to obtain. The dog also has problems with availability, and size of the structures can vary substantially among animals of the same weight

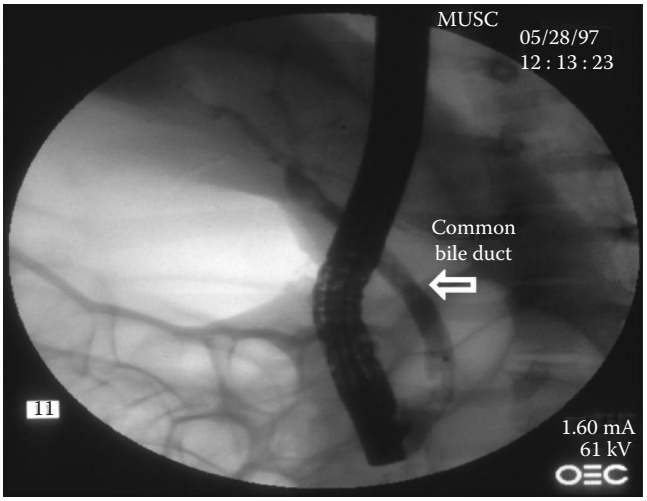

FIGURE 13.7 Retrograde cholangiogram of the common bile duct with retrograde flow into the intrahepatic biliary system.

because of breed differences. The pig has a unique ability to tolerate total biliary obstruction, which makes it a unique and ideal model for testing devices that relieve bile duct obstruction, such as stents (Sahai et al., 1998). The bile duct is ligated laparoscopically and dilates over the course of a couple of weeks. The ligation model, however, prevents endoscopic access to the bile duct and provides access only transhepatically. However, biliary stricture models have also been described in which a cautery probe (heater probe or bipolar probe) is passed into the bile duct via the biliary papilla, and then the mid-bile duct wall is cauterized. This reproducibly creates a bile duct stricture within a few weeks (Rumalla et al., 2003). One can then go on to test new types of biliary stents or devices to dilate strictures. Implantation of gallstones into the biliary tree has also been described to create an opportunity to teach biliary sphincterotomy and stone extraction, which is a common procedure performed in humans (Griffith et al., 1989).

ENDOSCOPIC HEMOSTASIS (GASTROINTESTINAL BLEEDING)

Diagnosis and treatment of GI bleeding are important applications of endoscopy in humans. Animal models are needed to help develop endoscopic techniques and therapeutic devices, as well as teach the techniques of endoscopic hemostasis. The two most common causes of GI bleeding are esophageal and gastric varices in patients with portal hypertension and bleeding from peptic ulcer disease. The dog has been found to be uniquely useful in the study of variceal bleeding because of its ability to produce portal hypertension and thus create esophageal and gastric varices (Jensen et al., 1983; Kulling et al., 1999). Swine, however, can be used to study ulcer bleeding and can develop portal hypertension (Chapter 5). A model has been described in which the short gastric artery is carefully exposed along a 2-cm segment on the side facing the stomach (Hu et al., 2005a,b,c). The artery is then anchored to a small gastrostomy made at the posterior wall near the vessel. An inflatable plastic cuff is placed around the base of the artery to control flow. This model is capable of simulating arterial hemorrhage from a gastric ulcer. In the report, an endoscopic suturing device (Eagle Claw®, Olympus, Tokyo, Japan) was tested to determine its capability to oversew and stop ulcer bleeding (Hu et al., 2005a,b). Recently, a model of delayed portal vein thrombosis has been created in swine following radiofrequency ablation procedures in the portal vein (Frich et al., 2006). The model has only been followed for less than a week, but it has the potential of developing chronic hepatic disease and portal hypertension.

COLONOSCOPY

Entering the animal rectally for colonoscopy requires the same caution as for oral entry to avoid rupture of the colon. The colon may be observed in the same manner as for other species, with the exception of the spiral colon, which is located in the left upper quadrant of the abdomen (Chapter 4). Currently, a number of technologies are being developed to perform screening colonoscopy in humans. These devices are self-propelling and automated. One such device was recently reported after preliminary testing in the pig colon (Pfeffer et al., 2006).

LARYNGOSCOPY AND BRONCHOSCOPY

Laryngoscopy and bronchoscopy can also be performed in swine. The anatomy is discussed in Chapter 9. Two precautions are necessary when using this technology. The epiglottis and larynx are very prone to spasm and edematous swelling with manipulation. The lateral ventricles of the larynx are also easily ruptured, resulting in subcutaneous emphysema. The larynx should always be sprayed with topical anesthetics, and appropriate-sized devices should be used to prevent trauma. Swine are also very susceptible to vagal stimulation and bradycardia with manipulation of the airway. Animals should always be atropinized during the entire procedure (Chapter 2). Isoproterenol is useful to prevent bronchospasm.

TRAINING IN FLEXIBLE ENDOSCOPY

The porcine model has proved very beneficial in endoscopic and laparoscopic training. In some circumstances, it remains the best model. Training in endoscopic ultrasound (EUS) is a case in point. This technology involves attaching a miniaturized ultrasound probe on the end of a flexible endoscope (Figure 13.8). The endoscope serves as the "hands" of the endoscopist and allows one to place the transducer in various positions within the upper GI track or rectum. The target organ is either the gut wall, structures (lymph nodes), or organs that lie adjacent to the gut (pancreas, bile duct, liver, etc.). With this technology, it is possible to place a needle down the biopsy channel of the endoscope, and then the needle can be advanced under real-time ultrasound guidance into a target outside the gut wall (lymph node, pancreatic mass, etc.) (Figure 13.9). Cells are then aspirated from the target and read by a cytopathologist. This technique has become very important in the practice of GI endoscopy but requires considerable training. The learning curve can be shortened by doing

FIGURE 13.8 A picture showing the distal end of a radial scanning echoendoscope. This is a standard endoscope with oblique-forward viewing optics with an ultrasound transducer mounted on the distal tip. The ultrasound transducer is rotated 360° around the long axis of the scope producing a 360° image.

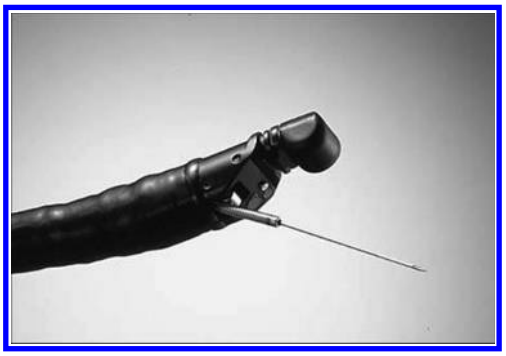

FIGURE 13.9 A picture showing a linear array echoendoscope. The transducer on this instrument is constructed of multiple piezoceramic elements aligned parallel to the shaft of the scope. When a needle is passed through the biopsy channel, it comes out in the plane of imaging allowing real-time, ultrasound-guided fine needle aspiration (FNA) cytology.

preliminary work on the swine model (Bhutani et al., 1998, 2000). Although computer and inanimate models have been developed to aid EUS training (Burmester et al., 2004, Sorbi et al., 2003), they do not provide an adequate substitute for the live pig model.

Although some training requires anesthetized swine, animal conservation is important. As a result, over the last 5 years, there has been an explosion in the use of *ex vivo* swine tissue for endoscopic training. This model was first conceived by Martin Neumann in Erlangen, Germany (Neumann et al., 2000). It was first developed as a potential laparoscopic training model, but it quickly became apparent that its greatest utility would be for flexible endoscopic training. The concept involves harvesting the upper GI tract (which can include the liver and biliary tree, as well as the respiratory tree with lungs and the colon) from a pig that has been slaughtered for meat. The specimen is cleaned and prepared followed by freezing. When needed, the specimen is thawed and tacked down with sutures within a plastic form that allows the anatomy to be displayed as if it were human (Figure 13.10). Various "pathologies" can be created, such as implantation of a small piece of the splenic artery into the gastric wall to simulate a bleeding ulcer. The extralumenal end of the artery is hooked to a pump, which instills red-dye-colored fluid to simulate blood. An endoscope is passed into the stomach, and the pump is started. From the endoscopist's perspective, this looks just

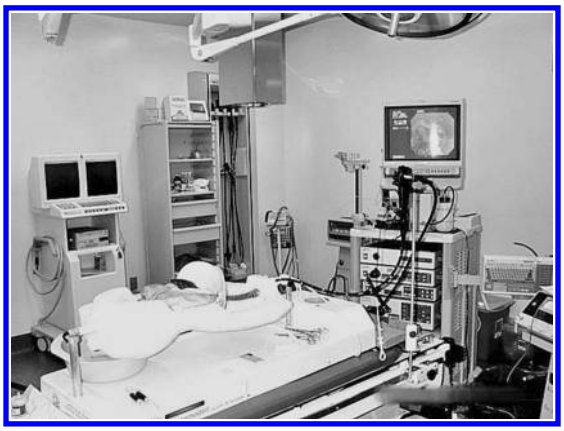

FIGURE 13.10 A picture taken from the Medical University of South Carolina (MUSC) Digestive Disease Center animal lab showing the Erlanger model, where the upper gastrointestinal (GI) tract of the swine has been mounted in the plastic tray to simulate the human anatomy. An endoscopic system has been set up in preparation for a teaching session on therapeutic endoscopy.

like arterial bleeding from an ulcer. Various therapeutic techniques can then be taught to stop the bleeding such as injection therapy, cautery with a heater or bipolar probe, or clips (Hochberger et al., 2004, 2005). One can create polyps, esophageal varices, and various pancreatobiliary pathologies to teach various therapeutic endoscopic procedures. The model serves as a very useful teaching tool and utilizes a readily available (and otherwise wasted) resource. The impact of these *ex vivo* training models is increasing (Hochberger et al., 2001, 2002; Matthes et al., 2005; Neumann et al., 2001). These same models can be used for preliminary testing of endoscopic devices as well.

LAPAROSCOPIC AND THORACOSCOPIC SURGERY

Minimally invasive surgical techniques have replaced many open surgical procedures in clinical practice, and complex operations are now routinely performed laparoscopically. The porcine model has proved to be invaluable in both training and development. The basic techniques involve insufflating a body cavity with CO_2, placing trocars, and using camera guidance to perform surgical techniques with specialized instruments through the trocars. Specialized suturing and instrument-handling techniques, different from those of open surgery, are required. Minimally invasive surgery can offer significant patient benefits including decreased postoperative morbidity and length of stay, earlier return to work, and potentially improved oncologic outcomes (KOOBY, Gutt et al., 2004).

When using swine for training, nonsurvival techniques are preferred because they allow the surgeon the chance to perform multiple procedures and do not require close attention to aseptic techniques. Training courses should include the use of inanimate models or VR simulators prior to performing the techniques on live animals. Most procedures and instruments can be utilized in swine weighing 25–35 kg. Reviews of recommendations for training of surgeons have been published (Bailey et al., 1991; Freeman, 1994; Kopchok et al., 1993; Lyons and Sosa, 1992; Soper and Hunter, 1992; Srinivasan et al., 1999).

Swine are usually positioned in dorsal recumbency for these procedures, but the positioning may have to be varied for retroperitoneal and thoracic procedures. The positioning of the trocars depends upon the procedure being performed. Standard positions are described here as an example (Figure 13.11). The most common and safest initial entry port is at the umbilicus, where a Veress needle is

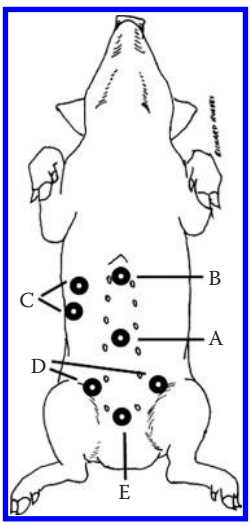

FIGURE 13.11 Drawing of a pig showing examples of laparoscopic trocar insertion points. A, Veress needle and camera trocar; B, large trocar site for upper abdominal procedures; C, small trocars in paramedian and lateral positions; D, small trocars for caudal abdominal procedures; E, large trocar for lower abdominal procedures.

utilized to insufflate the abdomen to 12–14 mmHg with CO_2. A trocar is then placed through this incision and the remaining trocars are placed under direct visualization with the laparoscope. The number and positioning of the secondary trocars are dependent upon the procedure at hand. Trocars are positioned in an arc at a distance sufficient from the operative field of interest so that it can be well visualized and accessed. Ports should be distant from one another by at least 10 cm to avoid interinstrumental interference while working. Proper port placement should not be underestimated as it does involve a learning curve and is essential to the success of complex operations.

Insufflation of the abdomen and thorax causes hemodynamic changes, and monitoring the intra-abdominal pressure is essential. Creating a pneumoperitoneum with CO_2 increases the arterial pCO_2 and reduces the pH. It has been demonstrated that using helium as the gas eliminates this respiratory acidosis (Leighton et al., 1993). Both the reverse Trendelenburg position and the increase in intra-abdominal pressure caused by insufflation reduce the venous blood flow from the lower limbs, which could predispose to venous thrombosis and pulmonary embolism (Jorgensen et al., 1994). The effects of insufflation during thoracoscopy are even more dramatic. Significant decreases in cardiac index, mean arterial pressure, stroke volume, and left ventricular stroke work index were noted with pressures greater than 5 mmHg. In the same study, central venous pressure increased (Jones et al., 1993). In pigs, 25–35 kg, which are routinely used for these procedures, the mediastinum is thin and easily ruptured, and precautions should be taken with both the use of insufflation and the placement of chest tubes (Chapter 9). The diaphragmatic communication between the peritoneal and pleural cavities in young swine is easily ruptured, and gas in either cavity is likely to penetrate the other cavity if the pressure is high enough (Freeman, 1994; Swindle et al., 1988). At the end of the procedure, the cavity is examined for hemostasis and proper tissue apposition. Following desufflation, the peritoneum, muscle, and skin layers penetrated by the trocars are closed with sutures or staples.

Freeman (1994) and Srinivasan et al. (1999) have written succinct reviews of the most common techniques used for training in swine; these publications, in conjunction with the technical descriptions provided by others, will be used as examples of the types of technical variations that are used in performing various laparoscopic surgical techniques.

Cholecystectomy

Three secondary trocars, two 5 mm and one 10 or 12 mm, are inserted into the cranial abdomen in addition to the primary umbilical camera port. The gallbladder (Figure 13.12) is bluntly dissected to the cystic duct and ligated. The gallbladder is removed through the large trocar and may be emptied

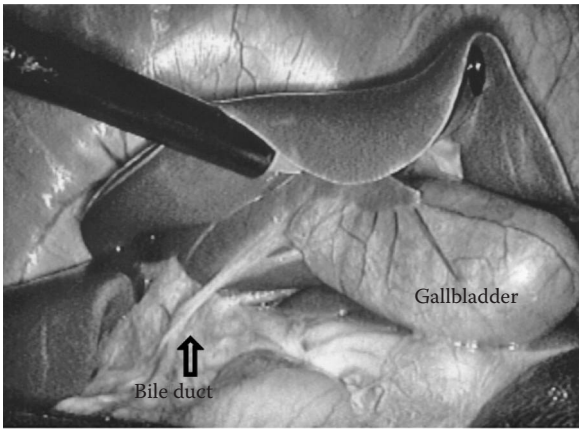

FIGURE 13.12 Laparoscopic view of the gallbladder and bile duct.

with suction prior to removal (Pasricha et al., 1995; Rodriguez et al., 1993; Soper et al., 1991, 1993; Srinivasan et al., 1999). The open technique is described in Chapter 5.

Small Intestinal Anastomosis

Four 10- or 12-mm trocars are inserted in the paramedian position over the area of interest in addition to the camera port. The standard techniques of identification of a segment, resection, and anastomosis are described in Chapter 4 and are performed with laparoscopic instruments, sutures, and staples. The excised segment is removed through one of the trocars prior to closure. Laparoscopic techniques have been demonstrated to be as effective as open techniques when wound healing and complications were compared (Fleshman et al., 1993; Noel et al., 1994; Olson et al., 1995; Pietrafitta et al., 1992; Soper et al., 1993; Srinivasan et al., 1999).

Colonic Anastomosis

As for small intestinal anastomosis, four secondary 10- or 12-mm trocars are positioned in the paramedian position; however, they are in the caudal abdomen. A staple anastomosis is performed after mobilization of the segment to be excised. Both intracorporeal and extracorporeal techniques have been utilized. For the extracorporeal technique, the proximal segment is externalized through the right lower quadrant. The circular stapler may be placed through the anus if the anastomotic site is distal. Colonic anastomosis using these techniques has been demonstrated to be as effective as the open techniques described in Chapter 4 (Fleshman et al., 1993; Noel et al., 1994; Olson et al., 1995; Pietrafitta et al., 1992; Soper et al., 1993; Srinivasan et al., 1999).

Vagotomy and Hiatal Procedures

Four 5-mm or 10- to 12-mm trocars are used in the cranial abdomen. The esophagophrenic ligament is thicker in the porcine model than in the human and must be carefully dissected. The vagus nerve can be identified along the margin of the esophagus, or selective vagotomy procedures at the level of the stomach can be performed (Figure 13.13). Hiatal hernias can be created and repaired as well as the performance of fundoplications. Procedures in this region require atropinization of the animal to prevent vagal-induced bradycardia. Pneumothorax is also a potentially fatal complication associated with the dissection and must be monitored by the anesthetist and relieved with chest tubes, if necessary (Freeman, 1994; Josephs et al., 1992; Srinivasan et al., 1999).

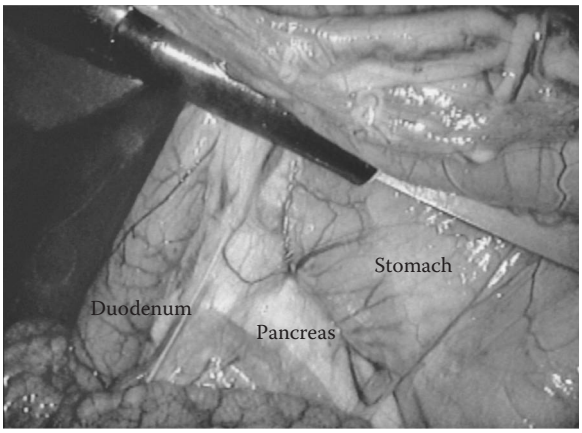

FIGURE 13.13 Laparoscopic view of the pancreas, duodenum, and stomach.

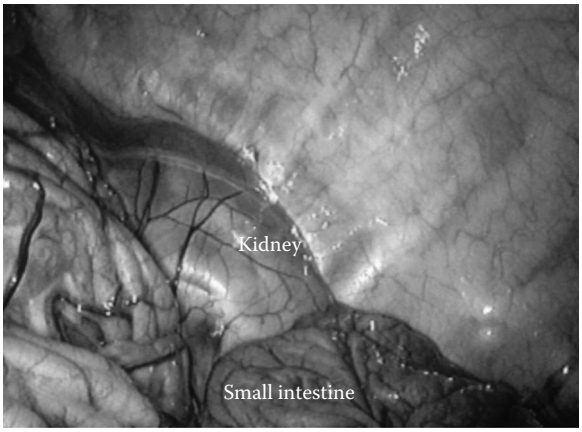

FIGURE 13.14 Laparoscopic view of the right kidney and small intestine.

Nephrectomy and Renal Ablation

Either a paramedian or a retroperitoneal approach may be made to the kidney with three 10- or 12-mm trocars (Figure 13.14). After dissection and ligation of the blood vessels, the kidney may be removed with a larger trocar or a specimen retrieval bag after morcellation (Chiu et al., 1992; Kerbl et al., 1993). Laparoscopic and percutaneous procedures of the kidney have also involved removal and ablation of tissue with radiofrequency (Bernardo and Gill, 2002; Desai et al., 2005; Gill et al., 2000; Hsu et al., 2000; Wagner et al., 2005). The open techniques are described in Chapter 7.

Inguinal Herniorrhaphy

Spontaneous inguinal hernias of swine or dissected inguinal rings (Figure 13.15) of male swine may be used as models. Two secondary 10- or 12-mm trocars are introduced in the caudal abdomen. For this procedure, the edges of the peritoneum around the inguinal ring are dissected free from the inguinal ring, and a prosthetic mesh is inserted and stapled in place. The insufflation pressure is reduced to facilitate proper alignment of the tissues prior to placing the last staples and closure of the peritoneum (Layman et al., 1993). The inguinal ring may also be repaired using a preperitoneal approach. From the midline, the trocars are inserted without penetration of the peritoneum. After

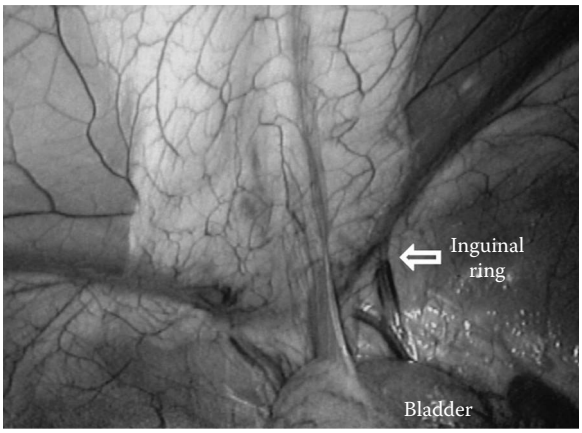

FIGURE 13.15 Laparoscopic view of the inguinal ring and urinary bladder.

insufflation, the operating trocars are inserted lateral to the midline below the umbilicus and cranial to the pubis (Freeman, 1994; Srinivasan et al., 1999). Inguinal hernias are discussed in Chapter 11.

Appendectomy

The pig does not have an appendix; however, one can be created as an acute model for training purposes from a loop of small intestine or uterine horn. For this procedure, two 10- or 12-mm trocars are introduced on either side of the midline. A loop of the structure has its blood supply ligated, and pretied loop ligatures are placed at the base of the loop (Freeman, 1994; Srinivasan et al., 1999).

Splenectomy

The spleen may be removed after placing four secondary 10- or 12-mm trocars in the cranial abdomen. The vascular supply is ligated or stapled and the organ reduced in size with a morcellation device prior to removal (Freeman, 1994; Srinivasan et al., 1999). Splenectomy is discussed in Chapter 6.

Adrenalectomy

With the pig in ventral recumbency, a unilateral adrenalectomy may be performed in the retroperitoneal space. Trocars are placed in the flank without penetrating the peritoneum, and the retroperitoneal space is insufflated. After ligation of the blood vessels and removal of the structure through 10- or 12-mm trocars, the retroperitoneal space is desufflated and closed in a routine fashion. Care should be taken to avoid penetration of the diaphragm with a trocar from this position (Brunt et al., 1993). Adrenalectomy is discussed in Chapter 7.

Urinary Bladder Procedures

Urinary procedures are discussed in Chapter 7. Laparoscopic urinary incontinence procedures have been described (Ou et al., 1993; Vancaillie, 1993). Two or three 10- or 12-mm trocars are placed in the right and left caudal quadrants of the abdomen. After tissue dissection to expose the urethra, a mesh or sutures are placed in the perivaginal tissue and anchored to Cooper's ligament.

Reproductive Procedures

The uterus of the pig is bicornuate with a small body (Chapter 8). The anatomy of the structure is dissimilar to human anatomy, and the goat is the preferred model for uterine body procedures and hysterectomy (Freeman, 1994). However, the porcine model may be used for ovarian and fallopian tube procedures, such as ovariectomy and tubal ligation.

Thoracoscopy

Because of the precautions described in the preceding text and in Chapter 9, the procedure should not be performed with insufflation of the thoracic cavity. Rather, single lung ventilation can be performed after unilateral intubation of the right main-stem bronchus. Auscultation can be used to confirm the correct placement of the tube. Pneumothorax on the left side will be produced when the trocars are inserted and the lung collapses. Depending upon the procedure that is performed, the trocars can be inserted into the appropriate intercostal spaces with the pig in dorsal, ventral, or lateral recumbency (Freeman, 1994). Techniques for slowing the heart rate include using high-dose opioid infusion anesthetic techniques and beta blockers. The heart rate should be maintained at >60 beats per minute (bpm) in swine. These techniques are discussed in Chapter 2.

Laparoscopic Ultrasonography

A semiflexible ultrasound transducer introduced through a laparoscopic port has been utilized to visualize abdominal structures. The transducer uses both gray-scale and color Doppler techniques to observe normal structures, to aid dissection, and also to diagnose abnormalities, such as gallstones. In one study, a 9.6-mm diameter catheter with a 5.0–7.5 mHz transducer was found to be applicable in both miniature swine and humans (Liu et al., 1995). Many modifications of imaging techniques, including CT, ultrasound, and MRI, have been utilized to aid in these types of procedures, and their use is demonstrated in the DVD attached with this book.

POSTOPERATIVE CARE FOR LAPAROSCOPIC AND ENDOSCOPIC PROCEDURES

If the surgeries are performed as survival procedures, appropriate postoperative care, including the use of postoperative analgesics, must be administered. Discomfort may be associated secondary to the introduction of CO_2 into the peritoneal cavity and gas in the intestines leading to ileus. Animals should also be monitored for respiratory acidosis and the potential of a tension pneumothorax.

NATURAL ORIFICE TRANSLUMINAL ENDOSCOPIC SURGERY

This is a rapidly emerging surgical discipline, which is a hybrid between flexible endoscopy and laparoscopic surgery. Originally, this involved passing a flexible endoscope into the stomach, creating a hole in the gastric wall and entering the peritoneal cavity. An intervention is then performed followed by withdrawal of the endoscope back into the gastric lumen and closure of the gastrotomy (see DVD for video of a nonsurvival demonstration). This was followed by development of the use of the technique to enter body cavities through the colon, vagina, umbilicus, and esophagus (Bernhardt et al., 2012; Cordova et al., 2013; Dubcenco et al., 2011; Grund and Lehmann, 2010; Kono et al., 2013; Leroy et al., 2012; Turner et al., 2011). The techniques will largely be done utilizing the porcine model, which has a thicker and more robust stomach than the human stomach (Hu et al., 2005a,b).

The perceived advantages include a "perfect" cosmetic surgery, more rapid healing, avoidance of wound infections, and less pain. The first report of this procedure, from a group at Johns Hopkins, was on transgastric peritoneoscopy in a pig (Kalloo et al., 2004). This was followed by a report of the long-term survival of a pig that underwent a gastrojejunostomy performed with a flexible endoscope by the transgastric approach (Kantsevoy et al., 2005). Since these two early reports, there has been considerable activity in this area. Experts in flexible endoscopy now see this as a potential expansion of therapeutic options; however, it has not become a mainstream therapy at this time. Numerous natural orifice transluminal endoscopic surgery (NOTES) procedures have been developed since these early initiatives. These have included abdominal procedures which have involved biopsies, surgical removal of numerous organs and structures, GI repair procedures, and gynecologic procedures (Jagannath et al., 2005; Kantsevoy et al., 2006; Park et al., 2005; Willingham and Brugge, 2007).

Many studies involve comparing NOTES to open surgery and laparoscopic surgery to determine if there are significant physiologic and outcomes differences between the various techniques (Azadani et al., 2012; Bingener et al., 2008a,b, 2009; Cordova et al., 2011, 2013; Dray et al., 2008; Earle et al., 2012; Giday et al., 2010; Ryou et al., 2012; Sohn et al., 2011; Soweid et al., 2012; Willingham and Brugge, 2007). Conclusions of most studies were that if aseptic equipment and technique were utilized, there were no clinically significant issues concerning infection, hemodynamics, or clinical chemistries associated with NOTES as compared to both laparoscopic and open surgical techniques.

There is also a need for the development of devices that enhance the ability of the surgeon to use NOTES techniques, and these are frequently studied in the porcine model as well (Earle et al., 2012; Moran et al., 2012; Park et al., 2010; Teoh et al., 2011; van Rentein et al., 2009; Voermans et al., 2011).

SURGICAL ROBOTICS

Surgical robotics is an evolving technology that embraces some of the aspects of laparoscopic and endoscopic procedures (Chitwood et al., 2001; Gourin and Terris, 2004; Kumar et al., 2005; Martinez and Wiegand, 2004; Oleynikov et al., 2005; Rentschler et al., 2004). The technology grew out of National Aeronautics and Space Administration (NASA) studies involving VR. Semiactive and passive computer-controlled robots have been used as an extension of minimally invasive surgery and laparoscopic techniques. Robotic techniques have proved to be helpful in increasing precision in micro tasks. Thus, the robot can make feasible the performance of complex surgical tasks laparoscopically. The robot involves the use of a remote console by the surgeon distant from the patient. As utilization of the robot entails unique skills, intense training must be performed using both *in vitro* and *in vivo* models to acquire mastery prior to clinical use. The pig has proved to be an applicable model for training and development of these procedures. It is expected that this technology will continue to evolve and improve (Figures 13.16 and 13.17).

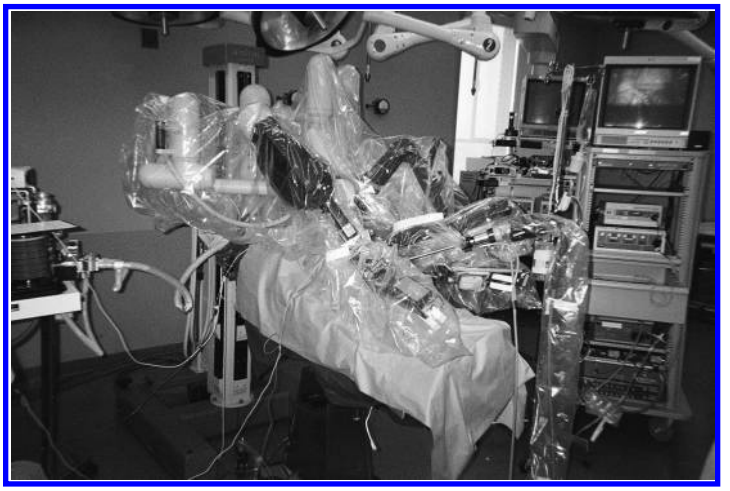

FIGURE 13.16 Advanced surgical training using the Da Vinci surgical robot. The pig is in the Trendelenberg position. (Courtesy of P. Rand Brown, DVM, Johns Hopkins University.)

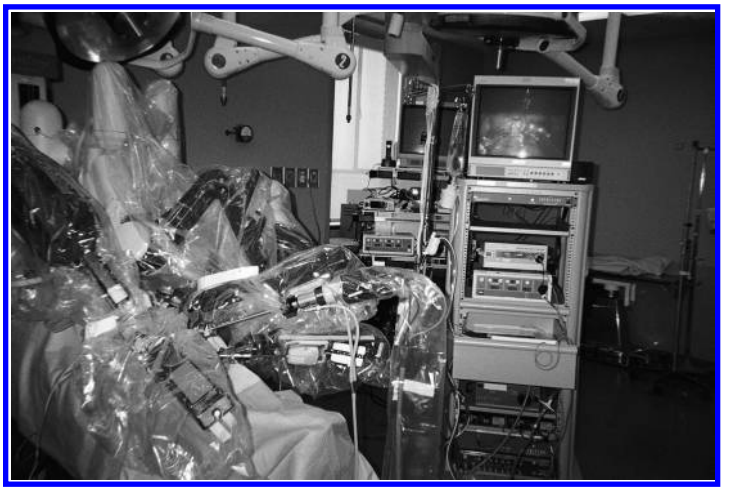

FIGURE 13.17 Surgical robot training using a porcine model. (Courtesy of P. Rand Brown, DVM, Johns Hopkins University.)

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14 Xenotransplantation and Transgenic Technologies

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CONTENTS

INTRODUCTION

Xenotransplantation, the use of organs or tissues from disparate species (pigs to humans in the case of this chapter), has been a focus of research by many groups for the past several decades. Xenotransplantation is a potential solution for the continued organ shortage in clinical transplantation. However, the immunological barriers that need to be overcome (discussed later in the chapter) continue to be significant despite advances in the prolongation of the survival of swine organs into nonhuman primates (NHPs). The scientific hype in the field of xenotransplantation of the past 20 years is becoming less positive; however, the use of xenogeneic cells (like pancreatic islets) and acellular tissues (such as heart valves) are currently showing promising results. While vascularized organs and tissues can conceptually reach clinical application (Ye et al. 1994, Dorling 2002, Alisky 2004), key immunological obstacles must first be overcome before translation into humans. The use of transgenic technologies, which humanize swine organs and minimize rejection, continues to be a major focus of research. Thus, successful xenogeneic organ acceptance will require a combined strategy using transgenic manipulation and improvement of immunosuppressive agents since the current level of immunosuppression required to prevent acute/hyperacute rejection in xenogeneic swine to baboon studies continues to be lethally toxic. The development of "humanized" swine using transgenic technologies in combination with immunological tolerance by the re-education of the immune system (Yang and Sykes 2007) is likely the most clinically promising approach. Conceptually, this would permit the recipient to be

weaned of all immunosuppressive agents, and thus eliminate the multiple side effects related to the use of these agents. This chapter will address these and other important elements necessary for the successful application of swine organs/cells for xenogeneic transplantation.

SWINE AS A XENOGENEIC DONOR

The best donor for human transplantation remains a human. However, severe organ shortage (Organ Procurement and Transplantation Network Database) requires other sources to be considered. The use of NHPs as xenogeneic organ donors, despite their similarities to humans, has not gained interest for several reasons. First, the use of NHPs has faced resistance from the public for ethical reasons. Second, the potential of NHP diseases, some of which are known to be lethal to humans (i.e., Herpes B), and the extended time required for breeding NHPs to reach the appropriate size to be used in transplantation have made them unlikely donors. Logistical difficulties of breeding NHPs in captivity, in addition to their overall smaller organ size (with the exception of apes), have made them a less favorable donor.

Swine on the other hand, though phylogenetically more distant to humans than NHPs, are utilized in clinical xenotransplantation for the following four reasons: (1) they can be grown quickly to reach similar organ size as humans, (2) their physiology is comparable to humans, (3) there is less public scrutiny for their use (e.g., they are already utilized as part of the food industry), and (4) their gestation length is short and production practices are well developed.

For animal organs/tissues to be used in the clinic, grafts must be demonstrated to be safe and long-lived in the new host. Swine tissues have been co-transplanted with human immune cells into well-established humanized mice in order to better understand the immunological barriers of xenogeneic rejection (Yang and Sykes 2007). Although these studies have proven to be valuable, they are far from perfect. In these rodent-based xeno-models, human, mouse and swine tissues are present and interpretation of results is challenging at best. A necessary yet imperfect pre-clinical approach is the use of NHPs as recipients of swine tissues. NHPs emulate human responses to swine xeno-antigens (Yang and Sykes 2007) with relatively high fidelity based on their immunological similarities. As an example, a major advantage can be seen in the cross-reactivity of a large majority of human biologicals (e.g., monoclonal antibodies and immunosuppressants) that facilitate the transition of the preparatory regimens developed in NHPs.

A short- and a long-term goal should be considered for xenotransplantation. Is the xeno-tissue required to last for many years or is the transplant just a temporary solution? While long-term function would be logically ideal, this is not currently achievable. Even with the best of our current technologies and knowledge of immunity, NHP recipients of swine organs rarely survive over two months (Yamada et al. 2005). The two major complications remain rejection of the graft (due to lack of control of immunological responses) and infections (due to much immunosuppression to treat for the former). Currently, a more realistic goal for xenotransplantation is the use of swine organs as a temporary life-saving procedure while patients wait for human organs to become available.

Use of Swine Tissues: Short-Term/Temporary Solutions

Despite the decrease of funding for xenotransplantation by the National Institutes of Health, other agencies, such as the U.S. military, remain interested in xenotransplantation. Developing an "off the shelf" biological dressing for severely burned/injured troops is needed (Albritton et al. 2014), and swine tissue may become available for that purpose. The most relevant obstacle of these skin grafting studies is whether swine skin induces the development of xeno-antibodies which could cross-react with human tissues and prevent multiple skin allografts to be transplanted. These studies showed that swine tissues were safe to be used for this purpose (Albritton et al. 2014).

Studies describing the similarities and differences in skin histology between swine and humans have been published (Lavker et al. 1991). Both species have well-defined dermal papillae and rete

ridges. Although pig skin is thicker and less vascular than humans, the overall characteristics of the cutaneous blood supply are similar (Lavker et al. 1991, Debeer et al. 2013). The size, distribution, and orientation of blood vessels in the dermis of the pig are similar to those found in human skin. While the subepidermal plexus, which supplies adnexal structures, is less developed, the adnexal structures found in swine and humans are similar. The epidermal turnover time, type of keratinous proteins found within the skin, and lipid composition of the stratum corneum (Nicolaides et al. 1968, Miller et al. 1998) are also similar. Based on these similarities, swine, rather than other species possessing fur, including mice, dogs, or NHPs, are the best candidates as donors for skin-specific xenotransplantation.

Use of Swine Cells/Tissues for Long-Term/Life-Long Solutions

Innovative approaches are still needed in order to make long-term xeno-organ survival a reality. To date, the most promising approach remains generation of immunological tolerance, in which the immune system is re-educated with the intention of organ acceptance without immunosuppression (discussed later). Cells/organs such as pancreatic islets, blood, heart, corneas, kidneys, and liver are being investigated. An in-depth discussion of every single organ is beyond the scope of this chapter. We will, however, briefly discuss several of the most promising and commonly investigated swine tissues.

1. The swine liver has been extensively studied. Studies using the liver illustrate that even if the anti-swine rejection response is avoided, questions still remain as to whether the swine liver can perform the expected metabolic life-preserving functions in a human host. Some studies claim that the metabolic function of swine liver may be more similar to humans than other species (Drougas et al. 1996). Moreover, grossly and histopathologically, the size and shape of the liver between human and swine are comparable (Swindle et al. 2012). Some differences pertaining to the structure of the hepatic septae that demarcate the hepatic lobes and lobules should not cause a problem. However, physiologically, one important function of the liver, the production of albumin, is not equivalent. There is 65% similarity in the amino acid composition between humans and swine albumin. Also, since serum albumin concentration is lower in pigs than in primates (Hammer 1998a,b, Platt 2000), it is possible that this could impact normal human fluid balance physiology potentially making a patient still require frequent hospital visits. The production of bile by the gallbladder is important for digestion. Interestingly, Kobayashi and colleagues found no significant difference in the composition of bile, including viscosity, between humans and pigs. Yet, it remains to be assessed whether long-term function of the liver and bile production of a swine liver in an NHP will allow for normal digestion in humans. The cytochrome P450 system has a similar activity between swine and humans (Skaanild and Friis 1997), however the mechanisms of cholesterol transport are different. The lipoprotein complexes used to transport cholesterol in the blood (LDL, HDL, and VLDL) differ by 40% between the two species (Hammer 1998a,b). The cardiovascular repercussions of these differences in humans and NHPs are unknown. In addition, swine also have a lower binding capacity of LDL, with a greater number of apoB receptors in the liver (Huff et al. 1993). Therefore, these differences, which have been used as a simple illustration, are some of the additional factors that must be considered when using the pig as an organ donor if and when the immunological barriers are solved.
2. Pancreatic islet cells: Swine insulin, which differs only by one amino acid when compared to humans, has been widely utilized. In order to free patients from chronic insulin injections, pancreatic islet transplantation of pig islets is being studied for type I diabetes mellitus. The biggest problem has been the loss of these islets as a result of instant blood-mediated inflammatory reaction (BMIR) (van der Windt et al. 2012). BMIR is a form of

hyperacute rejection involving the complement cascade. Promises for improved outcomes are expected from new swine expressing human transgenes such as the complement regulatory protein (CRP), CD46.

3. Red blood cell (RBC) transfusions: The use of swine blood is an alternative for an often exhausted blood bank. The use of β-galactosyl transferase knockout (GalT-KO) swine may provide an unlimited source of RBCs and potentially act as the "universal donor" (Long et al. 2009). However, despite lacking the Gal-antigen, other non-Gal swine-specific antigens continue to limit the use of the model. Development of novel transgenic swine expressing human clusters of differentiation (phenotype-determining proteins) may solve the problem of blood shortages.
4. Corneal transplants: The cornea is a relatively non-immunogenic tissue. Because of its immunoprivileged characteristics, corneas from pigs have survived months without rejection (Choi et al. 2014). Genetically engineered pigs (CD46-transgenic on a GalT-KO background) have shown promise *in vitro* and may soon be tested in NHPs (Hara et al. 2011).

IMMUNOLOGICAL CONSIDERATIONS

A plethora of reports discussing the immunological and physiological considerations associated with xenografic transplantation have been summarized in the scientific literature (Yang and Sykes 2007; Griesemer et al. 2014). The question remains as to whether we are immunologically closer to long-term acceptance of xenogeneic organs. Several decades ago, swine organs transplanted into baboons would last seconds to minutes (due to antibody-mediated hyperacute rejection). Today, swine organs can last several months (Cooper et al. 1991, 2014). Despite these advances, a long "immunological" road remains ahead before solid organs can be safely transplanted from swine into humans. Immunological barriers continue to prevent clinical trials. Below we discuss the most important immunological causes limiting xenotransplantation.

Antibody-Mediated Rejection of Swine Organs

Hyperacute rejection and acute humoral xenograft rejection are the primary cause for the loss of swine organs in NHPs early post-transplant. Hyperacute rejection is mediated by natural antibodies directed toward the Gal-α-1,3 Gal-β-1-4GlcNac (also known as α1,3-Gal) antigen. The carbohydrate epitope targeted by NHPs (and humans) is present in the vascular endothelium of the swine organ. Thus, organs can be rejected extremely fast (i.e., within minutes). Alpha 1,3-galactosyltransferase (α1,3-Gal) is the enzyme responsible for synthetizing α1,3-Gal. This enzyme is present in most species but not in humans (or old-world monkeys). As a result of this deficiency, and because α1,3-Gal is present in many bacteria, old-world monkeys and humans have developed antibodies to the Gal epitope.

When antibody binds to the endothelium, the clotting cascade is triggered which leads to thrombosis in transplanted organs. Death is directly due to hypoxia and cellular lysis (Galili 1993). Attempts to absorb these antibodies by using a "decoy" organ for several hours prior to transplantation or by filtering the recipient's blood through an α1,3-Gal affinity chromatography column has temporarily prevented hyperacute rejection. However if hyperacute rejection is prevented, development of low levels of anti-α1,3-Gal natural antibodies leading to acute humoral rejection can also induce graft loss days after transplant (Schuurman et al. 2003). Thus, even in Gal-KO swine that lack the α1,3-Gal epitope, antibodies nonspecific for Gal develop and rejection still occurs. The targets of the non-Gal antibodies, which develop within the first year of life, remain to be fully described (Rood et al. 2007). It has been suggested that these antibodies are directed toward the swine leukocyte antigen (Diaz Varela et al. 2003).

T Cell-Mediated Rejection

T cell-mediated rejection has been less well studied because antibody-mediated rejection has dominated the rejection crises. Thus, it has been very difficult to reach an antibody-free rejection platform where T cell-specific responses have been able to be studied in detail. CD4 and CD8 T cells recognize and kill foreign tissues via cell:cell interaction and degranulation. T cells are also known to play a role in lysis via cytokine production, recruitment, and activation of other cytotoxic cells (e.g., macrophages and neutrophils). The influence of T cell-mediated xeno-rejection was elegantly documented by Yamada et al. T-cell depletion significantly prolonged the survival of swine xenografts in NHPs in which natural antibody-mediated rejection was suppressed (Yamada et al. 2005). Control of T-cell responses will be key with pancreatic islet transplants. Swine pancreatic islet cells have been shown to be rejected by T cells via interferon-gamma (IFNγ)-mediated responses and depletion of T cells prolonged swine pancreatic islet cell tolerance in NHPs (Cardona et al. 2006).

Tolerance Induction for the Prevention of Swine Tissue Rejection

In order to control the rejection of swine tissues, immunosuppressive drug cocktails targeting B cells, T cells, macrophages, and natural killer (NK) cells are required. This level of systemic immunosuppression is therefore extremely high and may lead to unacceptable side effects. A viable option for making xenotransplantation a reality is the development of immunological tolerance. Here the host immune system is educated to accept swine tissues (without life-long immunosuppressive drugs). These approaches have stemmed from murine (Sykes et al. 1997, Yang and Sykes 2007) and large animal (Yamada et al. 2000) allogeneic studies, where thymus or BM have been used for tolerance induction. Using thymi can allow for negative selection of host (human or NHP) T cells to be educated in the swine thymus. This will eliminate T cells that will strongly react to swine antigens; thus, only non-swine reactive naive T cells will emigrate from the thymus to the peripheral circulation. The use of bone marrow is another viable approach. The goal is to induce a state of mixed chimerism leading to tolerance of both B and T cells.

Innate Immune System

The innate immune system is considered the body's first line of defense. It is governed by mechanisms which defend the host against ever-evolving microorganisms using a vast array of cells and molecules, including granulocytes, NK cells, monocytes/macrophages, and dendritic cells. Here, we will discuss the impact of NK cells and macrophages as they pertain to xenotransplantation.

Natural killer cells (Leavy 2012) are important participants in the adaptive immune system. These cells have potent anti-tumor and anti-viral effector functions and exert their biological functions by secreting lytic proteins, such as perforin and granzyme. NK cells also secrete potent inflammatory cytokines, such as tumor necrosis factor-alpha (TNFα) and IFNγ. The function of NK cells is tightly regulated by cell surface receptors that either provide inhibitory (CD85, CD158) or stimulatory (activating) signals. The inhibitory receptors recognize major histocompatibility complex I (MHC-I), antigen-presenting molecules constitutively expressed in nearly all cells. Deficiency of MHC-I (by viral infection) or inability to recognize MHC-I in swine cells induces NK cells to degranulate. NK cells can also kill target cells by antibody-dependent cell-mediated toxicity (Leavy 2012). Continued investigation may establish if this mechanism is a major component of xenogeneic rejection (Nikolic et al. 2001).

Macrophages are cells derived from monocytes and are found in many tissues (Murray and Wynn 2011). These cells can cause rapid rejection of xenogeneic bone marrow (Abe et al. 2002). Macrophages can phagocytose swine cells via both complement-independent and antibody-dependent mechanisms (Ide et al. 2005). An inhibitory receptor on the cell surface of macrophages (CD172) becomes activated when engaged with CD47. CD47 is a "do not eat" signal that cells express to avoid phagocytosis. If CD172 is not activated by CD47, then the target cell is phagocytosed

(Oldenborg et al. 2000). Therefore, species incompatibilities between CD47 and CD172 (such as in xenotransplantation) contribute to the phagocytosis of the xenogeneic cells.

Complement and Coagulation Factors

Complement and coagulation factors are crucial in host defense and clotting. Studies from several groups suggest that differences in complement formation and coagulation factors exist between swine and humans. On one hand, two studies in which baboons received Gal-KO swine livers documented that clotting parameters normalized after the transplant was placed (Ramirez et al. 2000, Ekser et al. 2010). *Banna*-inbred minipig clotting factors demonstrated that swine serum could activate the human intrinsic and extrinsic clotting pathways. Interestingly, certain factors (II, V, VII, XII) had a significantly higher activity than those of humans (Zhang et al. 2004, 2005). A different study by Adham et al. (1997) involving the use of pig liver xeno-perfusion to treat acute liver failure in humans detected higher levels of vitamin K-independent clotting factors (V and XII) and decreased levels of vitamin K-dependent factors (VII and X). CRPs are thought to be relatively species specific. However, experiments by Rees and colleagues found that human CRP was able to inhibit swine complement (Rees et al. 2005), thus making them relatively compatible. Immunologically, the complement system of the host can be activated by the classical pathway (antibody mediated), the alternative pathway, or via the lectin pathway (Sacks et al. 2003). The final outcome is the membrane attack complex, which creates pores in cell membranes leading to cell lysis. Xenoreactive antibodies attach to the endothelium of the discordant organ and the complement system is subsequently activated. Within a few hours, the vascularized graft develops edema and hemorrhage followed by clotting, which decreases the blood supply to the graft. Techniques that reduce circulating antibodies, such as plasmapheresis or immunoabsorption, prolong the period of time that is required for the hyperacute rejection to occur. Because key clusters of differentiation are necessary to inhibit complement activation (known also as CRPs), transgenic swine expressing human CD46, CD55, and CD59 have been developed (Klymiuk et al. 2010). Use of animals expressing human CRPs have been less susceptible to hyperacute rejection (McGregor et al. 2012).

Genetic Modification of Swine: The Development of Humanized Swine

Transgenic technologies involve manipulating genes of interest by adding a functional gene, an inhibitory gene, or disabling a gene. Gene transfer into the swine genome was first done in 1985 using DNA microinjection into the pronuclei of fertilized oocytes (Hammer et al. 1985). However, this technique in swine has been shown to be inefficient and can induce random integrations leading to mosaicism, insertional mutations, and variable expression of the desired genes. This has forced the field to develop new approaches for the production of genetically modified swine. Several techniques have allowed for the transfer of genes into germline and somatic cells. Briefly, pronuclear transfer involves injecting a DNA construct into the pronucleus of a fertilized zygote at the 2- or 4-cell stage. Oocyte transduction involves integrating a gene construct with a replication-deficient retrovirus into an unfertilized oocyte. Sperm-mediated transfer involves inserting a gene construct into sperm nuclei and then fertilizing the oocyte. Nuclear transfer involves integrating a gene construct into fetal cell-derived donor populations and replacing the nuclei of oocytes with a new nucleus. Each of these approaches has its limitations. Germline gene transfer uses techniques which can lead to either random or defined integration of transgenes into the DNA. However, random insertions occur with DNA micro-injection, the use of retroviral vectors, or the application of sperm-mediated gene transfer. The use of somatic cell nuclear transfer after gene targeting by homologous recombination has led to a better and more targeted gene insertion (Klymiuk et al. 2010). For decades, highly inbred miniature swine, similar in size to adult humans, have been desired. This would provide a homogeneous genetically defined herd of swine for the purpose of xenotransplantation. The laboratory of Dr. David Sachs (Massachusetts General Hospital) has

TABLE 14.1
Genetically Modified Swine for Xenotransplantation

Immune Arm	Protein/Gene	Published Year	Author
Complement	CD55 or hDAF (decay accelerating factor)	1998	Waterworth et al. (1998)
Complement	CD55/CD59 (membrane inhibitor of reactive lysis)	1998	Lin et al. (1998)
Anticoagulation	vWB	1999	Meyer et al. (1999)
Complement	CD55/H-Transferase	2000	Cowan et al. (2000)
Complement/antibody	CD55/CD59/H-Transferase	2000	Cowan et al. (2000)
Complement	CD46 (membrane cofactor protein)	2004	Schirmer et al. (2004)
Masking	GALT-KO	2005	Dor et al. (2005)
Complement	CD55/CD46	2005	Moscoso et al. (2005)
Complement	CD55/ENDO-B GALACTOSIDASE C	2009	Yazaki et al. (2009)
Anti-inflammatory	hA20 (TNFα-induced protein 3)	2009	Oropeza et al. (2009)
Masking/complement	GALT-KO/CD46	2010	Horvath et al. (2010)
Antibody/complement	GALKO/CD55/CD59/ CD39/H TRANSFERSE	2011	Le Bas-Bernardet et al. (2011)
Anti-inflammatory	hHO-1 (human hemeoxygenase-1)	2012	Yeom et al. (2012)
Masking/complement	GALT-KO/CD55	2011	Tazelaar et al. (2011)
Cellular immune response	CD47	2012	Scalea et al. (2012)
Antibody	NeuGc-KO	2011	Padler-Karavani and Varki (2011)

developed three homozygous swine leucocyte antigen lines of miniature swine. The most inbred line (the DD line) has reached >97% consanguinity (Hanekamp et al. 2009, Duran-Struuck et al. 2010) and has been chosen as the most likely line for xenotransplant studies. The GalT-KO pig was created in this highly inbred DD pig (even though it is not yet considered a strain as has not achieved 20 generations of brother sister matings). The GalT-KO pig was created using somatic cell nuclear transfer. This approach provided the required specificity to remove the GGTA1 gene encoding a 371 amino acid protein. GGTA1 belongs to the glycosyltransferase 6 family responsible for transferring galactose from UDP-galactose to an acceptor molecule. Many other genes, which target the different arms of the immune system and the coagulation cascade (summarized in Table 14.1), have been modified for xenotransplantation. For more information about available genetically modified swine, the reader is encouraged to consult with The National Swine Resource and Research Center at the University of Missouri. This center provides supportive services for the development and distribution of transgenic swine for xenotransplantation: (http://www.nsrrc.missouri.edu).

ETHICAL AND SOCIAL CONSIDERATIONS

While all immunological and physiological barriers to xenotransplantation are being overcome, the equally important issue of the ethical use of swine must also be addressed. There are more than 118,000 people currently waiting for organ transplants in the United States, and in 2012 a total of 6115 patients died while waiting for transplants. On average, 18 people died each day in 2012 because of the shortage of donated organs (United Network for Organ Sharing 2013). Many people

continue to die each year; thus, physicians and scientists must continue moving forward in finding viable solutions for their patients, and xenotransplantation has the potential to become one. That being said, unusual circumstances exist in xenotransplantation which make it different from other more common procedures. For instance, the risk is not borne by the patient alone, but also by others. The transplantation community continues to grow with the increasing demand for donor cells, tissues, and organs. With this in mind, the varying degrees of regulation of xenotransplantation research in different countries illustrates the need for public health protection from infectious risks and the need for cooperation among national and international agencies (Tisato and Cozzi 2012). Most important of all factors to be considered concerns *what is most right* for all (i.e., the Utilitarian perspective) in our new global community, including those directly involved (patients and immediate contacts), those indirectly involved (third parties, or individuals who come in contact with recipients at various intervals following transplantation of xenografts), and those seemingly uninvolved (the general public). So the question remains: where does the issue of ethics in xenotransplantation currently stand?

To answer this question, the reality that individuals may at some point be able to travel to countries with more relaxed regulations regarding health risks must be weighed against novel, yet ethically sound, perspectives regarding patient autonomy and informed consent. When for consideration of all moral and non-moral points of view, it is suggested that advisory councils (which preferably should contain professional bioethicists) be established to weigh arguments regarding potential benefits and risks. These educated councils would be involved in making challenging decisions and helping disseminate current developments to the public. In addition, explicit waivers of the right to withdraw from xenotransplantation trials, perhaps framed like *Ulysses Contracts* (Spillman and Sade 2007), would be necessary to overcome serious unsolved dilemmas for maintaining patient autonomy and informed consent, as well as public safety. It is important that husbandry practices and pre- and postsurgical monitoring procedures for both swine donors and human recipients should be acknowledged and accepted within our society. This will require governing bodies to continue to approve such novel approaches.

REGULATORY GUIDELINES

Many issues related to the use of xenogeneic organs have been addressed by The Institute of Medicine and the Food and Drug Administration in 1995 and 1998. Guidelines were drafted and subsequently updated (IOM 1996, PHS 1996, FDA 1999, 2002, 2003). The World Health Organization (WHO), U.S. Department of Health and Human Services (USDHHS) through its Secretary's Advisory Committee on Xenotransplantation (SACX), and the Organization for Economic Cooperation and Development (OECD) held a series of hearings and also drafted documents on the procedures (WHO 1998, 2001, USDHHS 2004a,b). Regulations must address the following key issues:

1. *Infectious risk to society.* This includes rights of informed consent, the right to privacy and the right of a recipient to remove himself from a research study where long-term infectious disease monitoring is required to protect the rest of society. The International Xenotransplantation Association (IXA) agreed on a set of principles that addressed the above-mentioned rights. Only if a high likelihood of success is anticipated with a novel xenotransplant protocol, and only under strict oversight by health authorities, will a clinical trial be permitted to take place. The IXA principles have been adopted by the WHO (Sykes 2005), whose goal is to implement these principles worldwide.
2. *Economics.* Private and public funds have supported xenotransplantation. However, due to the multiple potential infectious complications (and liability), the private sector has been prompted to withdraw from funding xenotransplant programs. Unfortunately, removal of these much-needed funds has come at a time when significant advances have

been achieved by the creation of genetically modified swine, such as the GalT-KO model described above. A point of contention has also been predicted regarding the claim of ownership of newly humanized swine. The cost of breeding transgenic swine destined for xenotransplantation and their continuous disease control monitoring will also need to be managed.

Since the Institute of Medicine conference in 1996 (IOM 1996), the international community has developed guidelines and regulations concerning potential public health issues, recommendations for protocol approval, animal sources, clinical issues, and the need for a national or international registry and archive. Many countries have also developed their own specific guidelines and regulations concerning xenotransplantation. Summaries of the general recommendations of these guidelines and regulations are discussed in this section, with most of the discussion related to the documents from the United States and WHO.

The protocol issues include the composition of the xenotransplant team, the site, the protocol review, health surveillance plans, and the need for informed consent. The transplant team should include an infectious disease physician with knowledge of zoonoses, a veterinarian with expertise in zoonotic disease and animal husbandry, a transplant immunologist, a hospital epidemiologist or infection control specialist, and the Director of the Clinical Microbiology Laboratory at the transplantation site, in addition to the transplant surgical team. In the United States, the transplantation site has to be accredited and associated with the Organ Procurement and Transplantation Network. The protocol has to be reviewed by the following institutions: the Institutional Animal Care and Use Committee, the Biosafety Committee, and the Institutional Review Board for Human Research. All these committees must have expertise in zoonotic diseases and disease control. In addition, these protocols may be subject to regulation by the Food and Drug Administration. The protocol must include a plan for screening of animals, organs, and the source herd. It must also include provisions for written informed consent and education of the recipient.

The major portion of many of these documents relate to the regulation of animal sources of xenotransplant tissues. Animals have to be bred and reared in captivity, herds have to be closed and serologically screened, and tissues and organs from slaughterhouses cannot be used. Biomedical research animal facilities should be accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International to ensure compliance with all federal guidelines. The facility has to maintain and follow detailed standard operating facilities for quarantine and disease surveillance. Standard laboratory animal procedures for sanitation of facilities need to be followed, and the feed source and quality should be controlled to prevent the use of rendered products. Herd health maintenance programs include both prevention and treatment of potential zoonotic problems. The use of sentinel animals is suggested for monitoring serology, microbiology, and necropsy studies. Individual animals to be used as donors must also be screened. In addition, biopsies of the actual xenograft are recommended.

These clinical issues also must deal with the safety of the recipient and the medical personnel and require lifetime monitoring and archiving. This requires educating the recipient about informing close contacts. The archived information must be made available to a national archiving registry.

The WHO (1998, 2001) has prepared similar documents. However, they recommend the use of gnotobiotic animals that have been cesarean-derived and maintained in barrier facilities. Recognizing the impracticality of this source of animals, they provide for the use of animals from a similar health and husbandry situation as that described in the PHS document. They provide a criteria list for developing an exclusion list of infectious agents for xeno-organ donation. Their criteria for the clinical arena are similar to the PHS document, however, it is not as detailed.

These guidelines are so rigorous as to preclude the conduction of xenotransplants in centers other than major medical institutions with accredited laboratory animal programs. Very few commercial producers of swine could provide the required husbandry without substantial modification of their

facilities and programs. Many of the existing guidelines and regulations are in draft form, and most are continuously modified as the science of xenotransplantation develops.

CROSS-SPECIES DISEASE TRANSMISSION AND PUBLIC HEALTH CONSIDERATIONS

Pig-to-human xenotransplantation carries little risk compared to other species, such as with NHPs. A proposed set of practical considerations on herd maintenance and surveillance has been published (Swindle 1996, 1998). Viruses with the highest potential for zoonotic infection in immunocompromised recipients or damage to tissue and cellular xenotransplants include swine influenza, human influenza, encephalomyocarditis virus, porcine rotavirus, porcine lymphotropic herpesvirus, porcine reovirus, parainfluenza virus, porcine adenovirus, porcine cytomegalovirus, porcine circovirus, porcine pneumovirus, hepatitis E, pseudorabies, and porcine retrovirus. The incidence of occurrence and potential of pathogenicity vary widely among these infectious agents. There is also concern that some of these viruses, such as herpesvirus, may lead to the development of lymphoproliferative disorders. The primary agents of concern however are the influenza viruses and most critical, the retroviruses. Porcine endogenous retroviruses (PERVs) have been shown to infect human cell lines *in vitro* (Martin et al. 2006). The example of HIV, where an NHP retrovirus has become a serious human public health problem, is one of the deterrents slowing down the application of xenotransplantation. This is especially true when significant immunosuppression of the host is required. Concerns remain whether PERVs can mutate or recombine with human retroviruses creating a novel and virulent virus. There are three known PERVs (PERV A, B, and C). PERV A and B recombinants with PERV C have been shown to infect human cells *in vitro* (Oldmixon et al. 2002). Despite these *in vitro* studies, no *in vivo* human or NHP xenotransplant has yet demonstrated the infection of the host with swine PERVs. Genetic engineering of pig herds that are free of PERVs (by the removal of retroviral genes) may minimize PERV transmission, however there is still the fear of viruses yet not identified which may be innocuous in swine but which can mutate and infect immunosuppressed patients and subsequently spread into the general population.

Bacterial pathogens and commensals of concern include those of the following genera: *Salmonella*, *Pasteurella*, *Brucella*, *Erysipelothrix*, *Streptococcus*, *Campylobacter*, *Staphylococcus*, *Coxiella*, *Leptospira*, and *Mycobacterium*. The pathogenicity of these organisms varies widely depending upon the species and serotype.

Parasitic organisms should also be considered in the screening. *Balantidium coli* is the primary protozoal organism of concern. Nematodes can generally be controlled by anthelmintics; however some, such as *Ascaris suum*, can also be associated with visceral larval migrans and damage of donor organs such as the liver.

The potential list of pathogens is much longer and includes organisms such as dermatomycoses. In addition, immunocompromised patients may experience infection from organisms not previously associated with zoonotic disease. Summaries of the infectious risks associated with xenotransplantation and strategies for eliminating them have been published (Cooper et al. 1991, Michaels and Simmons 1994, Ye et al. 1994, Swindle 1996, 1998, Fishman 2000, Tucker et al. 2004). Closed housing and selective breeding will likely minimize infectious risks. Specific pathogen-free (SPF) swine could be used as a starter herd source for animals; however, they would not be considered to have the pathogen-free status necessary for xenotransplantation by current guidelines. There is a suggestion that a term such as xenografic-defined flora animals should be used, rather than SPF, to differentiate these two standards (Swindle 1996, Le Bas-Bernardet et al. 2011).

Lastly, human surveillance will be necessary to minimize any infectious risks spread into the population. The longer the survival of the grafts, the higher these risks. Thus, the risk of swine-related zoonosis will never be completely eliminated and indefinite infectious surveillance will be necessary for the life of the recipient.

MANAGEMENT AND HUSBANDRY STRATEGIES

Management and husbandry strategies are discussed in the FDA (1999, 2002) and PHS (1996) guidelines, and by Swindle (1996, 1998). AAALAC-accredited biomedical research facilities with ABSL 2 facilities are more likely to be capable of handling the quarantine and disease control standards required for xenotransplantation. The standard operating procedures for husbandry and the construction of the facilities should be adequate to meet these guidelines in such accredited research facilities. Their standards could be used as a guideline for the development of xenografic transplant facilities for housing donor herds of animals.

The use of a sentinel animal system for monitoring herds, similar to those used in rodent facilities for biomedical research, should be instituted. The use of multiple rooms with an all-in/all-out management system for batch procurement of tissues and organs is probably the most practical method to ensure compliance.

OTHER CONSIDERATIONS

Besides the immunologic and zoonotic problems to be overcome through research and management techniques, there are other issues to be considered. Swine may have congenital defects or genetic diseases such as malignant hyperthermia; and physiological function may be poor. As an example, the inbred swine herd used to create the Gal-KO has exhibited a high incidence of angular limb deformities, cryptorchidism, heart defects (valvular anomalies and tetralogy of Fallot) and myeloid leukemias (Duran-Struuck et al. 2010). There are also inflexible regulations related to the shipment of swine and swine tissues between states and countries that are in effect for agricultural and economic control purposes. Swine used for xenografts will either have to receive an exception to these regulations or be in compliance, which will depend upon the destination locale (Swindle 1996). It is likely that guidelines will continue to be revised as additional scientific meetings and public forums are held on the subject.

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15 Toxicology

Kristi L. Helke

CONTENTS

INTRODUCTION

Toxicology is the study of the effect of differing doses of compounds on a living system. Paracelsus (1493–1541) is credited with the phrase: "All things are poison and nothing is without poison; only the dose makes a thing not poison." Even an overdose of water can be toxic.

To prevent toxicities, the current paradigm requires testing of xenobiotic compounds in animals prior to approval for use in humans. Testing in both rodent and nonrodent species is required by agencies which oversee approval of compounds and devices intended for human use. Currently, dogs and nonhuman primates (NHP) are the most commonly used nonrodent species, and consequently, there is a plethora of historical data on these species. The best species for toxicity testing is the one that has similar mechanisms of metabolism for the drug as humans, which is not necessarily the dog and NHP. There are several reported potential benefits resulting from use of minipigs instead of dogs and NHPs (Webster et al., 2010).

PIG AS A TOXICOLOGICAL MODEL

Historically, the pig has been used as a model to test food products and additives and is currently gaining favor as a model for testing of other compounds as well (Dorea, 2006; Ikeda et al., 1998).

A recent survey of 22 big pharmaceutical companies revealed that only two of the companies routinely test to determine if pigs would be acceptable and a more relevant model (than dog or NHP) for the compound they are testing (Bode et al., 2010). Pigs have been used as a toxicological model for agents which require dermal application, oral administration, and intravenous (IV) infusion (Bollen and Ellegaard, 1997; Nunoya et al., 2007).

Species Differences

It is well documented that there are species differences in protein binding, tissue uptake, receptor properties, and metabolic profiles (Helke and Swindle, 2013; ICH, 1995). The pig is considered a good model in biomedical research because of its anatomical, physiological, and biochemical similarity to humans (Puccinelli et al., 2011). Sequencing of the pig genome has recently been completed and comparative maps show extensive homology with the human genome (Archibald et al., 2010; Groenen et al., 2012; Merrifield et al., 2011; Puccinelli et al., 2011). The pig is appropriate for investigation of drug disposition since its physiology is similar to that of human and both species have important membrane transport and enzymatic proteins in common (Anzenbacher et al., 1998; Hannon et al., 1990; Kararli, 1995; Swindle and Smith, 1998; Thorn, 2012). Data available on biotransformation in pigs indicates that Cytochrome P450 (CYP) enzyme families CYP1A, CYP2A, and CYP3A are very similar between pigs and humans, which will be discussed in detail later in this chapter.

Pigs versus Other Species

Compared to rodents, minipigs enable collection of larger volumes of multiple samples of body fluids and tissue biopsies, helping to approximate studies to humans (Nunoya et al., 2007).

Cross-species comparisons of pharmacokinetics (PK) are rare, and the ones that have been completed use different testing methods, making them difficult to compare (Fink-Gremmels, 2008). Only a few studies have determined Michealis-Menten constant (K_m) and maximum rate of metabolism (V_{max}) of the compound being examined (Fink-Gremmels, 2008). Phylogenetic similarity (even among different dog breeds) does not guarantee similarities in metabolism, as differences often exist within CYP expression or substrate specificity. Similarity based on shared physiologic features such as herbivore or carnivore does not assume similarity in CYP either (Fink-Gremmels, 2008). However, there is considerable similarity in activities of the biotransformation enzymes, provided the animal under study has an ortholog to the pertinent human CYP enzyme (Kvetina et al., 1999). Rodents in general do not correspond well to the metabolic properties of human CYP enzymes (Anzenbacherova et al., 2003; Zuber et al., 2002). An isoform is defined as a different protein within the same family, whereas an ortholog is a similar protein across species. All orthologs are isoforms, but not all isoforms are orthologs.

Minipigs possess a CYP ortholog to the main human liver enzyme of drug biotransformation, CYP3A, both in similar amounts and activities, as well as orthologs to CYP2A and 2C, which have similar activities to the human enzymes (Anzenbacher et al., 1998, 2002; Nunoya et al., 2007; Soucek et al., 2001).

The dog's sensitivity to nonsteroidal anti-inflammatory substances is well known. At low-doses, often below human therapeutic doses, dogs develop severe gastrointestinal (GI) lesions, erosions, and ulcers, which limit the dose range that can be tested (Lehmann, 1998; Wiseman, 1978). Pigs have increased tolerance to nonsteroidal anti-inflammatory drugs (NSAIDs), antihypertensives, and sympathomimetics compared to dogs (Gad, 2012).

Dogs are prone to emesis following treatment with some drugs that may skew results by insufficient or erratic exposure following oral dosing (Clausing, 2011). Some drugs/compounds cause pseudo-allergy reactions by causing histamine release at initial exposure, especially in dogs (Clausing, 2011).

Similar plasma concentrations among species are found with administration of meloxicam, but only rats and minipigs had metabolites similar to humans (Busch et al., 1998). Another species difference is noted with organic acids. Pigs do not seem to have trouble with excretion, but dogs have decreased renal clearance, resulting in greater toxicity from these compounds (Timchalk, 2004).

When pigs are administered acetaminophen, they develop methemoglobinemia, not liver damage, as is typical in other species (Henne-Bruns et al., 1988).

Anatomy

The GI tract shows the greatest variation among species in both anatomy (multi- or single-chambered stomach, cecum size, appendix, etc.) and physiology (pH profile, water content, and distribution/number of lymphoid follicles), and these differences need to be considered in order to prevent unrealistic expectations when animal models are used in preclinical studies (Merchant et al., 2011; Swindle and Smith, 1998). However, minipigs have very similar anatomy and physiology compared to humans, not only of the GI tract, but also the hepatic and renal systems (Kvetina et al., 1999; Swindle and Smith, 1998).

Pigs are well-recognized models for several major human disease categories including cardiovascular (CV), metabolic, and neural diseases (Mikkelsen et al., 1999; Puccinelli et al., 2011; Smith et al., 1999; Swindle and Smith, 1998; Thim et al., 2010). This is advantageous when testing a compound or device to treat a specific disease entity, especially CV devices, as the porcine models of these diseases are well developed. Atherosclerosis can be induced by diet in many porcine breeds, providing a model useful for testing drugs/devices. Size and anatomical similarities make pigs an excellent large animal model for a variety of functional studies including medical device studies (Hannon et al., 1990; Swindle et al., 2012).

Physiology

When biological samples are compared from pigs and humans, most values are similar including blood gas, blood chemistry values, acid–base, hormone levels, renal function, hemodynamics, and pulmonary function. Differences are present in parameters such as plasma volume, arterial pH, core temperature, and others, which have been attributed to immaturity of the pigs studied (Hannon et al., 1990).

Breed/Strain Differences

There are numerous pig breeds and there is a phylogenetic split between European and Asian breeds that occurred about 1 million years ago (Groenen et al., 2012). Pigs were domesticated ~10,000 years ago in multiple areas across Europe and Asia (Groenen et al., 2012). While conventional domestic pigs are acceptable in toxicity studies, the size of the animal and amount of drug/compound to reach required blood concentration levels may make them cost-prohibitive in chronic studies. Also, handling and housing animals >700 lb. are no small feat.

Fortunately, beginning in the 1940s and continuing through present day, several groups had the foresight to generate smaller breeds of pigs. The minipig reaches sexual maturity between 3 and 4 months for males and between 4 and 5 months for females (Ellegaard Göttingen Minipigs, 2010). In the dog, sexual maturity is not reached until 7–8 months in the male and 8–14 months in the female. Many chronic toxicity studies begin with immature animals, which may lead to interpretation errors (Ellegaard Göttingen Minipigs, 2010).

There are the obvious variations in the size of conventional versus minipigs, but there are also variances among breeds. It is important to differentiate between conventional (farm) animals and minipig breeds (Fink-Gremmels, 2008; Sakuma et al., 2004; Vaclavikova et al., 2004). For example, conventional Yorkshire pigs and Yucatan minipigs show diametric cardiac responses to administration of cocaine (Miao et al., 1996). Also, Ossabaw swine make a better model for both metabolic syndrome and coronary artery disease than Yucatans (Neeb et al., 2010).

The numerous minipig breeds have distinct characteristics. While genetic and phenotypic differences exist among breeds, there are allelic differences between individuals. These allelic differences are also present between races and individual humans. It is well established that there are species differences with regard to drug metabolism, but there are also breed differences within species. This is true for all of the species used in biomedical research, including the rat, dog, and pig. Humans also show differences in drug metabolism among races, with high polymorphisms shown in some CYP genes (www.cypalleles.ki.se) (Fink-Gremmels, 2008). To minimize these factors, all animals in toxicological studies (including minipigs) should be of a defined breed, that is, mixed breed animals should not be used (Directorate, 2012).

Drug properties such as PK and pharmacodynamics (PD) should not be generalized to the entire species. As in the dog, some breeds carry a mutation in the ATP-binding cassette gene, resulting in animals that are more sensitive to certain antiparasitic agents and morphine derivatives (Toutain et al., 2010). Even within breeds, substrains have developed (beagle) and it has been shown that clearance of a COX-2 inhibitor can have up to 2.5× difference dependent on strain (Paulson et al., 1999).

Breeds of Minipigs

There are currently several breeds of minipigs readily available in the United States, European Union, Japan, and China, with more under development. The most common breeds in the United States are Yucatan, Hanford, Sinclair, and Göttingen; in European Union, Göttingen; in Japan, Ohmini and CLAWN; and in China, Bama, Banna, and Wuzhishan appear to be most popular (Kohn, 2012; Larsen and Rolin, 2004; Liu et al., 2008; Misfeldt and Grimm, 1994; Nakanishi, 1981; Nunoya et al., 2007; Oshima et al., 1973; Wu et al., 2008). Minipigs are a genetically defined model (unlike dogs and monkeys) with the entire population history documented from early development to present for many of the breeds (Puccinelli et al., 2010; Simianer and Kohn, 2010). Genetically defined is not the same as genetically identical. The Yucatan minipig was developed from the progeny of pigs imported from the Yucatan Peninsula and is native to the tropics (Panepinto et al., 1978). The Hanford minipig was produced by crossing Palouse gilts with a Pittman-Moore boar and bred for decreased size (Sinclair BioResources, 2009). The Sinclair minipig was bred for small size and subsequently noted to have a significant incidence of melanoma (Millikan et al., 1974). Göttingen minipigs were originally developed by crossbreeding the Minnesota minipig, Vietnamese potbelly pig, and German Landrace pigs, and are now barrier-bred and microbiologically defined (Ellegaard Göttingen Minipigs, 2010). There is also a Brno Göttingen substrain developed in the Czech Republic about which very little information is available, especially regarding its origin, although it has been well studied and reported on with regard to toxicology studies. Even less information is available in the Western literature regarding Asian breeds/strains. The Ohmini was developed from small pigs of Manchuria (Watanabe et al., 1986). CLAWN minipigs were developed by mating F1 progeny of Göttingen minipigs with Ohmini miniature swine (Nunoya et al., 2007; Yabuki et al., 2007). The Wuzhishan pigs are a native Chinese miniature breed that has been inbred (Wang et al., 2006). The Banna minipig inbred line was developed in 1981 at the Banna Mini-Pig Inbred Line Institute, and there are 18 sublines (Zeng and Zeng, 2005). Bama minipigs are also a genetically stable strain increasingly used in preclinical drug evaluation (Li et al., 2006). The Microminipig, recently introduced in Japan with a mature bodyweight of 7 kg (Kaneko et al., 2011), was developed from the offspring of a single female which produced only small offspring (Kaneko et al., 2011). The Guangxi Bama, Ohmini, CLAWN, Wuzhishan, and Banna minipigs are all inbred (Li et al., 2006; Liu et al., 2008; Nunoya et al., 2007; Yabuki et al., 2007; Zeng and Zeng, 2005).

DRUG METABOLISM

The primary objective of toxicokinetics is to provide information on the systemic exposure achieved in animals as well as the relationship between dose and rate, and duration of exposure (ICH, 1995; Takacs, 1995). A second objective is to determine the relevance of these findings to clinical safety

in humans (ICH, 1995). In addition to traditional drugs and compounds, biotechnology-derived pharmaceuticals including proteins, peptides, cytokines, plasma factors, growth factors, and so on are also tested for toxicokinetics. Preclinical studies are performed to characterize PK, PD effects, and toxicodynamics before use of the compound in humans (Olejniczak et al., 2001). All organisms metabolize xenobiotic compounds, but not necessarily equally. They may have different metabolites, may not metabolize the agent at all, or the animal species chosen for testing may experience toxicities from the drug, to which humans may or may not be susceptible; hence, the importance of choosing a model with a similar metabolism pathway to humans for the xenobiotic being tested.

Once drugs are ingested, the typical path to metabolism is passage across the small intestinal mucosa by a transporter, P-glycoprotein (P-gp) (Kim et al., 1998; Schinkel et al., 1994; Sparreboom et al., 1997) followed by metabolism within intestinal epithelia by resident CYP enzymes. The resultant or native compound is then passed through the portal circulation to the liver where it is further metabolized by more or different CYP enzymes (Dresser et al., 2000). It is the differences in transporters and CYP enzymes among species and individuals that lead to differences in metabolism.

Some drugs are not metabolized and may be excreted unchanged via the bile, feces, urine, or air. Excretion via the kidneys plays only a modest role in drug elimination. With the exception of exhalation, the water solubility of the compounds determines their ease of elimination. Drugs are absorbed very rapidly from large surface areas such as the lungs, intestine, or skin (Benet et al., 1996). Biotransformation reactions result in an increase in polarity to aid in elimination but may also inactivate the drug (Benet et al., 1996). Phase I and phase II biotransformation reactions occur primarily in the endoplasmic reticulum and cytosol, and often happen within the same cell (Benet et al., 1996).

The distribution of drugs or metabolites is not necessarily the same between species (Garattini, 1985). In humans, only the blood is readily measured, but it may not be where the chemical accumulates (Garattini, 1985). The formation of metabolites may also be different between cell types/organs of the same animal species due to allelic differences (Fink-Gremmels, 2008; Garattini, 1985). Age is often overlooked in toxicokinetic studies, yet in the aged, there is decreased serum albumin, reduced metabolic activity of the liver, and decreased renal excretion, all of which have an impact (Garattini, 1985).

There are three important tenets to remember when extrapolating data among species: (1) Species dispose of or metabolize chemicals in different ways; therefore, plasma concentration should be measured rather than dosage; (2) Biotransformation of chemicals can result in biologically active metabolites, and knowledge of which chemical species is responsible for which effect is important; (3) Equal plasma concentration does not necessarily mean equal effect across species due to differences in the sensitivity of organs, cells, enzymes, and receptors among species (Dahlem et al., 1995; Garattini, 1985). As an example, in a study that dosed caffeine similarly among species, humans had 9× increased exposure over mice and rabbits, 5× increased exposure over rats, and 2× greater exposure over NHP (Garattini, 1985). When the dose was increased 10×, plasma levels of caffeine increased 12× in mice, 13× in humans, 16× in rabbits, and 46× in rats (Garattini, 1985).

Enzymes

Compounds are often metabolized using more than one metabolic pathway. CYPs are the most common metabolizing enzymes and react with the majority of xenobiotics (Benet et al., 1996; Thorn, 2012). While all organisms, including bacteria, have CYP enzymes, there are differences among species regarding which CYP isoforms are present (Nelson et al., 1996).

Phase I reactions include oxidative, reductive, or hydrolytic reactions and either introduce or expose a functional group on the parent compound which typically will result in a loss of activity of the drug (Benet et al., 1996). Phase II reactions result in the formation of a covalent linkage between the functional group and one of the endogenous molecules, namely, glucuronic acid, sulfate, glutathione, amino acids, or acetate, which typically make the compounds more polar and result in

rapid excretion (Benet et al., 1996). The most important phase II metabolism reactions are sulfation, glucuronidation, and glutathione conjugation of the functional groups added or exposed by phase I reactions (Zamek-Gliszczynski et al., 2006). There are distinct interspecies variations in phase I and phase II xenobiotic metabolism. In the pig, for example, there is decreased sulfate conjugation (a phase II reaction), compared to other species (Toutain et al., 2010).

Phase I Reactions

Within the liver, both phase I and II enzymes are involved in the elimination of exogenous and endogenous compounds. CYP enzymes play a central role in the phase I oxidative pathway, providing the functional group for phase II reactions, mostly conjugations (Desille et al., 1999). CYP enzymes catalyze numerous reactions including the phase I oxidation, hydroxylation, and reduction reactions as well as many others (Lock and Reed, 1998). CYP is a large family of enzymes that are functionally conserved and are found in all mammals. In humans, only 3 CYP families (CYP1, CYP2, and CYP3) are involved in the majority of all drug biotransformation (Toutain et al., 2010). CYP3A4 is especially important as it is involved in the biotransformation of the majority of all drugs and is expressed both in the liver and extrahepatically (intestine, kidney, others) (Benet et al., 1996). Allelic differences of CYP enzymes are present among breeds. For example, beagles have higher CYP2B11 activity than grayhounds (Court et al., 1999; Toutain et al., 2010).

Each CYP family has subfamilies or isoforms and subsequent unique polypeptides. Based on genetic studies, two-thirds of an individual's proteins will have a single amino acid difference from unrelated neighbors (Nelson, 1999). Therefore, among 50 CYP genes, approximately 30 will bear polymorphic sites that affect protein sequence (Nelson, 1999).

No two species have the same complement of CYP isoforms; however, they often have orthologs, so a specific CYP isoform of humans may have an ortholog in swine, but the identical isoform is not found. In CYP, this is important as one amino acid change may prevent metabolism of a substrate or change substrate specificity, resulting in changes in drug metabolism or disease (Fink-Gremmels, 2008; Nelson, 1999). These differences in sequence result in variances within families of enzymes, making extrapolation among species difficult. Comparison of cDNA shows high homology between pigs and humans; however, response to chemical inducers varies (Anzenbacher et al., 1998; Desille et al., 1999; Lu and Li, 2001; Monshouwer et al., 1998; Myers et al., 2001; Toutain et al., 2010). There are polymorphisms between CYP enzymes, resulting in different metabolic profiles between individuals. Some CYP enzymes are more likely to have polymorphisms with different metabolizing capabilities than others.

There are many studies in the literature that discuss the induction or presence of CYP enzymes within pigs of varying breeds. When evaluating data in these reports, it is necessary to note age, breed, and sex of pigs utilized in the study. While it is important to know whether a specific CYP enzyme is present or absent, the activity of the enzyme in question is what is important in metabolism of compounds. Not all enzymes metabolize similar compounds in different species or even different breeds.

Differences between conventional pigs and minipig CYP sequences exist; however, the variability is less than 1% and should be considered allelic variants (Puccinelli et al., 2011). Even though sequences are similar, the difference in activity may be due to differences in expression levels (Fink-Gremmels, 2008; Vaclavikova et al., 2004).

The enzymes most likely to be involved in the metabolism of a specific compound can be determined prior to *in vivo* testing by using *in vitro* techniques. Microsomes and hepatocytes from Göttingen minipigs are commercially available to aid in determination of whether minipigs are the most appropriate species in each instance of toxicity testing.

Some porcine CYP enzymes have been characterized including substrate specificity, inhibition, and regulation. Porcine (conventional, mini-, and micropigs) CYP1A, 2A, 2C, and 3A metabolize the same test substrates as human enzymes, whereas pig 2B, 2D, and 2E are different from the

corresponding human enzymes regarding metabolism of well-known substrates (Anzenbacherova et al., 2003; Skaanild, 2006; Soucek et al., 2001). Total CYP activity is approximately the same for both pigs and humans, but may be dependent on the breed of the pig and the sex of the animal (Skaanild, 2006).

The presence of CYP in Landrace pigs was measured via Western blot, and proteins that correspond to CYP1A2, 2A6, 2B1, 2B2, 2B6, 2D6, 2E1, 3A1, 4A1, 4A3 were detected; however, presence of these enzymes did not always correspond with similar activity (Myers et al., 2001).

There is between 74% and 76% sequence homology between isozymes of CYP3A in pigs and humans (NLM, 2009a,b), suggesting there are similarities as well as differences between enzymes that need to be considered before using any animal as a model for human toxicity.

CYP1A2, CYP2C19, CYP2D6, CYP2E1, CYP3A4 protein levels and activity were measured in both Göttingen and conventional (Landrace–Yorkshire–Duroc cross) pigs (Skaanild and Friis, 1997). No CYP2C19 or CYP2D6 activity was detected (Skaanild and Friis, 1997). When minipigs (Göttingen) were compared with three breeds of conventional pigs and two races of humans, minipigs had higher CYP content overall (Myers et al., 2001; Nebbia et al., 2003; Shimada et al., 1994; Skaanild and Friis, 1999). Not only does content of CYP differ among breeds, but also the activity levels are different, with Göttingens and Yucatans having higher CYP activity versus farm breeds (Sakuma et al., 2004; Vaclavikova et al., 2004). In addition, gender differences also exist with some CYP enzymes (Skaanild and Friis, 1997). Inflammation and the age of the pig have also been shown to affect CYP protein levels (Freudenthal et al., 1976; Myers et al., 2010). Still, significant discrepancies in interpretation of data measuring CYP expression levels and substrate specificity in pigs result primarily from use of different testing methods and strategies between conventional pigs, minipigs, and micropigs.

CYP1A metabolizes approximately 13% of compounds and is involved in carcinogen metabolism such as aromatic and heterocyclic amines, estrogens, mycotoxins, xanthenes, some antidepressants, and analgesics (Nebbia et al., 2003; Rendic, 2002; Skaanild, 2006). CYP1A1 is found in many tissues, often only after induction. CYP1A2 is present within the liver with both constitutive and inducible expression (Omiecinski et al., 1999). The *O*-dealkylation of 7-ethoxyresorufin (a CYP1A activity) is gender-related in minipigs but not in conventional pigs (Skaanild and Friis, 1999). Female minipigs have two to four times higher activity than males; however, the opposite is true for Caucasian humans (Bogaards et al., 2000; Shimada et al., 1994; Skaanild and Friis, 1997). Both ethoxy- and methoxy-resorufin *O*-dealkylation (enzyme activity associated with CYP1A) are present in humans and pigs (Nebbia et al., 2003).

Pig CYP1A1 is 82% similar to human CYP1A1 and 74% similar to human CYP1A2 (Nelson, 2009). This data shows that there is tremendous sequence overlap within the families and between orthologs of differing species.

CYP1B1 is the predominant isoform found outside the liver, but has not yet been fully characterized in the pig (Chirulli et al., 2007; Puccinelli et al., 2011).

CYP2A enzymes, found primarily in liver (Omiecinski et al., 1999), are important in humans, where they metabolize compounds such as nicotine, nitrosamines, and aflatoxin B1 (Skaanild, 2006). Expressions of cytochromes of the CYP2A subfamily show marked species differences, being expressed in mouse but not in rat kidney, and only in intact males (Lock and Reed, 1998).

The only porcine member of the CYP2A subfamily that has been identified and cloned is CYP2A19, which was isolated from liver of conventional pigs (Kojima and Morozumi, 2004; Puccinelli et al., 2010). There is 88.6% homology between porcine CYP2A19 and human CYP2A13 (Kojima and Morozumi, 2004). There are large inter-individual variations in porcine CYP2A activity, which are due to not polymorphisms, but transcriptional regulation, whereas in humans, differences in activity are due to polymorphisms (Skaanild and Friis, 2005). In pigs, the specific reaction for CYP2A is coumarin7-hydroxylation (in humans, this reaction is specific for CYP2A6) (Skaanild and Friis, 2005). There are similar substrate recognition sites in porcine and human proteins (Lewis and Lake, 2002; Skaanild and Friis, 2005). Gender differences in levels of CYP are present in

Yucatans, Göttingens, and humans (Skaanild and Friis, 1999). Expression levels increase with castration, indicating that this gene is under the control of sex hormones in minipigs (Gillberg et al., 2006; Skaanild, 2006). CYP2A is also reversibly inhibited by androgens *in vivo* in pigs (Gillberg et al., 2006).

CYP2B metabolizes diazepam, lidocaine, and antineoplastic compounds such as cyclophosphamide, iphosphamide, and tamoxifen (Skaanild, 2006). Homology between porcine CYP2B22 and human CYP2B6 proteins is 81.1% (Kojima and Morozumi, 2004). CYP2B genes show species-specific constitutive expression and are induced by phenobarbital (rabbit, hamster, minipig) (Lock and Reed, 1998).

The *N*-demethylation activity of the enzyme was not detected in pig microsomes, but the CYP2B-specific 7-ethoxy-4-triflouromethylcoumarin *O*-dealkylase activity was detected in Yucatans (Bogaards et al., 2000; Skaanild and Friis, 1999). A different reaction, 7-pentoxyresorufin dealkylation, which also tests for CYP2B activity was not detected in pigs by some groups (Anzenbacher et al., 1998; Monshouwer et al., 1998; Skaanild and Friis, 1999), whereas other groups did detect this activity (Behnia et al., 2000; Desille et al., 1999).

CYP2B activity levels (measured in microsomes) are similar between male and female pigs and are much higher in pigs than humans (Bogaards et al., 2000; Myers et al., 2001).

These differences may be due to different sources of hepatocytes and microsomes, the use of separate assays to measure activity or assay sensitivity, sequence differences, or it may be related to the fact that porcine CYP2B has activity more akin to human CYP2D (Skaanild, 2006; Skaanild and Friis, 2002). Needless to say, the current data on porcine CYP2B is inconclusive.

The CYP2C family has several enzymes. In humans, they metabolize about 25% of drugs, including losartan, propofol, estrogens, testosterone, mephonotoin, and methadone (Rendic, 2002). CYP2C members catalyze epoxidation of arachidonic acid in the rat, rabbit, and human (Lock and Reed, 1998). The porcine CYP2C49 protein is 70% homologous to the CYP2C human enzymes (Skaanild, 2006). Different CYP2C enzymes show some cross-reactivity toward many of the test substrates, which makes extrapolation more difficult among species and also confounds study of CYP2C activities independently (Skaanild, 2006; Skaanild and Friis, 2008). It appears that all three enzymatic activity assays that are used to measure human CYP2C activity are present in pigs, but to varying degrees and may be mediated by CYP families other than CYP2C (Skaanild and Friis, 2008). Porcine microsomal metabolism of human CYP2C9 substrates showed both similarities and differences from activity with human metabolism (Thorn, 2012). In humans, CYP2C9 comprises about 17%–20% total CYP in liver (Omiecinski et al., 1999). In the pig, there is high expression of CYP2C49 in liver, and low expression in the kidney (Kojima and Morozumi, 2004; Puccinelli et al., 2010). Expression of porcine CYP2C33, CYP2C42, and CYP2C49 is found in small intestine and nasal mucosa and CYP2C33 is present in the ovary (Puccinelli et al., 2010, 2011). As with some other CYP enzymes, there is a gender difference, with females having higher activity of the enzyme than males (Skaanild and Friis, 2008).

Human CYP2D6 metabolizes about 16% of all drugs, including antidepressants, antipsychotics, and β-blockers (Rendic, 2002). Human CYP2D6 is 78% homologous with porcine CYP2D21 and CYP2D25 (Sakuma et al., 2004; Skaanild, 2006). In humans, high inter-individual variances in metabolic activity exist due to numerous polymorphisms. The dog ortholog CYP2D15 also shows abundant polymorphisms (Rendic, 2002; Skaanild and Friis, 2002).

The human CYP2D6 substrate dextromethorphan is rapidly and extensively metabolized in pig versus human (Thorn, 2012). Yet, metabolism of some human CYP2D substrates correlates with protein levels of a porcine CYP2B protein, suggesting that porcine 2B plays an important role in catalyzing these reactions, as suggested above (Skaanild, 2006). Because of these discrepancies, it is concluded that the pig is not a good model to study compounds that are metabolized by CYP2D in humans due to the inconsistent and erroneous results obtained in experiments (Skaanild and Friis, 2002). It is still not established whether pigs have CYP2D, but if they do have, the activity differs greatly from humans (Skaanild and Friis, 2002).

CYP2E1 is inducible by alcohol and catalyzes the metabolism or bioactivation of alcohols, ketones, anesthetics, and nitrosamines (Skaanild, 2006). CYP2E1 is responsible for metabolism of approximately 4% of compounds, and there is 75% homology between pig and human CYP2E1 proteins (Rendic, 2002; Skaanild, 2006). Porcine CYP2E1 is highly expressed in liver, with lower levels in the kidney (Kojima and Morozumi, 2004; Puccinelli et al., 2011).

The enzymatic activity of CYP2E1 is related to sex hormone levels of minipigs (Göttingen, Yucatan), with females having higher activity than males (Bogaards et al., 2000; Skaanild and Friis, 1999). When specific enzymatic reactions used to test for human CYP2E activities were examined in the pig, there was no correlation. However, the reactions could be inhibited with inhibitors commonly used for human CYP2A and CYP3B suggesting that there are many functional differences between human and pig CYP2E and, similar to CYP2D, the pig may not be a good model to study xenobiotics metabolized by CYP2E (Skaanild, 2006).

CYP3A in humans is the most important CYP enzyme metabolizing approximately 34% of compounds and comprising 30% of total CYP and 30%–40% of CYP in liver (Omiecinski et al., 1999; Rendic, 2002). In addition to drug metabolism, the CYP3 family is involved in steroid hydroxylation (Lock and Reed, 1998; Rendic, 2002). Of the several porcine CYP3A sequences that have been isolated, there is 75%–83% homology with human protein CYP3A sequences (Fink-Gremmels, 2008; Sakuma et al., 2004; Skaanild, 2006). Hepatic content can vary up to 20× and activity may vary 10× among individuals (Dresser et al., 2000). CYP3A4 is present in liver and small bowel, thereby potentially having an effect on both presystemic and systemic drug disposition (Dresser et al., 2000). Small bowel CYP3A4 is found in apical enterocytes and there, the levels may vary up to 11× between individuals (Dresser et al., 2000).

Tissue expression patterns and enzymatic profiles are similar between porcine CYP3A29 and human CYP3A4 (Yao et al., 2011). The porcine and human sequences are similar, but there are differences in transcriptional regulation of CYP3A4 in the pig (Fink-Gremmels, 2008). Castration of mature boars resulted in increased CYP3A levels (Gillberg et al., 2006). This phenomenon may also account for sex differences in protein levels seen with other isoforms.

There are breed differences as well. Yucatans have higher CYP3A enzyme activity than Göttingens, which have higher activity than conventional pigs, the reason for which is yet to be examined (Bogaards et al., 2000; Skaanild and Friis, 1997, 1999). In humans, CYP3A5 is the predominant isoform in lung and stomach and is also found in small bowel, liver, and kidney (Dresser et al., 2000). Of note, the rat is not a good model of metabolism of compounds dependent on CYP3A4 as the ortholog is not induced similarly, and it does not metabolize all of the same compounds (Zuber et al., 2002).

The CYP4 family catalyzes the metabolism of fatty acids and arachidonic acid and is inducible by hypolipidemic drugs and chemicals that cause peroxisome proliferation (Lock and Reed, 1998).

CYP4A21 is found in pig kidney, but not in heart, muscle, intestine, spleen, thymus, lung, or adrenal gland (Lundell et al., 2001). Porcine CYP4A24 and 4A25 have been reported in liver and kidney (Lundell, 2002).

Phase II Reactions

Both phase I and phase II metabolisms are important in compound distribution and are responsible for interspecies differences (Zamek-Gliszczynski et al., 2006). To date, not much research has been carried out on phase II reactions in pigs, since much of the attention has been focused on the phase I reactions.

Phase II enzymes are distributed widely in many tissues (Thorn, 2012). Glucuronidation and sulfation are the most important phase II reactions in the biotransformation of xenobiotics in humans (Kiang et al., 2005; Miners et al., 2004). Interspecies differences in Phase II enzymes include decreased sulfation capabilities in pigs and deficiency in acetylation in dogs (Anzenbacherova et al., 2003; Baggot, 1990; Caldwell, 1986; Martinez et al., 2002). Sulfation is a major conjugation

pathway for phenols and contributes to biotransformation of xenobiotic compounds including alcohols, amines, and thiols (Caldwell, 1986; Lohr et al., 1998). Pigs conjugate *p*-cresol (metabolite of tyrosine) with glucuronide rather than sulfate as in humans to account for this deficiency (Merrifield et al., 2011).

Acetylation is metabolized by *N*-acetyltransferases (NAT) of which there are two families (NAT-1 and NAT-2). Pigs have high acetylating capacity compared to the dog, which does not express functional NAT-1 and NAT-2, essential for excretion of sulfonamides (Toutain et al., 2010).

Not much is known to date about the uridine diphosphate-glucuronosyltranferase (UGT) expression and function in pig intestine and liver (Thorn, 2012). In humans, UGT is the most important phase II enzyme (Thorn, 2012). It is becoming more important to characterize the expression and activity of these enzymatic proteins in pigs, especially with their growing use in pharmaceutical testing.

ORGANS OF DRUG METABOLISM

Gastrointestinal Tract

In orally administered xenobiotics, the first organ to have an effect on metabolism is the GI tract. While the liver is a well-known site of drug metabolism, enzymes in the intestine also play a major role in first pass extraction (Thorn, 2012). The primary mechanism of intestinal drug absorption is passive transcellular diffusion across the apical membrane as the rate-limiting step (Lennernas, 2007; Sugano et al., 2010; Thorn, 2012). Absorption from the GI tract is typically passive and is favored when the drug is nonionized and lipophilic (Benet et al., 1996).

Interspecies differences in drug bioavailability are most often the consequences of anatomical and physiological differences in the GI tract among species, followed by differences in transporters and metabolic enzymes. Anatomical and physiological differences among species include GI transit time, *in vivo* dissolution, presystemic metabolism, physicochemical interactions with gut contents, bacterial digestion, and site-specific differences in absorptive surface area.

Salivary amylase is present in humans and pigs but not in carnivores or horses (Martinez et al., 2002). The pH of the pig stomach, duodenum, jejunum, ileum, and colon is comparable to that of the human. The effect of food on gastric pH also differs across species. The gastric pH of fasted dogs is highly variable and after a meal, gastric acid secretion in dogs exceeds that of humans and swine, whereas postprandial pH in humans exceeds that in dogs due to strong buffering effect of diet, and typically returns to baseline within an hour (Akimoto et al., 2000; Kararli, 1995; Martinez et al., 2002). All of these factors contribute to xenobiotic solubilization, bioavailability, and absorption.

Marked anatomical differences between herbivores and nonherbivores exist that result in substantial differences in both rate and extent of oral drug absorption (Toutain et al., 2010). The relative amounts of stratified squamous epithelium and cardiac regions in the stomach are similar in humans and dogs, but are much larger in pigs (Martinez et al., 2002). The pig stomach is lined by thick mucus that inhibits drug absorption, which closely recapitulates the human stomach (Benet et al., 1996; Varum et al., 2010, 2012). In monogastric animals, the stomach is important in disintegration and dissolution of drug compounds. The pylorus acts as a sieving gate leading into the duodenum; in the pig, the torus pyloricus acts as a physical barrier to large particles of ingesta. GI transit times are important in xenobiotic assays and have resulted in failure of dogs to adequately model the bioavailability of some drugs including acetaminophen-sustained release tablets, griseofulvin, valproic acid, and ampicillin (Jamei et al., 2009; Martinez et al., 2002; van Meer and Simons, 1986). In pigs, gastric transit time is affected by particle size, similar to humans. Ingested pellets egress the stomach at a rate parallel to liquids (15 min to 1.5 h). There is some discordance in the results for larger particles which have been shown to remain in the stomach anywhere from 6 h to 1.5 days (Davis et al., 1986; Giacomini et al., 2010; Hossain et al., 1990; Martinez et al., 2002). When comparing nondigestible granules and tablets, minipigs show

delayed gastric emptying compared to humans (Aoyagi et al., 1992). The viscosity of ingesta also affects the rate of gastric emptying (Martinez et al., 2002).

Gastric emptying is the most important factor controlling the rate of access of drug to the site of absorption in the proximal small intestine (Toutain et al., 2010). Any factor that accelerates gastric emptying will likely increase the rate of drug absorption. Conversely, factors that delay gastric emptying decrease rate of absorption (Benet et al., 1996). Of note, the digestive system of neonatal pigs is not fully mature (to allow for passive transfer of maternal antibodies) (Martinez et al., 2002).

There is high interanimal variability in the gastric pH in all species. In pigs, the mean gastric pH is 4.4 (Merchant et al., 2011), which is influenced by the size of food particles. Proximal stomach bile acid concentrations are significantly higher in pigs fed finely ground compared to coarsely ground feed (Martinez et al., 2002). Elevated hydrochloric acid and bile acid concentrations are found in the stomach of pigs after feed deprivation (Lang et al., 1998). The pH of the pig small intestine is 6.1–6.7, which is higher than in the cecum and colon (pH = 6.0–6.4) (Merchant et al., 2011).

Abundant lymphoid tissue is present in porcine stomach, small intestine, and cecum (Merchant et al., 2011).

In addition to gastric emptying, intestinal transit time is also critical in bioavailability. Similar to humans, as omnivores, pigs possess a well-developed small intestine, but unlike humans, they have a more complex lower intestine to allow dietary fiber fermentation (Martinez et al., 2002). Pigs excrete 7% of fluid markers and 2% of particulate markers in feces within 24 h, similar to humans (Martinez et al., 2002).

Numerous efflux transporters are present in the intestine including P-glycoprotein (P-gp), breast cancer resistance protein, and multidrug resistance protein. P-gp plays an important role in limiting bioavailability of oral dosing for many compounds (Giacomini et al., 2010; Johne et al., 2002; Kim et al., 1998; Lown et al., 1997; Sparreboom et al., 1997; Thorn, 2012). As there are dissimilarities between drug metabolizing enzymes among species, there are also species effects on P-gp (Zolnerciks et al., 2011). Upon administration of a compound, before metabolism occurs, the compound needs access to the cell, which is commonly gained via a transporter. The most common xenobiotic cross-membrane transporter in humans, p-glycoprotein, has numerous haplotypes, leading to different PK for compounds (Johne et al., 2002). Solubilization of lipophilic drugs by bile salts is also a critical step to ensure bioavailability. Pigs, humans, and dogs have an analogous bile flow, which is much lower than rodents (Martinez et al., 2002). Solubilization is more important than inhibition of P-gp for food-related effects on intestinal absorption kinetics of drugs (Persson et al., 2008).

CYPs are expressed not only in the liver, but also in mucosa of small intestine, lung, kidney, brain, olfactory mucosa, and skin (Ding and Kaminsky, 2003). Extrahepatic CYPs play important roles in activation of xenobiotics and the GI tract is the most important of the extrahepatic sites of drug biotransformation, since drug molecules must pass through enterocytes after oral administration. In humans, the distribution of CYP enzymes is not uniform along the length of small intestine (the upper small intestine has increased levels of CYP3A) (Ding and Kaminsky, 2003; Paine et al., 1997; Thorn, 2012), which also leads to variations in metabolism. These parameters are yet to be examined in the pig. CYP3A is the most abundant subfamily in the small intestine, but is found only in enterocytes so the total mass of CYP3A in the entire small intestine is only 1% of that in liver (Paine et al., 1997; Yang et al., 2004). Swine intestinal cells also express CYP2C similar to humans, which primarily express CYP3A and CYP2C9 in the GI (Martinez et al., 2002; Paine et al., 2006).

Currently, methods and enzymology to examine the UGTs lag behind that of the CYPs (Fisher et al., 2001), resulting in only limited information regarding the contribution of intestinal glucuronidation (Thorn, 2012). Most UGT isoforms have distinct tissue expression, with significant expression in intestine, kidney, and others (Fisher et al., 2001). Intestinal glucuronidation plays a major role in first pass metabolism and in the degree of interindividual variation in overall oral bioavailability (Fisher et al., 2001). Consideration should be given to significant genetic variability and tissue localization in first-pass organs (Fisher et al., 2001).

Intestinal microflora have a relatively minor role in metabolism of most drugs. In pigs, the large intestine is the most likely site for this to be an issue (Martinez et al., 2002). Overall, pigs have comparable gut physiology to humans (Merrifield et al., 2011), indicating that most orally administered drugs are absorbed/transported in the small intestine.

Liver

The sum of drug extraction that occurs in intestine and liver is referred to as first pass or presystemic metabolism, of which, the liver is the primary site (Murayama et al., 2009; Thorn, 2012).

The liver is the largest of the drug metabolizing organs and is rich in metabolism enzymes. Most metabolic enzymes present in human liver have a counterpart in the pig liver. There are of course exceptions, but CYP3A4, which is the most important human isoform, has porcine orthologs (Donato et al., 1999).

The liver of the pig expresses the most important enzymes of biotransformation (Desille et al., 1999).

In order to more easily study drug metabolism, microsomes are commonly isolated from hepatocytes. While the use of pig microsomes is a good way to model drug metabolism, cellular transport systems are often bypassed and thus unaccounted for by using these techniques. Differences in protein levels and activity of hepatic CYP enzymes are the most important factors in the variation in drug metabolism among species (Anzenbacher et al., 1998). The average total CYP in microsomes from conventional pigs is similar to humans, but levels are higher in Göttingens (Puccinelli et al., 2011). Phenobarbital increases expression and activity of several CYP enzymes in both porcine liver and hepatocyte cultures (Desille et al., 1999).

Hepatocyte microsomes from Brno Göttingen minipigs have enzyme-specific activities similar to human CYP2A6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4 (Anzenbacher et al., 1998; Soucek et al., 2001). Neither Brno Göttingen nor human microsomes display 7-pentoxyresorufin *O*-dependentalase activity (indicative of CYP2B) (Anzenbacher et al., 1998). Enzymatic activities specific to human CYP1A, CYP2B, CYP2C, CYP2D, CYP2E, and CYP3A were present in microsomes from small minipigs (Bland name, Micromini pig) (Murayama et al., 2009). In primary cultures, conventional pig hepatocytes express orthologs of human CYP1A, CYP2A, CYP2B, CYP2C, CYP2E, and CYP3A subfamilies and exhibit CYP-dependent oxidative metabolism similar to human hepatocytes (Donato et al., 1999). In porcine microsomes, there was low alkoxyresorufin *O*-dealkylation (sum of activity of CYP1A1, CYP1A2, CYP2A, and CYP2B), yet when human versus pig activities of CYP1A1/2 and 2B were compared, they were found to be similar (Donato et al., 1999). This indicates that while many similar enzymatic activities are present, they are not identical and may be mediated by different protein families, or different proteins altogether. Like humans, pigs have decreased CYP2B activity relative to CYP1A1 and CYP1A2 (Donato et al., 1999). Bama minipigs have decreased CYP1A2, CYP2A6, and CYP2E1 activities compared to human, but CYP2D6 activity is higher (Li et al., 2006).

In humans, CYP3A4 is the most abundant CYP enzyme, and it has been suggested that a porcine CYP3A is also the major hepatic CYP in the pig (Donato et al., 1999). Porcine CYP3A29 has considerable homology to human CYP3A4 (Jurima-Romet et al., 2000; Vaclavikova et al., 2004). Nifedipine oxidase activity and testosterone 6β-hydroxylating activity (specific markers for human CYP3A4 enzyme) are comparable between human and Brno Göttingen minipig microsomes (Anzenbacher et al., 1998). This demonstrates the presence of a CYP3A4-like form in Brno white Göttingen minipig liver microsomes (Anzenbacher et al., 1998). Activities of Nifedipine oxidation and testosterone 6β-hydroxylation are similar between human and Bama minipigs (Li et al., 2006). Testosterone oxidation (specific activity of CYP3A4) profiles are similar between human and conventional pig models (Donato et al., 1999). The metabolites 2β- and 15β-hydroxytestosterone (OHT) are actively formed in both human and pig liver compared to other species whereas 16α-hydroxylation is poorly catalyzed in human and conventional pig liver microsomes, compared

to rat, dog, and rabbit (Donato et al., 1999). While CYP3A4 activity is quantitatively comparable between pigs and humans, qualitative differences may exist (Thorn et al., 2011). Banna minipigs have similar metabolism/hepatic function as humans, but differences in drug elimination and bilirubin levels have been noted (Zhang et al., 2004).

Similar levels and activities of hepatic glutathione transferase (GST) and UGT (both phase II enzymes) were found in human and pig hepatocytes (Donato et al., 1999). Pigs express high amounts of GSTα RNA and protein in liver. The expression and activities of GST indicate expression of at least 11 distinct GSTs in pigs (Desille et al., 1999; Fraslin et al., 1985).

Data indicates that livers and hepatocytes from pigs fulfill most important biotransformation pathways, including phase I and II detoxification enzymes (Desille et al., 1999). Due to the presence of CYP3A in pigs and minipigs, which metabolizes the majority of known drug substrates in humans, and in spite of the aforementioned differences, the pig seems to be the most appropriate species for reproducing the human hepatic metabolism of drugs and other xenobiotics, provided they are not metabolized via CYP2B, CYP2D, or CYP2E families (Anzenbacher et al., 1998; Donato et al., 1999).

Kidney

The most important organ for elimination of drugs and their metabolites is the kidney (Benet et al., 1996). It must be remembered that the kidney is also metabolically active in biotransformation, and in some cases, surpasses the liver (Anders, 1980).

The kidney possesses most of the common metabolizing enzymes (Lock and Reed, 1998). CYP and phase II conjugation enzymes are primarily localized in the proximal tubules, making them particularly susceptible to insult (Lock and Reed, 1998). The cell types within the kidney vary in levels of CYP present and even areas of the proximal tubule differ. In kidneys of conventional pigs, 2*C*33*v*4 expression is similar to that in the liver, but *CYP1A1, CYP2A19*, and *CYP2B22* levels are lower than those found in the liver. The porcine kidney shows minimal expression of *CYP2C49* and *CYP2E1* (Kojima and Morozumi, 2004).

Anionic drugs are transported by renal epithelium and include diuretics and penicillin-family drugs (beta lactams). Compared to humans and pigs, dogs have decreased ability to clear organic ions (Mandal, 2006; Timchalk, 2004). Functionally and anatomically, the porcine kidney is a good model of the human kidney.

Urine is the most metabolically distinct compartment, which may be due to the reduced need for homeostatic control, and therefore, may be the best metabolic diagnostic matrix in pigs, as in other species (Bollard et al., 2005; Merrifield et al., 2011). Pigs have a low urine concentrating ability and urine pH is determined mainly by composition of the diet (Toutain et al., 2010).

Skin

Less is known about drug metabolism in skin compared with the classical drug metabolizing organs (liver, kidney, lung, and intestine) (Oesch et al., 2007). Similarities of pig skin to that of humans have long been recognized in overall morphology including epidermal thickness, cellular composition, and cutaneous blood supply characteristics (Nunoya et al., 2007; Swindle and Smith, 1998; Swindle et al., 2012). Skin contains both phase I and II enzymes within keratinocytes (Baron et al., 2001). The stratum corneum is the most important barrier in dermal administration of xenobiotics. Relative to haired animals, dermal permeability is decreased in sparsely haired animals, such as pigs and humans, because the stratum corneum is thicker (Mortensen et al., 1998). The site of application is also important, especially in pigs, since porcine skin tends to thicken with age in anatomically distinct locations. For this reason, piglets or young animals are often chosen for toxicity studies of topically applied compounds (Gupta et al., 1999; Schmook et al., 2001). The topical or dermal application dose depends on applied concentration, application vehicle, body surface area exposed, duration of exposure as well as skin thickness (Dresser et al., 2000).

Little is known about phase I drug metabolizing enzymes in porcine skin. Very few reports of CYP presence and activity in porcine skin exist in the literature. In Bama miniature pigs, CYP3A29 expression is present in the skin and other organs and levels increase with age in some organs including the skin (Shang et al., 2009). Phase II metabolism enzymes are highly active in the skin with GST, UGT, sulfotransferases, and NAT present in both human and pig skin (Kurihara-Bergstrom et al., 1986; Oesch et al., 2007; Rangarajan and Zatz, 2001). An active sulfation enzyme in porcine skin has also been identified (Dressler and Appelqvist, 2006).

The pattern of enzymes and their localization impacts properties of drugs for the skin, but also dictates whether a drug reaches the blood flow unchanged or as a metabolite (Oesch et al., 2007).

REGULATORY ISSUES

The goal of regulatory agencies associated with approval of human pharmaceuticals and medical devices worldwide is to protect the human population from undue harm caused by untested xenobiotics (pharmaceuticals) and medical devices. Therefore, toxicological studies are completed in laboratory animals before compounds or devices are allowed to be marketed for human consumption or use. There are several agencies within the United States alone; the agency to which the toxicology report will be submitted depends on its intended use. The United States Food and Drug Administration (FDA), Environmental Protection Agency (EPA), and Unites States Department of Agriculture/Animal and Plant Health Inspection Service (USDA/APHIS) all have separate requirements for toxicological testing, although there is tremendous overlap. The international conference on harmonization of technical requirements of pharmaceuticals for human use (ICH) was formed in 1989 comprising agencies from the European Union, Japan, and the United States in order to streamline approval in several countries at once and to reach a common market for medical products (Olejniczak et al., 2001). Agencies require testing in both rodent and nonrodent species. Rats are historically used as the rodent toxicologic model whereas dogs and NHP are currently the most commonly used nonrodent models. Pigs and minipigs are acceptable models in many cases, and in some cases, are better models for human toxicities. For instance, the skin of humans is better emulated by that of the pig than either dogs or NHP. The GI tract of pigs is more representative of the human GI system than is that of the dog in regard to both pH and transit time of ingesta, and there are some important drug metabolizing similarities between humans and pigs as discussed in this chapter.

There are different guidelines for acceptance of medical devices depending upon in which country the device is to be used. Unlike xenobiotic/toxicology testing which has achieved harmonization with the formation of the ICH, medical device regulators have yet to formalize any agreement. Typically, the most stringent guidelines are those of the FDA and European Conformité Européenne (CE).

Once a device has made it to the stage of being implanted for evaluation of efficacy or feasibility, many tests have already been performed on the device including biocompatibility of components used in the device. After the device has been implanted for a predetermined length of time, a complete necropsy is performed on all study animals, including animals with unexpected or unexplained mortalities and morbidities (Gosselin et al., 2011).

In order to obtain approval for device usage in humans, specific guidelines regarding which tissues to collect are provided by regulatory agencies based on the type of device being tested. Not only is evaluation of toxicity and injury important, but immunologic reaction and presence of biofilms within devices also need to be assessed, if present (Donlan and Costerton, 2002; Schuh, 2008).

The device itself also needs to be examined to determine if it is affected by the animal and whether the device remains structurally sound and functional to do what it is designed to do. The FDA also recommends scanning electron microscopy (SEM) of the implant site to characterize surface behavior of endothelium adjacent to the implant, explant radiography, and histology of local and downstream tissues (FDA, 2010).

Efficacy testing of most devices is done in nonrodent species due to the size of the device, and minipigs are ideal for this purpose, especially in CV device testing. The size of the adult human heart corresponds to that of a 40–50 kg pig, and porcine coronary artery blood supply to the heart is nearly identical to humans in both anatomy and function (Gad, 2012). Further information on the heart and CV system is found in Chapter 9.

A recent addition to the drug and device testing field are drug-eluting stents, which consist of both biomedical devices and xenobiotics. Drug eluting stents tend to be regulated by authorities for both xenobiotic and device categories as combination products.

As stated previously, dogs and NHP are historically the most commonly used nonrodent species in chronic toxicologic studies, but minipigs may provide a better model as their physiology better resembles that of man. A relevant species is defined as one in which the test article is pharmacologically active due to expression of receptor or epitope (ICH, 2011).

PIGS AS A TOXICOLOGY MODEL: SYSTEMS

Pigs have historically been used in skin toxicology studies, but they have also been used to study other systems. Pigs are good models for skin studies as the thickness and permeability are similar to humans (Gad, 2012).

The CV system has similar coronary artery distribution, and the pig has been used extensively as a model for atherosclerosis and myocardial infarction.

A porcine adapted inhalation model apparatus has been developed to more easily allow the use of pigs in inhalation studies (Koch et al., 2001). Although lung lobation is different between pigs and humans, the lungs are functionally and physiologically similar.

Pigs are an appropriate model for neurotoxicity testing as dopamine, serotonin, and other neurotransmitters are similar between pigs and humans and have been extensively described and validated as models in neuroscience as well as toxicology (Lind et al., 2007). To examine CNS toxicity, behavioral and learning tests are often part of study design (Clausing, 2011).

Pigs have a litter size of 5–6, compared to NHP, which typically have only one offspring, making pigs more efficient teratology models (Clausing, 2011). Minipigs are often used for juvenile toxicity studies for many reasons such as early sexual maturity and the ease of cross-fostering (Clausing, 2011). Porcine eyes are very similar to humans with only cones in the macula.

SAMPLING

Clinical

In a toxicity or medical device study, clinical data is part of the report and needs to be collected as the study progresses. During a safety study, animals should be observed twice daily and changes in skin, eyes, mucous membranes, secretions/excretions, activity, gait, posture, strength, or behavior should be noted (EPA, 1998). Initially, the animal's body weight should be measured and then repeated weekly following treatment. Clinical pathology consisting of hematology, clinical chemistry, and urinalysis should also be performed prior to treatment and then monthly or midway through exposure (EPA, 1998). A pre- and poststudy ophthalmological exam is required by some agencies (EPA, 1998).

When examining systems in safety studies, there is a hierarchical relevance that begins with CV, respiratory, and central and peripheral nervous systems (ICH Guideline, 2000). If any of these systems are acutely affected, there may be a negative effect on the ability to sustain life (Valentin et al., 2005). In the CV system, toxicities may present as hypo- or hypertension and arrhythmias; in the respiratory system, as asthma or bronchoconstriction; and in the CNS, as seizures. If any of these events occur, they may be life threatening (Bass et al., 2004). Clinical evaluations of these systems include measurement of blood pressure, heart rate, ECG, tidal volume, blood pH, hemoglobin,

oxygen saturation, motor activity, behavior changes, coordination, and body temperature (ICH Guideline, 2000). These parameters should be assessed throughout the study. The frequency of these measurements is mandated by the organization to which the product is being submitted for approval for use in humans. ECG techniques have been optimized for the unique anatomy of the Göttingen minipig (Nahas et al., 2002). Subtle neurological abnormalities caused by xenobiotics may be detected using behavior testing, positron emission tomography (PET) scans, and other techniques in pigs (Lind et al., 2005; Mosher and Court, 2010).

After assessing the core systems, studies then assess other systems, which may cause concern for human safety such as the GI system (Bass et al., 2004; Valentin et al., 2005). Additional testing includes acute toxicity, repeated dose toxicity (on all organs), adverse effects on male or female fertility, embryotoxicity/postnatal adverse events, genotoxicity, tumorigenicity, sensitization/immunosuppression and stimulation of the immune system (immunotoxicity), and local and other adverse events (Hinton, 2000; Olejniczak et al., 2001).

Use of the pig is not required for any of the aforementioned tests, but its use may be advantageous in that it makes some of these tests and sampling easier due to the size of the pig. Toxicokinetic data may be generated using samples collected from the main study animals if large animals are used, whereas if rodents are used, satellite groups are often necessary (ICH, 1995).

When preclinical studies are performed, the model chosen should be appropriate for risk assessment based on similarity of metabolism to humans but other considerations should include similarities in maturity, physiological state, and manner of delivery (Olejniczak et al., 2001; Use, 2011). When performing regulatory studies, the intended clinical route of administration of the xenobiotic is preferred rather than IV administration (unless that is the proposed route of administration) (Bass et al., 2004; ICH Guideline, 2000). Not only should the parent drug be measured in plasma and tissues, but metabolites and isomers of the test article should also be examined (ICH Guideline, 2000). See Chapter 20 for detailed necropsy procedures and tables of required tissue sampling.

NEW DIRECTIONS/CONCLUSIONS

MicroRNAs (miRNAs) are starting to be used as toxicity markers (Mikaelian et al., 2013). miRNAs have recently been shown to regulate drug metabolizing enzymes, specifically the CYPs (Tsuchiya et al., 2006). There is currently only one reference in the literature on the regulation of CYPs by miRNA in the pig (Xu et al., 2011).

Since pigs model many human diseases better than other species, their use in toxicologic studies examining proteomic outcomes may be more relevant than other species (Verma et al., 2011).

The use of pigs in toxicoproteomics or proteomic technologies to better understand toxic mechanisms/modes of action is important, especially with the completion of the porcine genome (Groenen et al., 2012; Verma et al., 2011).

Whenever performing toxicological or medical device studies, review of pertinent websites for the most current regulations and guidelines is recommended (Schuh, 2008). Gad has compiled a useful appendix of relevant online biomedical device guidelines/forms/publications (Gad, 2012).

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16 Radiobiology

M. Michael Swindle

CONTENTS

Swine were among the earliest large animal models used in radiobiology research (Brown and Johnson, 1970, 1971; Lefaix and Daburon, 1998) but were largely replaced by the dog in the 1950s and 1960s, because of the difficulties in handling swine in the laboratory. There is a resurgence of the use of swine for particular types of radiobiology research, in part, owing to the improved technical procedures in handling and anesthesia that have been developed since then. Specifically, the areas in which swine are utilized are for studies involving total body irradiation, immune suppression, skin and muscular damage, pulmonary fibrosis, central nervous system (CNS) paralysis, digestive system damage, tissue and bone marrow transplant, and vascular injury. This section provides an overview of the most significant areas of interest for using porcine models.

Some confusion may exist in comparing the older literature to the current publications, in that it was conventional in the United States for rad units (radiation dose) to be utilized rather than gray units. For practical purposes, 1 gray (Gy) = 100 rad or 1 cGy = 1 rad. One Gy is the absorption of 1 J of radiation energy by 1 kg of matter (1 Gy = 1 J/kg = 1 m^2/s). Typically, a total-body dose of approximately 10–20 Gy is uniformly fatal to humans and swine. There is variation in swine owing to age and source of radiation, but typically dosages less than 7 Gy are not fatal. Short-term death is usually due to bone marrow suppression and long-term death is usually due to infection following dermal or organ pathology, and some long-term survivors develop neoplasia (Brown and Johnson, 1970, 1971). Whole-body irradiation with 6 Gy demonstrated that lymphocytic and neutrophilic suppression in conjunction with alterations in LDH, ALT, AST, amylase, and urea can be useful in prediction of radiation injury (Donnadieu-Claraz et al., 1999; Zarybnicka et al., 2011). In recent work with the 4- to 5-month-old Göttingen minipig irradiated with gamma photons (^{60}Co, 0.5–0.6 Gy/min) in the dose range of 1.6–12 Gy, an analysis of biomarkers for prediction of survivors was studied (Moroni et al., 2014b). Moribund pigs had decreased lymphocytic/granulocytic counts, increased C-reactive protein, alkaline phosphatase elevations, increased citrulline and fever.

The same authors used the minipigs for evaluation of hematopoietic (H-ARS) and gastrointestinal (GI-ARS) acute radiation syndromes (Elliott et al., 2014; Moroni et al., 2013a,b, 2014a,b). They report that H-ARS is produced with 1.6–2.0 Gy) and GI-ARS at higher levels of irradiation.

Bone marrow transplantation and suppression may be useful as part of transplantation and xenotransplantation protocols (Dor et al., 2004; Gollackner et al., 2003; Pennington et al., 1986). Total-body irradiation was used by Pennington et al. (1986), who administered 900 rads (9 Gy) of irradiation for bone marrow transplant and Dor et al. (2004) and Gollackner et al. (2003), who administered 100 cGy for adjunct immunosuppression in organ transplantation. Thymic irradiation (700 cGy) was also used by the latter two authors for the same purpose.

Radiation fibrosis has been studied extensively in the pig (Douglas et al., 1985; Hopewell et al., 1994; Lefaix et al., 1993, 1996; Martin et al., 1993; Rezvani et al., 2000; Sabatier et al., 1992; van den Aardweg et al., 1990). Fibrosis of the skin, subcutaneous tissue, and muscle model is readily induced in swine with different sources of radiation usually applied focally in dosages ranging from 4 to 340 Gy. Epithelial, microvascular, vascular, and muscular changes have been demonstrated in

addition to alterations in biochemical, collagen, myoglobin, and other morphological changes. This particular model seems to be one in which swine can replace other large animal species in development of treatments.

Pulmonary fibrosis can be induced in swine with 6–40 Gy (ED_{50} 21–26 Gy) either as homogeneous sequential dosages or, in some cases, single dosage to produce the syndrome that occurs in humans (Baumann et al., 2000; Kasper et al., 1993, 1994; Takahashi et al., 1995). The model has been used for both treatment and diagnostic development. The syndrome is characterized by consolidation, thickening of bronchioalveolar tissues, thickened septum, edema, hemorrhage, and inflammation.

Radiation-induced nephropathy has also been studied in the pig (Robbins et al., 1989, 1991a,b, 1993; Zimmerman et al., 1995). Selective kidney damage, including arteritis, necrosis, scarring, and calcification, along with alterations in glomerular filtration rate, blood flow, and hematocrit can be induced with a wide range of localized dosages of radiation 3–100 Gy. The ED_{50} in mature pigs is approximately 11 and 8 Gy for immature pigs.

Central nervous system paralysis following radiation injury has also been studied (van den Aardweg et al., 1994, 1995). In sexually mature farm pigs, the ED_{50} dose was 27–28 Gy when delivered as a single dose to varying lengths of the cervical spinal cord. Paralysis occurred in 7–16 weeks postexposure. White matter necrosis leading to neuropathy was histologically detectable. The syndrome had a rapid onset of less than 48 h to paralysis, when the first clinical signs were noticed. Immature pigs less than 23 weeks old had spontaneous recovery.

Pigs have also been studied for the effects of radiation enteritis due to both ingestion and administration of radiation doses (Moroni et al., 2014a; Scanff et al., 1999). Increasingly, pigs are being utilized in the evaluation of radiation therapies and safety evaluation in a variety of organs and systems (Antoch et al., 2004; Baumann et al., 2000; Fajardo et al., 2002; Hom et al., 2005; Hopewell et al., 2000; Li et al., 2005; Radfar and Sirois, 2003; Stepinac et al., 2005; Yan et al., 2009). It is likely that the use of pigs in radiobiology-related research will increase in the future as the pig becomes recognized as a replacement for canine models in many of these areas (Moroni et al., 2011).

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17 Imaging Techniques with CT, MRI, PET, and SPECT

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CONTENTS

TYPES OF SCANNERS

This chapter and the attached DVD describe four different imaging techniques: computerized tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission CT (SPECT). Depending on the imaging methods and application, the data acquired can be interpreted to yield information not only about anatomy and structures, but also of many physiological processes such as glucose metabolism, oxygen utilization, blood volume, and receptor binding.

A CT scanner is a type of X-ray machine that sends several beams simultaneously from various angles. CT imaging is based on the attenuation of an X-ray beam as it passes through the body. This method permits reconstruction of a three-dimensional image of the internal body structures, since different tissue types can be distinguished based on a higher absorption of X-rays in tissues of a higher density. CT can image different types of tissues and organs, such as bones and blood vessels, with good detail and contrast, and the imaging of soft tissues can be improved by the use of contrast agents. Through its high spatial resolution and moderate differentiation of tissue contrast, it is a fast and exceptionally useful technique for visualizing general anatomy and diseases.

MRI, like CT, provides detailed images with millimeter spatial resolution. MRI involves the use of a magnetic field that forces hydrogen cellular nuclei to align in different positions in relation to the magnetic field. A radiofrequency pulse specific to hydrogen atoms is applied. When the magnetic field is removed and the atoms move back into place, they transmit radio waves. The MRI scanner analyzes these signals and produces an image. MRI has a much greater soft tissue contrast than CT, making it especially useful in neurological imaging. The MRI signal derived from blood depends on the level of oxygenation and is therefore based on blood oxygen level dependent (BOLD) contrast. Using this technique, it is possible to get functional images of the cerebral blood flow and oxygen consumption using MRI.

PET is a molecular imaging modality for measurements of the distribution of an intravenously injected positron-emitting tracer. The PET scanner consists of detector rings that detect the two parallel photons that are emitted when a positron annihilates upon contact with an electron.

SPECT, on the other hand, utilizes isotopes which emit gamma rays directly, and the gamma rays are detected by a SPECT scanner with energy windows optimized for SPECT isotopes. For both PET and SPECT, this allows reconstruction of three-dimensional images of the tracer concentration within the body as function of time. From these dynamic PET and SPECT data, the metabolism of the target organ can be modeled. It is also possible to image receptors or transporters for calculation of their density and affinity. Specific PET tracers can be synthesized for the study of specific metabolic processes by radiolabeling naturally occurring substances (e.g., ^{11}C-glucose), analogues thereof (e.g., ^{18}F-deoxy-2-glucose [FDG]), pharmaceuticals (e.g., ^{11}C-raclopride), etc. PET isotopes must generally be produced on-site, as their radioactive half-lives are short, limiting the availability of PET imaging to research centers with an on-site cyclotron. Compared to other imaging modalities, PET has a lower spatial resolution (2–4 mm). Although an increasing number of PET examinations are conducted every year, SPECT and other gamma camera examinations are by far more common, and the most utilized isotope is ^{99m}Tc. ^{99m}Tc is a generator-produced isotope and is considerably less expensive to use compared with the cyclotron-processed isotopes. Gamma camera examinations are widespread and used worldwide for many different purposes, for example, bone and bone marrow scans, myocardial perfusion imaging as well as infection, brain, kidney, lung, thyroid and liver scans. Today PET scanners are available as combined PET/CT scanners, and SPECT as combined SPECT/CT. This allows the production of fused images combining anatomy (CT) and function (PET and SPECT). Recently, combined PET/MRI scanners have also emerged.

Scanners are expensive and require space and a dedicated staff; consequently, human clinical scanners are often used for porcine studies at research sites instead of purchasing research-only scanners. This requires the scanners to be well cleaned and sanitized after animal scanning. Urine, dander, and zoonotic organisms are potential sources of contamination. Female swine are often used because it is possible to catheterize their bladders, which helps prevent urinary contamination. Bladder catheters cannot be placed in male swine, but instead diapers can be used to prevent urinary contamination. Anesthetized swine may be covered with blankets and plastic sheets to prevent contamination of the scanner and the scanner room. Due to the risk of offensive smells, swine should be cleaned prior to scanning and studies should be performed on days when imaging of patients is not planned, or after the clinical imaging. The institutional veterinarian should monitor protocols for sanitization and usage in order to prevent infections and allergies.

ANESTHESIA

Many types of anesthetics can be used to induce and maintain anesthesia in swine during scanning procedures. A complete discussion of anesthesia techniques is provided in Chapter 2. Where short-term anesthesia can be used for CT and brief MRI scans, long-term anesthesia is needed in most studies, including functional MRI, PET, and SPECT scans. Stable physiological function is essential during these procedures. Good sedation and muscle relaxation are important to prevent movement artifacts in the images. This is especially important for dynamic SPECT and PET scans where a radiolabeled tracer *in vivo* is observed over time for up to several hours. Bolus injections of neuromuscular-blocking agents are sometimes needed to prevent spontaneous respiration (e.g., after 2–4 h of isoflurane anesthesia); however, proper care must be taken to ensure the animal is deeply anesthetized before these agents are used. The physiological function being studied should not be compromised by the anesthesia protocol. This is especially important in functional brain studies. Studies have shown that binding potentials of PET tracers can differ twofold between commonly used anesthetics.

In many cases, isoflurane or propofol anesthesia can fulfill most of the requirements for the proper induction of long-term anesthesia in a scanning setting. Infusion protocols, as discussed in Chapter 2, may also be utilized to provide prolonged anesthesia. As an example, the PET Center at Aarhus University Hospitals in Denmark uses the following anesthesia procedure for PET studies in 40-kg domestic swine:

1. Premedication with 250 mg s-ketamine + 50 mg midazolam intramuscularly
2. Insertion of ear vein catheter (Venflon 20 or 22G)
3. Induction with 125 mg s-ketamine + 50 mg midazolam i.v.)
4. Intubation with a size 7.0 endotracheal tube
5. Maintenance of anesthesia with inhalation of 2% isoflurane in oxygen and N_2O (1:2) or oxygen and air (1:2.2), or infusion with 40 mL/h i.v. of a mixture containing 30 mL propofol (10 mg/mL), 10 mL s-ketamine (25 mg/mL), and 10 mL midazolam (5 mg/mL)
6. Bladder catheterization and saline-administered i.v.

Many of the i.v. infusion protocols discussed in Chapter 2 may also be useful for long-term anesthesia. s-Ketamine (ketamine) is the pure right-racemic isomer of ketamine and is used in order to decrease the dosage required by approximately 50% in the anesthesia protocol previously described. Either nonmagnetic anesthesia equipment must be utilized for MRI studies or the equipment must be shielded adequately from the magnetic field.

SURGERY

In most cases in PET studies, i.v. tracer injections are required (rarely i.p. or inhalation), and often i.v. contrast injections are also needed for CT and MRI scans. Venous catheters are also used for infusion of anesthetics, test drugs, and saline. It is possible to use the ear vein for tracer and contrast injection in swine, but often a surgically placed central venous catheter is preferred. During dynamic PET studies, arterial blood sampling may be necessary for image calculations (e.g., receptor studies and pharmacokinetics), and the blood volume of swine makes it possible to collect repeated blood samples. However, in survival studies, a maximum of 10% of the total blood volume can be sampled during a 3-week period. Arterial catheters are also used for monitoring blood gases and blood pressure. Catheters are often placed in the femoral artery and vein because both catheters can be placed at the same time. This site is ideal for liver and brain studies, because of the long distance from the vessel access to the target organs. However, the jugular vein and carotid artery may also be useful. During surgery and scanning procedures, the swine are placed on a heating blanket to prevent hypothermia. The vascular access sites and the surgical procedures are detailed in Chapters 1 and 9.

MONITORING

During functional scans, such as PET and SPECT, stable physiological conditions are required. Also, from an animal welfare point of view, the animal must be monitored to ensure that it is adequately anesthetized. In our laboratory, the following parameters are monitored during most PET and PET/CT scans: continuous blood pressure, heart rate, rectal temperature, oxygen saturation, blood gases every hour, blood glucose, and reflexes (interdigital, corneal, and palpebral). The monitoring of blood gases is often important during functional scans (PET, SPECT, and BOLD MRI) because the blood flow is primarily affected by the partial CO_2 concentration, secondary to the blood pressure. Special equipment is needed during MRI scans because metal will interact with the scanner. Alternatively, the equipment can be placed outside the scanner room and connected to the swine by long tubes. For MRI scans of the head or brain, the metal spring in the cuff of the tracheal tube must be removed prior to the scan. Even therapeutic iron injections or implants can be problematic. CT scanners are less sensitive to iron and other metals. In PET and SPECT studies, these substances are only a minor problem, and no special equipment is needed.

SCANNING PROCEDURES

CT scans can be performed very rapidly, in as short a time as 1 min, MRI scans require a few minutes to 1 h, and functional MRI scans often take up to 1 h. Dynamic PET and SPECT studies

frequently take many hours. PET/CT and SPECT/CT scanners require a low dose CT scan for photon attenuation correction of the emission recordings. Depending on the tracer used, the emission scans take from a few minutes to several hours. Tracers based on ^{15}O (e.g., ^{15}O-H_2O for blood flow measurements and ^{15}O-CO for blood volume measurements) take less than 10 min and ^{13}N (e.g., ^{13}N-NH_3 for blood flow measurements) take approximately 30 min. Tracers using ^{11}C-labels (e.g., ^{11}C-raclopride for dopamine $D_{2/3}$ receptor measurements) take 60–90 min and ^{18}F (e.g., ^{18}F-FDG for glucose uptake) may take up to several hours. We normally require 200–1000 MBq PET tracer for 40 kg Landrace swine.

CONCLUSION

The use of CT, MRI, PET, and SPECT imaging methods in swine may bridge the gap between preclinical and clinical research. Protocols used in preclinical research can be modified and used for clinical research in humans. Furthermore, swine have organ sizes similar to human organs, allowing the use of human equipment such as surgical instruments, catheters and medical devices.

A disadvantage of the large body weight of adult swine is the strain on the staff when handling the animals, which may increase the risk of back injuries. The staff should therefore be trained in the handling of large animals prior to the work. Also, the use of minipigs, which maintain a low body weight in adulthood, may be considered as an alternative animal subject to domestic swine.

IMAGES OF THE VARIOUS PROCEDURES

Images of pigs using the procedures described in this chapter are included in the DVD attached to this book. You will have to download a free copy of microdicom (http://www.microdicom.com/) to your computer to view some of the images.

18 Ossabaw Island Miniature Swine
Metabolic Syndrome and Cardiovascular Assessment

Michael Sturek, Johnathan D. Tune, and Mouhamad Alloosh

CONTENTS

INTRODUCTION

Ossabaw swine were deposited on Ossabaw Island, GA, in the 1500s by Spanish explorers (Mayer and Brisbin, 1991) and, since then, the ocean has remained an impenetrable barrier to emigration of Ossabaw pigs to the mainland. Natural models of disease that arise from adaptation of animals to unique selection pressures can give insights into similar complex, multifactorial diseases in humans. Ossabaw miniature swine may recapitulate the natural pathogenesis of type 2 diabetes because of their "thrifty genotype" that enabled survival in the feast and famine ecology of Ossabaw Island. The thrifty genotype hypothesis is that in the hunter–gatherer stages of human development the ability to store excess fat enabled survival during periods of famine (Neel, 1962).

In the 1970s and early 1980s, Ossabaw miniature swine were studied by ecologists for their unique adaptations in their natural habitat on Ossabaw Island (Mayer and Brisbin, 1991; Stribling et al., 1984) and, after establishment of colonies on the mainland, Ossabaws were studied by animal scientists for their propensity to obesity (Buhlinger et al., 1978; Martin and Herbein, 1976; Martin et al., 1973; Weiss et al., 1974), insulin resistance (Wangsness et al., 1977), plasma lipoproteins (Etherton and Kris-Etherton, 1980), and renal physiology (Zervanos et al., 1983).

Renewed interest in Ossabaw miniature swine was sparked in 2001 with the realization of the obesity and diabetes epidemic (Bellinger et al., 2006; Mokdad et al., 2001) and Brisbin's timely appeal to the scientific community to save feral Ossabaw Island swine from eradication by the Georgia Department of Natural Resources for environmental reasons (Brisbin and Mayer, 2001). Our laboratory obtained animals from the island in an expedition in 2002 and established a breeding colony at Indiana University. We have conducted studies involving obesity, metabolic syndrome, and diabetes and have made comparisons to the Yucatan miniature swine model (Boullion et al., 2003; Dixon et al., 1999, 2002; Edwards et al., 2006; Hainsworth et al., 2002; Kaser et al., 2004; Lloyd et al., 2006; Mokelke et al., 2003, 2005a,b; Sheehy et al., 2006; Witczak and Sturek, 2004; Witczak et al., 2005; Zafar et al., 2004).

The natural pathogenesis of type 2 diabetes involves a tendency to obesity with gradually increasing impairment of insulin action in a "prediabetes" condition, which has also been termed the

metabolic syndrome or *cardiometabolic risk* (Eckel et al., 2005, 2006; Grundy et al., 2005; Kahn et al., 2005). In later stages, there is a significant increase in fasting blood glucose, which defines diabetes. Intensive research is under way to meet the need for animal models to understand these comorbidities and develop therapies. The metabolic syndrome Ossabaw pig could be an outstanding large animal model. Strategies involving transgenic manipulations in other species and other breeds of minipigs are under development.

The metabolic syndrome in humans is actually a cluster of risk factors that includes: (1) central (intra-abdominal) obesity, (2) insulin resistance, (3) impaired glucose tolerance, (4) dyslipidemia as measured by decreased plasma high-density lipoprotein (HDL) cholesterol compared to low-density lipoprotein (LDL), that is, increased LDL/HDL ratio, (5) dyslipidemia as shown by increased plasma triglyceride, and (6) hypertension (Eckel et al., 2005; Ford et al., 2004; Grundy et al., 2004; McGill, Jr. et al., 2002). Although the definition and precise clinical utility have recently been controversial (Kahn et al., 2005), generally the presence of three of these characteristics renders a diagnosis of the cardiometabolic syndrome (Eckel et al., 2006; Grundy et al., 2004).

CHARACTERIZATION OF THE MODEL

The most critical question regarding the use of Ossabaw miniature swine was: Did removals from Ossabaw Island in 2002 have the thrifty genotype characteristics, that is, "metabolic syndrome," found in the early characterization during ~1970–1985? Data collected to study these characteristics definitively confirm the early data, thus providing a rationale for more cardiovascular characterization, which was not performed in the early studies. The attached DVD includes experimental methods and images of metabolic syndrome (e.g., glucose tolerance) and cardiovascular characterization. The main criterion for the use of the Ossabaw miniature swine is the natural occurrence of metabolic syndrome and progression to type 2 diabetes with concomitant cardiovascular disease, which is unique to the Ossabaw. We further emphasize and highlight that the miniature size (~30 kg) of the Ossabaw at sexual maturity provides another advantage for husbandry and for study of sex differences in metabolic syndrome, type 2 diabetes, cardiovascular disease, etc. Although outstanding work shows that a line of crossbred domestic pigs with familial hypercholesterolemia will develop MetS (Bellinger et al., 2006), use of the standard-sized domestic swine is not practical because they weigh >250 kg and are 2 years of age before metabolic syndrome occurs. Thus, in addition to the metabolic characteristics of Ossabaw miniature swine, the relatively small stature (30–80 kg) of Ossabaw pigs is essential (Dyson et al., 2006; Edwards et al., 2008; Langohr et al., 2008; Lloyd et al., 2008; Mattern et al., 2007).

Table 18.1 compares the major features of metabolic syndrome (items 1–6) present (Yes) or not (No) in Yucatan vs. Ossabaw miniature swine and their utility as cardiovascular disease models (item 7). The Yucatan is our comparison because of our extensive, ~25 years of work with this genetically leaner pig that is the predominantly used miniature swine for laboratory research; however, almost any other pig has characteristics of the Yucatan pig. We provide mainly the summary message here and leave detailed descriptions of our methods and experimental designs to the literature citations and the figures in the attached DVD. Ossabaws clearly show greater propensity to obesity than Yucatans and direct measures show a greater accumulation of visceral fat on rigorously controlled experimental diets. Despite intensive efforts to induce insulin resistance and glucose intolerance in Yucatan swine on high fat, high cholesterol, and high sucrose diets, we were not able to reproduce the findings of Phillips and colleagues in the early 1980s (Panepinto et al., 1982; Phillips et al., 1982a,b). We emphasize that currently available lines of Yucatans do not naturally develop obesity-associated insulin resistance (Otis et al., 2003). In contrast, Ossabaw swine fed a high calorie diet display a natural pathogenesis of all metabolic syndrome characteristics. Other miniature swine breeds currently available for laboratory animal medicine, for example, Yucatan and Göttingen, also do not progress to type 2 diabetes (e.g., Larsen et al., 2007; Otis et al., 2003; Phillips et al., 1982a). Göttingen pigs (Larsen et al., 2002, 2005, 2006, 2007), however, will develop mild

TABLE 18.1
Comparison of Metabolic Syndrome (Items 1–6) in Yucatan and Ossabaw Miniature Swine and Utility as Cardiovascular Disease Model (Item 7)

Characteristic	Yucatan	Ossabaw	Reference
1. Obesity	No	Oss > Yuc	Bell et al. (2010), Bender et al. (2009), Berwick et al. (2012, 2013), Bonin et al. (2012), Boullion et al. (2003), Bratz et al. (2008), Clark et al. (2011), Dincer (2011), Dyson et al. (2006), Edwards et al. (2010), Elmadhun et al. (2014a, 2013), Faris et al. (2012), Flum et al. (2007), Hamamdzic and Wilensk (2013), Handa et al. (2014a,b, 2015), Hanhineva et al. (2013), Kreutz et al. (2011), Lassaletta et al. (2012), Lee et al. (2009), Li et al. (2012, 2011), McKenney et al. (2014), Moberly et al. (2013), Neeb et al. (2010), Newell-Fugate et al. (2014), Owen et al. (2013), Padilla et al. (2013), Payne et al. (2010), Pedersen et al. (2012), Rodgaard et al. (2013), Sabe et al. (2014a), Sham et al. (2014), Talbott et al. (2006), Toedebusch et al. (2014), Trasino et al. (2013), Trask et al. (2012), Wastney et al. (2013), Witczak et al. (2005), Zhang et al. (2013)
2. Insulin resistance	No	Yes	Bell et al. (2010), Dincer (2011), Dyson et al. (2006), Edwards et al. (2010), Elmadhun et al. (2013), Faris et al. (2012), Fullenkamp et al. (2011), Habegger et al. (2012), Handa et al. (2014a,b, 2015), Kreutz et al. (2011), Lassaletta et al. (2012), Lee et al. (2009), Li et al. (2012, 2011), McKenney et al. (2014), Neeb et al. (2010), Newell-Fugate et al. (2014), Otis et al. (2003), Padilla et al. (2013), Pedersen et al. (2012), Potu et al. (2013), Sham et al. (2014), Trask et al. (2012), Witczak and Sture (2004), Zhang et al. (2013)
3. Glucose intolerance (or impaired glucose tolerance, [IGT])	No	Yes	Bell et al. (2010), Bender et al. (2009), Berwick et al. (2013), Boullion et al. (2003), Bratz et al. (2008), Dincer (2011), Dixon et al. (2002), Dyson et al. (2006), Edwards et al. (2010), Elmadhun et al. (2014a), Faris et al. (2012), Fullenkamp et al. (2011), Handa et al. (2014a,b, 2015), Hanhineva et al. (2013), Kreutz et al. (2011), Lassaletta et al. (2012), Lee et al. (2009), Li et al. (2012), McKenney et al. (2014), Mokelke et al. (2003), Neeb et al. (2010), Newell-Fugate et al. (2014), Otis et al. (2003), Payne et al. (2010), Potu et al. (2013), Sabe et al. (2014a), Sham et al. (2014), Trask et al. (2012), Witczak et al. (2005, 2006), Witczak and Sture (2004)
4. Dyslipidemia (↑LDL/HDL)	Yes	Yes	Bell et al. (2010), Berwick et al. (2012, 2013), Bratz et al. (2008), Clark et al. (2011), Dixon et al. (2002), Dyson et al. (2006), Edwards et al. (2010), Fullenkamp et al. (2011), Hanhineva et al. (2013), Kreutz et al. (2011), Lassaletta et al. (2012), Lee et al. (2003, 2009), Li et al. (2011), Long et al. (2010a,b), McKenney et al. (2014), Moberly et al. (2013), Neeb et al. (2010), Owen et al. (2013), Padilla et al. (2013), Potu et al. (2013), Rector et al. (2003), Trasino et al. (2013), Trask et al. (2012), Witczak et al. (2006), Zhang et al. (2013)
5. Dyslipidemia (↑triglycerides)	No	Yes	Bell et al. (2010), Bratz et al. (2008), Clark et al. (2011), Dincer (2011), Dixon et al. (2002), Dyson et al. (2006), Edwards et al. (2010), Fullenkamp et al. (2011), Handa et al. (2014a), Hill et al. (2003), Kreutz et al. (2011), Lassaletta et al. (2012), Lee et al. (2009), Long et al. (2010a), McKenney et al. (2014), Moberly et al. (2013), Mokelke et al. (2003), Newell-Fugate et al. (2014), Payne et al. (2010), Potu et al. (2013), Rector et al. (2003), Trask et al. (2012), Witczak et al. (2005), Witczak and Sture (2004), Zhang et al. (2013)

(*Continued*)

TABLE 18.1 (*Continued*)
Comparison of Metabolic Syndrome (Items 1–6) in Yucatan and Ossabaw Miniature Swine and Utility as Cardiovascular Disease Model (Item 7)

Characteristic	Yucatan	Ossabaw	Reference
6. Hypertension	No	Yes	Berwick et al. (2012, 2013), Bratz et al. (2008), Dincer (2011), Dyson et al. (2006), Edwards et al. (2010), Elmadhun et al. (2014a), Faris et al. (2012), Handa et al. (2014b), Kreutz et al. (2011), Lassaletta et al. (2012), Lee et al. (2009), McKenney et al. (2014), Moberly et al. (2013), Otis et al. (2003), Payne et al. (2010), Trask et al. (2012), Zhang et al. (2013)
7. Cardiovascular disease, atherosclerosis	Yes	Yes	Bender et al. (2009), Borbouse et al. (2009, 2010a,b), Bratz et al. (2008), Chen et al. (2011), Dincer (2011), Dixon et al. (2002), Dyson et al. (2006), Edwards et al. (2008, 2010), Hainsworth et al. (2002), Korte et al. (2005), Langohr et al. (2008), Le et al. (2007), Lee et al. (2003), Lloyd et al. (2008), Long et al. (2010a), Mokelke et al. (2003, 2005), Neeb et al. (2010), Payne et al. (2010), Sturek (2011), Turk et al. (2003), Wamhoff et al. (2002), Wang et al. (2008, 2009, 2013a), Ziegler et al. (2010), Berwick et al. (2012, 2013), Elmadhun et al. (2014a,b, 2012), Hamamdzic and Wilensk (2013), Handa et al. (2014b, 2015), Huo et al. (2013), Kreutz et al. (2011), Lassaletta et al. (2012), Li et al. (2012, 2011), Long et al. (2010b), McKenney et al. (2014, 2015), Moberly et al. (2013), Owen et al. (2013), Paderi et al. (2011), Sabe et al. (2014a,b), Scott et al. (2013), Spence and Weave (2013), Trask et al. (2012), Wang et al. (2011a,b, 2012, 2013b), Wastney et al. (2013), Zhang et al. (2013)

metabolic syndrome. Published data and those on the DVD show that Ossabaw pigs with metabolic syndrome have vascular calcification and extreme coronary atheroma (Table 18.1, item 7), thus it is feasible to stent natural atherosclerotic lesions, not balloon-injured healthy arteries (e.g., Edwards et al., 2008; Gal and Isner, 1992; Johnson et al., 1999; Lowe et al., 2003; Schwartz and Edelman, 2002; Touchard and Schwartz, 2006).

A very important message about Ossabaw miniature swine is the nearly ideal opportunity for achieving integration and translation to human clinical medicine. The need for a large animal, that is, swine, model of the metabolic syndrome, instead of rodent models, was reinforced poignantly by the European Union-funded RETHINK project addressing the need for large animal research (Dolgin, 2010) and other position statements and initiatives to increase large animal research (Arner, 2005; Schwartz Longacre et al., 2011; Sipido et al., 2009). Despite the significant advances in mouse models that have been facilitated by the Animal Models for Diabetic Cardiovascular Complications (AMDCC) (Hsueh et al., 2007), it is our opinion and others (Seok et al., 2013) that the use of animal models in translational research requires large, more human-like animal models such as swine to be complete.

Genetic studies were performed on a repeating domain in the regulatory γ3 subunit of the AMP-activated kinase (PRKAG3) gene. Hampshire pigs display a single amino acid difference at position 200 where arginine is mutated to glutamine (Arg200 → Gln). This gain-of-function genotype is associated with high muscle glycogen, low intramuscular fat, and overall leanness (Andersson, 2003; Milan et al., 2000). In contrast, sequencing of the PRKAG3 gene in Ossabaw Island pigs revealed the majority to be homozygous for a different mutation, Val199 → Ile, while the remainder of the pigs were heterozygous for the Val199 → Ile mutation and the wild-type allele Val199–Arg200 (Lloyd et al., 2006). The Val199 → Ile mutation is associated with impairment of AMP kinase enzyme activity, low muscle glycogen, and increased intramuscular fat, consistent with the obese Ossabaw pig phenotype (Andersson, 2003; Ciobanu et al., 2001). Selective breeding created

a distinct line of Ossabaw pigs that is homozygous for the wild-type AMP kinase allele (Chawla et al., 2012). Consistent with the pivotal role of AMP kinase in energy balance, the homozygous wild-type AMP kinase line tolerates myocardial ischemia substantially better than the homozygous Val199 → Ile mutation Ossabaw with impaired AMP kinase activity (Chawla et al., 2012).

Tables A.32 through A.34 in the appendix make systematic comparison of serum chemistry data from trapped pigs on Ossabaw Island and data derived from Yucatan and Ossabaw pigs housed long term (1 year) in a biomedical research facility on standard pig chow. Pigs were anesthetized with isoflurane to obtain blood samples for the latter group compared to the caval blood sampling that employed physical restraint for the clinical chemistry from trapped Ossabaw. For both breeds housed in captivity in the biomedical facility, the anion gap, potassium, total bilirubin, creatine kinase, and CO_2 are normal compared to the trapped wild pigs, thus probably indicating a less stressful environment overall and less stressful blood sampling procedure. Notable differences are the increased triglycerides and glucose in Ossabaws compared to Yucatans, which reinforce the more extreme cardiometabolic risk factor profile of the Ossabaws. Another intriguing difference is the increased creatinine in Ossabaws, which suggests some mild renal impairment even under these controlled conditions. The decreased urea nitrogen is consistent with less muscle mass in the Ossabaw (Ezekwe and Martin, 1975; Hausman et al., 1983; Kasser et al., 1981) and argues against the increased creatinine being driven by possibly increased muscle mass in the Ossabaws. It is completely unknown whether the increased ability of the Ossabaw kidney to concentrate urine (Zervanos et al., 1983) for adaptation to high salt consumption renders it more susceptible to subsequent damage. Overall, there was no difference in hematology between Ossabaw and Yucatan pigs. The only striking value in both breeds is the low hematocrit of 27. This is explained entirely by the isoflurane anesthesia, as Ossabaws sampled in the conscious state had a hematocrit of 41.8 ± 2.6 (SD; $N = 5$), similar to conscious Yucatans in other studies.

SUMMARY AND CONCLUSIONS

Ossabaw swine were rediscovered as a valuable animal resource after the removal, in 2002, of feral swine from Ossabaw Island, following the first removal in the 1970s. Ossabaw swine removed from the island must undergo a stringent quarantine to ensure health and absence of parasites and major infectious diseases. The pigs have thrived in captivity. The "thrifty genotype" has been maintained in captivity for almost 13 years and the analogy to modern day humans suggests that the genotype will continue to be maintained. Clearly, Ossabaw swine express the major components of the metabolic syndrome ("prediabetes," "cardiometabolic risk"), including extreme obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, and hypertension. Selective breeding has derived more general robust features of metabolic syndrome and distinct lines of the AMP kinase genotypes. This chapter has provided an overview of the evidence for metabolic properties and methods of assessment and has contrasted the Ossabaw with the characteristics of more genetically lean Yucatan swine and domestic swine. Vascular studies provide detailed methods and characterization of vascular anatomy and functional properties. Unique vascular calcification and excessive stenosis after coronary stenting suggest predisposition to vascular disease and utility of the Ossabaw swine model. Exercise training of obese pigs is described. These findings echo the consensus that pigs in general and Ossabaw swine specifically are anatomically and metabolically similar to humans. The cardiovascular system is almost indistinguishable from that of humans and knowledge gained has much relevance to human medicine. Thus, the Ossabaw pig provides a unique animal resource to gain insight into multiple, complex factors involved in development of obesity, metabolic syndrome (pre-diabetes), and type 2 diabetes in humans and the resulting morbidity and mortality from cardiovascular disease. It is hoped that the future will see more widespread availability and use of Ossabaw swine in biomedical research.

Note: The attached DVD contains descriptions of methods, images, and video clips of various aspects of the study of the metabolic syndrome in Ossabaw Island minipigs (Figures 18.1 through

18.28). Complete figure legends are included with the images. The outlined titles as follows provide a guide to the illustrations on the DVD.

FIGURE 18.1 Ossabaw Island pig and environment.

FIGURE 18.2 Standard Ossabaw swine housing facility for biomedical research.

FIGURE 18.3 Growth and adipose composition of lean and obese male Ossabaw pigs housed in biomedical research facility.

FIGURE 18.4 Noninvasive imaging of adipose distribution in Ossabaw swine.

FIGURE 18.5 Measurement of glucose regulation and cardiovascular parameters in conscious pig.

FIGURE 18.6 Catheterization supplies and angiography equipment.

FIGURE 18.7 Hind limb arteries, ventrodorsal view. Includes video clips 18.1 through 18.6.

FIGURE 18.8 Hind limb, superficial femoral artery access for catheterization, and formation of collateral femoral arteries in ventrodorsal view. Includes video clips 18.7 and 18.8.

FIGURE 18.9 Renal arteries in ventrodorsal view. Includes video clips 18.9 and 18.10.

FIGURE 18.10 Major abdominal arteries in ventrodorsal view. Includes video clips 18.11 and 18.12.

FIGURE 18.11 Forelimb and thoracic arteries in ventrodorsal view. Includes video clips 18.13 and 18.14.

FIGURE 18.12 Left carotid and cerebral arteries in ventrodorsal view. Includes video clips 18.15 and 18.16.

FIGURE 18.13 Schematic of heart and major epicardial coronary arteries and interventional devices.

FIGURE 18.14 Heart and right coronary artery in ventral views. Includes video clips 18.17 through 18.20.

FIGURE 18.15 Heart and left coronary arteries in ventral views. Includes video clips 18.21 through 18.26.

FIGURE 18.16 Ventricular angiography and echocardiography. Includes video clip 18.27.

FIGURE 18.17 Two-dimensional and M-mode echocardiograms. Includes video clip 18.28.

FIGURE 18.18 Intravascular ultrasound (IVUS) of coronary arteries. Includes video clips 18.29 through 18.32.

FIGURE 18.19 Transcutaneous femoral artery ultrasound. Includes video clip 18.33.

FIGURE 18.20 Coronary stent deployment in left anterior (ventral) descending coronary artery. Includes video clips 18.34 through 18.38.

FIGURE 18.21 Poststent stenosis of left anterior (ventral) descending coronary artery 4 weeks after stent deployment. Includes video clips 18.39 and 18.40.

FIGURE 18.22 Coronary blood flow. Includes video clip 18.41.

FIGURE 18.23 Angiograms obtained 4 weeks after placement of ameroid occluder on circumflex coronary artery. Includes video clips 18.42 and 18.43.

FIGURE 18.24 Superimposed positron emission tomography (PET) and computed tomography (CT) images.

FIGURE 18.25 Positron emission tomography (PET) measurement of regional myocardial blood flow.

FIGURE 18.26 Coronary conduit artery histology.

FIGURE 18.27 Treadmill exercise protocol for obese Ossabaw pig before and after femoral artery ligation and coronary stent placement. Includes video clip 18.44.

FIGURE 18.28 Recordings of aortic pressure and coronary blood flow obtained from a conscious pig during rest and treadmill exercise.

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19 Swine in Cancer Research

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CONTENTS

The early and effective translation of diagnostic and therapeutic modalities from bench to bedside is one of the major challenges in cancer research. Cancer remains one of the biggest killers in the United States while the FDA approval rate of new anticancer drugs from the time of first-in-human trials remains one of the lowest compared with the FDA approval rates of drugs for other diseases (Kola and Landis, 2004). The complexity and heterogeneity of cancer contribute to this situation, as do the limitations of the current animal models.

Most of the animal models widely used in cancer research are xenograft mouse models, but results in these models have limited translation to human patients. It has been shown that most rodent models of cancer, except those that recapitulate the genetic signature of native malignancy, lack predictive value for assessment of treatment efficacy in man (Kamb, 2005). Another limitation is that many mouse xenograft tumors do not metastasize or show very limited metastasis (Cespedes et al., 2006), whereas most human tumors have metastatic potential and the majority of cancer deaths occur from metastasis. It has also been shown that the genetic events necessary for tumorigenesis in mice differ from those in humans (Lim and Counter, 2004).

Swine models of cancer offer unique advantages for translational research. Pigs—because of their ability to breed quickly, variety of body sizes, and similarity to humans in anatomy as well as physiology—are a promising laboratory model that offers easier translation of complex novel diagnostic and therapeutic strategies for cancer. Pigs are already the preferred model for developing many cancer therapeutic techniques, including diagnostic imaging, interventional therapy, and surgical techniques. Recent advances in the genetic manipulation of pigs further strengthen the possibility that swine models will play an important role in cancer research.

This chapter reviews tumor models in swine, including naturally occurring tumors with a focus on cancer incidence and inherited tumor models, such as swine melanoma, and experimental tumor models in swine, with a focus on chemically induced tumor models such as hepatocellular carcinoma (HCC), tumor transplant models, and transgenic tumor models. Current trends that hold promise for significant growth in the use of swine models in cancer are also explored.

NATURALLY OCCURRING TUMOR MODELS IN SWINE

Cancer Incidence in Swine

Prior to 2000, the reported incidence of naturally occurring cancers in swine was low. Those reports led to the incorrect assumption that pigs have a much lower incidence of spontaneous cancers than

do many other animals or humans. It should be realized that domestic pigs, which are mainly used as food animals, are slaughtered at less than 1 year of age, much earlier than the comparative ages at which the incidence of cancer increases significantly in other species, including humans. Prior to 1970, most reports of spontaneous cancers in swine were from surveys and retrospective studies from abattoirs, swine production operations, or agricultural diagnostic services and were thus incidental findings drawn primarily from a population of young (<2 years old) pigs intended for food production. (Anderson and Jarrett, 1968; Anderson and Sandison, 1968a, 1969a,b; Anderson et al., 1969; Monlux et al., 1956; Plummer, 1956; Sandison and Anderson, 1968a,b, 1969). Congenital tumors are rare in pigs, except for the occurrence of congenital papillomatosis of the tongue in pigs in China (Misdorp, 2003; Xiong, 1992). Fisher and Olander (1978) reported a 0.2% incidence of cancer in swine specimens submitted to a laboratory over an 11-year period. This incidence rate is the same as that of cancer in humans younger than 20 years old (Jemal et al., 2013). While Fisher and Olander reported that the majority of tumors found in pigs were lymphosarcomas and melanomas, these researchers also reported a wide variety of tumor types including granulocytic sarcoma, hemangiosarcoma, hemangioma, papilloma, embryonal nephroma, lipoma, chondroma, and leiomyoma (Fisher and Olander, 1978). These findings suggest that the rates of naturally occurring cancers in swine may more closely resemble those in humans than previously thought.

The predominant spontaneous cancers in young swine are reported to be lymphoma, nephroblastoma, and melanoma. In humans, lymphoma also is one of the most common neoplasms reported in children, and the incidence of nephroblastoma (aka, embryonal nephroma, Wilms tumor) is much higher in children than in adults. Lymphomas, the most frequently reported swine tumors from abattoir surveys, typically occur with no breed predilection in young pigs of either sex but can also occur in mature pigs. Most lymphomas in swine are multicentric; thymic lymphoma is the second most common type. Infiltration of the liver, kidney, and spleen commonly occurs. Most lymphomas in swine are classified as lymphocytic, but lymphoblastic, histiocytic, and mixed types have been reported (Laber et al., 2002).

Congenital and hereditary tumors in pigs show some similarities to tumors in humans. Nephroblastoma is the second most common tumor and the most common primary renal neoplasm in pigs, which usually develops in fetal life (Laber et al., 2002). Embryonal nephromas occur in pigs less than 1 year old, occur more frequently in males than females, and typically are unilateral and arise in the renal parenchyma (Meuten, 2002). Many similarities of this swine tumor to nephroblastoma or Wilms tumor in children have been reported (Payton et al., 1988), and similarities in molecular changes have been suggested (Ghanem et al., 2001; Kanemoto et al., 2003). However, embryonal nephroma in swine differs from Wilms tumor in the swine tumor's lack of aggressiveness, predominant epithelial origin, underdeveloped neoplastic glomeruli, and lack of glomerular basal membrane. Unlike similar tumors in dogs and cats, most nephroblastomas in swine are asymptomatic and do not metastasize except for rare spread to regional lymph nodes, liver, and lungs. No evidence of a hereditary or familial nature has been found in swine nephroblastoma. Attempts to transplant cultured swine nephroblastoma tumor cells to hamster cheek pouches and to neonatal pigs have not been successful (Pirtle et al., 1970).

Pigs have a high incidence of melanocytic tumors—both melanocytomas and malignant melanomas. A high percentage (85%–90%) of these naturally occurring tumors regress spontaneously, but the remaining tumors are malignant, show progression, and metastasize. Melanoma models have been established by selective breeding of pigs and are discussed in detail elsewhere in this chapter.

Sporadic cases of other cancers have been reported in young swine. A single report of time-limited occurrences of skin tumors in multiple neonatal pigs, which was similar to rhabdomyosarcoma in young children, was made in a study from the Netherlands (Vos et al., 1993). The possibility of an inherited factor in swine rhabdomyosarcoma was suggested but not proven in another study (van der Loop et al., 1995). Very few swine tumors are classified as carcinomas. It was previously reported that tumors of epithelial origin are rare in pigs (Ramsay and Migaki, 1975). This finding may be related to the fact that most necropsy specimens are from young pigs while most carcinomas appear in older animals. A high incidence of congenital papillomatosis of the tongue was reported in young pigs in China (Xiong, 1992).

Surveys taken from older breeder animals suggest that pigs follow the normal pattern of increasing cancer occurrence and tumor spectrum with age. In a survey of 460 sows, 18 (3.9%) had ovarian hemangiomas, with the highest incidence occurring in sows 5–8 years old. Because pathological features of these tumors were similar to those in humans, the tumors were thought to be a useful model for ovarian hemangioma in women (Hsu, 1983). A similar survey of older sows with uterine tumors showed that the predominant tumor type reported was uterine leiomyoma; other types were nodular fibromyoma, endometrial polyp, and embryonic sarcoma.

The increasing number of reports of cancers in aging pet pigs provides further evidence that cancer incidence rates in pigs are comparable with those of other domestic and companion animals and humans. The popularity of Vietnamese potbellied pigs as companion pet animals in the early 1990s led to rapid increases in the population of this miniature breed. As pets, these animals were allowed to live out their natural life span and were more routinely given veterinary care. Several case reports and retrospective studies of cancers in this population began appearing in the literature from 2000 to 2012, as these pigs reached 10–20 years of age. Results of a University of Tennessee 10-year retrospective study of pet potbellied pigs demonstrated a 35% incidence (22 of 63 animals) of neoplasia. Of the 63 animals, 28 (44%) had metastatic disease. The average age of the pigs at diagnosis was 11.3 years. The predominant cancers were hepatic and intestinal carcinomas. Other cancers included multiple myeloma involving vertebral bodies, mast cell tumor, nasal adenocarcinoma, and nasal squamous cell carcinoma (Newman and Rohrbach, 2012). An endemic form of nasal adenocarcinoma has been reported in pigs from Brazil, China, and Ghana (Wilson and Dungworth, 2002). Squamous cell carcinoma in swine was first described by Kleinschmidt et al. (2006), who reported a 10-year-old potbellied pig with metastatic lesions in the retropharyngeal lymph nodes and lungs. A uterine adenocarcinoma was also reported in this pig, but the relationship of this tumor to the squamous cell carcinoma could not be determined. A comprehensive list of reports of naturally occurring tumors in swine is given in Tables 19.1 through 19.16.

TABLE 19.1
Tumors of the Alimentary System in Swine

Location	Tumor Type	Cases	References	Comments
Gastrointestinal tract	Lymphoma		Lombard and Granier (1959)	As cited by Bostock and Owen (1973)
Digestive system	Hemangiosarcoma	1	Brandly and Migaki (1963)	
Stomach	Carcinoma	1	Anderson et al. (1969)	
Intestine	Adenocarcinoma	6	Vitovec (1977a)	
Stomach (pylorus)	Leiomyoma	1	Fisher and Olander (1978)	
Oral	Ameloblastoma and papilloma	2	Fisher and Olander (1978)	
Tongue	Papillomatosis		Xiong and Chen (1992)	As cited by Misdorp (2003)
Ilium	Lymphoma	11	Tanimoto et al. (1994)	
Oral cavity	Squamous cell carcinoma	1	Kleinschmidt et al. (2006)	
Stomach, small intestine, spiral colon	Transmural gastric carcinoma, small intestinal adenocarcinoma, metastatic hepatocellular carcinoma, and carcinoma	3	McCoy et al. (2009)	
Oral cavity	Squamous cell carcinoma	1	Swenson et al. (2009)	
Small intestine	Adenocarcinoma and T-cell lymphoma	2	Corapi et al. (2011)	
Small intestine	Ganglioneuroma	1	Murakami et al. (2011)	
Spiral colon	Colonic carcinoma	2	Newman and Rohrbach (2012)	

TABLE 19.2
Tumors of the Circulatory System in Swine

Location	Tumor Type	Cases	Reference
Circulatory system		2	Misdorp (1967)
Heart	Rhabdomyomas		Kast and Hanichen (1968)
Heart	Rhabdomyoma	1	Omar (1969)
Heart	Rhabdomyoma		Szazados et al. (1973)
Heart	Rhabdomyoma	2	Bradley et al. (1980)
Heart	Rhabdomyoma		McEwen (1994)

TABLE 19.3
Tumors of the Endocrine System in Swine

Location	Tumor Type	Cases	Reference	Comments
Mammary	Adenocarcinoma	1	Feldman (1926)	As cited by Cotchin (1957)
Adrenal	Carcinoma	1	Davis et al. (1933)	
Adrenal	Cortical tumor	1	Brandly and Migaki (1963)	
Adrenal	Adenoma of cortex and pheochromocytoma	2	Anderson et al. (1969)	
Pancreas	Islet cell carcinoma	1	Anderson et al. (1969)	
Adrenal	Unknown	2	Brown and Johnson (1970)	
Mammary	Unknown	1	Vitovec (1977c)	
Mammary	Carcinoma	1	Musonda et al. (1990)	Metastasis to lung
Adrenal	Malignant pheochromocytoma	1	Martinez et al. (2012)	
Mammary	Adenoma	1	Newman and Rohrbach (2012)	

TABLE 19.4
Experimentally Induced Tumor Models In Swine

Location	Tumor Type	Reference	Comments
Systemic	Osteosarcoma, leukemia, lymphoma	Howard and Clarke (1970), Howard et al. (1968a–d, 1969)	90Sr-induced neoplasias
Attempted xenograft to hamster cheek pouches	Porcine embryonal nephroma	Pirtle et al. (1970)	Unsuccessful
Stomach	Unknown	Stavrou (1976)	Chemically induced
Colon	Unsuccessful	Wargovich (1991)	Unsuccessful
Liver	Human hepatocellular carcinoma	Rai (2005)	Unsuccessful
Liver	Hepatocellular carcinoma	Li et al. (2006)	Chemical induction (*N*-nitrosodiethylamine)
Subcutis (not orthotopic)	Sarcoma (transformed fibroblasts)	Adam et al. (2007)	Genetically defined tumors
Systemic	Myelogenous leukemia	Cho et al. (2007), Duran-Struuk et al. (2010)	Transplantable tumors from major histocompatibility complex (MHC) swine
Mammary	Transgenic mammary cancer	Luo et al. (2011)	Transgenic BRCA1 knockout
Subcutis (not orthotopic)	Human xenograft	Basel et al. (2012)	Naturally occurring immunodeficient pig
Colon	Familial adenomatous polyposis p53 mutation	Flisikowska et al. (2012) Leuchs et al. (2012)	Precursor to colon cancer Transgenic
Immune system	Il2rg mutation	Suzuki et al. (2012)	Transgenic SCID pigs

TABLE 19.5
Tumors of the Hepatobilliary System in Swine

Location	Tumor Type	Cases	Reference	Comments
Liver	Lymphoblastoma		Bowler (1948)	As cited by Bostock and Owen (1973)
Liver	Hepatocellular carcinoma and fibrosarcoma	2	Plummer (1956)	Hepatocellular carcinoma had metastasized to lungs.
Liver	Teratoma	1	Neemann (1959)	As cited by Misdorp (2003)
Liver	Hepatocellular carcinoma	7	Brandly and Migaki (1963)	
Gallbladder	Carcinoma	1	Anderson and Sandison (1968b)	
Liver	Liver cell tumor (4), hemangioendothelioma (1), lymphosarcoma (42), other secondary tumors (4)	51	Anderson and Sandison (1968b)	
Liver	Hepatocellular carcinoma	4	Anderson et al. (1969)	
Liver	Hepatoma	1	Brown and Johnson (1970)	
Liver	Hepatocellular carcinoma	1	Ramachandran et al. (1970)	
Liver	Hepatocellular carcinoma	5	Vitovec (1977c)	
Liver	Hepatocellular carcinoma and bile duct adenoma	2	Bastianello (1983)	
Liver	Primary hepatic neoplasia	1	Bundza et al. (1984)	
Liver	Hepatocholangioadenoma	1	Ohfuji et al. (1992)	
Liver	Hemangioma	1	Tanimoto and Ohtsuki (1992)	
Liver	Hepatocellular adenoma	124	Kashima et al. (1995)	As cited by Radkowski et al. (2010)
Liver	Hepatocellular carcinoma	1	Morrow (2002)	Metastases to omentum and spleen
Gallbladder	Cholangiocellular carcinoma	1	McCoy et al. (2009)	
Liver	Hepatocellular carcinoma	1	McCoy et al. (2009)	Metastasis
Liver	Hepatocellular carcinoma	22	Haddad (2012)	
Gallbladder	Cholangiocellular carcinoma	1	McCoy et al. (2009)	
Liver	Hepatocellular carcinoma	5	Newman and Rohrbach (2012)	

Inherited Tumor Models: Swine Melanoma

Melanoma is one of the few cancers for which the incidence rate has been on the rise worldwide for the past 30 years (MacKie et al., 2009). Unlike most other cancers, melanoma's incidence is high in young people, increasing the potential relevance of an animal model that allows long-term follow-up after therapy. Existing animal models of melanoma include Xiphophorus fish, rodents, the South American opossum (Monodelphis), and swine. Swine models of melanoma have important advantages over other animal models; these advantages include spontaneous occurrence, high incidence in early life, and similarity to human melanoma in origin of precursor melanocytes and tumor histology and biology. The larger body size of swine compared with the other model systems allows the use of interventional therapies in a clinical setting, while the longer life span allows long-term follow-up. In addition, the close similarity of swine skin to human skin allows better translation of diagnostic and therapeutic strategies for melanoma. These skin similarities include the ultrastructure of the epidermal–dermal junction, enzyme pattern of the epidermis, epidermal tissue turnover time, keratinous proteins, and thickness of the epidermis (Vincent-Naulleau et al., 2004). These features make swine melanoma models clinically relevant for studying host–tumor cell interactions throughout all stages of the disease: premalignant, malignant transformation, metastasis, and regression.

Surveys of naturally occurring lesions in agricultural animals describe a low occurrence rate of melanotic lesions in swine (Davis et al., 1933; Monlux et al., 1956; Nordby, 1933). However, a high

TABLE 19.6
Tumors of the Integumentary System in Swine

Location	Tumor Type	Cases	Reference	Comments
Unknown	Melanoblastoma	3	Feldman (1926)	As cited by Feldman (1928)
Unknown	Melanoma and fibroma	2	Davis et al. (1933)	
Unknown	Melanoma	8	Nordby (1933)	
Orbit	Squamous cell carcinoma	1	Plummer (1956)	Metastasis to parotid gland and lung
Skin	Melanoma	3	Monlux et al. (1956)	
Skin	Spindle cell carcinoma, fibroma, melanoma	3	Cotchin (1960)	
Skin	Malignant melanoma	1	Case (1964)	Extensive metastasis
Skin	Malignant melanoma		Hjerpe and Theilen (1964)	Littermates affected
Skin	Melanoma (5), adenoma (1), fibrosarcoma (1)	7	Brandly and Migaki (1963)	
Skin	Schwannoma	1	Misdorp (1967)	
Skin	Melanoma	21	Strafuss et al. (1968)	
Skin	Mastocytoma	6	Migaki (1969), Migaki and Langheinrich (1970)	
Skin	Malignant melanoma	20	Flatt et al. (1972)	
Skin	Malignant melanoma	3	Manning et al. (1974)	Extensive metastasis
Unknown	Malignant melanoma	1	Thirloway et al. (1977)	
Skin, subcutis	Unknown	3	Vitovec (1977c)	
Skin	Melanoma (7), hemangioma (2), hemangiosarcoma (1), papilloma (1)	11	Fisher and Olander (1978)	
Skin, head, and neck	Papillomatosis	1	Rieke (1980)	As cited by Misdorp (2003)
	Malignant melanoma	2	Baba et al. (1983)	
Skin	Squamous cell carcinoma	5	Bastianello (1983)	
Skin	Angioma	1	Prakash et al. (1983)	
Skin, ear	Fibroleiomyoma	1	Nakamura et al. (1987)	
Skin, lymph nodes	Melanotic lesions	217	Bundza (1990)	
Skin	Plexiform schwannoma	1	Tanimoto and Ohtsuki (1993)	
Subcutis	Leiomyofibrosarcoma		Whyte (1996)	As cited by Misdorp (2003)
Skin, head, and back	Fibropapillomatosis	1	Vitovec et al. (1999)	
Subcutaneous	Fibrosarcoma	1	Jeong et al. (2003)	
Subcutis	Neurofibrosarcoma	1	Misdorp (2003)	
Skin	Mastocytoma	1	Martinez et al. (2011)	
Skin, head, and back	Fibropapillomatosis	1	Nishiyama et al. (2011)	No evidence of papillomavirus infection
Skin	Mast cell tumor, squamous cell carcinoma	2	Newman and Rohrbach (2012)	

incidence of melanomas were reported in a strain of Hormel miniature pigs, known as Sinclair pigs, that was developed at the University of Missouri's Sinclair Comparative Medicine Research Farm. After finding that 21% of the progeny had melanotic tumors, selective inbreeding was successfully performed to increase tumor incidence (Millikan et al., 1974). Additional melanoma models have been developed by the selective breeding of swine breeds with noted low natural incidences of congenital melanomas—these include the Hormel and Sinclair pigs, Munich miniature swine Troll

TABLE 19.7
Lymphosarcoma in Swine

Location	Tumor Type	Cases	Reference	Comments
Unknown	Lymphosarcoma		Bostock and Owen (1973)	As cited by Bostock and Owen (1973)
Unknown	Lymphosarcoma	2	Feldman (1926)	As cited by Feldman (1928)
Unknown	Lymphosarcoma		Manegold and Machens (1927)	As cited by Bostock and Owen (1973)
Multicentric	Lymphocytoma	1	Davis et al. (1933)	
Unknown	Lymphoblastoma	1	Kernkamp (1945)	As cited by Bostock and Owen (1973)
Multicentric	Follicular lymphoma (1), lymphocytoma (4), lyphoblastoma (4)	9	Monlux et al. (1956)	
Multicentric	Lymphosarcoma	13	Plummer (1956)	
Unknown	Lymphoblastoma		Domizio (1959)	As cited by Bostock and Owen (1973)
Multicentric and thymic	Lymphosarcoma	15	Cotchin (1960)	
Unknown	Lymphosarcoma	178	Englert and Krüger (1965)	As cited by Bostock and Owen (1973)
Multicentric and thymus	Malignant lymphoma (67) thymoma (1)	68	Brandly and Migaki (1963)	
Unknown	Lymphosarcoma	45	Renier (1966)	As cited by Bostock and Owen (1973)
Multicentric	Lymphatic leucosis	11	Misdorp (1967)	
Multicentric or thymus	Malignant lymphoma (200), thymoma (5)	205	Migaki (1969)	
Thymus and multicentric	Lymphosarcoma (92), follicular lymphoma (1)	94	Anderson et al. (1969)	
Multicentric	Lymphosarcoma	1	Stevenson (1973)	
Multicentric	Lymphosarcoma	53	Head et al. (1974)	
Hematopoietic tissue	Lymphoid	71	Vitovec (1977c)	
Multifocal and thymus	Lymphosarcoma	8	Fisher and Olander (1978)	
Unknown	Malignant nodular lymphosarcoma	1	Seno et al. (1980)	
Multicentric	Lymphosarcoma	11	Bastianello (1983)	
Unknown	Lymphoid neoplasms	2	Saito (1982)	As cited by Ogihara et al. (2012)
Lymph nodes, Thymus	Lymphoma, T-cell lymphoma	7	Kadota and Niibori (1985), Kadota et al. (1986, 1987, 1990), Kadota (1987), Kadota and Nakajima (1988)	
Multicentric	Lymphoma	1	Skavlen et al. (1986)	
Unknown	Lymphoid and myeloid neoplasms	78	Marcato (1987)	
Unknown	Malignant lymphomas	36	Hayashi et al. (1988)	
Unknown	Malignant lymphoma	14	Nakajima et al. (1989)	
Multicentric	Lymphoma	16	Tanimoto et al. (1994), Tanimoto and Ohtsuki (1998)	
Multicentric	Lymphosarcoma	1	Vo et al. (2004)	
Multicentric	Lymphoma	2	Hejazi and Danyluk (2005)	
Unknown	B-cell lymphoblastic leukemia	1	Rafferty et al. (2007)	
Multicentric	B-cell lymphoma	1	Rocha et al. (2011)	
Unknown	Lymphoid neoplasia	17	Ogihara et al. (2012)	

TABLE 19.8
Miscellaneous Tumors in Swine

Location	Tumor Type	Cases	References	Comments
Unknown	Myxoma	1	Feldman (1926)	As cited by Feldman (1928)
Pericardium	Fibrosarcoma	1	Davis et al. (1933)	
Region of the patella	Fibroma	1	Monlux et al. (1956)	
Abdomen	Ganglioneuroma	1	Monlux et al. (1956)	
Musculoskeletal tissues	Fibrosarcoma	1	Brandly and Migaki (1963)	
Spleen	Hemangioma	2	Brandly and Migaki (1963)	
Diffuse	Undifferentiated carcinoma	4	Brandly and Migaki (1963)	
Systemic	Carcinomatosis	1	Lyhs (1967)	
Miscellaneous sites	Unknown	6	Misdorp (1967)	
Connective tissues	Fibrblastic (2), hemangioendothelioma (1), capillary hemangioma (1)	4	Anderson et al. (1969)	
Multifocal	Carcinoma (6) and sarcoma (1)	7	Vitovec (1977c)	Undetermined primary site
Mesentery	Lipoma	1	Fisher and Olander (1978)	
Multifocal	Hemangioma	1	Wells and Morgan (1980)	
Spleen	Malignant fibrous histocytoma	1	Tanimoto et al. (1988)	
Intracranial	Ossifying lipoma	1	Turnquist and Miller (1993)	
Multifocal	Rhabdomyosarcoma	11	Vos et al. (1993)	Likely genetic
Spleen	Hamartoblastoma	1	Kadota et al. (1994)	
Pleura	Mycroblastoma sarcoma	1	Kubota et al. (2000)	
Abdominal cavity	Peripheral neuroblastoma	1	Diessler et al. (2002)	
Perineal (3), mesentery (1)	Leiomyoma	4	Newman and Rohrbach (2012)	

(MMS), and melanoblastoma-bearing Libechov minipig (MeLiM)—resulting in herds that have a high incidence of melanoma by 3 months of age (Vincent-Naulleau et al., 2004). Selective breeding in the Sinclair and Munich Troll breeds has resulted in herds that have 50% incidence of melanomas at birth, with 85% developing melanomas by 1 year of age.

MeLiM and Sinclair pigs have common ancestry in the Hormel strain. There is no sex predilection for melanoma occurrence in these breeds, but a higher tumor incidence occurs in pigs with black hair coats than in red-coated pigs. Two gene loci are involved in the Sinclair swine model of melanoma, and similar genetic involvement was reported in MeLiM models. One involved genotype is the B haplotype located within the swine leukocyte antigen (SLA) complex; animals that are homozygous for the B haplotype have an increased incidence of melanoma. The second genotype is unlinked to SLA and appears to be related to the retinoblastoma (*Rb*) locus in humans. Deletion or inactivation of this allele increases susceptibility to melanoma. This trait is not sex linked, although oophorectomy (but not castration) has been shown to reduce tumor incidence in animals with the trait (Carson III and Walker, 2002).

Following early mapping studies that indicated that at least these two loci were involved in melanoma predisposition (described above), genome-wide mapping done in the MeLiM model revealed that predisposition to melanoma was an autosomal dominant trait with incomplete penetrance and that candidate melanoma genes were located in numerous loci in five distinct chromosomal regions. Further, it was demonstrated that known risk susceptibility genes in humans, including *CDK4* and *BRAF*, were not involved in melanoma development in this model (Geffrotin et al., 2004). Quantitative trait loci mapping of a genome-wide scan of melanoma-bearing MeLiM swine showed that the MeLiM *MC1R*2* allele, which determines black coat color in pigs, predisposes pigs

TABLE 19.9
Myelogenous, Unspecified and Miscellaneous Lymphoid Neoplasms in Swine

Location (if Known)	Tumor Type (if Known)	Cases	Reference	Comments
Unknown	Unknown		Hodgson (1903)	As cited by Bostock and Owen (1973)
Unknown	Lymphoid leukemia	1	Biester and McNutt (1926)	As cited by Bostock and Owen (1973)
Unknown	Unknown		Bostock and Owen (1973)	As cited by Bostock and Owen (1973)
Unknown	Chronic granulomatous disease (resembled Hodgkin's lymphoma)	35	Forbus and David (1946)	As cited by Migaki (1969)
Unknown	Unknown		Bostock and Owen (1973)	As cited by Bostock and Owen (1973)
Unknown	Unknown		Pyke (1955)	As cited by Bostock and Owen (1973)
Unknown	Unknown		Salomon (1955)	As cited by Bostock and Owen (1973)
Unknown	Giant cell sarcoma, reticulum cell sarcoma	2	Plummer (1956)	
Unknown	Unknown		Bostock and Owen (1973)	As cited by Bostock and Owen (1973)
Unknown	Unknown		Bostock and Owen (1973)	As cited by Bostock and Owen (1973)
Unknown	Malignant myeloma (1) chronic granulomatous disease (38)	39	Brandly and Migaki (1963)	
Unknown	Lipid reticulosis	1	Anderson et al. (1969)	
Unknown	Granulocytic sarcoma	2	Migaki (1969)	
Unknown	Unknown		Wittman (1969)	As cited by Bostock and Owen (1973)
Unknown	Unknown		Marcato and Andreucci (1971)	As cited by Bostock and Owen (1973)
Hematopoietic tissue	Myeloid	5	Vitovec (1977c)	
Multicentric	Granulocytic	1	Fisher and Olander (1978)	
Unknown	Myeloid leukosis	1	Allsup et al. (1981)	
Unknown	Myeloid leukemia (eosinophilic)	1	Kashima et al. (1982)	
Leukemia	Leukemia	1	Bastianello (1983)	
Leukemia	Myeloid	1	Kadota et al. (1984)	
Unknown	Myeloid leukemia	7	Kadota (1987), Kadota et al. (1987)	
Systemic	Mast cell leukemia	1	Bean-Knudsen et al. (1989)	
Unknown	Myelogenous leukemia	18	Duran-Struuk et al. (2010)	In MHC swine, potential leukemia model
Meninges and bone	Multiple myeloma	1	Rintisch et al. (2010)	
Unknown	Mast cell leukemia	1	Sipos et al. (2010)	
Multicentric	Eosinophilic granulocytic sarcoma	1	Brum et al. (2012)	

to melanoma (Du et al., 2007). Gene expression analysis via DNA microarray was performed in melanoma-bearing and nonmelanoma-bearing Sinclair pigs during tumor development, metastasis, and regression. Differential expressions were found for genes known to be involved in human melanoma pathogenesis, including *SILV, TYR*, and *RAB28*. The *SILV* gene encodes melanocyte protein PMEL17. The *TYR* gene encodes the tyrosinase protein that catalyzes multiple steps in melanin biosynthesis in melanocytes and melanoma cells. The analysis also found increased expression

TABLE 19.10
Tumors of the Nervous system in Swine

Location	Tumor Type	Cases	References	Comments
Nervous tissue	Benign tumors	3	Brandly and Migaki (1963)	
Spine, intradural	Lipoma	1	Johnson and Brown (1969)	
Meninges	Hemangioma	1	Fisher and Olander (1978)	
Brain	Glioblastoma	1	Fisher and Olander (1978)	
Brain	Astrocytoma	1	Ziemer et al. (1985)	
Frontal leptomeninges	Multiple myeloma	1	Rintisch et al. (2010)	Also sternum and pelvis
CNS	B-cell lymphoma	1	Rocha et al. (2011)	Also in abdomen, head, and nose
Meninges	Hemangiosarcoma	1	Spitzbarth et al. (2011)	

TABLE 19.11
Tumors of the Female Reproductive System in Swine—Ovary

Location	Tumor Type	Cases	Reference	Comments
Ovary	Cavernous hemangioma	1	Davis et al. (1933)	
Ovary	Hemangiosarcoma	2	Laszio (1940)	As cited by Teige and Karlberg (1984)
Ovary	Cystadenoma (10), sarcoma (5), teratoma (2), fibroma (1)	18	Dobberstein (1953)	As cited by Nelson et al. (1967)
Ovary	Seminoma	1	Lombard (1962)	As cited by Nelson et al. (1967)
Ovary	Granulosa cell tumors	2	Brandly and Migaki (1963)	
Ovary	Hemangiosarcoma		Jubb (1963)	As cited by Nelson et al. (1967)
Ovary	Cystadenocarcinoma	1	Smith (1966)	As cited by Nelson et al. (1967)
Ovary	Hemangioma (2) papillary cystadenoma (1) granulosa cell tumor (1)	4	Nelson et al. (1967)	
Ovary	Carcinoma (2), granulosa-cell tumor (1)	3	Anderson et al. (1969)	
Ovary	Hemangiosarcoma	2	Maeda (1973)	As cited by Teige and Karlberg (1984)
Ovary	Carcinoma, granulosa cell tumor, leiomyoma, fibroma	5	Vitovec (1977c)	
Ovary	Hemangiosarcoma	1	Yamagata (1977)	As cited by Teige and Karlberg (1984)
Ovary	Granulosa cell tumor and lymphosarcoma	2	Bastianello (1983)	
Ovary	Hemangioma	18	Hsu (1983)	
Ovary	Carcinoma (1), granulosa cell tumor (2)	3	Teige and Karlberg (1984)	
Ovary	Malignant luteoma	1	Hashimoto et al. (1989)	Metastatic lesions in the diaphragmatic peritoneum and heart
Ovary	Malignant luteoma	1	Hashimoto et al. (1989)	Metastatic lesions in the diaphragmatic peritoneum and heart
Ovary	Cystadenocarcinoma	1	Leder et al. (1990)	
Ovary	Anginoma	69	Kashima (1995)	As cited by Radowski et al. (2010)
Ovary	Leiomyoma	1	Baumwart et al. (2010)	

TABLE 19.12
Tumors of the Male Reproductive System in Swine

Location	Tumor Type	Cases	Reference	Comments
Scrotum	Hemangioma	1	Szczech et al. (1973)	
Testicle		1	Vitovec (1977c)	
Penis	Papilloma	1	Fisher and Olander (1978)	
Testicle	Hemangiosarcoma	1	Fisher and Olander (1978)	
Scrotum	Hemangioma		Wells and Morgan (1980)	As cited by Sheikh-Omar and Jaafar (1985)
Scrotum	Hemangioma		Munro and Munro (1982)	
Testicle	Intratubular germ cell tumor	3	Wekerle et al. (1987)	
Testicle	Mixed Sertoli and Leydig cell tumor	1	Mabara et al. (1990)	
Testicle	Leydig cell tumor	1	Weaver et al. (2000)	
Scrotum	Hemangioma	12	Teankum et al. (2008)	Three boars had concurrent testicular tumors (see below)
Testicle	Hemangioma (1); hemangioma with intratubular germ cell tumor (1); mixed hemangioma, intratubular germ cell-like tumor and Sertoli cell tumor (1)	3	Teankum et al. (2008)	Concurrent with scrotal hemangiomas
Testicle	Interstitial cell tumor and Leydig cell tumor	2	Newman and Rohrbach (2012)	

of genes associated with cellular transcription in melanoma cells, including the proto-oncogenes c-Fos and c-jun. Antiapoptotic genes were overexpressed, including genes known to be important in other cancers, such as *BCL2*. Continued characterization of the swine model of melanoma may help elucidate candidate genes and molecular markers for development as novel therapeutic targets or biomarkers in humans (Okomo-Adhiambo et al., 2012).

Progressive and regressive melanomas have similar clinical and histologic features in Sinclair and MeLiM pigs (Okomo-Adhiambo et al., 2012). Melanomas develop throughout life, starting *in utero*; a proportion of these become malignant. Cutaneous malignant melanomas may have multiple primary sites, are frequently large, exophytic, and may become ulcerated. Up to 25% of these tumors metastasize to lymph nodes and distant organs. Despite this, morbidity remains low because most primary and many metastatic lesions undergo regression and loss of pigmentation. *In vitro*, malignant melanoma cells (which are of neural crest origin) exhibit similar characteristics to childhood tumors of neural crest origin in humans, such as retinoblastoma or neuroblastoma (Okomo-Adhiambo et al., 2012).

Melanoma development occurs in five distinct stages in swine. Stage I lesions are flat black macules with single or nests of melanocytes characterized by heavy pigmentation. State II lesions are raised, pigmented nodules with melanocytes that invade the superficial dermis. Stage III lesions are highly proliferative, become exophytic, and frequently ulcerate. The melanocytes exhibit cellular atypia with frequent mitotic figures and have an epithelioid or spindle-shaped appearance. These neoplastic cells are invasive—infiltrating the deep dermis—and may metastasize. Stage III tumors, which are similar to melanomas in humans both histologically and ultrastructurally, are 70% consistent with the human melanoma classifications of acral lentiginous melanoma, superficial spreading melanoma, or nodular melanoma. In the Sinclair swine model, 89% of the melanoma lesions have been reported to be deep and invasive, corresponding to Clark levels IV and V in human disease, with frequent invasion of blood vessels and nerves (Das Gupta et al., 1989). Stage IV is the first stage of regression. The tumors become smooth and bluish and may have a depigmented halo. Pigment-laden macrophages infiltrate the tumor. Stage V is the final stage of regression. Malignant

TABLE 19.13
Tumors of the Female Reproductive System in Swine—Uterus, Cervix, and Vagina

Location	Tumor Type	Cases	Reference	Comments
Uterus	Sub-serous nodular fibromyoma	1	Boucek (1906)	As cited by Cotchin (1964)
Uterus	Leiomyoma	1	Genest and Trepanier (1952)	As cited by Cotchin (1964)
Uterus	Endometrial polyp	1	Gimbo (1955)	As cited by Cotchin (1964)
Vagina	Embryonic sarcoma	1	Monlux et al. (1956)	
Uterus	Leiomyoma (5), sarcoma (1)	6	Kronberger (1960)	As cited by Cotchin (1964)
Uterus	Leiomyosarcoma	1	Brandly and Migaki (1963)	
Broad ligament	Leiomyoma	1	Von Winterfeldt (1964)	
Uterus	Smooth muscle tumor, leiomyoma and adenomyoma	3	Anderson et al. (1969)	
Uterus	Apparent leiomyoma	6	Brwon and Johnson (1970)	
Uterus	Carcinoma	1	Werdin and Wold (1976)	
Uterus	Leiomyoma	5	Vitovec (1977c)	
Cervix, vagina	Fibroma	1	Akkermans (1984)	
Uterus	Leiomyoma (6), Fibroma (3), cystadenoma (1), fibroleiomyoma (1)	11	Akkermans (1984)	Of 1445 breeding animals
Uterus	Myoma	33	Kashima (1995)	As cited by Radowski et al. (2010)
Uterus	Carcinosarcoma	1	Bedenice et al. (2000)	
Uterus	Leiomyoma	2	Munday and Stedman (2002)	
Uterus	Adenocarcinoma	1	Harmon et al. (2004)	Metastasis to lymph nodes, liver and lung
Uterus	Leiomyoma (11), leiomyosarcoma (1), undifferentiated sarcoma (1)	17	Mozzachio et al. (2004)	Seventeen tumors identified but tissue only available for 13
Uterus	Adenocarcinoma	1	Cannon et al. (2009)	Metastasis to lungs and lymph nodes
Cervix	Leiomyoma	1	Seva et al. (2009)	
Uterus	Adenocarcinoma	1	Augustijn et al. (2010)	Also in cervix
Uterus	Adenocarcinoma	1	Golbar et al. (2010)	
Uterus	Leiomyoma (9), leiomyosarcoma (7), adenoma (8), adenocarcinoma (2)	20	Ilha et al. (2010)	
Uterus	Leiomyoma (1), Leiomyosarcoma (3)	4	Newman and Rohrbach (2012)	Some metastasis to lymph nodes

melanocytes disappear and are replaced with depigmentation and dermal fibrosis. The tumor becomes extensively infiltrated with macrophages and lymphocytes.

Oxenhandler et al. (1982) performed histopathologic analyses of 104 biopsy specimens collected from melanoma-bearing Sinclair pigs. Growth and regression were characterized by a series of cellular events culminating in regression, depigmentation, and scar formation. The melanomas in these pigs showed mononuclear cell infiltration with similar temporal and topographic distribution patterns as those seen in human melanoma (Oxenhandler et al., 1982). This pattern suggests that immune-mediated mechanisms play the primary role in tumor regression (Carson III and Walker, 2002). Additional evidence for the role of the immune system in tumor regression was demonstrated in studies showing that tumor-bearing pigs developed uveitis that correlated with the destruction of melanocytes in the fundus and iris (Lentz et al., 1983) and that swine with melanoma developed cell-mediated immune reactivity against tumor-associated antigens on melanoma cells (Hook et al., 1983). Other studies suggest that γdT cells may mediate the cytotoxic response. These studies have

TABLE 19.14
Tumors of the Respiratory System in Swine

Location	Tumor Type	Cases	Reference	Comments
Lung	Carcinoma, fibrosarcoma	2	Plummer (1956)	
Lung	Chondrosarcoma	1	Brandly and Migaki (1963)	
Lung	Lymphosarcoma (12), ovarian (1)	13	Anderson (1968)	All tumors are secondary
Nose and nasal cavity	Unknown	1	Vitovec (1977c)	
Trachea	Chondroma	1	Fisher and Olander (1978)	
Mucosa of the ethmoid	Carcinoma		Rajan et al. (1981)	Likely viral etiology
Lung	Mammary carcinoma	1	Musonda et al. (1990)	Metastasis form mammary gland
Nasal	Squamous cell carcinoma and nasal carcinoma	2	Newman and Rohrbach (2012)	

TABLE 19.15
Tumors of the Skeletal System in Swine

Location	Tumor Type	Cases	Reference	Comments
Mandible	Sarcoma	1	Cotchin (1960)	
Thoracic vertebrae (3), lumbar vertebrae (1)	Osteogenic sarcoma	4	Harcourt (1973)	
Bone	Lymphosarcoma	1	Owen (1974)	
Bone	Unknown	1	Vitovec (1977c)	
Oral cavity	Osteoma	1	Rosendal (1979)	
	Osteoblastic osteosarcoma	1	Seva et al. (2001)	
Mandible	Osteosarcoma	1	Williamson and Byrne (2006)	
Sternum and pelvis	Multiple myeloma	1	Rintisch et al. (2010)	Also in spinal cord
Vertebrae	Plasma cell tumor	2	Newman and Rohrbach (2012)	One with metastasis to liver, kidney, lung, and spinal cord

established the swine melanoma model as an especially good model in which to study mechanisms of spontaneous tumor regression (Carson III and Walker, 2002).

Despite numerous similarities between the two models, some differences exist between the Sinclair and MeLiM melanoma models. MeLiM pigs have a higher incidence of fast-growing Stage III lesions in young pigs than do Sinclair pigs. Despite this, overall mortality due to metastasis remains low, at 5%–10%, and is similar to mortality rates seen in the Sinclair MMS models (Vincent-Naulleau et al., 2004). One advantage of the MeLiM model is a high incidence (50%) of melanoma early in life. Also, lesions in MeLiM pigs have a similar histologic appearance (i.e., heavily pigmented) to human melanoma and similarities with three human melanoma types: superficial spreading melanoma, nodular melanoma, or unclassified melanomas. Melanomas in MeLiM swine show similar biologic behavior to human melanoma—with some parameters, such as early ulceration or deep dermal invasion, correlating with aggressive behavior—and similar patterns of metastatic spread as seen in humans, with most initial metastatic lesions seen in regional lymph nodes.

Some important differences exist between all swine melanoma models and human melanoma. In humans, complete regression of metastatic melanomas occurs occasionally in cases with regional metastasis to the lymph nodes, but complete regression is very rare in visceral or pulmonary

TABLE 19.16
Tumors of the Urinary System in Swine

Location	Tumor type	Cases	Reference	Comments
Kidney	Embryonal adenosarcoma	Unknown	Day (1907)	As cited by Davis et al. (1933)
Kidney	Embryonal nephroma	9	Feldman (1926)	As cited by Feldman (1928)
Kidney	Embryonal adenocarcinoma (7), embryonal adenosarcoma (4)	11	Feldman (1928)	
Kidney	Embryonal nephroma	15	Davis et al. (1933)	
Kidney	Embryonal nephroma	1	Keller (1933)	As cited by Sullivan and Anderson (1959)
Kidney	Embryonal nephroma	13	Monlux et al. (1956)	
Kidney	Embryonal nephroma (6), hypernephroma (1)	7	Plummer (1956)	
	Embryonal nephroma	44	Smith and Jones (1957)	As cited by Sullivan and Anderson (1959)
Kidney	Embryonal nephroma	229	Sullivan and Anderson (1959)	
Kidney	Embryonal nephroma	6	Cotchin (1960)	
Kidney	Adenocarcinoma	1	Sandison and Anderson (1968a)	
Kidney	Embryonal nephroma	43	Brandly and Migaki (1963)	
Urinary system	Embryonal nephroma (11), not described (1)	12	Misdorp (1967)	
Kidney	Nephroblastoma (13), Carcinoma (3)	16	Anderson et al. (1969)	
Kidney	Embryonal nephroma	205	Migaki et al. (1971)	
Kidney	Nephroblastoma (8), not described (1)	9	Vitovec (1977b)	
Kidney, renal pelvis	Carcinoma	1	Vitovec (1977b)	
Kidney	Embryonal nephroma	2	Fisher and Olander (1978)	
Kidney	Embryonal nephroma (2), lymphosarcoma (1)	3	Bastianello (1983)	
Kidney	Nephroblastoma	74	Hayashi et al. (1986)	
Kidney	Nephroma	15	Guarda (1995)	As cited by Radokowski et al. (2010)
Kidney	Nephroma	121	Kashima (1995)	As cited by Radokowski et al. (2010)
Kidney	Nephroblastoma	1	Newman and Rohrbach (2012)	

metastasis. However, swine melanoma has high incidence of complete regression, even in metastatic disease. In swine melanoma models, it is difficult to classify tumors or stage the disease because of the continuum seen from fully benign to malignant lesions and because of the occurrence of different types of tumors on same animal.

EXPERIMENTAL TUMOR MODELS IN SWINE

Chemically Induced Tumor Models: Hepatocellular Carcinoma

One of the better characterized swine tumor models is the HCC model. HCC is the most rapidly increasing cancer in the United States, with an average survival rate of less than 1 year. Most HCC

incidence is related to hepatic cirrhosis, which results from chronic liver damage as a result of infection, hepatotoxins, alcohol use, or other inflammatory liver diseases. More recently, metabolic syndrome and associated nonalcoholic steatohepatitis (NASH) have been reported as a potential cause of liver cancer. Due to the high similarity of the swine liver to the human liver at the anatomical, physiological, and cellular levels, swine models are among the most suitable large-animal models for HCC. A swine HCC model can provide an ideal platform for translational research in areas ranging from early diagnosis and treatment to long-term follow-up for recurrence and treatment-related complications.

There have been two studies of experimentally induced HCC in swine. In both cases, *N*-nitrosodiethylamine (DENA), which is a potent hepatocarcinogen, was used to induce the tumors. DENA has been widely used in different species to induce liver cancer and is the gold standard for such use in mouse models of HCC.

In 1977, Graw and Berg reported feeding Göttingen minipigs DENA at a dose of 0.4 mg/kg for 5 years, yielding swine liver tumors (Graw and Berg, 1977). The animals were evaluated at the end stage of cancer and demonstrated multiple stages of HCC as well as other types of liver neoplasia, including Kupffer cell sarcoma. Metastatic tumors were found in regional lymph nodes and lungs. Tumors were also found in kidneys and the brain of some of the pigs. Though the study established the possibility of DENA use in pigs to create HCC models, the tumors' origin, progression, and molecular similarities to the human disease were not well characterized. In addition, the long period required to generate the model did not fit to a typical research cycle.

More recently, Li and colleagues further refined and characterized the swine HCC model in China Taihu piglets (Li et al., 2006). In this study, 10 mg/kg of DENA was administered weekly for 3 months. Ten months after administration, early tumor development was detected. The tumors' radiological characteristics were similar to those of human HCC. The tumors were hypervascular, showed significant enhancement in arterial-phase scans, and demonstrated a blood supply mainly from the hepatic artery as in human HCC. Histochemical staining showed that the tumors were positive for alpha-fetoprotein and Hep-Par-1 expression as seen in human HCC. Although the authors mentioned the presence of cirrhosis in this model, any evidence of generalized, extensive cirrhosis seems to be lacking in the report.

The authors of this chapter (RKU and PTT) conducted a pilot study (under publication) on the possibility of further accelerating the swine model described by Li et al. Tumor-like lesions were observed in contrast-enhanced computed tomography images as early as 7 months after DENA was administered using a modified technique. Yucatan minipigs were used for the study. Early observations found potential differences between the cirrhotic lesions in the swine livers compared with HCC in humans. For example, the cirrhotic lesions were more nodular than the generalized, diffuse cirrhosis seen in other animal models and in human HCC.

The hepatic manifestation of metabolic syndrome is NASH, a progressive liver disease that might be a major etiological factor in HCC formation in developed countries (Hashimoto and Tokushige, 2012; White et al., 2012). HCCs arising from metabolic syndrome as the only risk factor have distinct morphological characteristics and may not cause fibrosis of the liver (Hashimoto and Tokushige, 2012). Multiple pig breeds reported to be effective animal models for metabolic syndrome, including Ossabaw minipigs (Lee et al., 2009), Yorkshire pigs (Elmadhun et al., 2012), and the Iberian pig (Torres-Rovira et al., 2012), which has leptin resistance. Leptin is currently linked to the origin of HCC in humans (Wang et al., 2010). The Ossabaw minipig demonstrated NASH (Lee et al., 2009) as part of the metabolic syndrome. *GLP1* transgenic swine have also been found to be a good model for metabolic syndrome, expanding the possibility of developing a very relevant pig model for metabolic syndrome/NASH-based HCC.

Many opportunities exist to continue refining and improving the DENA model to yield a faster, efficient HCC swine model. One weakness in the current swine DENA model is that HCC arises in a background devoid of many of the concurrent comorbidities commonly seen in the human disease, including hepatic inflammation, diffused and generalized fibrosis, and cirrhosis. One option for improving the model is to incorporate multiple background diseases that typically lead to HCC in

humans or other animal models. For example, Ossabaw pigs with NASH, transgenic pigs with oncogene interference (e.g., p53 knockout pigs), or leptin-resistant pigs could be used for DENA-based HCC model development. Promoters such as barbiturates are commonly used to accelerate DENA-based rodent HCC models, but they were not used in any reported pig DENA models. The direct induction of a cirrhotic background (Avritscher et al., 2011) in pigs, to simulate the effect of partial hepatectomy (part of the classic HCC model in rodents), providing a rapidly regenerating liver for more effective carcinogenesis by DENA may help to accelerate the model. In swine models, the technique will also provide a more extensive cirrhotic background as seen in human HCC patients.

Tumor Transplant Models

Multiple attempts to create implanted tumor models in pigs, with various levels of success, have been reported. Unlike rodents, in which various levels of immunocompromised animals are available for the implantation of viable xenografts, swine with a naturally occurring tolerance to xenografts or allografts are rare. To overcome the immune rejection of foreign tissues, Adam et al. (2007) tried to transform normal fibroblasts collected from a healthy pig to tumor cells by utilizing retroviruses to insert transgenes that result in the disruption of the p53, Rb, c-Myc, Ras, and telomerase pathways. The transformed cells were then implanted orthotopically to subcutaneous tissue behind each ear of the donor pig. With partial immunosuppression of the animal, these cells formed rapidly growing tumors that maintained transgene expression *in vivo*. Histopathology and immunohistochemical analysis identified these as poorly differentiated sarcomas with regions of cells expressing markers of epithelial origin. There was likely some innate immune response despite the partial immunosuppression, as evidenced by large numbers of inflammatory cells mixed with abundant dying tumor cells and dead tumor cell debris (Adam et al., 2007). The tumors were small relative to size of the animal and did not invade blood vessels or lymphatics. Although no further work has been reported on this model, the model may prove valuable in combination with other tumor models.

More recently, potential tumor allograft and xenograft models in swine have emerged. These models are mainly the result of the development of inbred lines of pigs as well as identification of a line of pigs that have a compromised immune system. One highly inbred line of pigs was reported to have multiple incidences of leukemia. The cancer cells were isolated, and cell lines were established (Cho et al., 2007). Tumor allograft models were produced later, using a cell line derived from an animal that had been diagnosed with chronic myeloid leukemia (Duran-Struuck et al., 2010). Successful growth of tumor cells injected intravenously and subcutaneously occurred after the administration of total body irradiation at a dose of 500 cGy to recipient pigs, while a dose of 300 cGy was sufficient for tumor growth following subcutaneous injection. Immunohistochemistry of the tumors was consistent with the phenotype of the cultured cells and a lack of cellular infiltrate, suggesting the host did not mount an immune response. While this study has demonstrated the ability to successfully transplant cell lines in histocompatible swine with total body irradiation preconditioning, there has been no success in achieving tumor growth without immunosuppression. More work is necessary to achieve tumor growth in host animals without any preconditioning or concurrent therapy.

Basel et al. reported the first successful human tumor xenograft model in swine (Basel et al., 2012). A line of Yorkshire pigs being bred for increased feed efficiency was observed exhibiting severe combined immunodeficiency (SCID)-like symptoms. These pigs were found to have extremely low levels of circulating lymphocytes and significantly atrophied thymus and lymph nodes. Although the exact mutation has yet to be discovered, the inheritance appears to be simply autosomal recessive. Two human cell lines, one melanoma and one pancreatic cancer, were injected subcutaneously behind each ear. Histopathology and immunohistochemistry revealed tumors of human cell origin in all injection sites with no indication of rejection. One challenge in working with this model is the pigs' severely immunocompromised status. All the immunodeficient pigs used in this study were euthanized due to infections unrelated to their tumors within 22 days after injection. However, if these complications can be mitigated, this model shows potential in a

variety of preclinical applications. Since swine anatomy and physiology are very similar to those of humans, the immunodeficient swine tumor model could be used for testing multiple types of therapy with more translatable results than those yielded by mouse models.

Transgenic Tumor Models

The emergence of transgenic technology in swine holds the promise for powerful genetically engineered cancer models in swine. The study and production of transgenic mice have been well established in cancer research. However, in addition to reflecting human biology more closely than the classic animal models, pigs are easy to breed and produce large litters (Bendixen et al., 2010). Due to advances in cloning and transgenics, the use of cloned pigs in preclinical research has increased (Prather et al., 2003). The reported gene targets for cancer models in pigs include *BRCA1*, *FAP*, *P53,* and *IL2RG* Gene-targeted SCID.

The transgenic swine models that target cancer genes have been developed using various techniques with varying degrees of success. Recombinant adeno-associated virus (rAAV)-mediated gene targeting and handmade cloning were used to develop a transgenic pig with a *BRCA1* knockout. Seven piglets were produced that were confirmed to be *BRCA1* knockouts using polymerase chain reaction and Southern blot-based genotyping. Unfortunately, none of these piglets survived past 18 days (Luo et al., 2011). Fibroblasts containing a targeted disruption of the X-linked *IL2RG* chain were used to generate cloned pigs by serial nuclear transfer to create a SCID model in pigs. Thirty-one cloned piglets carrying the targeted mutation were produced, but only four lived past 1 year of age. One of these surviving carrier females was bred to a wild-type male, producing F1 and F2 generations that resulted in both carrier females and affected males. Bone marrow cells from wild-type siblings were transplanted to affected males via intravenous injection. Engraftment was achieved both with and without conditioning. If similar results are observed in cells transplanted from other species, SCID pigs promise to become a valuable xenograft model (Suzuki et al., 2012).

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder in humans characterized by formation of colonic adenomas that often become malignant when left untreated (Morpurgo et al., 2004). The creation of transgenic swine carrying genetic mutations analogous to those found in human FAP shows promise in creating a large-animal model for colorectal cancer. Two variations of the disease are being studied involving mutations at two different codons orthologous to those found in human FAP. Histologic analysis of lesions taken from these pigs at 1 year of age showed similarities to those observed in human FAP. Recently, F1 piglets have been born, which should aid in further characterizing the model and its progression to cancer (Flisikowska et al., 2012). This work also may be combined with work being done in swine with a gene target mutation of *p53* gene. Mesenchymal stem cell isolates from adipose tissue from male Landrace pigs were used to prepare clones with *P53* mutations. The resulting 15 piglets appeared phenotypically normal and are being monitored closely for any abnormalities. The next phases will involve Cre activation of the latent allele *in vivo* (Leuchs et al., 2012).

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20 Necropsy on Research Swine

Kristi L. Helke

CONTENTS

This chapter has been added as a guide and contains suggestions for performing a complete necropsy on a pig. As proficiency is gained with these techniques, individuals generally develop routine methods that best suit their work environment and practices. The techniques presented here are useful in an academic or research setting. Many laboratories already have necropsy standard operating procedures (SOPs) to guide and standardize sampling. The DVD attached with the textbook contains color images of gross necropsy techniques and histology of the various tissues.

Necropsies are performed on experimental animals to determine outcome of drug administration (toxicity) or to determine the outcome/feasibility/efficacy of a medical device or treatment. If an animal displays any spontaneous disease or dies unexpectedly, a necropsy should be performed to determine the cause.

Unlike necropsies in the field or on agriculture animals, those performed on laboratory research animals are more detailed (USDA, 2014). Necropsies for research reasons, device testing, or treatment studies need to be thorough and every organ needs to be examined for unexpected toxicities or effects of the device. By the time a compound is used in a device, it should have already gone through rigorous biocompatibility testing, so only the site of device implant and surrounding tissues needs to be examined, especially for cases where a new use for a previously approved product is being developed. Tissue responses such as injury, inflammation, and wound healing including encapsulation of the device are all examined. The minimal tissues to be collected include those immediately surrounding the device. If the device is placed internally, refer to Table 20.1 as a reference for tissue evaluation considerations.

Tissues to be sampled are determined based on length of time the device is implanted as well as the anatomic location of implantation. For example, a device that is in contact only with skin for less than 24 h will have different sampling requirements than a permanent device in contact with circulating blood (FDA, 2009a,b) (Table 20.1).

THE NECROPSY REPORT

An initial necropsy description is composed of the animal's signalment, history, and gross findings. Signalment is defined as species, breed/strain, color, sex, age (or date of birth), body weight, and

TABLE 20.1
Testing to be Done Based on Contact Tissue and Time

Body Contact		Contact Duration A < 24 h B 24 h to 30 d C > 30 d (Permanent)	Cytotoxicity	Sensitization	Irritation or Intracutaneous Activity	Systemic Toxicity (Acute)	Sub-chronic Toxicity	Genotoxicity	Implantation	Hemocompatibility	Chronic Toxicity (Supplemental Test)	Carcinogenicity (Supplemental Test)
Surface devices	Skin	A	X	X	X							
		B	X	X	X							
		C	X	X	X							
	Mucosal membrane	A	X	X	X							
		B	X	X	X	O	O		O			
		C	X	X	X	O	X	X	O		O	
	Breached or compromised surfaces	A	X	X	X	O						
		B	X	X	X	O	O		O			
		C	X	X	X	O	X	X	O		O	
External communicating device	Blood path–indirect	A	X	X	X	X				X		
		B	X	X	X	X	O			X		
		C	X	X	X	X	X	X	O	X	X	X
	Tissue/bone/dentine communicating	A	X	X	X	O						
		B	X	X	O	O	O	X	X			
		C	X	X	O	O	O	X	X		O	X
	Circulating blood	A	X	X	X	X		^		X		
		B	X	X	X	X	O	X	O	X		
		C	X	X	X	X	X	X	O	X	X	X
Implant device	Tissue/bone	A	X	X	X	O						
		B	X	X	O	O	O	X	X			
		C	X	X	O	O	O	X	X		X	X
	Blood	A	X	X	X	X			X	X		
		B	X	X	X	X	O	X	X	X		
		C	X	X	X	X	X	X	X	X	X	X

Source: Based on requirements published by US FDA 2009a. #G95-1 Attachment B-Table 2 Supplementary Evaluation Tests for Consideration. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm080752.htm; FDA 2009b. #G95-1 Table 1 Initial Evaluation Tests for Consideration. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm080742.htm

X: ISO evaluation test for consideration.

O: Additional test which may be applicable.

^: For all devices used in extracorporeal circuits.

animal identification number. Animal history includes a complete history of experimental manipulations and any clinical signs along with duration, which were observed prior to the animal's death. Clinical history should include laboratory or clinical pathology data (if available). The time between death or euthanasia and necropsy as well as cause of death or method of euthanasia should also be noted since method of euthanasia may result in necropsy artifacts (King et al., 2005; Silva and Sundberg, 2012).

Any lesions should be described and include the following information: Organ/tissue (be specific), location (medial, ventral, caudal, thoracic, etc.), pattern (focal, multifocal, diffuse), specific alteration (hemorrhage, abscess, mass, etc.), color, shape, size, severity, degree (mild, moderate, severe), and so on (King et al., 2005; Silva and Sundberg, 2012). Interpretation of lesions is done in the comments or diagnosis portion of the report. Once histological analysis has been performed, a full necropsy report may be completed.

Information required in the report to the evaluating agency includes gross necropsy information with detailed description of the device site and photographs of lesions and device site. Evidence of responses to the device including inflammation (acute or chronic), and other physiologic changes should also be noted (FDA, 2010). When evaluating a cardiovascular device, the degree of vessel disruption, cellularity, mineralization, and disruption of lamina are all required to be included in the final report (FDA, 2010).

FIXATION

Formalin and paraformaldehyde are both commonly used fixatives in tissue sampling. Other fixatives are also available, or tissues may be frozen. Tissue penetration rate depends on fixative chosen. If immunohistochemistry will be performed, fixation can be important. When sampling tissues, specimens should be cut to no larger than 1 cm^3 to allow for proper fixation. Anything larger will not permit infiltration of fixative before autolysis occurs. Some tissues undergo autolysis much more rapidly than others. For instance, cells with numerous lysosomes such as exocrine pancreatic cells undergo autolysis much more quickly than cells with fewer lysosomes. Tissues should be placed into a volume of fixative that is at minimum 10× greater than the size of the tissue, but ideally 20× or more fixative than tissue is preferred. If formalin is being used, tissues should be kept in fixative 48–72 h before processing.

In device studies, embedding tissue samples with the device intact in resins is preferred to paraffin for histological evaluation of materials which are not easily sectioned (e.g., metal stents) (Schuh, 2008). This allows evaluation of tissue reaction without disturbing the device/tissue interface.

There are many different decalcifying agents, which may be used to decalcify bone and allow for easy sectioning to permit histological analysis. Description of decalcifying solutions is beyond the scope of this chapter. Fragments of bone may be immersed in decalcifying solution for varying amounts of time (depending on agent used) until bone is pliable and easily cut or sectioned.

Some protocols require frozen sections in which fresh tissue is placed in embedding media and then flash frozen.

Occasionally, protocols require perfusion fixation of the entire animal, a technique most common in neuroscience studies to quickly fix the brain. Due to their size, pigs require a large amount of formalin or paraformaldehyde for this procedure. Perfusion can be completed in one of many ways. After anesthesia and a sternotomy, the heart apex can be punctured with immediate insertion of a cannula attached to a pump that will pump paraformaldehyde or saline solution through the animal followed by a puncture to the right atria to allow for blood outflow. Alternatively, the abdomen may be opened, abdominal organs displaced, and a cannula placed into the descending aorta distal to a site that is cross-clamped. A stab incision is made proximal to the clamp and outflow can be suctioned or removed. The site of cannulation depends on the organ most important in the study. For example, if brain is the primary organ of interest, use cardiac cannulation, whereas the pancreas is the primary organ of interest, use abdominal aorta cannulation. For smaller pigs (<25 kg) 10 L of

4% paraformaldehyde has been used successfully in our lab followed by 10 L PBS to ensure clearing of blood cells from the tissues. If the goal is to eliminate blood cells, the animal can be perfused with saline only and tissues submersion fixed in formalin.

PREPARATION

Before beginning, ensure that the appropriate equipment, materials, and instruments are on hand including materials for note taking and a camera for photography. Table 20.2 lists common instruments and equipment used in necropsies. Prelabeled jars of formalin for specific organs are helpful to ensure no organs are omitted and to identify organs after fixation. A checklist helps to make sure all organs are collected and nothing is forgotten.

When performing a necropsy, many organs should be both weighed and measured for changes indicative of toxicity. Any abnormalities should be noted, measured, and photographed before being placed into fixative. Important organs to measure and weigh include testes, liver, kidney, spleen, and adrenal glands, since these organs will often show changes in weight with toxicities. Occasionally, brain, ovary, thyroid, uterus, heart, and spleen are also measured/weighed. Infrequently, weights of lung and lymph nodes are obtained (Michael et al., 2007).

Other organs can also be weighed depending on protocol. All organ weight measurements should be completed as soon as possible followed by fixation to prevent dehydration artifact and autolysis. If lungs are weighed, ensure that the same amount of tissue is weighed (remove esophagus and same amount of trachea/bronchus). Lungs may artifactually increase in weight with increased period from time of death to organ removal. The spleen, kidney, and adrenal glands should be measured in three dimensions. Testes and ovaries should also be weighed and measured if present.

IMMUNOLOGICAL ASSESSMENT

The most proximal regional lymph tissues that drain the drug application site should be examined histologically. Peripheral or distant lymph nodes are too variable to be of diagnostic use (Haley et al., 2005). Hematology and lymphoid organ (thymus, spleen, draining, and distant lymph nodes) weights should be assessed when evaluating immunotoxicology (Schuh, 2008).

Histological analysis of lymph tissues, including bone marrow, should also include analysis of lymphocyte subset distribution. If the compound is administered orally, Peyer's patches and mesenteric lymph nodes should be evaluated (Haley et al., 2005). Histologically, lymph nodes of pigs have a unique structure, with centrally located cortical tissue and germinal centers that appear inside out when compared to other species. The follicles are arranged centrally and not peripherally and the lymph flow is reversed with lymph entering at the hilus (Riquet et al., 2000; Spalding and Heath, 1987b; Figure 20.1).

Examination of lymph tissues is best done systematically by tissue compartment, that is, in the spleen, examine red pulp, white pulp, marginal zone, and then examine entities within each

TABLE 20.2
Equipment for Necropsy

Required Equipment	Suggested or Optional Equipment
Scalpel plus extra blades	Towel clamps
Forceps	Allis tissue forceps
Fixative (Formalin/paraformaldehyde)	Hemostats
Camera	Colon clamp
Note-taking/record-keeping materials	Perfusion pump (if perfusion fixing)
Bone saw	
Virchow skull breaker	

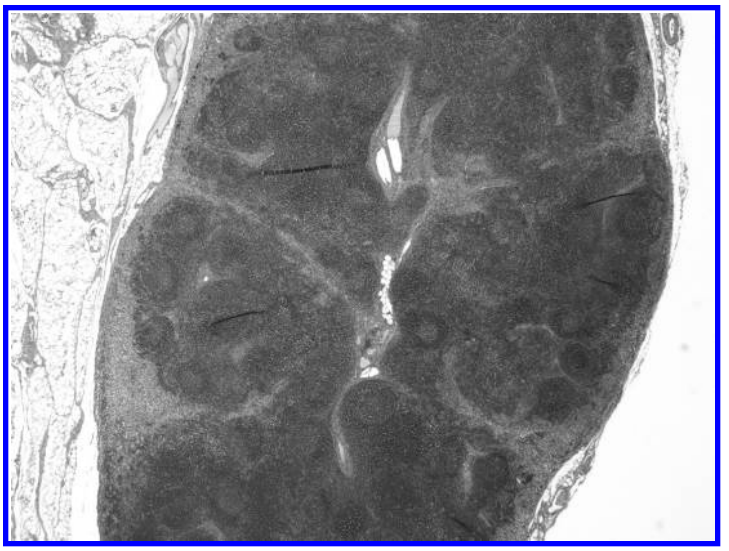

FIGURE 20.1 Porcine lymph node 20×.

compartment; in white pulp, examine periarteriolar lymphoid sheaths (PALS), lymphoid follicles, and germinal centers. This technique is recommended for all lymphoid tissues (Haley et al., 2005).

There are numerous lymph nodes throughout the pig as in all other species. Numerous resources are available to determine which lymph node is draining the site of interest.

THE NECROPSY

Collection of specific tissues and histological analysis are required for preclinical testing. The required tissues are dependent primarily on the agency that grants approval for the drug/device being tested. A group of veterinary toxicologic pathologists has created a best practices guideline on tissues to sample based on observations and the likelihood of lesions presenting in a specific organ (Bregman et al., 2003). The suggested tissues from several agencies are presented in Table 20.3.

Gross necropsy should include visual examination of external surfaces of the body, including all orifices, cranial, thoracic, and abdominal cavities, and their contents.

In the pig, there are some anatomical differences of which the prosector should be aware. The thymus consists of cervical and thoracic lobes. The parathyroid glands are resident within the cranial portion of the thymus (not the thyroid as in other species), which lies on either side of the trachea at the level of the larynx and can be found using the branch of the carotid that enters the cranial thymus (Soshin et al., 2010). The thyroid lobes are fused into one gland that lies on the ventral aspect of the trachea just cranial to the thoracic inlet. The stomach contains both glandular and squamous portions with the squamous portion being in the area of the cardia, and pigs have a torus pyloricus, which is a raised area in the pyloric outflow region of the stomach.

Organ systems are listed in the order of methodical examination. The order of the investigation can be changed based on the study, but the following order is easy to follow and allows observation of the complete animal.

Examine the outside of the animal for any abnormal discolorations or lesions. If any are noted, photograph and save a sample for histology. The oral cavity should be thoroughly examined including the teeth, tongue, soft palate, and tonsils. Ensure ear canals do not contain excess secretory material**.** Ensure the eyes are clear and not cloudy.

The skin thickness varies in different anatomic areas of the pig. The skin thickness, cornification, and underlying dermis that most closely recapitulates human skin histologically on the pig are

TABLE 20.3
Suggested Tissues to Sample from Various Agencies

	Society of Toxicologic Pathlogy a (Bregman et al., 2003)	European Agency for the Evaluation of Medicinal Products (EMA, 2002)	Society of Toxicologic Pathology b (Jacobs et al., 2003)	US Food and Drug Administration Redbook (FDA, 2007)	US Environmental Protection Agency (EPA, 1998)
Adrenal gland	X	X	X	X	X
Aorta	X	X	X	X	X
Bone (cartilage)	X	X (femur)	X	X (femur)	
Bone marrow	X	X*	X	X (sternum)	X
Brain (3 levels)	X	X (including cerebellum)	X	X	X (cerebrum, cerebellum, medulla/pons)
Cecum	X	X	X	X	X
Cervix			X	X	
Colon	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymis	X	X	X	X	X
Esophagus	X	X	X	X	X
Eye	X	X	X	X	X
Gallbladder	X	X	X	X	X
Harderian gland	X	X*	X	X	
Heart	X	X	X	X	X
Ileum	X	X	X	X	X
Jejunum	X	X	X	X	X
Kidney	X	X	X	X	X
Lachrymal gland			X		
Larynx		X			X
Liver	X	X	X	X	X
Lung	X	X	X	X (with mainstem bronchi)	X
Lymph node	X	X	X	X (1 related to route of administration, 1 distant)	X (1 related to route of administration, 1 distant)
Mammary gland	X	X	X	X	X
Nasal cavity/ turbinates		X*	X	X	X
Optic nerve		X	X		X
Ovary	X	X	X	X	X
Oviduct		X	X	X	
Pancreas	X	X	X	X	X
Parathyroid gland	X	X	X	X	X
Peripheral nerve	X	X	X	X (sciatic)	X (sciatic or tibia)
Pharynx			X		X
Pituitary	X	X	X	X	X
Preputial/clitoral glands		X			
Prostate	X	X	X	X	X

(*Continued*)

TABLE 20.3 (*Continued*)
Suggested Tissues to Sample from Various Agencies

	Society of Toxicologic Pathlogy a (Bregman et al., 2003)	European Agency for the Evaluation of Medicinal Products (EMA, 2002)	Society of Toxicologic Pathology b (Jacobs et al., 2003)	US Food and Drug Administration Redbook (FDA, 2007)	US Environmental Protection Agency (EPA, 1998)
Rectum		X*	X	X	X
Salivary gland	X	X	X	X	X
Seminal vesicle	X	X	X	X	X
Skeletal muscle	X	X	X	X	
Skin	X	X	X	X	X
Spinal cord	X	X	X	X (cervical, mid-thoracic, lumbar)	X (cervical, mid-thoracic, lumbar)
Spleen	X	X	X	X	X
Stomach	X	X	X	X	X
Testis	X	X	X	X	X
Thymus	X	X	X	X	X
Thyroid	X	X	X	X	X
Tongue		X	X		
Trachea	X	X	X	X	X
Urinary bladder	X	X	X	X	X
Uterus	X	X	X	X	X
Vagina	X	X	X	X	
Zymbal's gland		X*	X (with external ear)	X	
Other tissues with lesions/ abnormality	X	X	X	X	X
Tissue masses	X	X	X		X

Note: * indicates to be saved in case needed for later study. Not necessarily included in initial histological analysis.
Society of Toxicologic Pathlogy (STP) a is letter to editor with added tissues for examination. STP b is original publication.
X: Tissue required by agency/reference.

on the flank. The skin over the shoulders thickens with age, especially in males, and the skin on the abdomen is the thinnest (Figures 20.2 through 20.5).

It is convenient to use a curved incision (resembling a backwards question mark; Figure 20.6) on the abdomen for several reasons. This approach avoids the male genitourinary tract, is easy to perform alone, no retractors are needed (uses gravity as an assistant), and results in a more tidy necropsy. Towel clamps can be used initially to grasp the skin and assist in reflection of the abdominal flap (Figure 20.7).

Abdominal Cavity, Spleen and Pancreas, Liver, Gastrointestinal Tract, Urogenital System (Male, Female), Kidneys, and Adrenal Glands

This approach allows for clear visualization of the abdominal organs *in situ* (Figure 20.8). Initially, assess the urinary bladder and remove urine from bladder using a syringe attached to a large bore needle. A urinalysis may be performed if required or urine may be discarded. The bladder is easily

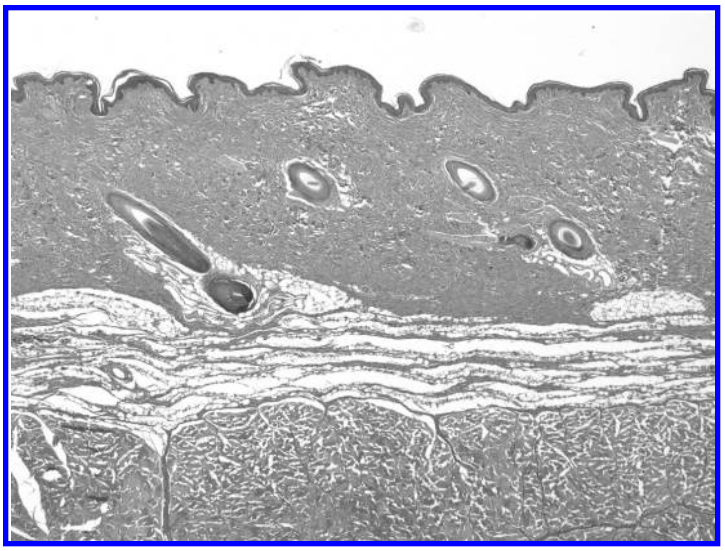

FIGURE 20.2 Skin of flank showing epidermis, dermis, and subcutis 20×.

sampled at this time. In males, collection of accessory sex glands is convenient to do immediately after assessing the bladder (Figures 20.9 and 20.10).

The pancreas readily autolyzes and should be sampled next. It is found adjacent to the proximal duodenum, pylorus, and greater curvature of the stomach (Figure 20.11).

Following collection of these initial organs and after examination but before removal of remaining abdominal organs, it is useful to double cross-clamp the caudal vena cava and portal vein, distal colon/rectum, and vessels at the root of the mesentery. After the caudal vena cava/portal veins are cross-clamped, examine the abdominal surface of the diaphragm for any irregularities and ensure that it has a concave surface (indicating negative pressure in the thorax; Figures 20.12 and 20.13). Cut between the clamps in the three locations and carefully lift out the abdominal organs to a clean work surface. Cutting between the clamps in the three locations results in minimal loss of fluids into the body cavity, making it easy to examine retroperitoneal organs (Figure 20.14).

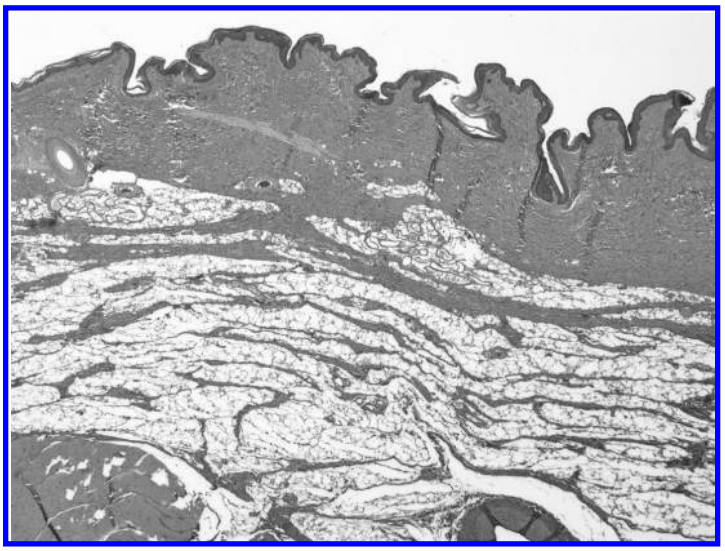

FIGURE 20.3 Skin of ventral abdomen showing epidermis, dermis, and subcutis 20×.

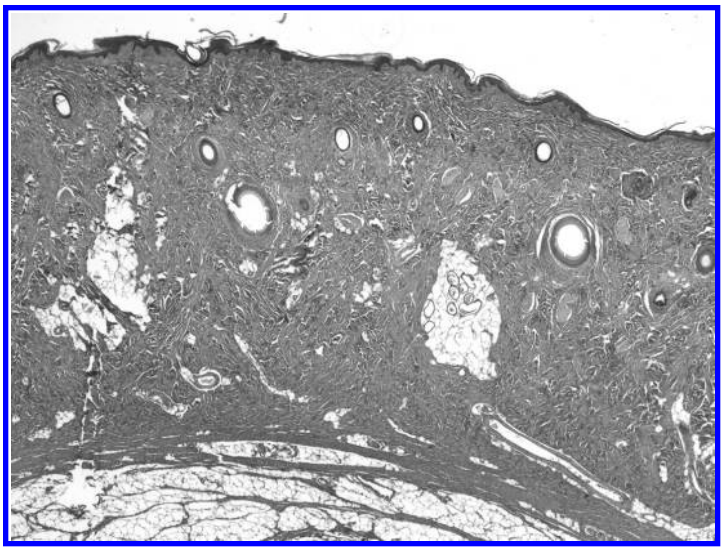

FIGURE 20.4 Skin of anterior foreleg showing epidermis, thick dermis, and subcutis 20×.

After removal of the organs from the abdomen, they should be separated. The spleen should be removed, measured, and weighed. If barbiturates are used in euthanasia, the spleen may be markedly enlarged (up to 20× normal size; King et al., 2005). The spleen should be examined grossly on all surfaces and then sliced multiple times on both surfaces looking for any irregularities (Figure 20.15). When the spleen is cut, if the enlargement is from the euthanasia, blood will flow freely from the cut surface. Remove the liver from surrounding gastrointestinal (GI) organs. Examine all liver surfaces and note any irregularities and liver weight. Slice through the liver on both diaphragmatic and abdominal surfaces several times to examine parenchyma for any abnormal areas which should be sampled. If there are no irregularities, continue to sample several lobes, being consistent in the areas you sample between animals. Examine the gallbladder and incise it to examine the bile, which should be clear yellow-green and viscous. Make sure a section of gallbladder is placed into fixative.

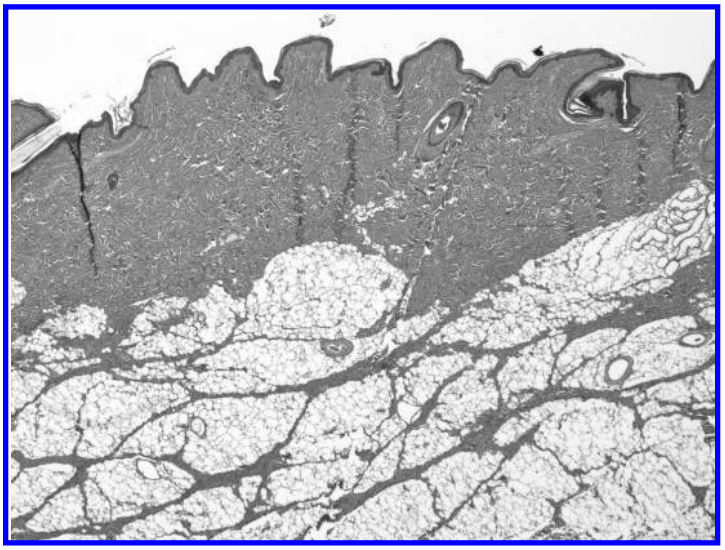

FIGURE 20.5 Skin caudal to ear showing epidermis, dermis, subcutis 20×.

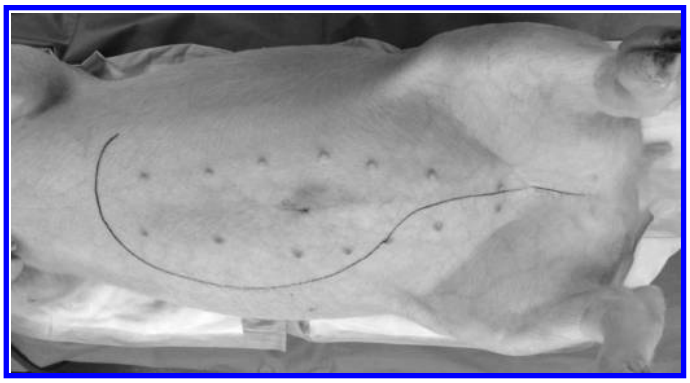

FIGURE 20.6 Porcine ventrum with planned incision shown.

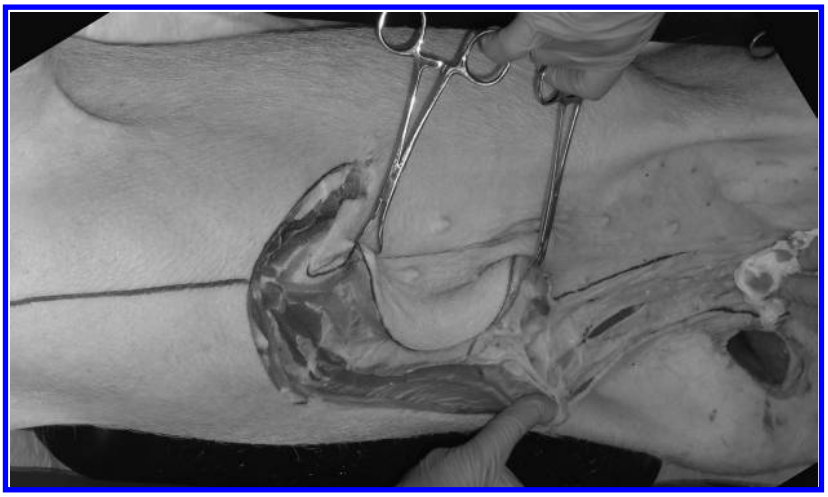

FIGURE 20.7 Abdomen after initial incision is made. Towel clamps are used to help retract skin and allow for greater exposure.

FIGURE 20.8 Abdominal organ exposure. The spiral colon is seen in the left ventral aspect of the abdomen (at top of image). The spleen is markedly enlarged due to method of euthanasia.

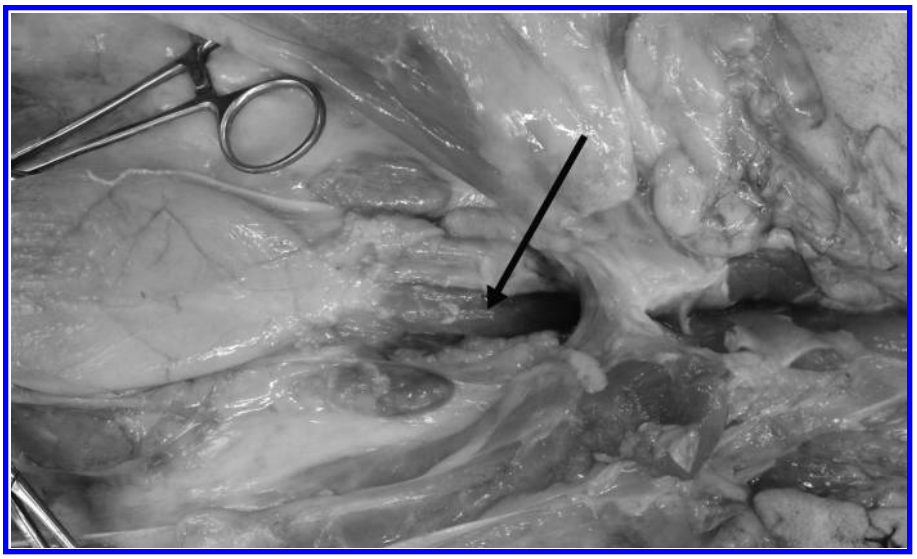

FIGURE 20.9 Bulbourethral glands (arrow) on ventral aspect of urethra of castrated male pig.

The GI tract extends from the mouth to the anus. It is typically removed in more than one section. The esophagus is often removed with the thoracic organs, and the abdominal portion is removed along with abdominal organs. The GI tract should be opened along its entire length to examine for any abnormalities. In the pig GI tract, there are a few anatomical differences compared to other species. The stomach has a squamous portion adjacent to the esophagus (pars oesophagea) that readily ulcerates with administration of fine ground feed, or feed withholding

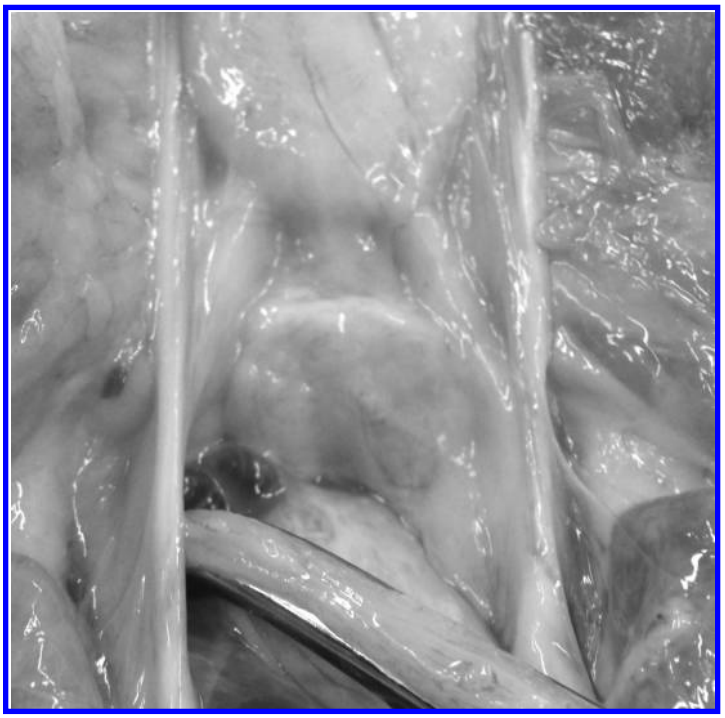

FIGURE 20.10 The urinary bladder is at the top of the image and the prostate is in the center.

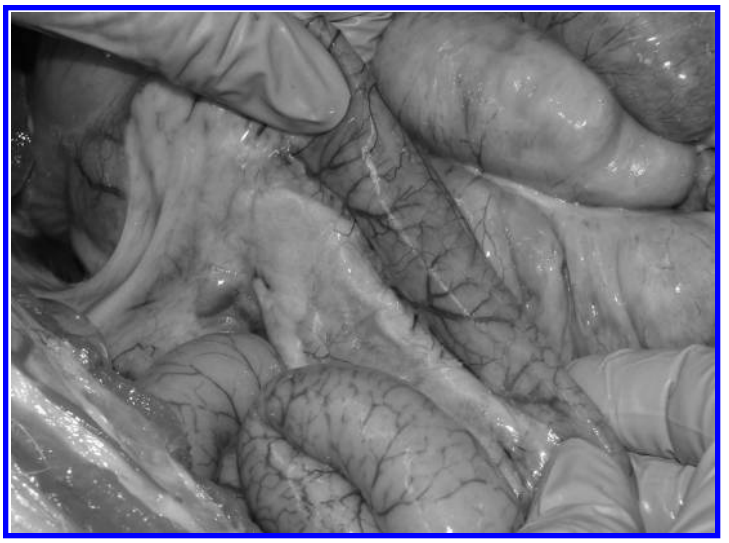

FIGURE 20.11 The pancreas lies adjacent to the duodenum and is easily found after locating the pylorus.

(Lawrence et al., 1998; Figure 20.16). While other areas of the stomach may ulcerate, it is most common in the pars oesophagea. In the pig, the fundus and corpus regions are similar in size due to the large outpouching of the fundus (Egbuji et al., 2010). The corpus or body of the stomach and the pyloric regions are similar between pig and human with the exception of the torus pyloricus. The torus pyloricus is a muscular protrusion of the pyloric sphincter into the pyloric outflow canal to maintain a homogenous size of ingesta entering the small intestine (Figures 20.16 and 20.17).

The small intestine is similar between pigs and humans. For necropsy, it should be removed and the entire length opened and examined. The major duodenal papilla is 2–5 cm from the pylorus and releases secretions from the bile duct (Yen, 2001). The minor duodenal papilla is 14–25 cm distal to

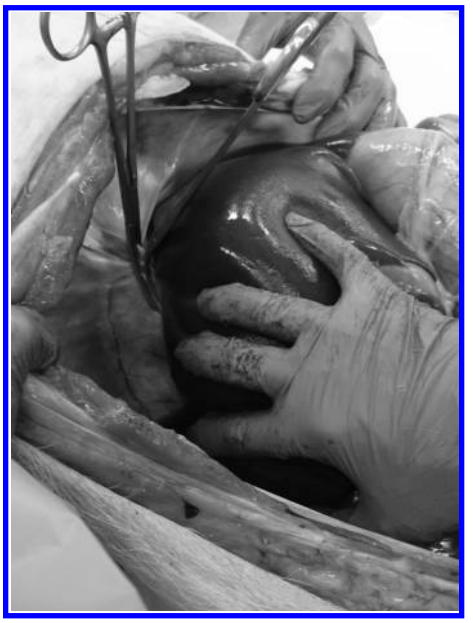

FIGURE 20.12 Caudal vena cava and portal veins are double cross-clamped to prevent blood flow into the abdominal cavity.

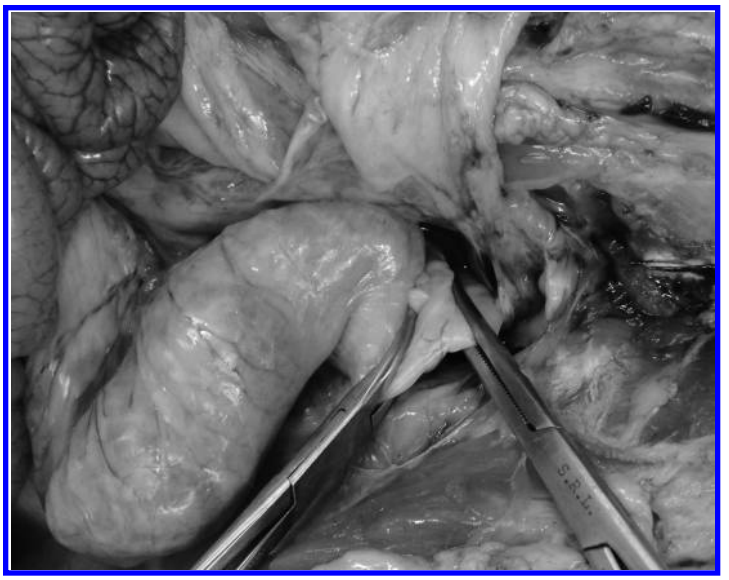

FIGURE 20.13 The rectum is double cross-clamped using doyen or mayo-robson intestinal clamps to retain fecal matter within the rectum and colon.

the pylorus and releases pancreatic enzymes from the accessory pancreatic duct (Yen, 2001). There are Peyer's patches scattered in the mucosa of both the small and large intestine. The mesenteric lymph node is quite prominent and easily identified (Figure 20.18). There is a large continuous Peyer's patch, sometimes called the cecal tonsil, in the terminal ileum believed to function primarily as a B-cell lymphoid organ (Burkey et al., 2009). This continuous Peyer's patch can be seen grossly and is a normal structure in the pig (Figure 20.19). The enteric blood supply is composed of numerous small radiating arteries and veins in the mesentery that supply the small intestine instead of arterial arcades which are found in other species (Spalding and Heath, 1987a; Figure 20.20).

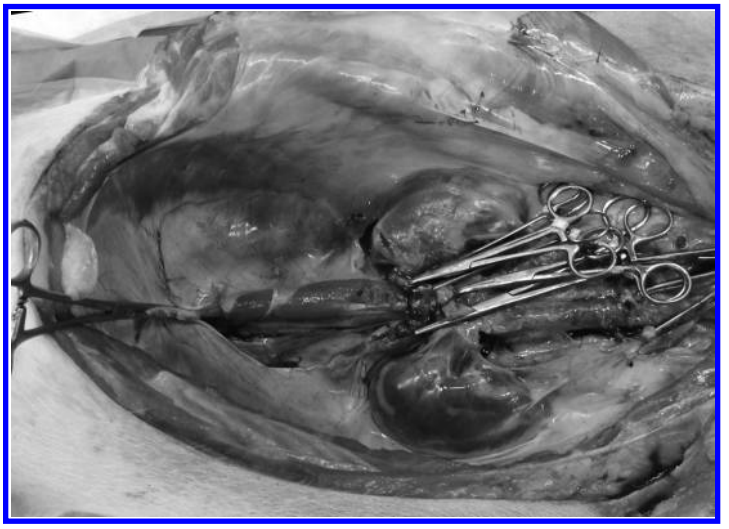

FIGURE 20.14 Easy visualization of retroperitoneal organs after removal of gastrointestinal tract, liver, and spleen.

FIGURE 20.15 Spleen with surface sliced multiple times. Blood exuding from cut surfaces is indicative of use of barbituate euthansia solution.

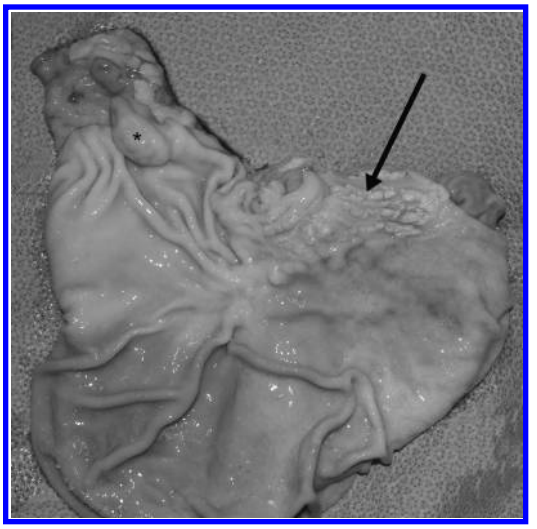

FIGURE 20.16 Stomach, mucosal surface. Pars esophogea is indicated by arrow. The torus pyloricus (*) is located in the pylorus.

FIGURE 20.17 The torus pyloricus is a muscular outpouching in the pyloric region.

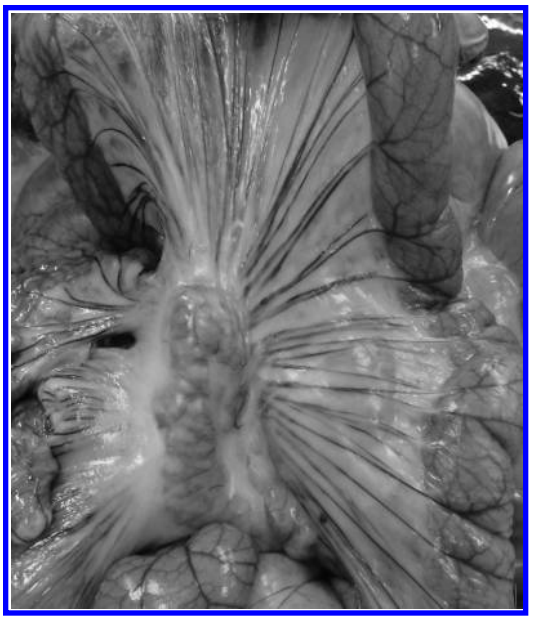

FIGURE 20.18 Mesenteric lymph node is evident at root of mesentery.

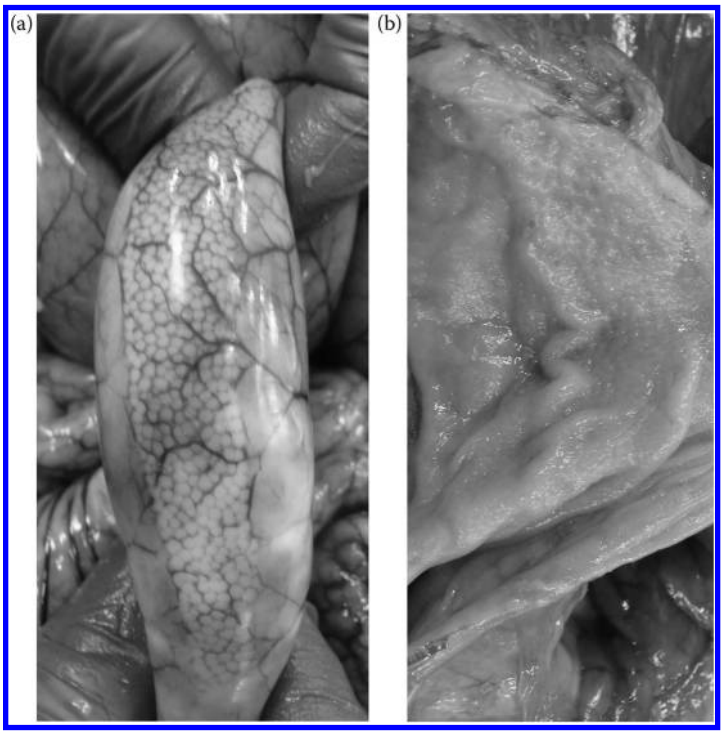

FIGURE 20.19 Serosal (a) and mucosal (b) views of large Peyer's patch in terminal ileum (cecal tonsil).

FIGURE 20.20 Blood supply to the small intestine is composed of radiating arteries and veins in the mesentery.

The large intestine comprises ascending, transverse, and descending portions similar to humans. The ascending colon of the pig is arranged in four centripetal coils, a central flexure, and 3.5 centrifugal coils, and is commonly known as the spiral colon (Yen, 2001; Figure 20.8). Fecal material should be removed from the GI tract before fixation.

At a minimum, samples should be collected from the stomach, duodenum, jejunum, ileum, cecum, and colon. Sections of the intestine can be removed, placed flat on a surface with the mucosal side up, and a tongue depressor maneuvered under the intestine and then lifted, allowing the intestine to wrap around the depressor (Figure 20.21). This allows for flat fixation of the intestine and prevents curling of the section when placed into fixative. This same technique can be used for several tissues, which are small and have a tendency to curl or fold upon themselves.

Following removal of the liver, spleen, and GI tract from the abdomen, the retroperitoneal organs are easily visualized. The kidneys of the pig are similar to the human in location and anatomy. Similar to humans, the porcine left kidney is most cranial. After examination of the ureters, and confirmation that there is not hydronephrosis, the kidneys may be removed from the abdomen, measured, and weighed. The renal capsule should be removed to examine the cortical surface of the kidney, and ensure there are no adhesions of the capsule to the cortex. The kidneys should then be sliced to examine the cortex and medulla. The left kidney should be cut longitudinally (along the long axis) to show the renal papillae and cortex. The right kidney may be cut similarly, or it may be cut transversely (Figure 20.22) so that the left and right kidneys can be differentiated after fixation. The normal cortex:medulla ratio is 1:2 or 1:3 in the sagittal plane, whereas in a coronal section, the cortex comprises approximately 80% of the total mass (Maxie and Newman, 2007). The porcine kidneys are similar to humans in structure in that they are multireniculate and multipapillate, and as such, make good surgical urologic models (Dalmose et al., 2000). The porcine renal vessels are comparable in diameter to many human vessels, and due to the porcine renal blood flow are often used to study vascular devices.

The porcine adrenal glands are approximately $3–4 \times 1 \times 0.5$ cm and lie along the abdominal great vessels at the cranial pole of the kidney. The right adrenal gland is often very closely associated with the vena cava and in some cases integrates into the vessel wall.

In females, the ovaries are located just caudal to the kidneys. The fallopian tubes are prominent and tortuous and lead into the uterine horns. The uterus is bicornuate and extends from near

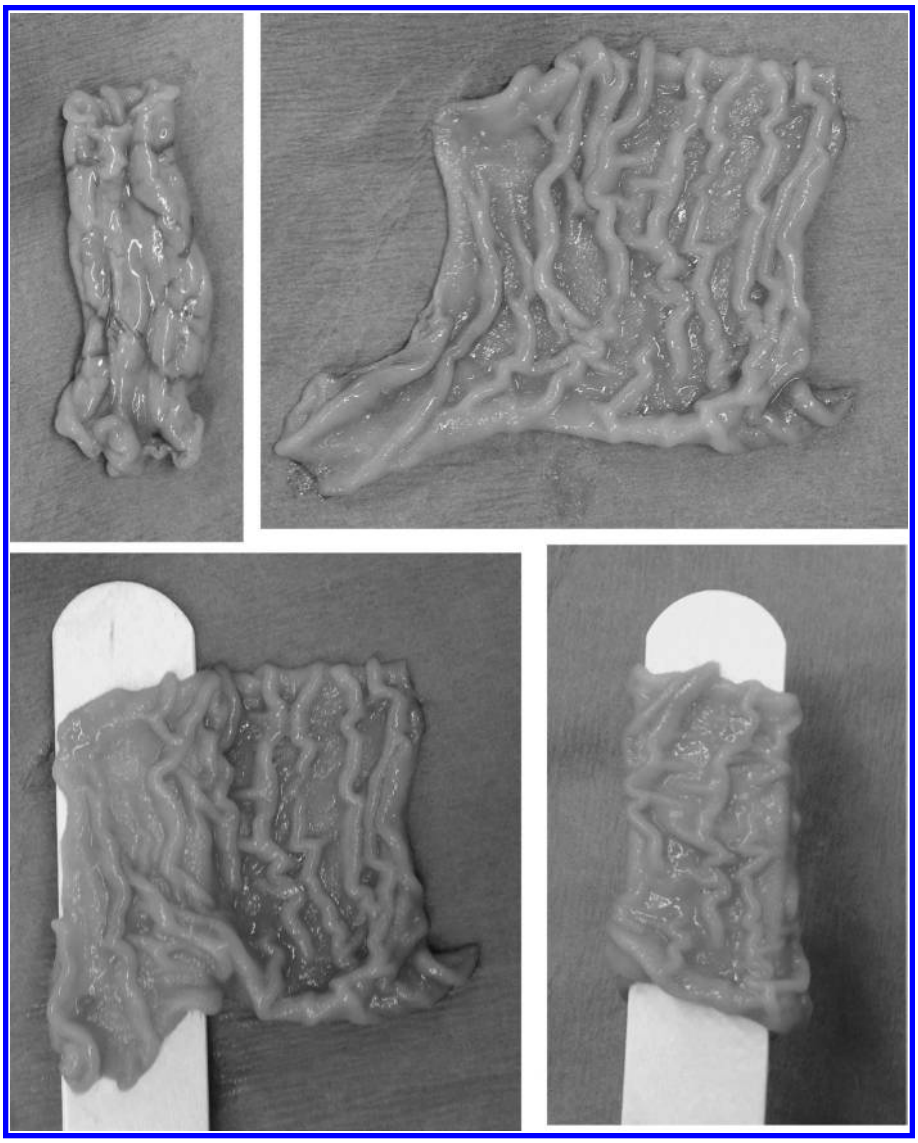

FIGURE 20.21 Small intestine. Example of method to handle tissue to prevent folding.

the ovaries on both sides to the cervix where the horns meet. The cervix is 10–14 cm long, which terminates into the vagina. Mammary tissue is also sampled and examined histologically. Teat number in pigs is variable, so collect mammary tissue from the same gland for all animals (most caudal or cranial).

There are several accessory sex glands in the male. These include vesicular glands (previously seminal vesicles), bulbourethral glands, and the prostate gland. The vesicular glands are paired and lie around the ureters at the base of the bladder, the prostate surrounds the urethra at the base of the bladder as in humans, and the bulbourethral glands lie distal to the bladder along the ventral urethra (Figure 20.9). Many animals used in research settings have been castrated and it is therefore uncommon to see testes or epididymides; however, animals in toxicological testing studies remain intact. To test for testicular and epididymal toxicities, sexually mature animals are required. Both left and right organs should be weighed. Davidson's fixative will help prevent shrinkage artifact

FIGURE 20.22 Kidney showing method of sectioning with left kidney cut longitudinally (right) and right kidney cut transversely (left).

seen with formalin. The testicular artery can be used for perfusion fixation to help avoid further fixation artifacts (Lanning et al., 2002)

The abdominal aorta is easily visualized and should be opened along its length to examine for lesions (Figure 20.23).

Thoracic Cavity: Removal of Lungs, Heart, and Neck Organs

Once the abdominal organs are removed, the thorax is exposed by cutting through the skin from the chin to the xyphoid (Figure 20.24). After removal of muscles attached to the ribs, the thorax may be entered by cutting the diaphragm. If the diaphragm is still intact after the liver has been removed, ensure the surface is concave before making an incision. If the diaphragm has not been cut previously and does not have a concave surface, this should be noted along with whether there is fluid or excess air within the thorax. Incise the diaphragm adjacent to the sternum, beginning under the xyphoid cartilage. Extend the cut bilaterally. Lift the xyphoid cartilage and use the scalpel to cut along the costochondral junctions bilaterally cranial toward the manubrium. This will allow the sternum to be easily lifted from the rib cage and the chest opened after cutting the pericardium. The costochondral junctions can easily be cut with a scalpel in adult animals, negating the need for a rib cutter and exposing sharp bony edges (Figure 20.25).

The pericardium surrounds the heart and contains approximately 5–10 cm^3 of clear fluid in health. The pericardium is severed upon removal of the sternum and care should be taken to examine this fluid upon opening the chest cavity and ensure there are no abnormalities in amount, color, or consistency.

In young pigs, the thymus extends from the cricoid cartilage caudally into the thorax (Figures 20.26 and 20.27). At one year of age, the thymus begins to involute. At 20 months of age, thymic involution is complete in the minipig (Nobori et al., 2006). The thyroid is a bilobed organ and in the

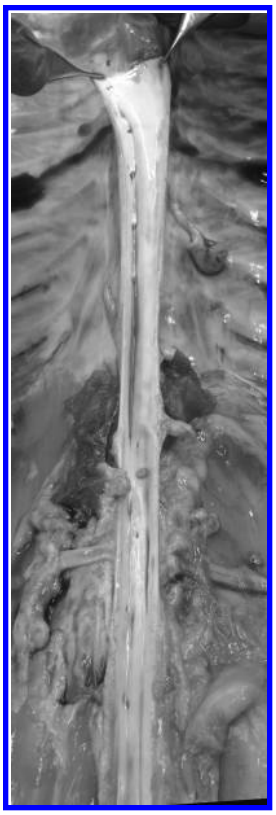

FIGURE 20.23 Abdominal aorta opened along length to examine for any abnormalities.

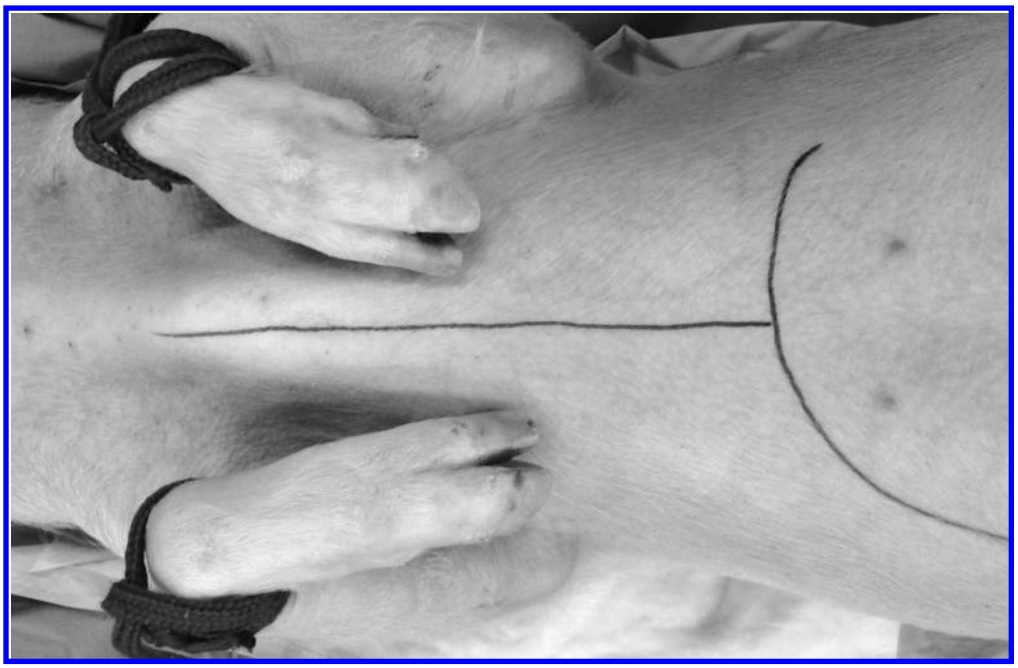

FIGURE 20.24 Suggested thoracic skin incision on ventrum of pig.

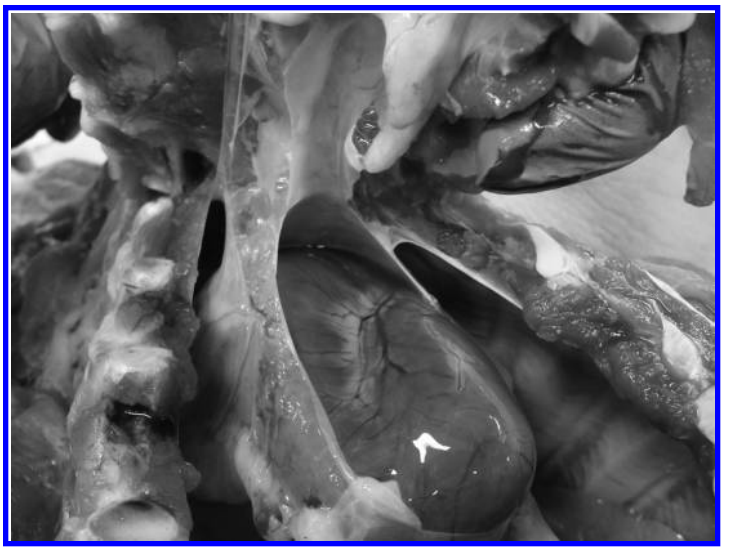

FIGURE 20.25 Thorax with sternum lifted off but pericardium still attached.

pig, the lobes are fused along the ventral trachea under the thymus (Figure 20.26). The thyroid should be removed, measured, and weighed (Figure 20.27). Unlike other species, in the pig the parathyroid organs are found within the cranial thymus and not adjacent to the thyroid glands. While embryologists suggest that there are two pairs, only one pair has been observed grossly (Sisson et al., 1975; Soshin et al., 2010).

Upon opening the thorax, examine the color of the lungs, especially dorsally. Depending on the time between euthanasia or death and examination, blood may be pushed into the lungs because of rigor mortis in the extremities, pushing the blood centrally where there is less resistance (King et al., 2005). If the animal is in dorsal recumbency, then the dorsal lungs may be severely congested and artifact needs to be considered.

In rodent inhalation studies, examination of the nasal cavity, nasopharynx, paranasal sinus, larynx (three levels), trachea (include bifurcation), and lung (all lobes) is required (OECD, 2010). In pigs, these tissues are all readily apparent and should also be sampled. However, the entire lung lobe is too large to examine histologically, so consistency of sampling sites of lobes among animals is necessary.

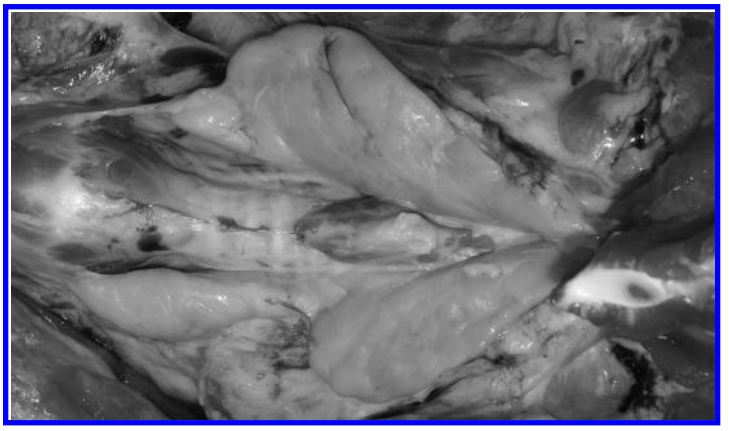

FIGURE 20.26 Ventral view of cervical organs. The thyroid is located centrally on the ventral trachea. The thymus gland is adjacent to the trachea and covers the thyroid prior to dissection. It extends from larynx caudally into thorax.

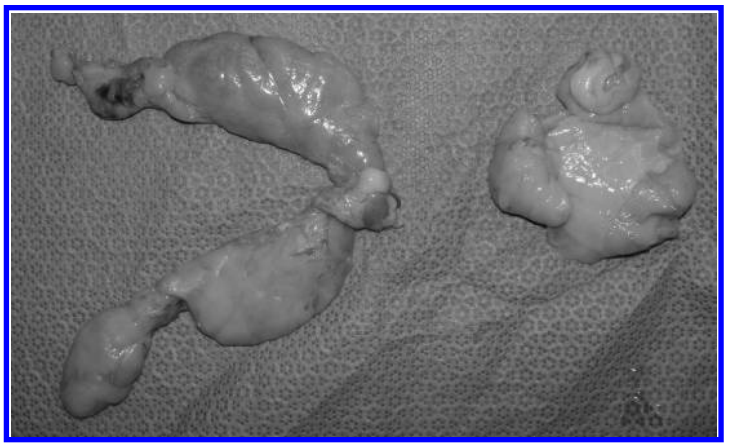

FIGURE 20.27 Thymus. The cervical region is on the left with the thoracic portion on the right.

Be sure to sample several similar lung regions from each animal in addition to sampling any gross lesions evident at necropsy. Lungs should be palpated for consistency. They should be soft and collapse easily. If they are firm or do not collapse, this is indicative of disease. The sampled lungs may be perfused with formalin by inserting an 18 gauge needle attached to a 20–30 cm^3 syringe filled with fixative and clamping with a hemostat at the top of a small bronchus. Slowly inflate the sampled lobe. Cut away a portion of lung distal to the needle that is perfused with fixative and allow to drop into specimen container filled with fixative (Figure 20.28). Sample distal, apical, and cardiac lobes

FIGURE 20.28 Section of lung being perfused via syringe with distal most section cut and placed into fixative.

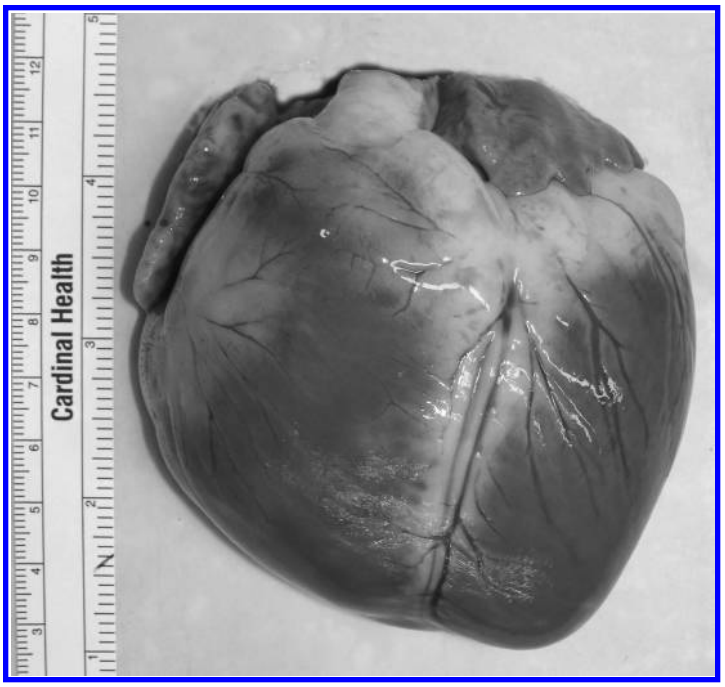

FIGURE 20.29 Heart.

bilaterally along with the proximal diaphragmatic lobe. Pulmonary intravascular macrophages in states of health in pigs are normal, and their presence does not represent a disease state or toxicity.

Bronchi and large vessels should be opened to examine for any abnormalities. The lungs should then be completely examined by slicing through at approximately 1 inch intervals.

The heart lies within the pericardium adjacent to the sternum. The coronary vessels are similar to those of human. Examination of the epicardium should be complete before incising the cardiac muscle (Figure 20.29). The heart is easily examined by cutting along the cardiac groove to free the right ventricle and allow inspection of the endocardium, the papillary muscles, and the tricuspid valves. Continue the incision into the atrium to allow inspection of the pulmonary artery and semilunar valves. The left ventricle may be bisected to include the apex and this will allow inspection of the internal structures of the left ventricle including the mitral valve (Figure 20.30). After inspection of the mitral valve, follow the outflow tract with scissors cutting through the atrial wall and valve leaflets and exposing the aorta and semilunar valves (Figures 20.31 and 20.32). The endocardium should be smooth and shiny with no irregularities. Once the aorta is exposed, the coronary vessels may be followed and examined for any irregularities. Subendocardial hemorrhages may be evident as multifocal random red brushstrokes under the endocardium. These lesions may be an artifact of the euthanasia method. If barbiturate euthanasia is prolonged, endothelial damage may occur and lead to hemorrhages most evident on the endocardial surface (Grieves et al., 2008).

Head, Skeletal System, and Bone Marrow

If necessary, cerebrospinal fluid may be sampled from the spinal cord after removing the neck organs and before removing the head by inserting a 23 gauge needle between the vertebrae from the ventral aspect. Removal of the skull makes access to the brain much more manageable. Transect the spinal cord before manipulating the skull to remove it to prevent neuronal artifacts. After all connecting tissues and muscles are transected, a scalpel may be used to easily cut between the occiput and the atlas, thus removing the skull.

FIGURE 20.30 Endocardial surface of the left ventricle with exposure of the mitral valve.

The eyes are easily removed using Allis tissue forceps to hold the palpebrae or eyelids shut (Figure 20.33). An incision is then made through the skin around the eyelid but smaller than the orbit using a scalpel. Curved Metzenbaum scissors can then be used to cut through the remaining connecting tissues and muscle along with the optic nerve while carefully lifting the globe out of the socket using the Allis tissue forceps (Figure 20.34). The porcine and human eye both lack a tapetum lucidum within the choroid. The macula overlies the optic nerve rather than being distant to it as in humans (Sanchez et al., 2011). There are no other ocular differences to note. When trimming the eye for histological examination, it should be bisected in such a manner to include both the optic nerve and the macula.

The tongue may be removed with the esophagus, trachea, and lungs, or left intact and examined as part of the head. The tongue should be examined grossly and a section saved for histological examination.

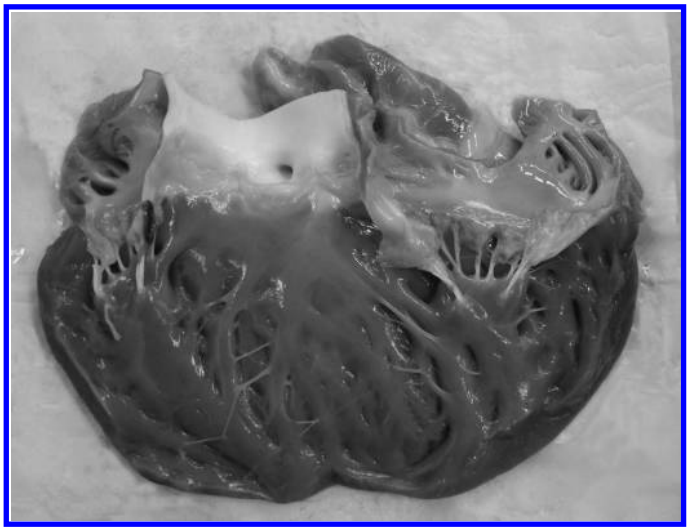

FIGURE 20.31 Left ventricular outflow tract with exposure of the aorta.

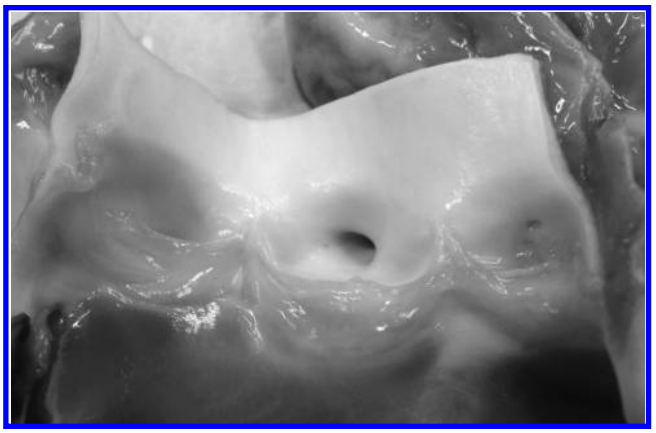

FIGURE 20.32 Aortic semilunar valves with origin of coronary artery.

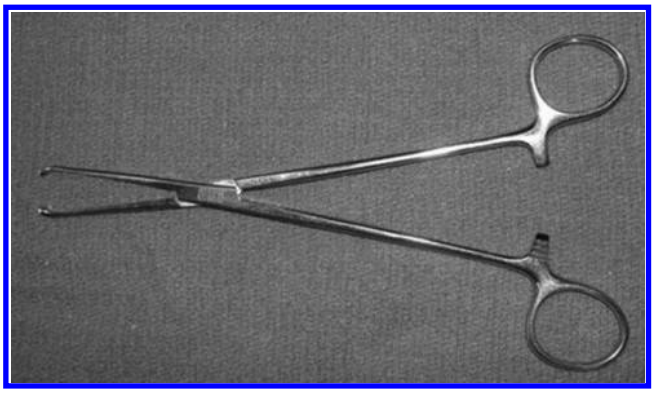

FIGURE 20.33 Allis tissue forceps.

FIGURE 20.34 Removal of the globe is made easier using Allis tissue forceps to grasp the palpebrae and a metzenbaum scissors to cut connective tissue within the orbit.

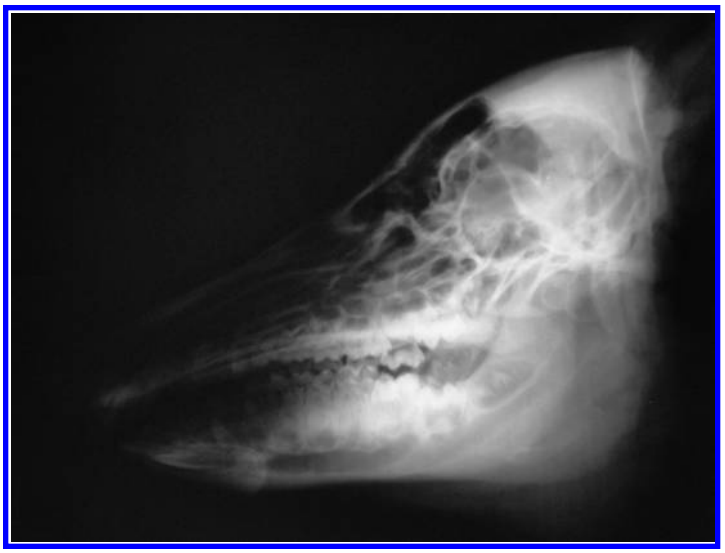

FIGURE 20.35 Lateral view radiograph of skull of Sinclair minipig.

The brain is not difficult to remove but does require some skill and anatomic knowledge. Depending on the age of the pig, there may be large caudal frontal sinuses that must be traversed before entering the calvarium and these sinuses enlarge with age (Honig et al., 2002; Koppe et al., 2000; Figures 20.35 through 20.37). Once the muscle is removed from the skull in the area that will be cut using the oscillating bone saw, the scalpel can be used to remove the remaining periosteum using a scraping motion. The cuts to open the calvarium are as follows: connect the dorsal occipital condyle to the

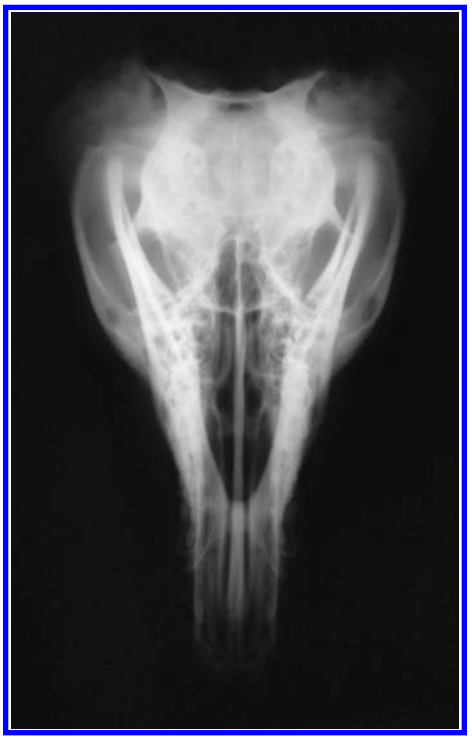

FIGURE 20.36 Ventral view radiograph of Sinclair minipig.

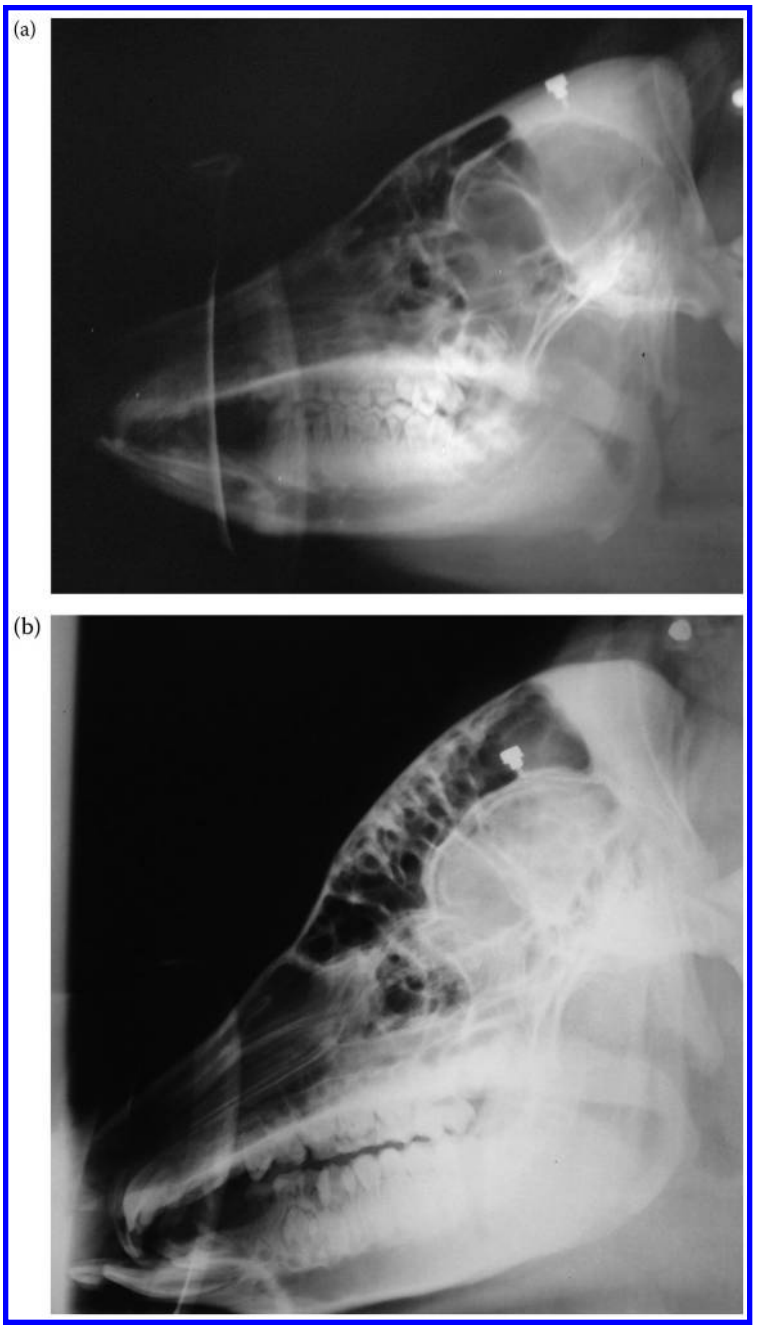

FIGURE 20.37 Lateral view radiographs of Göttingen minipig. (a) 7 months old and (b) same animal at 14 months of age. Note greatly enlarged frontal sinuses.

temporal line, cut across the temporal fossa connecting the temporal line to the orbital ridge line, and then across the ridge of the orbit at approximately 60° angle to midline. Use Virchow Skull Cracker to loosen and remove the calvarium exposing the brain (Figure 20.38). Rongeurs may be needed to remove all of the bone, especially if the sinuses are large, or the bone saw may be used after removing the bony trabeculae to cut through the calvarium (Figure 20.39). The dura is a tough layer that needs to be carefully cut with a scalpel to remove it. An incision is made through the olfactory lobes, and

FIGURE 20.38 Virchow skull breaker.

FIGURE 20.39 Bacon cranial rongeur forceps.

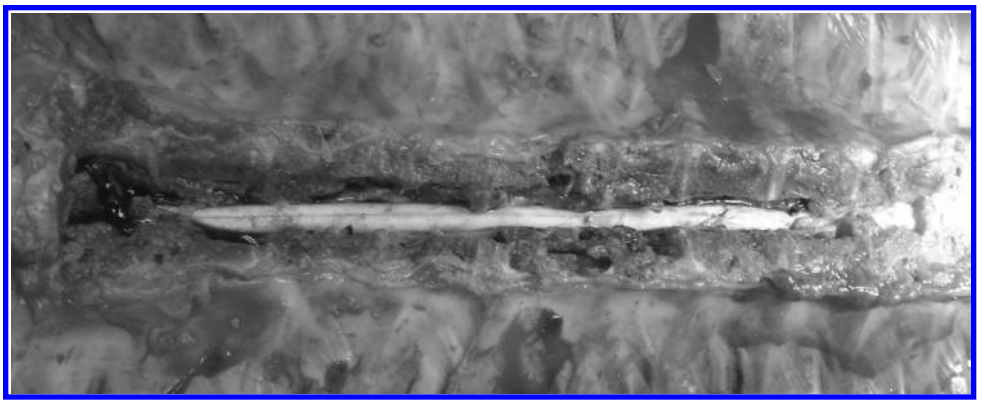

FIGURE 20.40 Exposed thoracic spinal cord, ventral view. Spinal cord was approached from the body cavity, not from the dorsal surface.

the brain is gently lifted out of the calvarium while cutting the optic nerves, the pituitary stalk, and remaining cranial nerves. Transect the brain along the midline which will allow fixative to penetrate the ventricles and place into formalin. The pituitary can be removed after removal of the sella turcica.

The brain is similar to that of the man in that it contains abundant sulci and gyri. Similarities have been shown between human and pig in hippocampus, cortical and subcortical structures, limbic, diencephalic, and brainstem structures (Lind et al., 2007; Sauleau et al., 2009). Variability in neocortex and gyral patterns has been noted between individuals and genders in the pig (Lind et al., 2007). For basic examination of the brain, six to seven sections should be taken in order to examine the following structures: caudate/putamen, cerebellum, cerebral cortex, choroid plexus, hippocampus, hypothalamus, medulla oblongata, midbrain, pons, and thalamus which can be identified using an atlas (Felix et al., 1999).

The spinal cord should be examined in both longitudinal and transverse sections at cervical, thoracic, and lumbar regions. The spinal cord can be accessed from the ventral surface by using a bone saw and cutting through the spine at a 45° angle to the frontal plane (Figure 20.40).

Sections of peripheral nerve should be examined in both longitudinal and transverse sections. The sciatic nerve is one of the largest and easiest to access. A skin incision from the ischiatic spine toward the patella along the femur allows visualization of the superficial gluteal and biceps femoris muscles. Separation of these two muscles reveals the sciatic nerve (Figure 20.41). Careful handling of the nerve will prevent stretch artifacts. Placing an approximately 2-inch (5 cm) piece of tongue depressor under the nerve helps to manipulate the nerve without disrupting the fibers. The tongue depressor along with nerve can be placed directly into formalin.

Skeletal muscle should be examined during necropsy. The skeletal muscle in the pig is similar to other species. Many sites may be sampled, although the psoas muscle is one of the most likely muscles to have lesions associated with porcine stress syndrome (Goedegebuure, 1987). Ensure the sampling site is consistent between animals in a study. Bone is typically sampled by saving a piece of the sternum which also provides a sample of bone marrow. Hematopoietic bone marrow in pigs contains binucleated and multinucleated erythroid precursors in health (Wilcox et al., 2012).

Necropsy Artifacts and Background Lesions

There are several conditions specific to pigs that may be encountered while doing pathological analysis.

Systemic

Thrombocytopenic purpura has been reported in Göttingen pigs in both the United States and European Union. There is no sex predilection (Carrasco et al., 2003; Dincer and Skydsgaard, 2012).

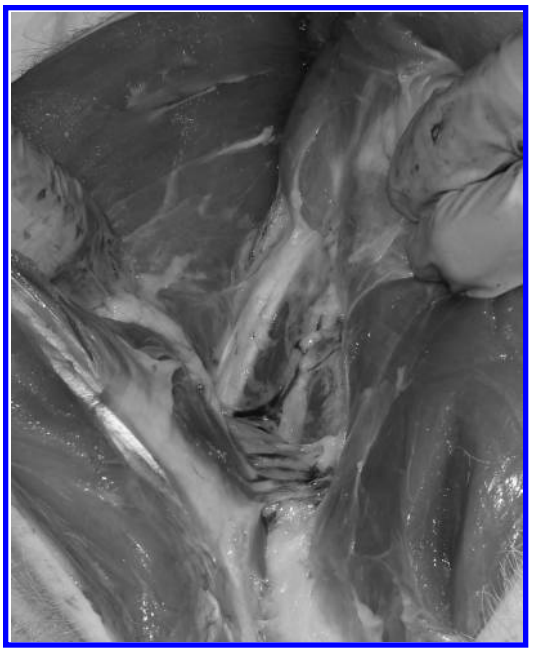

FIGURE 20.41 Separation of superficial gluteal and biceps femoris muscles to visualize the sciatic nerve.

Grossly, animals have extensive multifocal subcutaneous hemorrhage. They have thrombocytopenia and anemia, leading to subcapsular hemorrhage of lymph nodes and hemorrhages of the urinary bladder urothelium (Carrasco et al., 2003). Other lesions include ulceration of the torus pyloricae and hemorrhages in numerous tissues (Carrasco et al., 2003). Vascular lesions are consistently present in renal pelvis and coronary arteries, primarily within small-to-medium muscular arteries which display neointimal proliferation, thrombosis, and medial deposits of myxoid matrix. (Maratea et al., 2006).

Renal glomeruli display membranoproliferative lesions. There are increased numbers of immature and apoptotic megakaryocytes within the bone marrow (Carrasco et al., 2003).

Nasal Cavity

In Yucatans there may be minimal generalized rhinitis with occasional multifocal hyperplasia/degeneration of turbinate epithelium which may interfere with interpretation of results in inhalation studies (Jones et al., 1999).

Thyroid/Thymus

Due to the anatomical location within the cervical region, the thymus and thyroid may be injured during blood collection (Dincer and Skydsgaard, 2012; Rinke, 1997). Thymic granulomatous lesions composed of epithelioid cells, multinucleated giant cells, and lymphocytes are occasionally present. These lesions may be associated with pathogens, but have also been reported to be idiopathic (Baba et al., 2006).

Liver

In the liver of nearly 10% of female Yucatan pigs, there are intralobular hepatocyte nodules which lack normal liver architecture/organization and appear to be an incidental finding (Garlick et al., 2001).

Chronic necrotizing cholecystitis/hypoplasia/aplasia—Animals with this entity display no associated clinical signs or changes in clinical pathology parameters. The gallbladder is slightly diminished in size with thickened walls. Bile is absent, or if present is thickened. Histology shows diffuse necrotizing, hemorrhagic mucosal layer with granulomatous inflammation extending into

TABLE 20.4
Common and Rare Background Lesions in Research Pigs

System	Lesion	Notes	References
Cardiovascular Respiratory	Arteritis and periarteritis	Especially in vessels of the mesentery, epididymides, intestines, kidney, lung, spleen, heart, stomach, and urinary bladder	Dincer and Skydsgaard (2012)
	Minimal chronic interstitial pneumonia		Dincer and Skydsgaard (2012)
	Bronchopneumoniae		Svendsen et al. (1998)
	Alveolar hemorrhage		Svendsen et al. (1998)
	Presence of alveolar macrophages		Svendsen et al. (1998)
	Focal mineralization		Svendsen et al. (1998)
Musculoskeletal	Myositis of the tongue, quadriceps, others	Degen, regen	Dincer and Skydsgaard (2012) Svendsen et al. (1998)
	Bone marrow may have serous atrophy of fat cell	Common	Svendsen et al. (1998)
Gastrointestinal	Erosions ulcerations of glandular stomach	Especially at pyloric-duodenal junction	Dincer and Skydsgaard (2012)
	Acute to chronic inflammation of intestinal mucosa		Dincer and Skydsgaard (2012)
Liver	Liver often has focal to multifocal mononuclear cellular infiltrates, cytoplasmic vacuolation possible		Dincer and Skydsgaard (2012)
	Focal inflammatory cell infiltrate		Svendsen et al. (1998)
	Hematopoiesis	In young (<4 wks old)	Svendsen et al. (1998)
	Interlobular hepatocyte nodules that lack normal hepatic architecture	Primarily in female Yucatans	Garlick et al. (2001)
Gallbladder	Edema, hypoplasia, and cholecystitis	All entities are rare	Svendsen et al. (1998), Dincer and Skydsgaard (2012)
Renal	Mononuclear infiltrates; tubular basophilia (regeneration) common glomerulosclerosis (ref).		Svendsen et al. (1998), Vezzali et al. (2011)
Hematopoietic and lymphoid systems	Sinusoidal hemorrhage is common in mandibular LN	Likely due to blood sampling	Dincer and Skydsgaard (2012)
	Minimal reactive histiocytosis		Dincer and Skydsgaard (2012)
	Eosinophils are commonly seen especially in the mesenteric lymph nodes	Levels can be increased when there is a hematopoietic demand.	Dincer and Skydsgaard (2012)
	Spleen—atrophy is rare, but may occur with stress or weight loss	Congestion can be agonal dependent on mode of death	Dincer and Skydsgaard (2012)
Adrenal glands	Cortex may have extramedullary hematopoiesis		Dincer and Skydsgaard (2012), Svendsen et al. (1998)
Skin	Mild focal infiltrates—edema, crusts on epidermal surface		Dincer and Skydsgaard (2012) Svendsen et al. (1998)
Brain	Mineralized foci particularly in cerebral/cerebellar leptomeninges		Dincer and Skydsgaard (2012) Svendsen et al. (1998)

the muscular layer (Dincer and Skydsgaard, 2012). While this is a background finding, bile is important in drug metabolism and this entity may affect toxicological outcomes.

Kidney

Focal, mild spontaneous glomerulonephritis is present in some Göttingen pigs (4/154) (Vezzali et al., 2011).

Skeletal Muscle

Myositis/myonecrosis in skeletal muscle—The quadriceps femoralis, which is routinely sampled in toxicology studies, is commonly affected along with other skeletal muscles. Changes include degenerate and regenerating myofibers (Dincer and Skydsgaard, 2012).

Adipose

Serous atrophy of fat cells in bone marrow—In Göttingens, bone marrow may contain homogenous eosinophilic material between diminished or degenerated adipocytes. Presence of this eosinophilic material is accompanied by decreased amounts of hematopoietic tissue (Dincer and Skydsgaard, 2012).

Reproductive Tract

Male reproductive tract—Unilateral or bilateral testicular tubular hypoplasia/atrophy is a common background finding in pigs. Small foci of interstitial mononuclear cells, primarily lymphocytes, are often found within the prostate.

Female reproductive tract—Uterine lesions in aging pet miniature pigs are common, and since many of the minipigs have similar genetic background, it is not unreasonable to assume similar lesions will develop in minipigs in research or toxicology studies. Diffuse endometrial hyperplasia is the most common lesion (75%), with nearly 50% having smooth muscle tumors of the uterus; 30% have nodular lesions (Ilha et al., 2010).

Congenital

The most common malformation in Göttingens is cryptorchidism, followed by syndactyly, muscular-joint contracture, cleft palate, ventricle septal defects, and diaphragmatic hernia. Cryptorchidism, syndactyly, and septal defects were determined to be heritable defects (Berggren and Jensen, 2008). Background lesions in many pig breeds include those found in Table 20.4 (opposite page).

CONCLUSIONS

This is only an overview of the techniques and methods to be used in a porcine necropsy. Every situation is different and may call for more diligence or tissues to be sampled than presented here. There are many techniques for doing a necropsy and those presented are the preference of the author. Knowledge of porcine anatomy is essential in order to complete successful necropsies and prevent unnecessary tissue artifacts.

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FDA (United States Food and Drug Administration) 2009b. #G95-1 Table 1 Initial Evaluation Tests for Consideration. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm080742.htm

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21 Use of Swine in Biomedical Research

Niels-Christian Ganderup

CONTENTS

INTRODUCTION

This chapter will provide the reader with an introduction to the pig as a biomedical research animal model, providing an overview of their historic uses in biomedical research leading up to the beginning of the last century. From that point onward (ca. 1900), the focus will shift from a historical, and somewhat anecdotal, overview to a meta-analysis of the use of pigs in research, as documented by the published literature with a focus on major areas of use, including geographical and institutional patterns of publication and funding patterns. Finally, the number of pigs used in research in four major geographic regions will be presented.

Domestication of Pigs

The classification of pigs is covered in Chapter 1 of this book and shall not be repeated here. There is general consensus that the domesticated pig, and hence all forms and variations of pigs/swine (mini/micro/miniature) descend from the Eurasian wild boar. The Eurasian wild boar (*Sus scrofa scrofa*) and the Asia wild board (*Sus scrofa vittatis*) are considered to be the two key strains in the domestication of the pig (Groenen et al., 2012; Pfeiffer, 1978). Domestication took place in distinct geographical locales and historical times. The first evidence of domestication is dated 9000–10,000 years ago in what is now the Middle East. From there pigs spread with humans throughout northern Africa, Greece and finally throughout what is now eastern and central Europe (Bökönyi, 1974; Groenen et al., 2012). In East Asia evidence of domestication dates back 7000 years (Jones, 1998; Porter, 1993). The main driver for domestication of pigs was man's gradual shift from a nomadic life as hunter-gatherers to a more settled life where the pig served as a staple source of protein (Jones, 1998). However, pigs were used for much more that meat. In fact, most of the pig's body was used: bones and tusks were turned into tools and jewellery; leather was used to make saddles and clothing; and pig fat was turned into lamp oil (Doll, 2003; Xylouri-Frangiadaki et al., 1997). In recent times pigs have been used for truffle hunting (Sullivam, 1982) and sniffing out narcotics (Burke, 1993).

Even though the pig is a very robust and adaptable species, it is not indigenous to all regions of the world. In some regions, for example, Denmark, wild pigs have been hunted to extermination and thus do not exist in the wild, while in regions without a native pig population domestic pigs have escaped from captivity to form feral pig populations. Some of these feral populations can be traced back to pigs introduced by explorers, who brought them on ships for long journeys as living provisions. They subsequently escaped captivity or were left in a certain locale to be recaptured on the return journey (see e.g. the Ossabaw Island Miniature Swine elsewhere in this book). Adaptability and hardiness enables pigs to survive in most regions of the world, even in places where it is not indigenous, placing swine among the 100 most invasive species (Anonymous, 2010). The reader is referred to Giuffra et al. (2000), Larson et al. (2005, 2007) for additional information regarding domestication of pigs. Information about phylogenetic relationships of pig populations and genetic diversity can be found in Scandura et al. (2011), Célio Alves et al. (2010), Kim et al. (2002), and Rothschild and Ruvinsky (2011). Pig genetics is covered in Rothschild and Ruvinsky (2011) and a detailed account of the wild pigs of North America can be found in Mayer and Brisbin (2008).

Breeds of Pigs

The exact number of pig breeds in existence is uncertain and depends on how a breed is precisely defined. An overview of existing domestic pig breeds follows. A unique entry point for learning more about the various breeds of domestic pigs is maintained under the auspices of The Pig Site (http://www.thepigsite.com/info/swinebreeds.php). It contains detailed information about 73 breeds of domestic pigs from all over the world (see Table 21.1). For most breeds the site presents a short overview of its history and unique characteristics, pictures, and references. The majority of pigs in Table 21.1 are primarily used for consumption rather than biomedical research. This leads to a significant confounder when domestic pigs are used in biomedical research since descriptions often refer to "domestic pigs" rather than specification of a particular breed. This makes analysis of the specific breeds of domestic pigs used in research uncertain, thus the analysis below encompasses all swine without respect to breed.

Breeds of Minipigs

Although this chapter focusses on pigs, the minipig plays a pivotal role in biomedical research primarily for chronic studies and safety assessment of medicinal products. A recent publication names 16 minipig breeds (Köhn, 2012), but numbers exceeding 20 have been reported (Panepinto, 1996). The most commonly used minipig breeds are listed in Table 21.1. All minipig breeds are classified as *Sus scrofa* and are treated as a single breed. There are obvious differences (e.g., size) but basic anatomy and physiology remain constant between the minipig and large breeds.

Minipigs are not as widely available as domestic pigs. In fact, compared to domestic pigs their availability is limited (see Figure 21.1). To learn more about minipigs breeds, their history, and for a list of major distributors, see Köhn (2012).

Use of Pigs in Early Biomedical Research

Dissection of animal bodies to better understand "the human situation" has been practiced for approximately two millennia as has the use of human subjects (Bouchet, 1996; Dooley, 1973). Galen of Pergamum (ca. 129–199), viewed by some as the founder of the discipline of experimental anatomy (Bouchet, 1996) and physiology (Marketos and Skiadas, 1999), was a highly prolific author of several hundred volumes, many of which deal with anatomy, physiology, and pharmacology. His investigations into the brain and spine (Gross, 1998; Marketos and Skiadas, 1999; Pearce, 2008) are noteworthy and include the following concepts: (1) that the brain and spinal cord initiate muscle contraction through nerves (Bennett, 1999; Lopez-Munoz and Alamo, 2009); (2) the location of the pituitary and thyroid glands and the notion of "brain–thyroid" interaction (Toni, 2000); and

(3) ventricular theory of cognition (Rose, 2009). His work included the use of both apes and pigs (Galen of Pergamon, 1962; Maehle and Tröhler, 1987; Paton, 1993). Even though he is claimed to be the first to conduct anatomical studies on human subjects, the extent of which is disputed, much of his knowledge is said to have come from tending wounded gladiators rather than dissection of human cadavers (Figure 21.2) (Dooley, 1973).

None the less, his work remained pivotal to the understanding of the human body for centuries until the appearance of Flemish anatomist Andreas Vesalius (1514–1565) and his famous work *De Humani Corporis Fabrica Libri Septem* (*Seven Books on the Structure of the Human Body*, Vesalius, 1543). Vesalius revolutionized the understanding of the human body through systematic and meticulous dissection of human and animal (dog, pig, and ape) cadavers but also through vivisection of animals (Figure 21.3). As an additional note in this treatise, Vesalius claims that Galen could not have performed dissections on human cadavers because of Galen's errors, which became evident to Vesalius during his dissections (Benini and Bonar, 1996).

Since the time of Vesalius, pigs have been used continuously in research. A detailed account of landmarks in medicine and biomedical research involving pigs is beyond the scope of this chapter. The reader interested in learning more about vivisection and animal experimentation is referred to Leffingwell (2012), entitled "Vivisection," a reprint of a landmark publication on the topic from 1923. This book is complemented by "Vivisection in a historical perspective" by Rupke (1990), which covers the topic from antiquity to the 1920s.

TABLE 21.1
Pure Breeds of Domestic Pigs

A
American Landrace
American Yorkshire
Angeln Saddleback
Arapawa Island

B
Ba Xuyen
Bantu
Banza
Beijing Black
Belarus Black Pied
Belgian Landrace
Bentheim Black Pied
Berkshire
Black Slavonian
British Landrace
British Lop
Bulgarian White

C
Cantonese
Chester White
Czech Improved White

D
Danish Landrace
Dermantsi Pied
Duroc
Dutch Landrace

F
Fengjing
Finnish Landrace
French Landrace

G
German Landrace
Gloucestershire Old Spot
Guinea Hog

H
Hampshire
Hereford
Hezuo

I
Iberian
Italian Landrace

J–K
Jinhua
Kele
Krskopolje
Kunekune

L
Lacombe
Large Black
Large Black-White
Large White (Yorkshire)
Lithuanian Native

M
Mangalitsa
Meishan
Middle White
Minzhu
Mong Cai
Mukota
Mora Romagnola
Moura
Mulefoot

N
Neijiang
Ningxiang
Norwegian Landrace

O
Ossabaw Island
Oxford Sandy and Black

P
Philippine Native
Pietrain
Poland China

R–S
Red Wattle
Saddleback
Spots
Swabian-Hall Swine
Swedish Landrace
Swallow Bellied Mangalitza

T
Tamworth
Thuoc Nhieu
Tibetan
Turopolje

V–W
Vietnamese Potbelly
Welsh
Wuzhishan

Source: Created for www.thepigsite.com by The British Pig Association and Nebraska State University. With permission.

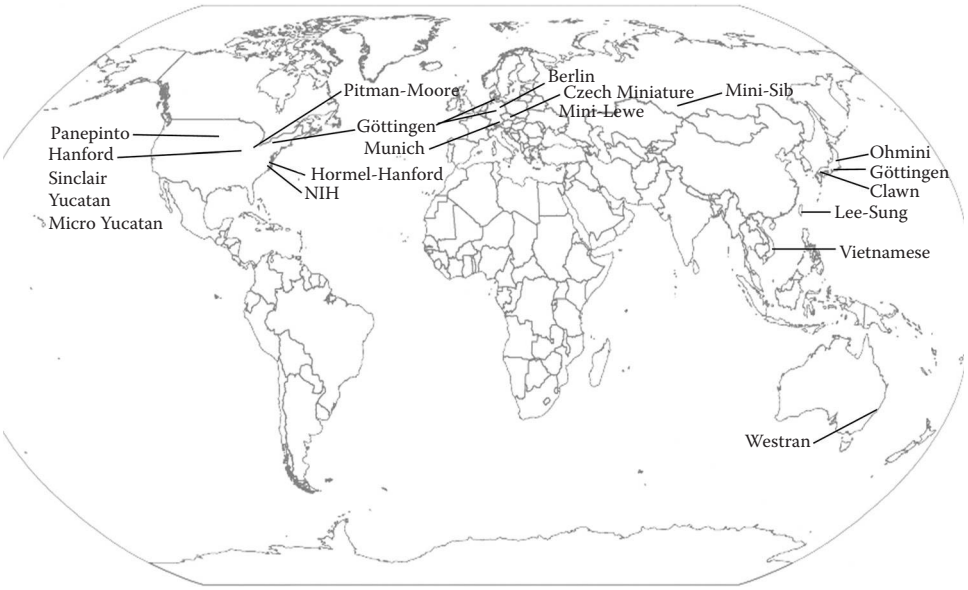

FIGURE 21.1 Global distribution of minipig breeds used in biomedical research. (From Köhn, F., 2012. *The Minipig in Biomedical Research*, Boca Raton: Taylor & Francis, pp. 3–16. With permission.)

The use of pigs after 1900 can be documented based on citations for published scientific literature through searchable online databases from around 1900 onwards. A meta-analysis of the published literature that included the pig as an animal model has been performed to provide a better understanding of research use of swine.

Analysis of Publications Using the Pig Model

The following section describes foci of swine research geographically, by journal, and by organization. Furthermore, an overview of major areas of research involving pigs is presented.

Unless otherwise noted, analysis was done using Science Citation Index Expanded (SCI-E, part of the Web of Science, © 2013 Thomson Reuters) database, which covers over 8300 major journals

FIGURE 21.2 Galen performs a surgical procedure on a pig tied to a table while several men (some identified by name) observe. On the right another pig is carried toward the operating area. (From *Galeni opera ex sexta Juntarum editione*, 1586. Open Source. Reprinted from the National Library of Medicine. With permission.)

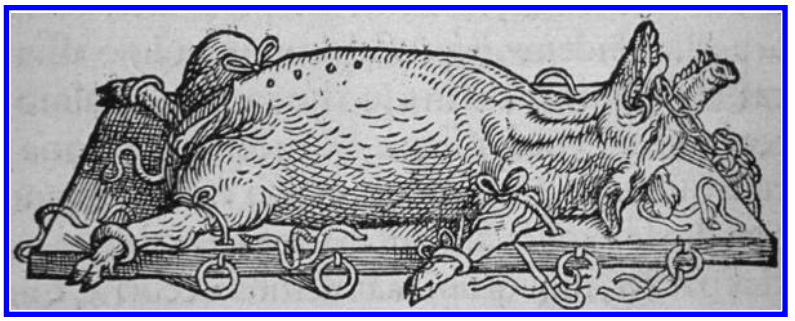

FIGURE 21.3 Pig on dissection table (From *De humani corporis fabrica* (Liber VII, p. 661) 1543. Open Source. Reprinted from the National Library of Medicine. With permission.)

within the natural sciences with searchable coverage from 1900 to the present. Data analysis was done using the filters and options available in the Science Citation Index Expanded database and results were exported for further analysis and graphical representation.

Anecdotally, the oldest article referenced in PubMed is from 1851 (Bree) and recounts observations of parasites recovered from a pig's lung. In 1852 a book was published which contained information on housing, management, and diseases of swine (Martin, 1852) that was agricultural in context. Although not comparable to housing and welfare practices of today, the topic of this early text underscores the fact that the need for information about proper care of pigs is not new.

Data was extracted from SCI-E search for "pig" while excluding citations with "NOT guinea" in the topic field. The analysis was done over a period of two weeks, during which time publications were added, causing the number of citations to increase from 124,981 to 125,380. This small increase does not affect the outcome of the calculations, and for convenience, the number of citations will simply be referred to as 125,000. The data set is analyzed from different perspectives, beginning with basic characteristics such as number of publications per year, citation types, and language.

For languages used in publications involving swine, the data confirms that English is the dominant language for communication of scientific research (see Table 21.2).

The message to researchers is clear: to be read and recognized in a global research community one has to write in English. Although almost one in 20 papers is written in German, it is not likely that the global research community comprehends German to the same extent as it does English.

TABLE 21.2
Top-10 Languages Used in Publications Using Swine

Language	%
English	91.7
German	4.3
French	1.2
Portuguese	<1
Czech	<0.5
Spanish	<0.5
Hungarian	<0.5
Polish	<0.5
Dutch	<0.5
Russian	<0.5

TABLE 21.3
Top-5 Document Types Cited in SCI-E (1900–2012) Using the Pig as Model Animal

Document Type	%	Comment
Article	79.1	Original research
Meeting abstract	9.9	Summation presented at a symposium or conference
Proceedings paper	4.9	Published literature based on conferences, symposia, etc.
Review	2.6	Renewed study of material not presenting any new information
Note	1.6	A paper that mentions or remarks on a published paper
Total	98.1	

The percentages presented in Table 21.2 cover many categories of scientific literature within SCI-E, and there may well be specific areas where there is a slight difference in percentages, but overall, English is the de facto primary language used in scientific communication.

From the 125,000 citations identified on SCI-E, original research is the main type of scientific communication followed by meeting abstracts, proceedings papers, and reviews (see Table 21.3).

The Top-5 document types cover 98.1% of all publications involving pigs, and the 1.9% not covered includes book reviews, editorial material, and corrections. It is evident from the numbers in Table 21.3 that primary research dominates the publications involving swine, accounting for almost 80% of all citations across different subject areas.

The number of citations for pig on SCI-E can be seen in Figure 21.4. The period includes a total of 125,380 citations dating from 1/31/1900 to 12/31/2012. For clarity, and because prior to 1950 the number of citations per year was low, only citations from 1950 to 2012 are depicted. It is evident that in the 1950s, there was a stable but low output of pig citations, probably as a result of the research community regaining momentum after the Second World War. Throughout the 1960s, there was

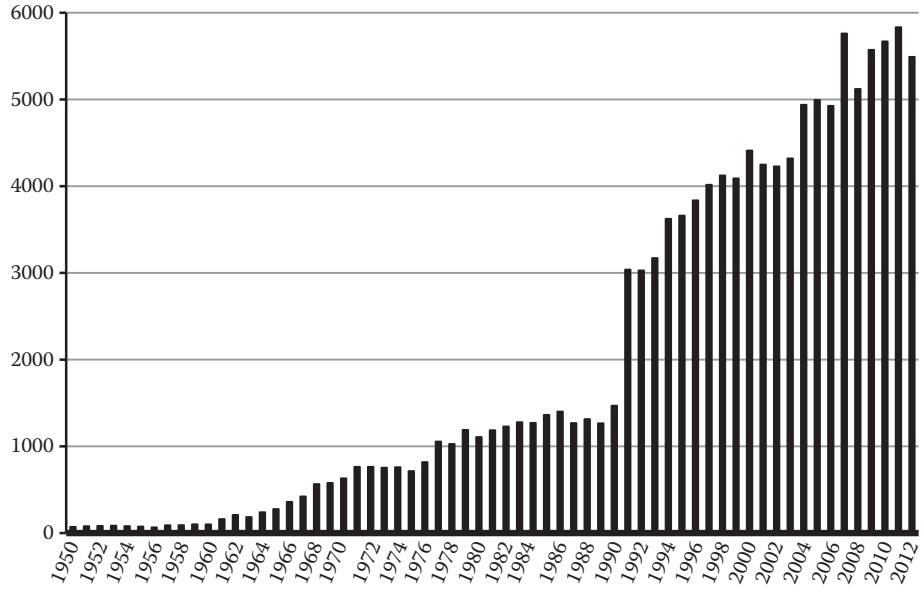

FIGURE 21.4 Number of citations (*Y*-axis) for pig by year (1950–2012) on SCI-E (*X*-axis).

a steady increase in the number of citations, exceeding 1000 citations/year in 1977 and all years subsequently. There was a sharp increase in the number of citations from 1468 in 1990 to 3038 in 1991. The exact reason for this is unknown, although it seems likely to have resulted from a significant expansion of the number of sources (i.e., journals) included in the SCI-E. Other publication milestones were attained in 1991 with over 3000/year, more than 4000 in 1997 and over 5000/year since 2007 and all subsequent years.

The number of publications using pigs has grown significantly over the past decades and in recent years has produced between 5121 and 5833 citations per year, corresponding to 14–16 citations per day.

Figure 21.5 depicts the geographical distribution of citations from the top 20 contributing countries. The largest single contributing country is the United States followed by Germany and England, with the subsequent 17 countries in decreasing order. Of the top 20 countries, the United States has an aggregate contribution of 29.6%, Europe 43.5% while remaining countries contribute 12.9%, for a total of 86.0% of all citations. Even though the United States is the single largest contributing country in terms of total activity, research involving pigs is skewed towards Europe.

The numbers presented in Figure 21.5 are absolute numbers. Converting the numbers to per capita would change the order of contributions as some of the countries in the Top-20 have relatively small populations, for example, Denmark, the Netherlands and Sweden. The smaller countries would receive a higher ranking, while China would be ranked near the bottom. The United States has a large contribution to the total number of citations and a large population, so would be in the mid-range.

The Top-20 academic research institutions measured as percentage of total citations originating from a given academic institution are depicted in Figure 21.6. It may seem contradictory that so few U.S. institutions are in the Top-20 since the United States is the number one contributor to the number of citations. This is because the United States has many universities which aggregate to a high number of citations but the contributions of individual institutions are not sufficient for inclusion in the Top-20.

It is noteworthy that all institutions in the Top-20 are based in North America or Europe. The aggregated per cent output of the total citations from North America and Europe is close, 12.9% and 9.7%, respectively. The geographical bias (North American and European dominance) seen in Figure 21.6 is likely caused by the extensive use of pigs as a consumption animal which gave rise to a voluminous body of research in this area (see Figure 21.7). This in turn facilitated the use of pigs

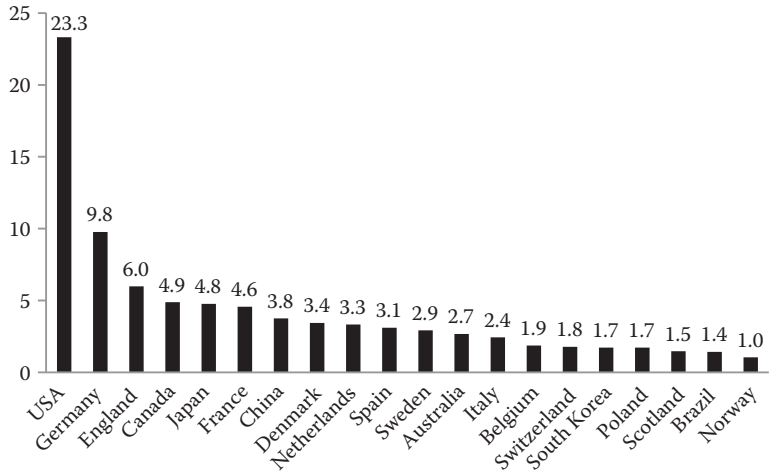

FIGURE 21.5 Top-20 contributor countries (*X*-axis) to pig citations (as per cent of total, *Y*-axis). The numbers are based on total numbers of publications originating from individual countries.

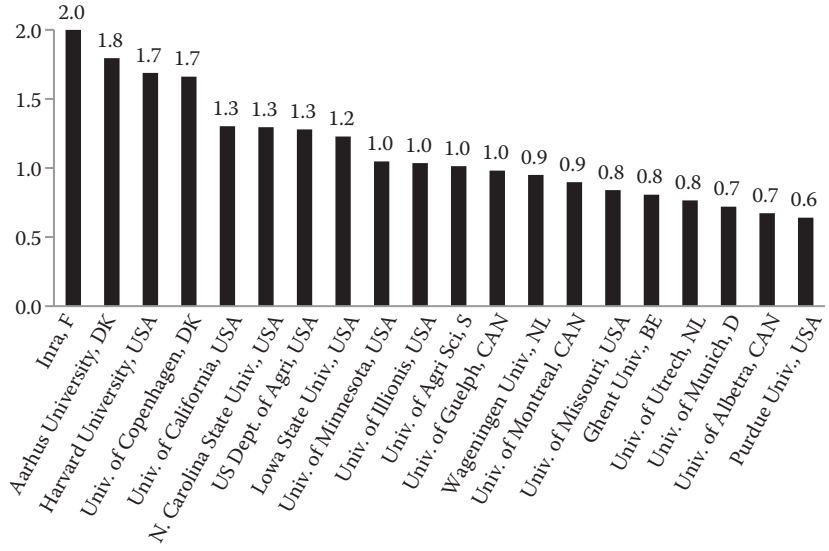

FIGURE 21.6 Top-20 citations by academic institution (*X*-axis) as percent of total publications (*Y*-axis). INRA: French National Institute for Agricultural Research; US Dept. of Agri: US Department of Agriculture; Univ. Agri Sci: Swedish University of Agricultural Sciences; F: France; DK: Denmark; USA: the United States of America; S: Sweden; NL: the Netherlands; CAN: Canada; BE: Belgium; D: Germany.

in other areas of research since practices for housing, handling, and experimental manipulation had been established for agricultural research.

Most citations are attributed to the *Journal of Animal Science*, which publishes articles on animal production and fundamental aspects of genetics, nutrition, and physiology not directly related to life science research (see Figure 21.8). Biomedical research is also heavily represented in the list

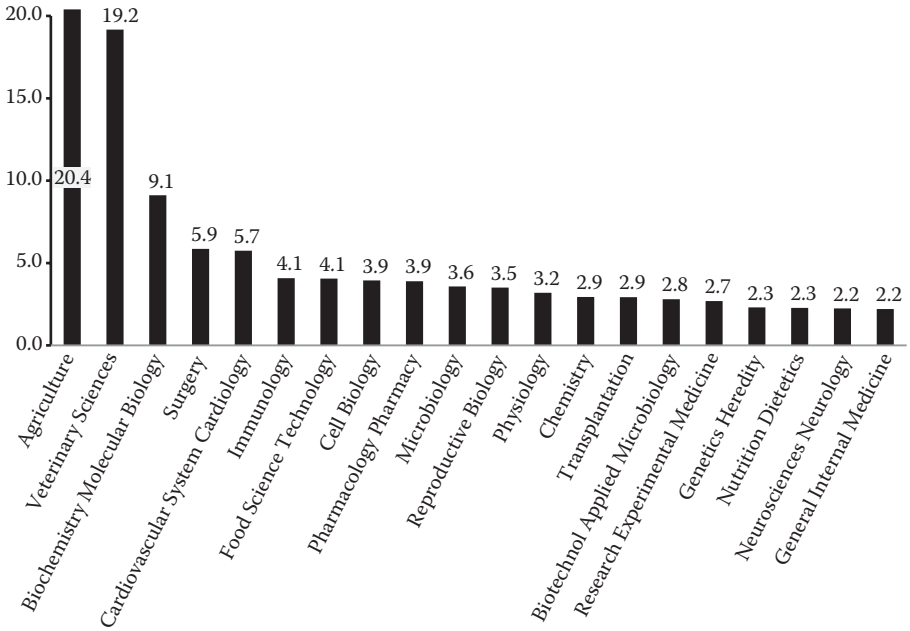

FIGURE 21.7 Top-20 research areas (*X*-axis) per cent of total citations (*Y*-axis).

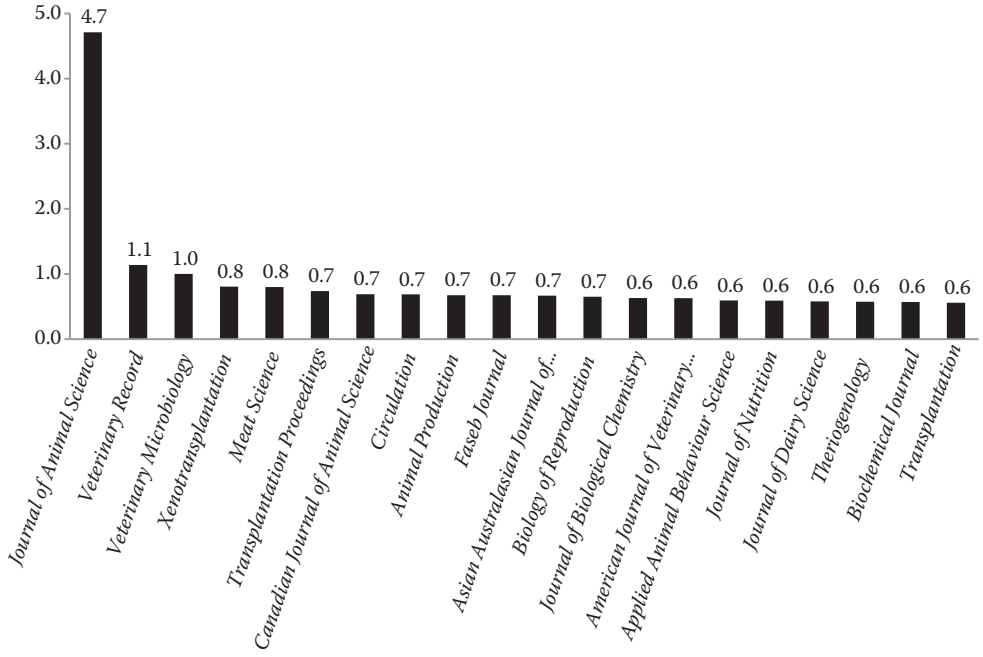

FIGURE 21.8 Top-20 journals (*X*-axis) with pig citations per cent of total number of citations (*Y*-axis). (*Asian Australasian Journal of Animal Science*; *American Journal of Veterinary Research*.)

covering topics such as xenotransplantation, circulation, and transplantation. However, many biomedical research journals are not included in the Top-20 journals.

Areas of research employing pigs are described in Figure 21.7. Research areas are predefined by SCI-E and each citation is categorized into one or more areas. This makes it possible to break down the total output into areas and rank them. As the same citation can be allocated to several areas, the total number of citations by research area is larger than the number of actual citations (articles, reviews, etc.; see Table 21.3).

The single largest area of swine research is in agriculture. The importance of domesticated swine produced for consumption is evident in the research areas where they are used as well as the number of citations in those areas. Within biomedical research, use of swine in surgery accounts for 5.9% of all citations. Other areas in biomedical research which uses swine extensively include cardiovascular research, cell biology, pharmacology, and physiology. Interest in utilizing the pig in xenotransplantation has driven the emphasis on studies of the porcine immune system.

The pig has a strong presence in biomedical research and is a versatile and relevant model which can help researchers probe, describe and understand the human situation. Europe and North America are hot spots for porcine research due to the number of citations by country and distribution of academic institutions which serve as power houses for swine research.

Increased Use of Pigs in Biomedical Research

In biomedical research, it is incumbent upon researchers to use the most relevant animal model and the fewest number of animals anticipated to provide meaningful results. Experiments should be designed to minimize pain, suffering and distress while meeting scientific aims and ensuring study integrity. In all instances animal experiments should be sanctioned by an independent entity, for example, an IACUC and/or national animal research council. Such entities ask the "what" and the "how" but, most importantly, they ask the "why" behind any animal experiment, advocating on

behalf of the animal. This ensures the conduct of only animal experiments likely to produce relevant and valuable knowledge for human health and safety, and that all procedures are performed as humanely as possible. Given the aforementioned considerations, it seems reasonable to assume that the use of any experimental animal contains an explicit screening of its scientific relevance, a de facto authorization of its suitability and likelihood to provide answers to the questions asked. With this in mind it is evident that the number of experimental animals used, regardless of species, implicitly tells a story about the scientific relevance and suitability of that species in biomedical research. This following discussion gives an overview of the use of pigs over time in three geographical regions.

The use of pigs in biomedical research in the European Union, North America (Canada and the United States) and Japan has been published (Ganderup et al., 2012). Data for non-human primates, dogs and swine have been collated for comparison (see Figure 21.9).

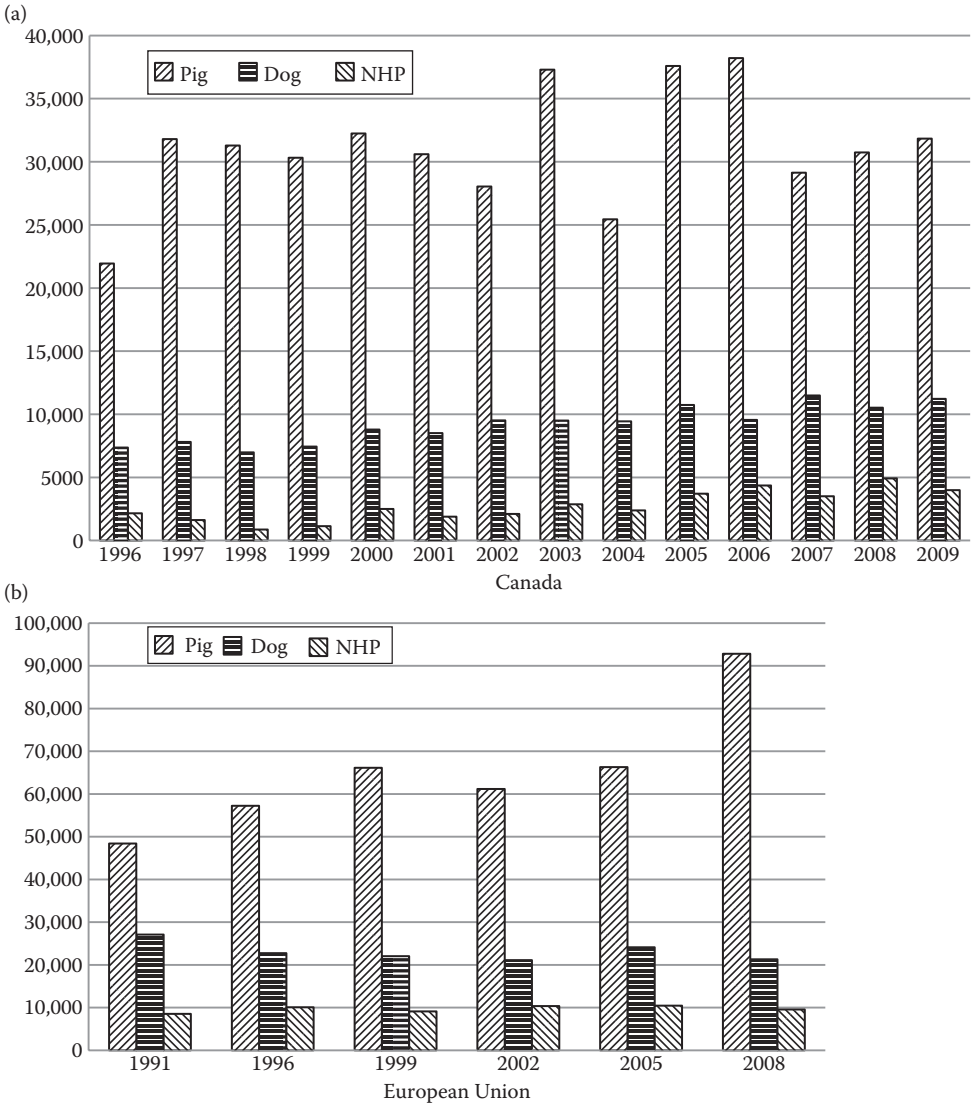

FIGURE 21.9 Use of dog, pig, and nonhuman primate (NHP) in Canada (a), European Union (b). (From Ganderup, N.C. et al., 2012. *International Journal of Toxicology*, 31:507–528. With permission.) (*Continued*)

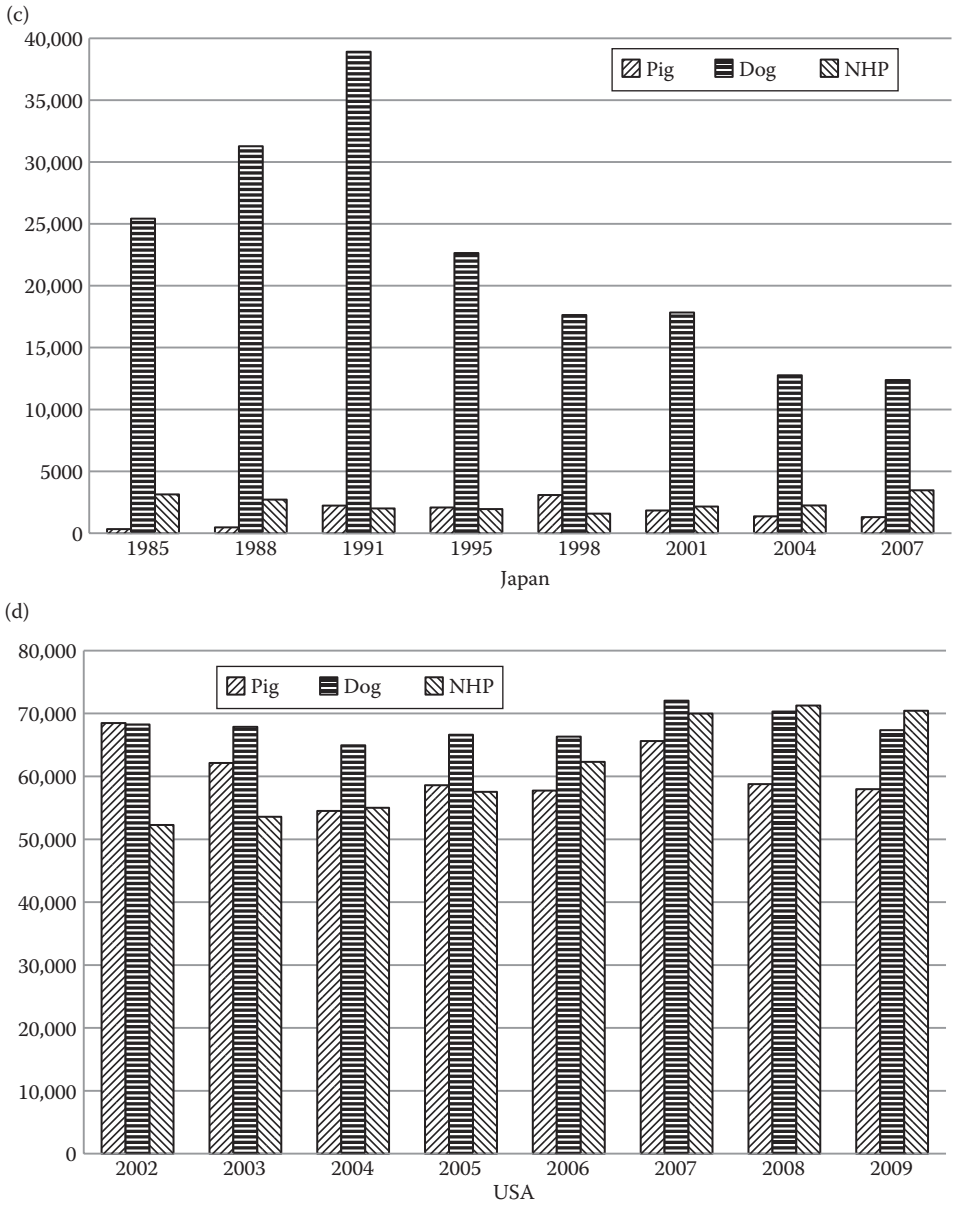

FIGURE 21.9 (*Continued*) Use of dog, pig, and nonhuman primate (NHP) in Japan (c), and the United States (d). (From Ganderup, N.C. et al., 2012. *International Journal of Toxicology*, 31:507–528. With permission.)

The key point is that pigs are used extensively throughout Europe and North America, but much less so in Japan. Tens of thousands of swine are used annually in North America and Europe. There is no indication of future decreased usage of pigs and as the corpus of data continues to grow the use of pigs continues to expand. For selected years, data were captured which compare the use of pigs to non-human primates and dogs in the three regions (Figure 21.10); a clear growth in the use of pigs is evident. For a detailed discussion about data acquisition and limitations, the reader is referred to the original paper (Ganderup et al., 2012).

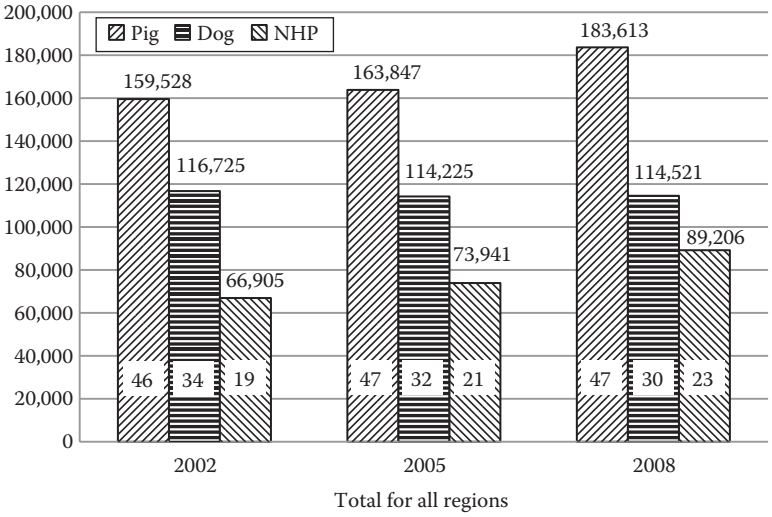

FIGURE 21.10 The total use of dog, pig, and NHP in the four regions for the years 2002, 2005, and 2008. From 2002 to 2008, the use of pigs increased by 15% and the use of dogs was more or less constant (down 2%) while the use of NHPs grew 33%. (Numbers above columns are number of animals; numbers inside columns are per cent of a given year, total for each year equals 100%.) (From Ganderup, N.C. et al., 2012. *International Journal of Toxicology*, 31:507–528. With permission.)

A Word about Minipigs

Minipigs are, from a basic anatomical and physiological perspective, similar to domestic swine raised for agriculture, except for their size. By virtue of their small size, minipigs offer distinct advantages compared to domestic farm swine for use in chronic investigations. Specifically, they require less feed, smaller pen sizes for housing, are easier to manipulate, and require less test substances. Furthermore, certain strains of minipigs are bred to much stricter health standards than domestic farm breeds. For detailed coverage of the different breeds of minipigs, the reader is referred to Köhn (2012).

Minipigs are predominantly used in safety (toxicology) and efficacy (pharmacology) testing of potential new medicines and medical devices. Detailed coverage of their use in this field is beyond the scope of this text. Additional information on this topic is available (Ganderup et al., 2012; McAnulty et al., 2012).

An indication of the extensive use and versatility of the minipig in toxicologic and pharmacologic testing is provided in Table 21.4, which lists more than 40 marketed drug products where the minipig was used as the non-rodent species.

A detailed analysis of the predictive value of the minipig, that is, how well findings in the minipig translate to findings in humans, can be found in Ganderup (2012) which also describes type and severity of findings and compares them where possible.

CONCLUSION

Domestic swine used in agriculture and pigs used in the biomedical research laboratory have served man in different but equally important ways historically and into the present. Use of swine may still increase significantly, as the body of evidence supporting their suitability for studies in comparative medicine steadily increases. They do have their limitations but as with any animal model the key in successful utilization of the animal model is a thorough understanding of its limitations.

TABLE 21.4
Indications for Which Minipig Was Used to Varying Levels in Toxicological and/or Pharmacological Assessment

Indications	Generic Name	Comments
Psoriasis	Calcipotriene; Calcitriol	Vitamin D analogues
	Calcipotriene–Betamethasone	Vitamin D analogue-corticosteroid
	Clobetasol; Fluocinonide	Corticosteroids
	Tazarotene	Retinoid
Atopic dermatitis	Pimecrolimus; Tacrolimus	Calcineurin inhibitors
Acne vulgaris	Adapalene–Benzoyl Peroxide	Retinoid-oxidising agent
	Tazarotene	Retinoid
	Clindamycin-Benzoyl Peroxide	Antibiotic-oxidizing agent
	Clindamycin-Tretinoin	Antibiotic-retinoid
Actinic keratosis and renal carcinoma	Diclofenac	Cyclooxygenase inhibitor
	Fluorouracil	Thymidylate synthase inhibitor
	Methyl aminolevulinate	Photosensitiser
	Everolimus	Kinase inhibitor (mTOR)
Anti-viral	Kunecatechins (HPV)	Immunomodulator
	Enfuvirtide (HIV)	Fusion inhibitor
Lentigines and melasma	Mequinol–tretinoin	Tyrosinase inhibitor-retinoid
	Fluocinolone Acetonide-hydroquinone-tretinoin	Corticosteroid-tyrosinase inhibitor-retinoid
Hirsutism and androgenic alopecia	Eflornithine	Ornithine decarboxylase inhibitor
	Minoxidil	Vasodilator
Diabetes	Insulin detemir	Insulin analogue
Parkinson's and Alzheimer's disease	Rotigotine; pramipexole	Dopamine agonists
	Selegiline	Monoamine oxidase inhibitor
	Rivastigmine	Cholinesterase inhibitor
Osteoporosis	Calcitonin; alendronate; risedronate	Osteoclast-mediated bone resorption
Rheumatoid arthritis	Meloxicam	Non-steroidal anti-inflammatory
Hypertension, heart failure and arrhythmia	Carvedilol	Vasodilator (anti-adrenergic beta blocker)
	Dronedarone	Antiarrhythmic
Anaesthesia and analgesia	Lidocaine	Local anaesthetic (sodium channel blocker)
	Tapentadol	Centrally acting analgesic
Antibiotics	Retapamulin	Pleuromutilin antibiotic
	Quinupristin–dalfopristin	Streptogramin antibiotics
	Telavancin	Glycopeptide antibiotic
Imaging	Sulfur hexafluoride	Contrast agent for cardiac and vascular imaging
Prevention of sunburn	Anthelios®	UV-protection
Miscellaneous	Lanreotide	Somatostatin analogue
	Ecallatide	Kallikrein inhibitor
	Iron sucrose	Iron supplement

Source: From Ganderup, N.C., 2012. *The Minipig in Biomedical Research*, Boca Raton: Taylor & Francis, pp. 573–594. With permission.

Note: Multiple products are separated by semicolon (;), for combination products the active pharmaceutical ingredients are joined by hyphen (-).

Something Galen is cited for having said, albeit with different words: "the structure of the bodies of the animals … resembles the structure of the human body **in some degree**" taken from Marketos and Skiadas (1999).

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Appendix

SECTION I: GROWTH CHARTS, NUTRITION, AND PHYSIOLOGICAL PARAMETERS

TABLE A.1
Minipig Age–Weight Correlations

Hanford		Yucatan		Yucatan	
Age (in Months)	**Weight (kg)**	**Age (in Months)**	**Weight (kg)**	**Age (in Months)**	**Weight (kg)**
1	4–7	1	3–6	1	3–5
2	8–11	2	7–9	2	6–8
3	12–19	3	10–15	3	9–12
4	20–27	4	15–20	4	12–14
5	25–33	5	20–25	5	14–16
6	34–42	6	25–30	6	16–20
8	40–50	8	35–45	8	25–35
10	45–55	10	45–55	10	30–40
12	55–70 (male)	12	55–65 (male)	12	55–65 (male)
	50–65 (female)		45–50 (female)		35–45 (female)

Source: Reprinted from Sinclair Research, Auxvasse, MO. With permission.

TABLE A.2
Reproductive Physiological Parameters

	Microswine	Miniswine
Breed		
Growth	Yucatan	Hanford, Yucatan
Birth weight	600–700 g	600–1000 g
Weight as sexual maturity	15–20 kg	Hanford 28–42 kg; Yucatan 20–30 kg
Adult weight	40–60 kg (at 12–14 months)	68–80 kg (at 2 years)
Life span	10–15 years	10–15 years
Reproduction		
Gestation period	111–114 d	111–114 d
Average litter size	5–6	Hanford 6–8; Yucatan 5–6
Weaning age	28–35 d	28–35 d
Sexual maturity	5–6 months	5–6 months
Breeding age	6–8 months	6–8 months

Source: Reprinted from Sinclair Research, Auxvasse, MO. With permission.

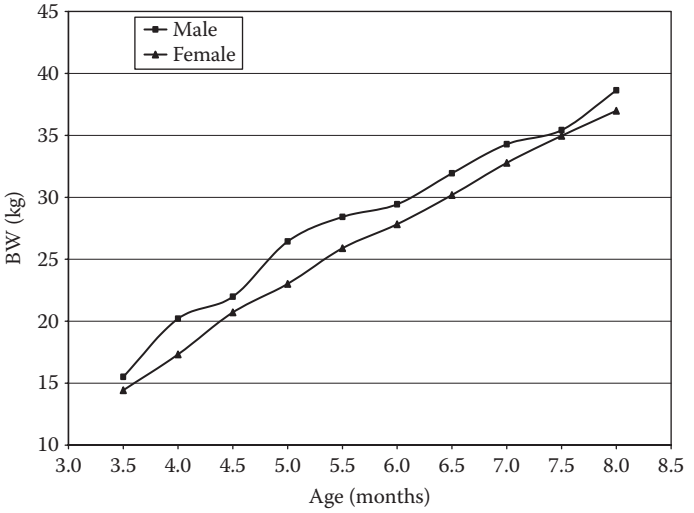
TABLE A.3
Recommended Diets

Age	Model	Recommended Diet[a]
Pigs 1–2 months old	Micro and mini	0.25–0.75 lb/d of a 50/50 mix of starter and maintenance and breeder diet
Pigs 2–3 months old	Micro and mini	0.75–1.25 lb/d, maintenance and breeder diet
Pigs over 3 months old	Micro	1.25–1.75 lb/d, maintenance and breeder
	Mini	1.75–2.2 lb/d, maintenance and breeder

Source: Reprinted from Sinclair Research, Auxvasse, MO. With permission.

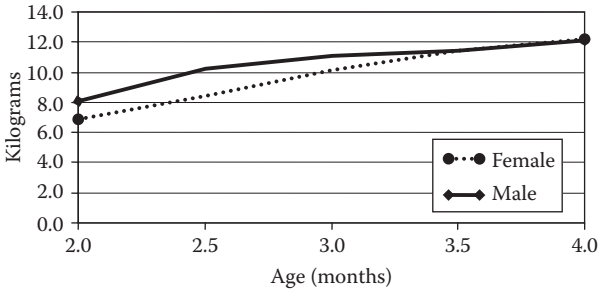
[a] Individual animals and conditions vary, so new users of swine should be observant, especially at first, to get a feel for optimum feed levels. Weight loss and weight gain are the best indicators.

TABLE A.4
Body Weight of Juvenile and Young Adult Hanford Miniature Swine



Source: Reprinted from Sinclair Research Center, Auxvasse, MO. With permission.

TABLE A.5
Body Weight for Juvenile Sinclair Miniature Swine



Source: Reprinted from Sinclair Research Center, Auxvasse, MO. With permission.

TABLE A.6
Weight Development (kg) of the Göttingen Minipig at the Full-Barrier Breeding Facility in Dalmose

Year	Body Weight (kg) 1995
Birth	0.45
1 month	2.7
2 months	5.3
3 months	7.2
6 months	13.7
9 months	17.7
12 months	23.9
18 months	32.4
24 months	34.9

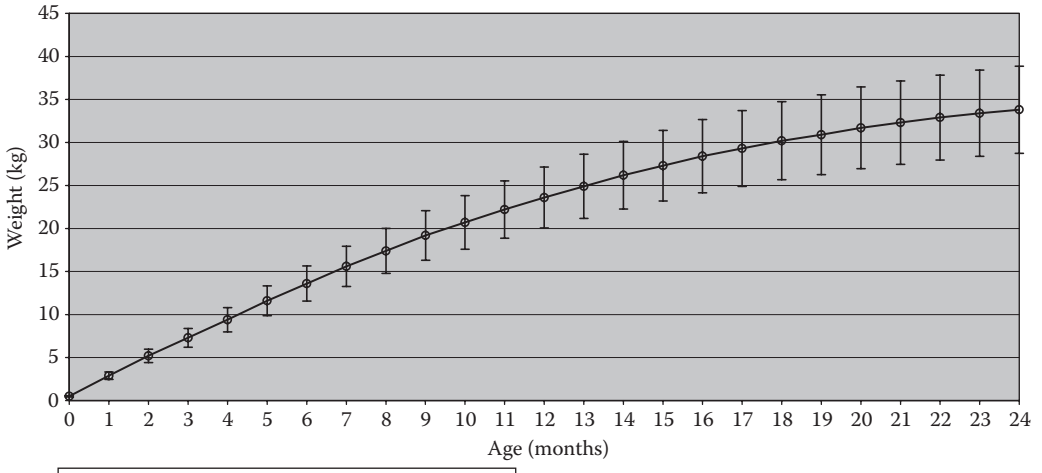
Source: Courtesy of Peter Bollen, Ellegaard Göttingen Minipigs ApS, Dalmose, Denmark. Reprinted from *Scand. J. Lab. Anim. Sci.* With permission.

TABLE A.7
Breeding Characteristics and Timing of Early Development in the Göttingen Minipig

Length of gestation	114 d
Placentation	Diffuse, epitheliochorial
Puberty	140–170 d
Cycle length	21–22 d
Oestrus length	3 d
Blastocyst formation	Day 5–6
Implantation	Day 11–13
Organogenesis period	Day 11–35

Source: Reprinted from Jørgensen, K.D. 1998. *Scand. J. Lab. Anim. Sci.* 25 (Suppl. 1): 63–75. With permission.

TABLE A.8
The Göttingen Minipig Growth Curve: Mean Weight and Standard Development Birth through Two Years of Age



Weight at birth: 0.45 kg
Age of weaning: 4 weeks (3 kg)
Sexual maturity boars: 3–4 months (7–9 kg)
Sexual maturity sows: 4–5 months (9–12 kg)
Closure of growth lines: 18–22 months (30–33 kg)

Source: From Ellegaard Göttingen Minipigs ApS, Dalmose, Denmark. With permission.

TABLE A.9
Feed Composition and Amount for Normal Growth of Ossabaw Miniature Swine

Age (Months)	Feed Type	Feed (g)	kcal
Up to 1–1.5	Typical Creep Feed or LabDiet Mini-Pig Starter #5080	Ad libitum	up to ~920
1.5	Lab Diet Mini-Pig Grower #5L80 or Starter #5080	270	920
2	Lab Diet Mini-Pig Grower #5L80	300	990
3	Lab Diet Mini-Pig Grower #5L80	350	1155
4	Lab Diet Mini-Pig Grower #5L80	400	1320
5	Lab Diet Mini-Pig Grower #5L80	450	1485
6	Lab Diet Mini-Pig Grower #5L80	500	1650
7	Lab Diet Mini-Pig Grower #5L80	550	1815
8	Lab Diet Mini-Pig Breeder #5082 or Mini-Pig Grower #5L80	800	2400
>8	Lab Diet Mini-Pig Breeder #5082 or Mini-Pig Grower #5L80	~800	~2400–2700

Source: Courtesy of Michael Sturek, PhD, Indiana University.

SECTION II: HEMATOLOGY AND SERUM CHEMISTRY

TABLE A.10
Biochemical and Hematologic Values (Farm Pigs, 3–4 Months)

Parameter	Units	Baseline	Intraop	POD 1	POD 3	POD 7	POD 10
Na	mEq/L	142 ± 1	141 ± 1	142 ± 1	137 ± 4	143 ± 1	139 ± 1
K	mEq/L	4.8 ± 0.2	4.2 ± 0.1	4.7 ± 0.3	4.2 ± 0.3	4.1 ± 0.2	4.2 ± 0.2
Cl	mEq/L	101 ± 1	102 ± 1	98 ± 1	95 ± 6	103 ± 1	99 ± 2
Ca	mg/dL	10 ± 0.1	9.7 ± 0.1	8.8 ± 0.2	9.1 ± 0.3	9.4 ± 0.4	9.4 ± 0.0
P	mg/dL	9.5 ± 0.4	8.8 ± 0.4	8.6 ± 0.5	9.3 ± 1.6	7.5 ± 0.5	7.6 ± 0.7
Fe	µg/dL	137 ± 20	101 ± 21	40 ± 17	152 ± 105	78 ± 17	61 ± 6
Total protein	g/dL	5.6 ± 0.1	4.7 ± 0.1	5.3 ± 0.1	5.6 ± 0.8	5.3 ± 0.2	5.4 ± 0.1
Albumin	g/dL	3.1 ± 0.1	2.5 ± 0.1	2.8 ± 0.1	2.8 ± 0.2	2.7 ± 0.1	2.4 ± 0.1
Globulin	g/dL	2.5 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.8 ± 0.5	2.5 ± 0.1	3.0 ± 0.0
Urea	mg/dL	10 ± 1	10 ± 1	12 ± 3	30 ± 19	11 ± 2	12 ± 1
Creatinine	mg/dL	1.2 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.8 ± 0.9	1.0 ± 0.1	1.1 ± 0.2
Bicarbonate	mEq/L	26 ± 1	25 ± 1	30 ± 0.4	25 ± 1	28 ± 1	33
Hemoglobin	g/dL	11.2 ± 0.3	8.8 ± 0.2	12.2 ± 0.4	9.5 ± 0.8	9.0 ± 0.6	8.7 ± 0.7
Bilirubin	mg/dL	0.15 ± 0.04	0.09 ± 0.02	0.18 ± 0.02	0.15 ± 0.02	0.13 ± 0.02	0.05 ± 0.05
Alkaline phosphatase	IU/L	164 ± 12	148 ± 14	164 ± 9	152 ± 23	126 ± 15	93 ± 4
ALT	IU/L	38 ± 3	32 ± 2	40 ± 7	36 ± 4	43 ± 8	28 ± 13
AST	IU/L	39 ± 3	35 ± 6	60 ± 17	137 ± 4	39 ± 13	24 ± 6
LD	IU/L	622 ± 51	446 ± 39	732 ± 155	654 ± 61	493 ± 18	365 ± 87
Prothrombin time	s	11.2 ± 0.3	—	12.4 ± 0.7	10.8	—	—
Hematocrit	%	40 ± 1	30 ± 1	41 ± 1	37 ± 5	31 ± 2	30 ± 2
Leukocytes	$\times 10^3/\mu L$	21 ± 1	15 ± 1	20 ± 3	19 ± 2	17 ± 1	18 ± 2
Neutrophils	%	57 ± 4	57 ± 3	60 ± 6	65 ± 7	51 ± 6	48 ± 19
Lymphocytes	%	39 ± 4	41 ± 3	37 ± 6	33 ± 8	41 ± 6	46 ± 16
Monocytes	%	3 ± 1	4 ± 1	1 ± 0	1 ± 1	5 ± 2	1 ± 0.3
Eosinophils	%	1 ± 0.4	2 ± 0.2	1 ± 1	2 ± 1	2 ± 0.3	2 ± 1
Band neutrophils	%	1 ± 0.4	1 ± 0.4	1 ± 1	0 ± 0	4 ± 2	—

Source: Reprinted from Drougas et al. 1996. *Lab. Anim. Sci.* 46(6): 648–655. With permission.
Note: POD = postoperative day. Intraoperative values were from isoflurane-anesthetized pigs.

TABLE A.11
Blood Coagulation, Platelet, and Hematocrit Reference Values for Göttingen Minipigs

Parameter	Unit	Males	Females[a]	Fetal	Pregnant
Number of animals (*n*)		9	16	9	22
Age	Months	8 ± 2	11 ± 8	Gestation day 105 of 110	11 ± 3[b]
Hematocrit	%	32 ± 4	33 + 5	30 + 3	31.0 + 5
Platelets	10^9/L	410 ± 90	420 ± 120	n.a.	440 ± 100
Fibrinogen	g/L	7.0 ± 2.0	7.5 ± 3.3	0.4 ± 0.1	4.0 ± 0.5
Fibrinmonomere	mg/L	60 ± 20	—	15 ± 10	40 ± 10
PTT	s	30 ± 7	30 ± 4	45 ± 5	30 ± 8
PTI (Quick)	%human	160 ± 30	180 ± 30	110 ± 20	190 ± 30
AT III	%human	100 ± 10	110 ± 10	35 ± 10	110 ± 20
Factor II	%human	70 ± 10	—	40 ± 10	80 ± 15
Factor V	%human	370 ± 60	450 ± 150	360 ± 120	460 ± 160
Factor VII	%human	80 ± 20	140 ± 40	50 ± 10	100 ± 20
Factor VIII	%human	340 ± 60	340 ± 90	300 ± 160	380 ± 110
Factor IX	%human	200 ± 30	—	90 ± 10	190 ± 50
Factor X	%human	115 ± 15	—	55 ± 10	175 ± 50
Factor XI	%human	80 ± 20	—	20 ± 5	70 ± 20
Factor XII	%human	55 ± 20	—	20 ± 10	80 ± 40
Factor XIII	%human	40 ± 10	—	15 ± 5	40 ± 10
Protein C	%human	50 ± 10	76 ± 18	60 ± 5	80 ± 10
α2-APL	%human	80 ± 10	—	55 ± 5	70 ± 10

Source: From Petroianu, G.A. et al. 1998. *Scand. J. Lab. Anim. Sci.* (Suppl. 1): 211–219. With permission.
Note: Table entries are of the form: Mean ± SD.
[a] Petroianu, G.A., Maleck, W.H. 1997. Blood coagulation, platelets and haematocrit in male, female, and pregnant Göttingen minipigs. *Scand. J. Lab. Anim. Sci.* 24: 31–41.
[b] Samples were taken on gestation day 105 of 110.

TABLE A.12
Hematology of the Göttingen Minipig

Parameter	Abbreviation	Unit		Male (3 Months)	Female (3 Months)	Male (6 Months)	Female (6 Months)
Eosinophils	EOS	%	Mean	0.87	0.93	1.33	2.13
			SD	1.25	1.53	1.18	2.26
Eosinophils	EOS	10^9/L	Mean	0.10	0.10	0.12	0.19
			SD	0.15	0.17	0.11	0.23
Basophils	BASO	%	Mean	0.67	0.53	0.73	0.33
			SD	0.90	0.74	0.88	0.62
Basophils	BASO	10^9/L	Mean	0.08	0.07	0.06	0.02
			SD	0.12	0.1	0.07	0.04
Monocytes	MONO	%	Mean	1.13	1.53	2.00	1.47
			SD	1.25	1.51	1.07	1.19
Monocytes	MONO	10^9/L	Mean	0.14	0.20	0.17	0.14
			SD	0.15	0.21	0.09	0.13
Platelet count	THROMB	10^9/L	Mean	513.1	490.3	348.3	364.5
			SD	88.34	115.2	79.47	51.72
Activated partial thromboplastin time	APTT	s	Mean	45.86	44.08	42.65	43.22
			SD	5.77	9.99	8.17	9.83
Thrombin time	TT	s	Mean	25.8	28.36	23.91	23.69
			SD	4.16	5.00	3.59	4.55
Prothrombin time	ptt	s	Mean	11.71	11.54	11.94	11.65
			SD	0.34	0.46	0.62	0.41
Fibrinogen	FIBR	g/L	Mean	6.5	5.37	6.85	4.80
			SD	1.39	0.66	1.24	0.59

Source: Courtesy of Ellegaard Göttingen Minipigs ApS, Dalmose, Denmark. Reprinted from Ellegaard, L. et al. 1995. *Scand. J. Lab. Anim. Sci.* 22(3): 239–248. With permission.

TABLE A.13
Red and White Blood Cell Parameters for Juvenile (7 Weeks), Young (3 Months)[a] and Adult (Sexually Mature—6 Months)[a] Male and Female Göttingen Minipigs and Pregnant Göttingen Minipigs in Gestation Weeks 8–9, 10–11, and 12–13

			7 Weeks		3 Months		6 Months		Pregnant		
Parameter	**Abbreviation**	**Unit**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Weeks 8–9**	**Weeks 10–11**	**Weeks 12–13**
Number of animals	*n*	—	20	20	15	15	15	15	5	5	5
Hemoglobin	Hb	mmol/L	7.7 ± 0.9	7.9 ± 0.8	7.5 ± 0.4	7.4 ± 0.5	7.9 ± 0.5	7.7 ± 0.6	7.9 ± 0.2	8.8 ± 0.4	7.0 ± 0.5
Red blood cell count	RBC	10^{12}/L	7.7 ± 0.8	7.7 ± 0.8	8.0 ± 0.4	8.1 ± 0.7	7.9 ± 0.6	8.0 ± 0.6	7.5 ± 0.4	7.8 ± 0.8	8.4 ± 0.5
Hematocrit	PCV	mL/100 mL	41 ± 4	42 ± 4	36.8 ± 1.5	36.7 ± 2.3	38.2 ± 1.8	37.6 ± 2.4	38 ± 1	42 ± 2	39 ± 2
Reticulocyte count	RETIC	%	—	—	1.3 ± 0.5	1.7 ± 0.8	1.7 ± 0.9	1.1 ± 0.5	1.1 ± 0.6	1.1 ± 0.4	1.0 ± 0.3
Reticulocyte count	RETIC	10^{12}/L	—	—	0.10 ± 0.04	0.13 ± 0.06	0.13 ± 0.07	0.09 ± 0.04	—	—	—
Mean cell volume	MCV	10^{15}/L	54 ± 4	55 ± 5	46.0 + 3	45.8 ± 4.3	48.4 ± 3.1	46.9 ± 2.3	51 ± 2	54 ± 5	57 ± 4
Mean cell hemoglobin	MCH	fmol	—	—	0.94 ± 0.06	0.93 ± 0.10	1.00 ± 0.09	0.97 ± 0.06	—	—	—
Mean cell hemoglobin concentration	MHCH	mmol/L	19 ± 1	19 ± 1	20 ± 1	20.2 ± 0.5	20.7 ± 0.7	20.4 ± 0.3	21 ± 0	21 ± 0	21 ± 0
White blood cell count	WBC	10^9/L	14.4 ± 3.6	13.8 ± 3.0	12.0 ± 2.5	11.4 ± 1.4	8.7 ± 1.5	8.6 ± 2.2	7.0 ± 1.0	8.2 ± 2.3	7.3 ± 2.7
Neutrophils	NEUTRO	%	35 ± 14	38 ± 11	29 ± 10	29 ± 14	34 ± 8	30 ± 10	36 ± 10	29 ± 9	34 ± 11
Lymphocytes	LYMPHO	%	61 ± 14	58 ± 11	68.8 ± 10.3	67.6 ± 13.9	61.8 ± 7.8	66.1 ± 8.8	59 ± 9	64 ± 9	59 ± 8
Lymphocytes	LYMPHO	10^9/L	—	—	8.19 ± 2.0	7.98 ± 1.4	5.34 ± 1.0	5.65 ± 1.4	—	—	—
Eosinophils	EOS	%	0.7 ± 1.2	0.5 ± 0.8	0.9 ± 1.3	0.9 ± 1.5	1.3 ± 1.2	2.2 ± 2.3	2.0 ± 2.1	2.4 ± 1.9	5.0 ± 3.3
Eosinophils	EOS	10^9/L	—	—	0.10 ± 0.15	0.10 ± 0.17	0.12 ± 0.11	0.19 ± 0.23	—	—	—
Basophils	BASO	%	0.4 ± 0.7	0.1 ±.0.2	0.7 ± 0.9	0.5 ± 0.7	0.7 ± 0.9	0.3 ± 0.6	1.2 ± 0.8	1.0 ± 1.0	0.6 ± 0.5
Basophils	BASO	10^9/L	—	—	0.08 ± 0.12	0.07 ± 0.10	0.06 ± 0.07	0.02 ± 0.04	—	—	—
Monocytes	MONO	%	2.9 ± 1.4	3.3 ± 1.6	1.1 ± 1.3	1.5 ± 1.5	2.0 ± 1.1	1.5 ± 1.2	1.4 ± 1.7	3.0 ± 1.9	0.6 ± 0.9
Monocytes	MONO	10^9/L	—	—	0.14 ± 0.2	0.20 ± 0.2	0.17 ± 0.1	0.14 ± 0.1	—	—	—
Platelet count	THROMB	10^9/L	532 ± 338	556 ± 173	513 ± 88	490 ± 115	349 ± 79	365 ± 52	320 ± 49	293 ± 53	313 ± 88
Activated partial thromboplastin time	APTT	s	34 + 8	39 ± 11	45.9 ± 5.8	44.1 ± 10.5	42.7 ± 8.2	43.2 ± 9.8	33 ± 4	32 ± 13	26 ± 10
Thrombin time	TT	s	23 ± 4	25 ± 4	25.8 ± 4.1	28.4 ± 5.0	23.9 ± 3.6	23.7 ± 4.6	21 ± 2	23 ± 3	19 ± 4
Prothrombin time	Prothr	s	12 ± 1	11 ± 1	11.7 ± 0.3	11.5 ± 0.5	11.9 ± 0.6	11.7 ± 0.4	12 ± 0	12 ± 0	12 ± 1
Fibrinogen	Fibr	g/L	6.0 ± 1.3	5.2 ± 1.0	6.5 ± 1.3	5.4 ± 0.7	6.9 ± 1.3	4.8 ± 0.6	5.8 ± 0.8	5.6 ± 0.7	5.5 ± 0.9

Source: Reprinted from Jørgensen, K.D. et al. 1998. *Scand. J. Lab. Anim. Sci.* (Suppl. 1): 181–190. With permission.

Note: Table entries are of the form: Mean ± SD.

[a] Reprinted from Ellegaard, L., Damm Jørgensen, K., Klastrup, S., Kornerup Hansen, A., Svendsen, O., 1995. *Scand. J. Lab. Anim. Sci.* 22 (3): 239–248. With permission.

TABLE A.14
The Characteristics of Juvenile and Pregnant Göttingen Minipigs Compared with Young and Adult Individuals

	Hematology	Clinical Chemistry
Juvenile	High hemoglobin	Low serum carbamide
	Low red blood cell count	Low serum creatinine
	High hematocrit	High alkaline phosphatase
	High mean cell volume	High inorganic phosphorous
	Low mean cell hemoglobin	Low serum albumin
	Low percentage of eosinophils	High serum α_2-globulin
	High platelet count	Low albumin/globulin ratio
	Short-activated partial thromboplastin time	
	Short thrombin time	
Pregnant	High hemoglobin	Low cholesterol
	Low red blood cell count	High triglyceride
	High mean cell volume	High creatine kinase
	Low white blood cell count	Low lactate dehydrogenase
	High percentage of eosinophils	High serum carbamide
	Low platelet count	High serum creatinine
	Short-activated partial thromboplastin time	High serum total protein
	Short thrombin time	High serum β-globulin
	High plasma fibrinogen	High serum γ-globulin
		Low albumin/globulin ratio
		Low inorganic phosphorous

Source: Courtesy of Ellegaard Göttingen Minipigs ApS, Dalmose, Denmark.

TABLE A.15
Hematology Results for Juvenile and Young Adult Hanford Miniature Swine

		Male[a]				Female[a]			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Red Blood Cell									
Hematocrit	%	45.5	4.3	35.2	55.2	47.3	4.3	38.6	56.8
Hemoglobin	g/dL	14.5	1.3	11.2	17.5	15.0	1.3	12.3	17.9
Mean corpuscular hemoglobin	pg	18.4	1.1	16.4	21.1	18.8	1.0	15.8	20.3
Mean corpuscular hemoglobin concentration	g/dL	31.8	0.7	30.2	33.7	31.8	0.8	30.0	33.3
Mean corpuscular volume	fl	58.0	3.4	52.6	67.7	59.2	3.5	50.8	66.2
Red blood count	$\times 10^6/\mu L$	7.87	0.74	6.16	9.46	8.01	0.64	6.82	9.63
White Blood Cell									
Basophil	$\times 10^3/\mu L$	0.11	0.04	0.05	0.21	0.12	0.07	0.05	0.50
Eosinophil	$\times 10^3/\mu L$	0.32	0.30	0.01	1.49	0.28	0.22	0.01	1.19
Lymphocyte	$\times 10^3/\mu L$	11.98	2.41	7.19	17.98	11.36	2.75	5.59	17.33
Monocyte	$\times 10^3/\mu L$	0.68	0.22	0.24	1.32	0.66	0.24	0.14	1.47
Neutrophil	$\times 10^3/\mu L$	5.42	2.68	0.41	18.03	4.49	1.61	1.67	9.15

(*Continued*)

TABLE A.15 (*Continued*)
Hematology Results for Juvenile and Young Adult Hanford Miniature Swine

		Male[a]				Female[a]			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Large unstained cell	$\times 10^3/\mu L$	0.12	0.08	0.02	0.31	0.14	0.07	0.04	0.29
White blood count	$\times 10^3/\mu L$	18.6	3.6	13.0	30.4	17.1	3.1	11.1	25.8
Clotting Potential									
APTT	s	18.3	2.6	13.9	26.0	18.3	2.8	13.3	26.0
Platelets	$\times 10^3/\mu L$	471.3	130.1	172.0	845.0	458.0	125.0	152.0	751.0
Prothrombin time	s	14.2	1.0	11.8	17.1	14.1	1.1	12.1	17.6
Others									
Reticulocyte	$\times 10^9/L$	113.1	51.7	18.9	235.4	115.7	55.2	18.4	251.0

Source: Courtesy of Sinclair Research Center, Auxvasse, MO.
[a] $N = 28$ per gender group; age = ~ 4–8 months.

TABLE A.16
Range of Observed Hematologic Reference Values[a–d] for Sinclair Pigs

Analyte	Reference Value Range
WBC	4700–15,300/μL
RBC	4.97–8.69 × 106/μL high
	9.8–17.3 g/dL
PCV (calculated)	27–49 vol%
PCV (centrifuged)	28–50 vol%
MCV	52–61 fl
MCH	18.6–21.4 pg
MCHC	34.0–35.7 g/dL
Neutrophil (S)	46.2–5661/μL neutrophil (B)
	0–153/μL
Lymphocyte	3102–8840/μL
Monocyte	0–1572/μL
Eosinophil	0–544/μL
Basophil	0–162/μL platelets ($n = 19$)
	311–585 × 10^3/μL
Plasma T. Protein	6.7–7.8 g/dL

Source: Courtesy of Guy Bouchard, D.V.M., Sinclair Research Center, Auxvasse, MO. Values from the Clinical Pathology Laboratory, Department of Veterinary Pathology, University of Missouri.

Note: $N = 20$ for all analytes except platelets; 10 males and 10 females; all were 1.5 years old.

[a] Reference intervals were established by determining the nonparametric central 0.95 interfractile interval as recommended by the International Federation of Clinical Chemistry.

[b] Analtyes were measured using Kodak clinical chemistry systems except for serum osmolality, which was measured by a freezing point osmometer; some reference values were calculated from measured value.

[c] Too few reference values to establish reference interval by either parametric or nonparametric methods.

[d] WBC, RBC, Hgb, MCV, and platelets measured with Coulter 6-Plus 4; PCV (centrifuged) via microhematocrit method; 100 cell WBC differential counts; plasma total protein by refractometry; remaining values were calculated.

TABLE A.17
Hematology Results for Juvenile Sinclair Miniature Swine

		Male				Female			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Red Blood Cells									
Hematocrit	%	42.4	3.8	35.3	51.3	43.0	4.2	32.8	54.8
Hemoglobin	g/dL	13.4	1.3	11.2	16.4	13.6	1.3	10.7	17.2
Mean corpuscular hemoglobin	pg	17.9	1.1	15.0	19.4	18.7	1.0	16.4	20.7
Mean corpuscular hemoglobin concentration	g/dL	31.7	0.9	30.1	33.9	31.6	0.8	29.6	33.0
Mean corpuscular volume	fl	56.4	3.3	49.4	62.0	59.3	3.1	52.0	68.4
Red blood count	$\times 10^6/\mu L$	7.54	0.7	6.00	9.79	7.27	0.8	5.45	9.29
White Blood Cells									
Basophil	$\times 10^3/\mu L$	0.10	0.1	0.00	0.30	0.07	0.1	0.00	0.23
Eosinophils	$\times 10^3/\mu L$	1.44	1.3	0.00	6.00	1.38	1.1	0.00	5.00
Lymphocytes	$\times 10^3/\mu L$	11.62	3.8	4.95	20.46	9.96	2.8	5.41	17.65
Monocytes	$\times 10^3/\mu L$	0.98	0.5	0.31	2.69	0.82	0.4	0.13	1.77
Neutrophils	$\times 10^3/\mu L$	6.03	3.6	2.07	20.03	5.23	2.4	2.22	11.07
Large unstained cells	$\times 10^3/\mu L$	0.30	0.3	0.00	0.94	0.28	0.3	0.00	1.38
White blood count	$\times 10^3/\mu L$	19.31	5.2	9.21	30.59	16.62	4.1	9.44	26.73
Clotting Potential									
APTT	s	16.3	2.0	12.1	21.8	16.9	2.8	12.1	29.3
Platelets	$\times 10^3/\mu L$	459	123.4	149	669	440	135.0	184	728
Prothrombin time	s	14.4	1.6	10.9	18.4	14.4	2.0	10.5	19.1
Others									
Nucleated red cells	%	0	0.1	0	1	0	0.4	0	3
Reticulocyte	$\times 10^9/L$	130.4	61.8	37.3	276.3	136.7	57.6	52.2	326.8

Source: Courtesy of Sinclair Research Center, Auxvasse, MO.
Note: $N = 47$, age = ~ 3–4 months.

TABLE A.18
Hematologic Values of 30 Healthy, Mature Yucatan Miniature Swine

Value	Mean	Reference Range[a]	Range
RBC ($10^6/\mu L$)	7.0	5.4–8.6	5.6–8.8
Hemoglobulin (g/dL)	14.9	12.5–17.3	13.1–17.0
Hematocrit (%)	44.6	36.4–52.8	36.3–53.7
MCV[b] (fl)	64.4	57.0–71.8	58.2–72.5
MCH[c] (pg)	21.4	18.8–24.0	18.9–24.3
MCHC[d] (g/dL)	33.2	31.6–34.8	31.1–34.5
RDW	19.4	16.2–22.6	15.7–23.8
Platelets ($10^3/\mu L$)	440.6	201.4–679.8	217.0–770.0
WBC ($10^3/\mu L$)	12.6	6.6–18.6	6.9–21.2
100 cell manual differential			
% segmented neutrophils	41.9	17.5–66.3	18.0–64.0
% bands	0.2	0.0–1.2	0.0–2.0

(*Continued*)

TABLE A.18 (*Continued*)
Hematologic Values of 30 Healthy, Mature Yucatan Miniature Swine

Value	Mean	Reference Range[a]	Range
% lymphocytes	45.6	19.2–72.0	21.0–71.0
% monocytes	7.5	1.1–13.9	2.0–15.0
% eosinophils	4.1	0.0–10.0	0.0–13.0
% basophils	0.5	0.0–2.5	0.0–5.0

Source: Reprinted from Radin, M.J. et al. 1986. *Lab. Anim. Sci.* 36(4): 425–427. With permission.

[a] ×2 SD observed.

[b] Mean corpuscular volume.

[c] Mean corpuscular hemoglobin.

[d] Mean corpuscular hemoglobin concentration.

TABLE A.19
Clinical Chemistry of Göttingen Minipig

Parameter	Abbreviation	Unit		Male (3 Months)	Female (3 Months)	Male (6 Months)	Female (6 Months)
Alanine	ALAT	pkat/L	Mean	1.12	1.00	0.92	0.96
			SD	0.16	0.17	0.07	0.27
Ornithine carbamyl transferase	OCT	10/L	Mean	4.49	4.13	4.43	4.79
			SD	0.28	0.53	0.44	0.74
Sorbitol dehydrogenase	SDH	pkat/L	Mean	0.01	0.01	0.01	0.01
			SD	0.01	0.01	0.01	0.01
Aspartate aminotransferase	ASAT	pkat/L	Mean	0.38	0.34	0.36	0.34
			SD	0.13	0.09	0.10	0.07
Alkaline phosphatase	ALKPH	pkat/L	Mean	4.29	3.88	3.49	2.71
			SD	0.92	1.11	0.75	0.98
Bilirubin	BILI	pmol/L	Mean	2.69	2.27	2.32	1.87
			SD	0.49	0.74	0.48	0.56
γ-glutamyl transferase	GGT	pkat/L	Mean	0.80	0.79	0.89	0.77
			SD	0.10	0.12	0.12	0.18
Cholesterol	CHOL	mmol/L	Mean	1.72	2.40	1.33	0.96
			SD	0.33	0.49	0.20	0.43
Creatine kinase	CK	pkat/L	Mean	5.99	1.17	7.89	9.38
			SD	2.25	2.20	4.85	3.88
Lactate dehydrogenase	LDH	pkat/L	Mean	16.68	17.45	13.59	15.27
			SD	2.34	2.32	2.03	5.39
Amylase	AmYL	IU/L	Mean	49.04	50.08	49.33	52.17
			SD	11.59	13.97	10.53	15.23
Protein (total)	PROT	g/L	Mean	58.43	57.58	61.02	61.66
			SD	3.97	3.63	3.33	3.60
Triglicerides	TRIG	mmol/L	Mean	0.40	0.59	0.36	0.47
			SD	0.07	0.12	0.07	0.12
Carbamid	UREA	mmol/L	Mean	2.49	2.37	2.12	2.35
			SD	0.56	0.59	0.50	0.59

Source: Courtesy of Ellegaard Göttingen Minipigs. Reprinted from Ellegaard, L. et al. 1995. *Scand. J. Lab. Anim. Sci.* 22(3): 239–248. With permission.

TABLE A.20
Serum Chemistry from Göttingen Breeding Facility (Grouped by Age)

				Values Grouped by Age of Animals					
				6 Months		7 Months		8 Months[a]	
Parameter	Abbreviation	Unit	Sex	Average	SD	Average	SD	Average	SD
Sodium	Na	mmol/L	Female	149.12	5.02	149.53	6.42	150.69	4.11
			Male	146.15	1.95	149.43	4.52	148.77	3.37
Potassium	K	mmol/L	Female	5.23	0.95	5.55	0.73	4.70	0.56
			Male	5.04	0.47	5.06	0.47	5.09	0.44
Chloride	Cl	mmol/L	Female	103.56	3.84	103.79	4.42	103.81	2.51
			Male	101.69	2.06	104.14	3.37	102.31	2.72
Alanine aminotransferase	ALAT	µkat/L	Female	0.83	0.14	0.78	0.25	0.78	0.14
			Male	0.87	0.15	0.89	0.15	0.85	0.12
Albumin	Albumin	g/L	Female	47.91	3.71	48.64	4.21	51.03	4.43
			Male	46.28	3.45	46.97	2.78	48.23	2.62
Alkaline phosphatase	AP	µkat/L	Female	2.95	1.03	3.23	0.90	3.21	0.72
			Male	3.34	0.85	3.64	0.66	3.91	0.78
Amylase	Amylase	µkat/L	Female	54.81	6.88	45.77	9.15	56.34	10.26
			Male	47.79	5.07	43.87	7.29	44.03	12.40
Aspartate aminotransferase	AST	µkat/L	Female	0.46	0.14	0.38	0.08	0.49	0.15
			Male	0.47	0.07	0.47	0.14	0.50	0.22
Calcium	Ca	mmol/L	Female	2.76	0.15	2.88	0.13	2.65	0.08
			Male	2.69	0.14	2.79	0.12	2.79	0.12
Cholesterol	Chol	mmol/L	Female	2.37	0.36	2.41	0.52	2.67	0.60
			Male	1.68	0.26	1.51	0.32	1.43	0.27
Inorganic phosphorus	Phosphor	mmol/L	Female	2.79	0.33	2.60	0.25	2.61	0.49
			Male	2.58	0.45	2.29	0.24	2.21	0.15
Total protein	T. Prot	g/L	Female	62.95	6.69	64.95	4.62	70.42	7.59
			Male	61.85	4.46	62.90	3.98	65.15	3.83
Triglycerides	Trigl.	mmol/L	Female	0.46	0.12	0.64	0.16	0.76	0.19
			Male	0.39	0.18	0.42	0.12	0.64	0.24
Carbamide	Urea	mmol/L	Female	2.59	0.53	3.68	0.51	3.96	0.90
			Male	2.56	0.75	3.22	0.48	4.12	0.93
Magnesium	Mg	mmol/L	Female	1.13	0.10	1.17	0.15	1.23	0.13
			Male	1.07	0.12	1.13	0.12	1.16	0.07

Source: Courtesy of Altana Pharma AG, Barsbüttel, Germany.

Note: Parameters measured on the following instrument: Advia 1200, Bayer Company. Blood samples obtained from the Ellegaard Göttingen Minipig ApS breeding facility, Dalmose, Denmark, 2006. The population of animals is the same in Table A.20, Table A.21, and Table A.22. It is the same data grouped differently.

[a] Also includes animals 8 months and 1 week of age.

TABLE A.21
Serum Chemistry from Göttingen Breeding Facility (Grouped by Gender)

Parameter	Abbreviation	Unit	Gender	Average	SD	*N*
Sodium	Na	mmol/L	Female	149.67	5.25	57
			Male	148.15	3.68	40
Potassium	K	mmol/L	Female	5.19	0.84	57
			Male	5.06	0.45	40
Chloride	Cl	mmol/L	Female	103.70	3.68	57
			Male	102.75	2.92	40
Alanine aminotransferase	ALAT	μkat/L	Female	0.80	0.18	58
			Male	0.87	0.14	38
Albumin	Albumin	g/L	Female	48.97	4.20	57
			Male	47.16	3.00	40
Alkaline phosphatase	AP	μkat/L	Female	3.10	0.91	57
			Male	3.64	0.78	40
Amylase	Amylase	μkat/L	Female	52.36	9.61	57
			Male	45.19	8.73	40
Aspartate aminotransferase	AST	μkat/L	Female	0.44	0.14	57
			Male	0.48	0.15	39
Calcium	Ca	mmol/L	Female	2.77	0.15	57
			Male	2.76	0.14	40
Cholesterol	Chol	mmol/L	Female	2.46	0.49	57
			Male	1.54	0.30	40
Inorganic phosphorus	Phosphor	mmol/L	Female	2.68	0.36	57
			Male	2.36	0.34	40
Total protein	T. Prot	g/L	Female	65.57	6.98	57
			Male	63.29	4.22	40
Triglycerides	Trigl.	mmol/L	Female	0.60	0.20	56
			Male	0.48	0.21	40
Carbamide	Urea	mmol/L	Female	3.31	0.89	56
			Male	3.32	0.96	39
Magnesium	Mg	mmol/L	Female	1.17	0.13	57
			Male	1.12	0.11	40

Source: Courtesy of Altana Pharma AG, Barsbüttel, Germany.

Note: Animals are from 6 months to 8 months +1 week of age. Blood samples obtained from the Ellegaard Göttingen Minipig ApS breeding facility, Dalmose, Denmark, 2006; measured on the following instrument: Advia 1200, Bayer Company.

TABLE A.22
Serum Chemistry from Göttingen Breeding Facility (Grouped by Parameter)

Parameter	Abbreviation	Unit	Average	SD	*N*
Sodium	Na	mmol/L	149.06	4.72	97
Potassium	K	mmol/L	5.14	0.71	97
Chloride	Cl	mmol/L	103.32	3.41	97
Alanine aminotransferase	ALT	µkat/L	0.83	0.17	97
Albumin	Albumin	g/L	48.25	3.86	97
Alkaline phosphatase	AP	µkat/L	3.32	0.90	98
Amylase	Amylase	µkat/L	49.49	9.88	96
Aspartate aminotransferase	AST	µkat/L	0.46	0.14	95
Calcium	Ca	mmol/L	2.77	0.15	97
Cholesterol	Chol	mmol/L	2.09	0.62	97
Inorganic phosphorus	Phosphor	mmol/L	2.55	0.39	97
Total protein	T. Prot	g/L	64.66	6.11	97
Triglycerides	Trigl.	mmol/L	0.55	0.21	97
Carbamide	Urea	mmol/L	3.31	0.91	97
Magnesium	Mg	mmol/L	1.15	0.12	96

Source: Courtesy of Altana Pharma AG, Barsbüttel, Germany.

Note: Animals are from 6 months to 8 months +1 week of age. Average sex distribution: females 59%, males 41%. Serum chemistry measured on the following instrument: Advia 1200, Bayer Company. Blood samples obtained from the Ellegaard Göttingen Minipig ApS breeding facility, Dalmose, Denmark, 2006.

TABLE A.23
Clinical Chemistry Results for Juvenile and Young Adult Hanford Miniature Swine

		Male				Female			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Electrolyte Balance									
Calcium	mg/dL	10.6	0.5	9.5	12.9	10.6	0.4	10.0	11.4
Chloride	mEq/L	102.6	3.3	96.0	120.0	102.1	1.9	97.0	107.0
Phosphorus	mg/dL	8.1	0.7	6.8	9.7	8.2	0.5	7.1	9.3
Potassium	mEq/L	6.0	0.8	4.3	7.8	6.0	0.8	4.7	8.6
Sodium	mEq/L	145	5	134	169	144	3	137	151
Carbohydrate Metabolism									
Glucose	mg/dL	89.2	11.0	72.0	123.0	89.5	11.9	68.0	131.0
Liver Function—Hepatocellular									
Alanine aminotransferase	U/L	41.4	8.9	26.0	74.0	44.2	10.6	26.0	75.0
Aspartate aminotransferase	U/L	50.2	31.6	20.0	164.0	58.4	50.7	20.0	286.0
Lactate dehydrogenase	U/L	564.7	239.9	338.0	1845.0	635.1	264.5	365.0	1768.0
Liver Function—Hepatobiliary									
Alkaline phosphatase	U/L	133.4	31.3	67.0	216.0	134.6	37.8	76.0	244.0
Total bilirubin	mg/dL	0.1	0.0	0.1	0.3	0.1	0.1	0.1	0.4

(*Continued*)

TABLE A.23 (*Continued*)
Clinical Chemistry Results for Juvenile and Young Adult Hanford Miniature Swine

		Male				Female			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Kidney Function									
Creatinine	mg/dL	1.0	0.2	0.7	1.4	1.1	0.2	0.8	1.5
Urea nitrogen	mg/dL	8.2	2.2	4.0	14.0	9.5	2.4	5.0	14.0
Others									
Albumin	g/dL	3.7	0.4	2.8	4.5	3.7	0.3	3.1	4.5
Globulin	g/dL	2.7	0.5	2.0	3.8	2.9	0.4	2.1	4.0
A/G ratio		1.4	0.3	0.8	2.0	1.3	0.2	0.8	1.8
Total protein	g/dL	6.4	0.5	5.1	7.4	6.6	0.5	5.6	7.8
Cholesterol	mg/dL	73.3	12.0	45.0	108.0	90.6	16.2	56.0	142.0
Triglycerides	mg/dL	27.0	8.4	9.0	52.0	37.3	11.5	11.0	70.0
CO_2	mEq/L	26.5	3.3	21.0	35.0	24.3	2.5	17.0	28.0

Source: Courtesy of Sinclair Research Center, Auxvasse, MO.
Note: $N = 28$ per gender group; age = ~ 4–8 months.

TABLE A.24
Reference Intervals for Clinical Chemistry Values of Sinclair Pigs

Analyte	Reference Interval	Analyte	Reference Interval
A/G ratio	0.9–1.7	CO_2 (total)	26–35 mol/L
Albumin	3.3–4.8 g/dL	Creatinine	0.8–1.9 mg/dL
ALP	42–89 U/L	GGT	19–86 U/L
ALT	23–62 U/L	Glucose	48–290 mg/dL
AMS	365–1871 U/L	LDH	883–1450 U/L
Anion gap[a]	13.2–24.0 mmol/L	LPS	14–343 U/L
AST	16–43 U/L	Magnesium	1.7–2.3 mg/dL
Bilirubin (total)	0.1–0.3 mg/dL	Osmolality (meas)	285–324 mOsm/kg
Bilirubin (dir)	0.1–0.3 mg/dL	Osmolality (calc)[b]	276–307 mOsm/kg
Bilirubin (conj)	0.0–0.0 mg/dL	Osmolality gap[c]	3–20 mOsm/kg
Bilirubin (delta)	0.1–0.3 mg/dL	Phosphorus	5.8–7.8 g/dL
Calcium	10.0–11.3 mg/dL	Potassium	3.9–5.6 mmol/L
Chloride	98–106 mmol/L	Protein (total)	6.7–7.8 g/dL
Cholesterol	47–124 mg/dL	Urea nitrogen	7–13 mg/dL
CK	219–1411 U/L		

Source: Courtesy of Guy Bouchard, D.V.M., Sinclair Research Center, Auxvasse, MO. Values from the Clinical Pathology Laboratory, Department of Veterinary Pathology.
Note: $N = 40$ for all analytes; 20 males and 20 females; all were 1.5 years old.
[a] Anion gap = (Na + K) (Cl + CO_2).
[b] Calculated osmolality = 1.86 (Na + K) + UN/2.8 + Glucose/18.
[c] Osmolality gap = measured osmolality – (minus sign) calculated osmolality.

TABLE A.25
Clinical Chemistry Results for Juvenile Sinclair Miniature Swine

		Male				Female			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Electrolyte Balance									
Calcium	mg/dL	10.6	0.4	9.8	11.4	10.6	0.4	9.3	11.7
Chloride	mEq/L	103	3.2	96	110	104	3.3	97	110
Phosphorus	mg/dL	9.5	0.8	7.3	10.6	9.4	0.9	7.8	11.7
Potassium	mEq/L	5.8	0.9	4.1	8.0	5.8	0.8	4.3	8.3
Sodium	mEq/L	144	2.9	137	151	144	3.7	138	154
Carbohydrate Metabolism									
Glucose	mg/dL	86	12.1	58	112	80	17.6	42	118
Liver Function—Hepatocellular									
Alanine aminotransferase	U/L	32	8.3	20	55	31	8.0	19	55
Aspartate aminotransferase	U/L	37	17.0	18	91	37	16.9	13	89
Lactate dehydrogenase	U/L	538	172.0	338	1198	561	146.4	367	942
Liver Function—Hepatobiliary									
Alkaline phosphatase	U/L	102	26.4	51	168	96	18.5	51	140
Total bilirubin	mg/dL	0.2	0.1	0.1	0.6	0.2	0.1	0.1	0.8
Kidney Function									
Creatinine	mg/dL	0.9	0.2	0.6	1.4	0.9	0.2	0.5	1.3
Urea nitrogen	mEq/L	8	2.9	5	17	9	3.0	4	19
Others									
Albumin	g/dL	16.3	2.0	12.1	21.8	16.9	2.8	12.1	29.3
Globulin	g/dL	2.8	0.5	2.0	4.2	2.7	0.4	1.9	3.8
Bicarbonate	mEq/L	24	3.6	16	31	24	3.2	16	36
A/G ratio	mg/dL	1.28	0.3	0.59	1.90	1.31	0.3	0.71	2.05
Total protein	g/dL	6.2	0.5	5.1	7.2	6.1	0.5	4.6	8.0
Cholesterol	mg/dL	79	13.7	47	110	81	15.8	49	118
Triglycerides	mg/dL	50	26.8	18	139	56	31.2	22	150

Source: Courtesy of Sinclair Research Center, Auxvasse, MO.
Note: $N = 51$, age = ~3–4 months.

TABLE A.26
Clinical Chemistry Reference Values for Fasted Yucatan Miniature Pigs

		Mean			Pooled Values Observed	
Parameter	**Unit**	**Male**	**Female**	**SD**	**Median**	**Range**
Glucose	mmol/L	3.65	3.85	64	3.66	2.28–5.11
Urea	mmol/L	7.00	8.68	2.64	7.14	5.00–16.1
Creatinine	µmol/L	106.1	123.8[a]	15.9	114.9	88.4–150.3
Uric acid	µmol/L	3.6	7.1	7.1	5.9	0.0–29.7
Total protein	g/L	77.0	71.0	9.0	76.0	44.0–83.0
Albumin	g/L	53.0	46.0[a]	6.0	51.0	26.0–57.0
Globulin	g/L	24.0	25.0	4.0	24.0	18.0–36.0
Albumin/globulin		2.2	1.9[a]	0.3	2.1	1.3–2.7
Bilirubin total	µmol/L	3.42	3.42	1.37	3.42	1.7–6.84
Triglyceride	mg/L	192	341[a]	134	235	100–530
Total cholesterol	mmol/L	1.81	1.89	0.38	1.85	1.03–2.67
Alkaline phosphatase	U/L	90.1	87.7	21.5	84.5	39.0–128.0
γ-glutamyl transferase	U/L	61.1	62.1	11.2	61.5	41.0–86.0
Alanine aminotransferase	U/L	71.9	73.0	13.6	71.0	49.0–106.0
Lactate dehydrogenase	U/L	450.8	556.7[a]	88.0	492.0	389.0–727.0
Aspartate aminotransferase	U/L	38.7	41.8	5.9	39.0	32.0–55.0
Sodium	mmol/L	139.3	141.7	4.2	141.0	132.0–149.0
Potassium	mmol/L	4.0	4.2	0.3	4.0	3.5–4.8
Chloride	mmol/L	101.2	105.0	4.3	102.0	96.0–111.0
Iron	umol/L	22.7	23.6	3.5	23.1	11.8–37.6
Calcium	mmol/L	2.72	2.52	0.18	2.65	2.08–2.92
Phosphorus	mmol/L	2.42	2.39	0.26	2.33	1.97–2.91

Source: Reprinted from Parsons, A.H., Wells, R.E. 1986. *Lab. Anim. Sci.* 36(4): 428–430. With permission.

[a] Significant difference ($p < 0.05$) between sexes, $N = 24$.

TABLE A.27
Serum Biochemical Values of 30 Healthy Mature Yucatan Miniature Swine

Value	**Mean**	**Reference Range[a]**	**Range**
Glucose (mg/dL)	79.8	36.4–123.2	56.0–153.0
SUN (mg/dL)[b]	19.2	9.2–29.2	10.0–29.0
Creatinine (mg/dL)	1.6	1.2–2.0	1.2–2.0
Total protein (gm/dL)	7.5	6.1–8.9	6.3–9.4
Albumin (gm/dL)	4.7	3.9–5.5	4.1–5.6
Globulin (gm/dL)	2.8	1.6–4.0	1.4–3.6
A–G[c]	1.8	0.8–2.8	1.11–3.49
Sodium (mEq/L)	147.0	144–152.6	142.0–153.0
Potassium (mEq/L)	4.6	4.0–5.2	3.9–5.2
Chloride (mEq/L)	104.2	94.4–114.0	95.0–114.0
Calcium (mg/dL)	10.6	9.6–11.6	9.3–11.6
Phosphorus (mg/dL)	6.9	5.1–8.1	5.0–8.3
Total bilirubin (mg/dL)	0.1	0.0–0.3	0.0–0.3

(*Continued*)

TABLE A.27 (*Continued*)
Serum Biochemical Values of 30 Healthy Mature Yucatan Miniature Swine

Value	Mean	Reference Range[a]	Range
AST (IU/I)[d]	28.2	10.4–56.0	15.0–53.0
ALT (IU/I)[e]	33.6	20.4–46.8	20.0–48.0
CPK (IU/I)[f]	168.0	48.0–288.0	37.0–270.0
Cholesterol (mg/dL)	101.8	38.4–165.2	47.3–173.0

Source: Reprinted from Radin, M.J. et al. 1986. *Lab. Anim. Sci.* 36(4): 425–427. With permission.

[a] X ± 2 SD observed.

[b] Serum urea nitrogen.

[c] Albumin globulin ratio.

[d] Aspartate aminotransferase.

[e] Alanine aminotransferase.

[f] Creatine phosphokinase.

TABLE A.28
Hematology for Male, Adult Yucatan, and Ossabaw Miniature Swine

		Yucatan				Ossabaw			
		Mean	SD	Min	Max	Mean	SD	Min	Max
Erythrocytes	($\times 10^6$/mL)	4.4	0.2	4.0	4.7	4.8	0.5	4.2	5.4
Hematocrit[a]	%	26.6	1.6	24.0	29.0	26.6	2.7	23.0	29.0
Hemoglobin	g/dL	9.2	0.7	8.1	10.3	9.2	1.1	7.8	10.2
Mean corpuscular volume (MCV)	Fl	60.7	2.2	58.0	64.0	55.7	1.3	54.0	57.0
Mean corpuscular hemoglobin (MCH)	Pg	21.1	1.1	19.9	22.6	19.1	0.6	18.6	20.0
Mean corpuscular hemoglobin concentration (MCHC)	g/dL	34.6	0.6	33.9	35.5	34.5	0.7	33.6	35.2
Leukocytes	($\times 10^3$/mL)	8.2	1.4	6.5	10.7	10.5	2.2	8.0	13.8
Semented neutrophils	($\times 10^3$/mL)	3.43	0.88	2.48	4.92	4.32	1.58	2.68	6.90
Banded neutrophils[b]	($\times 10^3$/mL)	0.09	0.02	0.08	0.11	0	0	0	0
Lymphocytes	($\times 10^3$/mL)	4.54	0.91	3.49	6.08	5.29	1.07	3.29	6.21
Monocytes	($\times 10^3$/mL)	0.29	0.17	0	0.50	0.79	0.44	0.37	1.40
Eosinophils	($\times 10^3$/mL)	0.04	0.05	0	0.11	0.07	0.10	0	0.27
Basophils	($\times 10^3$/mL)	0	0	0	0	0.11	0.20	0	0.40
Platelets[c]	($\times 10^3$/mL)	427.1	83.8	300	553	562.0	150.3	422.0	692.0

Source: Courtesy of Michael Sturek, PhD, Indiana University.

Note: $N = 7$ per breed group, except where noted; age = ~12–15 months.

[a] Hematocrit under anesthesia.

[b] Undetectable in all Ossabaws; detectable in 3 Yucatans.

[c] $N = 4$ Ossabaw.

TABLE A.29
Clinical Chemistry for Male, Adult Trapped Ossabaw Island Miniature Swine

		Mean	SD	Min	Max
Electrolytes					
Anion gap	mmol/L	28.5	5.5	21	40
Calcium	mg/dL	9.6	1.3	7.5	11.5
Chloride	mmol/L	95.2	9.1	80.0	107.0
Magnesium	mg/dL	2.8	0.6	2.1	4.5
Phosphorus	mg/dL	7.2	1.3	4.8	9.5
Potassium	mmol/L	7.3	1.2	4.8	9.6
Sodium	mmol/L	139.1	13.5	117	157
Carbohydrate Metabolism					
Glucose	mg/dL	136.5	75.4	82	436
Liver Function—Hepatocellular					
Alkaline phosphatase (ALP)	U/L	57.2	21.0	32.0	107.0
Aspartate aminotranferase (AST)	U/L	51.2	20.0	25	92
γ-glutamyl transferase (GGT)	U/L	59.0	9.6	42	77
Liver Function—Hepatobiliary					
Unconjugated bilirubin	mg/dL	0.005	0.02	0	0.1
Total bilirubin	mg/dL	0.27	0.16	0.1	0.6
Kidney Function					
Creatinine	mg/dL	1.8	0.4	1.1	2.7
Urea nitrogen	mg/dL	8.2	2.4	4.0	13.0
Others					
Albumin	g/dL	3.6	0.6	2.7	4.6
Globulin	g/dL	3.8	0.4	3.1	4.6
Albumin/globulin ratio		0.94	0.16	0.59	1.19
Total protein	g/dL	7.4	0.8	6.0	9.0
Creatine kinase	U/L	1031	638	179	2112
Total cholesterol	mg/dL	77.9	23.7	45	127
Triglycerides	mg/dL	33.2	13.2	18	74
Total CO_2	mmol/L	22.8	3.1	17	30
Body weight	kg	46.7	11.9	29.1	73.6

Source: Courtesy of Michael Sturek, PhD, Indiana University.

Note: $N = 19$; estimated age >9 months. Mean, standard deviation (SD), and minimum (Min) and maximum (Max) values and units of measure are provided.

TABLE A.30
Clinical Chemistry for Male, Adult Yucatan, and Ossabaw Miniature Swine

		Yucatan				Ossabaw			
		Mean	SD	Min	Max	Mean	SD	Min	Max
Electrolytes									
Anion gap	mmol/L	10.9	2.1	6.6	13.0	9.7	2.4	6.0	12.0
Calcium	mg/dL	10.0	0.44	9.5	10.6	10.0	0.18	9.8	10.2
Chloride	mmol/L	101.1	2.12	99.0	105.0	100.4	1.4	98.0	102.0
Magnesium	mg/dL	2.4	0.2	2.2	2.6	2.2	0.1	2.1	2.3
Phosphorus	mg/dL	6.63	0.5	6.4	7.2	7.1	0.4	6.5	7.5
Potassium	mmol/L	3.8	0.32	3.4	4.4	4.2	0.2	4.0	4.4
Sodium	mmol/L	142.7	2.1	139.0	145.0	142.0	1.5	140.0	143.0
Carbohydrate Metabolism									
Glucose	mg/dL	93.1	31.9	47	150	126.0	12.3	107	145
Liver Function—Hepatocellular									
Alkaline phosphatase (ALP)	U/L	58.1	13.5	42.0	83.0	67.0	7.6	62.0	75.0
Aspartate aminotranferase (AST)	U/L	29.1	5.1	19	34	24.0	4.2	19.0	26.0
γ-glutamyl transferase (GGT)	U/L	45.9	13.9	23	61	60.4	15.8	45.0	83.0
Liver Function—Hepatobiliary									
Unconjugated bilirubin	mg/dL	0.04	0.08	0.0	0.2	0	0	0	0
Total bilirubin	mg/dL	0.11	0.04	0.1	0.3	0.1	0.0	0.1	0.1
Kidney Function									
Creatinine	mg/dL	1.3	0.1	1.1	1.5	1.7	0.3	1.1	1.9
Urea nitrogen	mg/dL	15.1	2.4	11.0	18.0	10.7	2.6	7.0	14.0
Others									
Albumin	g/dL	3.3	0.2	2.9	3.5	3.4	0.3	3.1	3.7
Globulin	g/dL	3.0	0.2	2.9	3.2	2.8	0.2	2.6	3.0
Albumin/globulin ratio		1.11	0.15	0.94	1.4	1.20	0.08	1.13	1.35
Total protein	g/dL	6.3	0.3	6.0	6.6	6.2	0.4	5.8	6.7
Creatine kinase	U/L	303.1	114.4	203.0	469.0	450.3	94.6	397.0	570.0
Total cholesterol	mg/dL	73.3	12.0	45.0	108.0	90.6	16.2	56.0	142.0
Triglycerides	mg/dL	27.0	8.4	9.0	52.0	37.3	11.5	11.0	70.0
Total CO_2	mmol/L	33.3	1.11	32.0	35.0	35.4	3.2	32	40
Body weight	kg	60.7	6.2	53.2	68.9	74.1	4.8	68.6	81.4

Source: Courtesy of Michael Sturek, PhD, Indiana University.

Note: $N = 7$ per breed group; age = ~12–15 months. Mean, standard deviation (SD), and minimum (Min) and maximum (Max) values and units of measure are provided.

SECTION III: ORGAN WEIGHTS AND MEASUREMENTS

TABLE A.31
Percentage of Body Weight of Various Tissues and Organs in the 12-Week-Old Pig

Organ	Percentage of Body Weight
Body weight	100
Cerebrum	0.31 ± 0.06
Cerebellum	0.06 ± 0.02
Spinal cord	0.14 ± 0.08
Thyroid gland	0.02 ± 0.004
Larynx-trachea	0.21 ± 0.03
Lungs	1.09 ± 0.18
Heart	0.49 ± 0.06
Aorta	0.11 ± 0.05
Esophagus	0.13 ± 0.05
Stomach	1.22 ± 0.16
Small intestine	4.46 ± 0.38
Large intestine	2.74 ± 0.59
Liver	3.16 ± 0.41
Spleen	0.19 ± 0.05
Pancreas	0.29 ± 0.18
Kidneys	0.55 ± 0.01
Adrenal glands	0.0104 ± 0.0018
Skeletal muscle	35.97 ± 2.3
Fat	12.52 ± 1.04
Skeleton	16.73 ± 0.89
Rind	6.32 ± 0.54
Blood	5.53 ± 0.42
Gastrointestinal tract content	5.61 ± 2.22
Various connective tissues	3.33 ± 0.62

Source: Reprinted from Elowsson et al. 1997. *Lab. Anim. Sci.* 47(2): 200–202. With permission.

Note: Data are expressed as mean ± 1 SD; mean weight = 22.74 ± 3.45 kg. GI = stomach, small, and large intestine.

TABLE A.32
Percentage of Body Weight of Specified Muscles (Left and Right Sides) of the 12 Week Old Pig

Muscle	Percentage of Body Weight
Longissimus dorsi	3.17 ± 0.27
Biceps femoris	2.14 ± 0.12
Quadriceps femoris	1.91 ± 0.17
Semitendinosus	0.66 ± 0.06
Semimembranosus	2.58 ± 0.21

Source: Reprinted from Elowsson et al. 1997. *Lab. Anim. Sci.* 47(2): 200–202. With permission.

Note: Mean weight = 22.74 ± 3.45 kg.

TABLE A.33
Selected Organ Weights for Juvenile and Young Adult Hanford Miniature Swine

	Male				Female			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Brain								
Absolute wt (g)	91.7	8.1	78.3	107.7	87.7	7.2	74.5	104.2
% BW	0.24	0.03	0.17	0.32	0.25	0.04	0.18	0.32
Adrenal (2)								
Absolute wt (g)	2.66	0.70	0.05	3.62	2.33	0.45	1.66	3.19
% BW	0.007	0.002	0.000	0.010	0.007	0.001	0.005	0.010
% Brain wt	2.93	0.89	0.06	4.60	2.68	0.57	1.80	4.10
Epididymis (2)								
Absolute wt (g)	86.3	10.8	65.7	105.0	NA	NA	NA	NA
% BW	0.22	0.03	0.16	0.27	NA	NA	NA	NA
% Brain wt	94.3	10.2	77.4	116.1	NA	NA	NA	NA
Heart								
Absolute wt (g)	159.1	20.8	128.0	215.4	144.4	19.9	110.7	178.5
% BW	0.41	0.05	0.34	0.51	0.40	0.03	0.36	0.47
% Brain wt	174	25	137	222	165	22	121	201
Kidney (2)								
Absolute wt (g)	153.9	22.3	115.9	198.6	123.0	13.9	96.9	146.1
% BW	0.39	0.05	0.31	0.49	0.35	0.04	0.27	0.41
% Brain wt	168	24	133	224	141	19	110	178
Liver								
Absolute wt (g)	672.9	61.1	533.2	798.7	677.5	67.8	561.1	866.7
% BW	1.7	0.2	1.4	2.2	1.9	0.2	1.7	2.4
% Brain wt	737	73	617	895	775	87	655	988
Lung								
Absolute wt (g)	263.0	38.3	222.5	354.9	230.5	43.5	184.1	348.3
% BW	0.67	0.09	0.52	0.88	0.65	0.12	0.52	1.03
% Brain wt	290	55	224	443	265	56	183	428
Pituitary Gland								
Absolute wt (g)	0.21	0.05	0.11	0.37	0.16	0.04	0.07	0.22
% BW	0.00050	0.00001	0.00030	0.00100	0.00050	0.00010	0.00020	0.00070
% Brain wt	0.23	0.07	0.14	0.47	0.19	0.05	0.08	0.24
Prostate								
Absolute wt (g)	4.0	1.4	2.1	6.6	NA	NA	NA	NA
% BW	0.010	0.004	0.01	0.02	NA	NA	NA	NA
% Brain wt	4.4	1.5	1.9	7.7	NA	NA	NA	NA
Seminal Vesicle (2)								
Absolute wt (g)	148.2	49.0	72.3	252.7	NA	NA	NA	NA
% BW	0.38	0.12	0.20	0.59	NA	NA	NA	NA
% Brain wt	163	56	70	301	NA	NA	NA	NA

(*Continued*)

TABLE A.33 (*Continued*)
Selected Organ Weights for Juvenile and Young Adult Hanford Miniature Swine

	Male				Female			
	Mean	**SD**	**Minimum**	**Maximum**	**Mean**	**SD**	**Minimum**	**Maximum**
				Spleen				
Absolute wt (g)	202.6	105.2	53.3	414.5	148.0	94.9	38.3	379.5
% BW	0.51	0.25	0.15	0.99	0.43	0.29	0.12	1.12
% Brain wt	222	118	62	480	171	114	50	466
				Testis (2)				
Absolute wt (g)	211.8	35.1	133.6	273.3	NA	NA	NA	NA
% BW	0.54	0.09	0.37	0.68	NA	NA	NA	NA
% BW	0.14	0.05	0.08	0.30	0.11	0.04	0.05	0.18
% Brain wt	57.9	19.7	31.5	121.4	46.0	20.0	20.0	85.8
				Thyroid (2)				
Absolute wt (g)	3.3	1.0	2.0	6.1	2.5	0.7	1.5	4.7
% BW	0.0083	0.0027	0.0000	0.0200	0.0071	0.0016	0.0000	0.0100
% Brain wt	3.6	1.3	2.1	7.4	2.9	0.9	1.7	5.3
				Uterus				
Absolute wt (g)	NA	NA	NA	NA	262.5	97.9	117.5	551.0
% BW	NA	NA	NA	NA	0.72	0.23	0.35	1.24
% Brain wt	NA	NA	NA	NA	302	115	121	608
				Ovaries				
Absolute wt (g)	NA	NA	NA	NA	6.4	2.4	1.8	10.2
% BW	NA	NA	NA	NA	0.018	0.006	0.006	0.030
% Brain wt	NA	NA	NA	NA	7.4	2.8	2.0	11.5

Source: Courtesy of Sinclair Research Center, Auxvasse, MO.
Note: $N = 28$, age = ~ 6–8 months, NA = not applicable.

TABLE A.34
Age-Related Whole Body Composition and Body Weight of the Female Sinclair Miniature Swine[1]

Age (in years)	Bone Mineral Content (kg)[2]	Lean Mass (kg)[2]	Total Fat (kg)[2]	Body Weight (kg)[2,3]
1.0	0.806[a]	25.000[a]	9.014[a]	34.819[a]
1.5	1.216[b]	33.812[b]	16.702[a,b]	51.730[b]
2.0	1.556[c]	43.853[c]	24.396[b,c]	69.806[c]
2.5	1.745[c,d]	44.710[c]	30.384[c,d]	76.839[c,d]
3.0	1.849[d,e]	49.552[d]	37.071[d,e]	88.472[d,e]
3.5	1.804[d,e]	49.319[d]	28.140[b,c,d]	79.262[c,d,e]
4.0	1.991[e]	52.427[d]	37.665[d,e]	92.083[e,f]
>6.0	2.021[e]	52.115[d]	47.892[e]	102.029[f]

Source: Reprinted from Bouchard, et al. 1995. *Lab. Anim. Sci.* 45(4): 408–414. With permission.
[1] Whole-body composition measured using a Dual Energy X-ray Absorptiometer (QDR 2000-Plus, Hologic, Waltham, MA).
[2,a,b,c,d,e,f] Means with different superscripts within the same column are different at $p < 0.05$.
[3] Body weights of miniature swine older than 2.5 years of age are exaggerated due to obesity.

TABLE A.35
Selected Organ Weights for Juvenile Sinclair Miniature Swine

	Male				Female			
	Mean	**SD**	**Minimum**	**Maximum**	**Mean**	**SD**	**Minimum**	**Maximum**
				Brain				
Absolute wt (g)	76.7	4.78	67.7	85.2	72.2	3.16	64.9	77.9
% BW	0.61	0.086	0.51	0.77	0.59	0.068	0.47	0.71
				Adrenal (2)				
Absolute wt (g)	1.39	0.197	1.04	1.71	1.23	0.150	1.04	1.48
% BW	0.011	0.002	0.009	0.015	0.010	0.002	0.008	0.015
% Brain wt	1.81	0.26	1.32	2.30	1.71	0.22	1.42	2.06
				Epididymis (2)				
Absolute wt (g)	11.4	3.69	5.16	15.6				
% BW	0.087	0.019	0.058	0.113				
% Brain wt	14.8	4.70	7.61	21.1				
				Heart				
Absolute wt (g)	59.4	8.75	43.4	73.1	53.3	7.31	39.2	64.8
% BW	0.47	0.033	0.39	0.52	0.43	0.029	0.39	0.50
% Brain wt	77.3	9.67	62.0	89.2	73.8	9.64	60.4	88.4
				Kidney (2)				
Absolute wt (g)	58.9	6.92	48.3	72.6	53.2	4.10	46.2	59.3
% BW	0.47	0.10	0.34	0.71	0.43	0.05	0.36	0.52
% Brain wt	77.1	9.98	66.1	93.8	73.8	5.56	63.0	80.7
				Liver				
Absolute wt (g)	324	44.5	246	394	312	29.1	268	363
% BW	2.59	0.52	1.76	3.67	2.53	0.31	2.09	3.13
% Brain wt	423	56.1	332	516	433	42.6	365	513
				Lung				
Absolute wt (g)	165	64.8	115	354	202	98	103	448
% BW	1.29	0.44	0.92	2.57	1.65	0.83	0.80	3.44
% Brain wt	215	85.7	161	469	280	135	140	611
				Pituitary Gland				
Absolute wt (g)	0.103	0.034	0.072	0.191	0.081	0.019	0.057	0.118
% BW	0.001	0.0002	0.001	0.001	0.001	0.0002	0.000	0.001
% Brain wt	0.13	0.047	0.090	0.26	0.11	0.028	0.082	0.17
				Prostate				
Absolute wt (g)	1.00	0.412	0.595	1.74				
% BW	0.008	0.003	0.004	0.015				
% Brain wt	1.32	0.56	0.71	2.35				
				Seminal Vesicle (2)				
Absolute wt (g)	8.93	3.81	2.03	12.6				
% BW	0.069	0.028	0.023	0.11				
% Brain wt	11.6	5.01	3.00	17.3				

(*Continued*)

TABLE A.35 (*Continued*)
Selected Organ Weights for Juvenile Sinclair Miniature Swine

	Male				Female			
	Mean	**SD**	**Minimum**	**Maximum**	**Mean**	**SD**	**Minimum**	**Maximum**
				Spleen				
Absolute wt (g)	44.6	19.7	12.5	86.1	48.6	17.2	22.2	72.5
% BW	0.34	0.13	0.14	0.58	0.40	0.15	0.17	0.62
% Brain wt	57.4	23.6	18.4	105	67.6	24.4	30.2	104
				Testis (2)				
Absolute wt (g)	49.9	19.6	21.2	78.5				
% BW	0.38	0.11	0.21	0.52				
% Brain wt	64.6	24.3	31.4	95.8				
				Thymus				
Absolute wt (g)	26.4	12.0	9.85	48.6	22.5	8.58	13.1	44.1
% BW	0.20	0.089	0.11%	0.41%	0.18	0.061	0.10	0.30
% Brain wt	34.4	16.2	14.5	65.2	31.3	12.3	18.3	62.2
				Thyroid (2)				
Absolute wt (g)	1.22	0.274	0.744	1.73	1.04	0.282	0.653	1.51
% BW	0.010	0.002	0.007	0.014	0.008	0.002	0.006	0.012
% Brain wt	1.58	0.31	1.10	2.15	1.44	0.38	1.00	2.06

Source: Courtesy of Sinclair Research Center, Auxvasse, MO.
Note: $N = 12$, age = ~ 4 months.

TABLE A.36
Visceral Composition of Male Yucatan and Ossabaw Miniature Swine

	Diet		
Breed	**Control Chow**	**46% kcal Fat**	**75% kcal Fat**
Ossabaw	$N = 7$	$N = 8$	$N = 3$
% Protein	12.2 ± 0.4	11.7 ± 0.3	9.9 ± 0.5
% Fat	19.9 ± 1.9	23.4 ± 0.8	28.9 ± 0.6
Yucatan	$N = 6$	$N = 5$	$N = 3$
% Protein	13.2 ± 0.5	13.4 ± 0.5	11.9 ± 0.2
% Fat	12.5 ± 2.1	16.2 ± 2.8	20.3 ± 2.0

Source: Courtesy of Michael Sturek, PhD, Indiana University.
Note: Values are mean ± standard error.

SECTION IV: REPRODUCTIVE PARAMETERS

TABLE A.37
Effect of Parity on Reproductive Parameters of Sinclair Miniature Swine

Parity	*N*	Litter Size	Stillborn H	Weaned Piglets	*N*	Litter Weight (kg)
1	150	6.5 ± 2^{a}	$0.64 \pm 09^{a,b}$	5.0 ± 0.2^{a}	124	3.5 ± 0.1^{a}
2	100	$7.6 \pm 0.2^{b,c}$	$0.47 \pm 0.11^{a,b}$	$6.4 \pm 0.2^{b,c}$	85	$4.4 \pm 0.2^{b,c}$
3	60	$7.9 \pm 0.3^{b,c}$	$0.52 \pm 0.14^{a,b}$	6.7 ± 0.3^{c}	55	4.5 ± 0.2^{c}
4	35	$7.2 \pm 0.4^{a,b}$	0.91 ± 0.18^{b}	5.4 ± 0.4^{a}	34	$3.9 \pm 0.2^{a,b}$
5	16	$7.5 \pm 0.5^{a,b,c}$	$0.63 \pm 0.27^{a,b}$	$5.5 \pm 0.6^{a,b}$	13	$4.0 \pm 0.4^{a,b,c}$
>6	10	8.9 ± 0.7^{c}	1.90 ± 0.34^{c}	$5.8 \pm 0.7^{a,b,c}$	9	$4.3 \pm 0.5^{a,b,c}$

Source: Reprinted from Bouchard, G. et al. 1995. *Lab. Anim. Sci.* 45(4): 408–414. With permission.
Note: Data collected between 1985 and 1993, expressed as mean ± SEM; *N*, number of litters applied to the columns on the right; under certain circumstances some litters were not weighted.
^{a,b,c} Means with different superscripts within column are different at $p < 0.05$.

TABLE A.38
Effect of the Sow Age on Reproductive Parameters of Sinclair Miniature Swine

Sow	*N*	Litter Size	Stillborn H	Weaned Piglets	*N*	Litter Weight (kg)
1	72	6.4 ± 3^{a}	0.39 ± 0.13^{a}	5.2 ± 0.3^{a}	69	3.4 ± 2^{a}
2	164	$7.4 \pm 0.2^{b,c}$	$0.54 \pm 0.8^{a,b}$	6.2 ± 0.2^{b}	142	4.2 ± 0.1^{b}
3	88	$7.2 \pm 0.2^{b,c}$	$0.72 \pm 0.12^{b,c}$	$5.7 \pm 0.2^{a,b}$	68	4.1 ± 0.2^{b}
4	34	$7.7 \pm 0.4^{b,c}$	1.09 ± 0.19^{c}	$5.6 \pm 0.4^{a,b}$	30	4.1 ± 0.3^{b}
5	6	$6.0 \pm 0.9^{a,b}$	$0.67 \pm 0.44^{a,b,c}$	$4.5 \pm 0.9^{a,b}$	5	$3.8 \pm 0.6^{a,b}$
>6	7	8.6 ± 0.8^{c}	2.00 ± 0.41^{d}	4.4 ± 0.9^{a}	6	$3.3 \pm 0.6^{a,b}$

Source: Reprinted from Bouchard, G. et al. 1995. *Lab. Anim. Sci.* 45(4): 408–414. With permission.
Note: Data collected between 1985 and 1993, expressed as mean ± SEM; *N*, number of litters applied to the columns on the right; under certain circumstances some litters were not weighted.
^{a,b,c} Means with different superscripts within column are different at $p < 0.05$.

TABLE A.39
Correlation between Morphology and Motility of Göttingen Minipig Sperm

	Motility		
Morphology	Vigorous Progressive (%)	Weak Progressive (%)	No Progressive (%)
Normal ($n = 520$)	95	4	1
Distal drop ($n = 200$)	10	83	7
Coiled flagellum and distal drop ($n = 200$)	0	20	80

Source: Reprinted from Jørgensen, K.D. et al. 1998. *Scand. J. Lab. Anim. Sci.* (Suppl. 1): 161–169. With permission.

TABLE A.40
Minipig Sperm Morphology of Göttingen Minipig Breeding Boars

	From Ejaculates (n = 37) (%)	From Cauda Epididymis (n = 9) (%)
Normal sperm	50 ± 23	17 ± 7
Proximal drop	2 ± 2	15 ± 22
Distal drop	6 ± 9	24 ± 17
Coiled flagellum and distal drop	10 ± 11	34 ± 15
Coiled flagellum	23 ± 10	6 ± 5
Extremely coiled flagellum	6 ± 7	1.4 ± 1.0
Bent flagellum	4 ± 4	0.9 ± 0.9
Detached flagellum	0.5 ± 1.0	0.5 ± 0.4
Detached normal head	1.8 ± 4.3	1.8 ± 1.7
Detached abnormal head	0.01 ± 0.08	0 ± 0
Small head	0.2 ± 0.4	0.1 ± 0.2
Abnormal shape of head	0.1 ± 0.3	0.1 ± 0.2
Acrosom changes	0.01 ± 0.08	0 ± 0

Source: Reprinted from Jørgensen, K.D. et al. 1998. *Scand. J. Lab. Anim. Sci.* (Suppl. 1): 161–169. With permission.

TABLE A.41
Reproductive Data for Göttingen Minipig Breeding Boars (n = 6)

	Ejaculate (mL)	Sperm Count in Ejaculate $\times 10^6$/mL	Gilts Mated	Gilts Pregnant (%)	Number of Mating Days (Mean)	Testes Weight (g)	
						Left	Right
	91	52.4	17	11 (65)	2.6	38.81	38.91
	120	68.9	12	8 (67)	2.5	50.15	48.71
	81	38.8	16	9 (56)	2.6	42.88	43.36
	89	108.5	13	10 (77)	1.8	43.03	46.44
	63	43.7	7	6 (86)	2.3	—	—
	116	73.1	7	5 (71)	1.9	—	—
Mean	93	64.2	12	8 (70)	2.3	43.72	44.36
SD	22	25.6	4	2 (10)	0.4	4.71	4.24
Range	81–120	38.8–108.5	7–17	5–11 (56–86)	1.8–2.6	38.81–50.15	38.91–48.71

Source: Reprinted from Jørgensen, K.D. et al. 1998. *Scand. J. Lab. Anim. Sci.* (Suppl. 1): 161–169. With permission.

TABLE A.42
Reproductive Data for Cryptorchid Göttingen Minipigs

	Ejaculate (mL)	Sperm Count in Ejaculate ($\times 10^6$/mL)	Weight of Testis in Scrotum (g)	Weight of Epididymis in Scrotum (g)	Weight of Testis in Abdominal Cavity (g)	Weight of Epididymis in Abdominal Cavity (g)
	72	18.7	25.80 (l)	12.50	6.03 (r)	4.76
	49	0.3	17.68 (l)	14.90	6.59 (r)	8.09
	88	39.9	19.09 (r)	7.64	5.01 (l)	3.37
	43	31.7	31.56 (r)	17.33	10.12 (l)	12.23
	83	13.7	24.70 (l)	9.56	7.75 (r)	4.76
	—	—	42.13 (r)	12.39	1.04 (l)	3.48
	—	—	37.95 (l)	12.43	7.72 (r)	5.45
	69	16.3	31.73 (r)	10.83	5.06 (l)	7.13
	—	—	24.54 (l)	10.61	— (r)	—
	86	31.9	29.68 (r)	13.26	3.34 (l)	5.28
Mean	70	21.8	28.49	12.15	5.85	6.06
SD	18	13.5	7.74	2.73	2.66	2.77
Range	43–88	0.3–39.9	17.68–42.13	7.64–17.33	1.04–10.12	3.37–8.09

Source: Reprinted from Jørgensen, K.D. et al. 1998. *Scand. J. Lab. Anim. Sci.* (Suppl. 1): 161–169. With permission.
Note: $N = 11$, l = left, r = right.

SECTION V: FETAL PARAMETERS

TABLE A.43
Fetal Skeletal Diagnoses in Göttingen Minipigs

M	Palatoschisis	1%
M	Gnathoschisis	1%
m	Short nasal bone	1%
m	Incomplete ossification of frontal and parietal bones	1%
m	Four sternebrae	3%
m	One or more sternebrae small	5%
m	Irregular shape of one or more sternebrae	4%
m	Misaligned sternebrae	1%
m	Incomplete ossification of vertebrae	1%
m	Extra ossification centre between vertebrae	2%
m	Fused vertebrae	2%
m	Irregular shape of one or more vertebrae	2%
m	Kinky tail	3%
m	Wavy ribs	1%
m	Misaligned ribs	2%
m	Three metacarpals	1%
m	Five or less carpals	4%

(*Continued*)

TABLE A.43 (*Continued*)
Fetal Skeletal Diagnoses in Göttingen Minipigs

m	Seven carpals	1%
M	Hexadactyly, forelegs	1%
M	Tridactyly, forelegs	1%
m	Incomplete ossification and irregular shape of sacral bones	1%

Source: Reprinted from Jørgensen, K.D. 1998. *Scand. J. Lab. Anim. Sci.* 25 (Suppl. 1): 63–75. With permission.

Note: Day 110–112; $n = 220$. M = Major malformations/abnormalities (obviously detrimental). m*** = Minor malformations/abnormalities (of little consequence).

TABLE A.44
Fetal Skeletal Diagnoses in Göttingen Minipigs

	Day 68–70 *n* = 78 (%)	Day 110–112 *n* = 220 (%)
Sternebrae		
6 Sternebrae	13	30
5 Sternebrae		67
4 Sternebrae	60	3
3 Sternebrae	23	
<3 Sternebrae	4	
One or more sternebrae small	63	5
Irregular shape of one or more sternebrae	23	4
Misaligned sternebrae	1	1
Ribs		
15 Ribs or rudimentary 15th rib	18	15
14 Ribs	51	68
13 Ribs	6	6
Cervical rib	25	11
14th Rib rudimentary	15	20
One cleft rib	16	10
Fused ribs	6	8

Source: Reprinted from Jørgensen, K.D. 1998. *Scand. J. Lab. Anim. Sci.* 25 (Suppl. 1): 63–75. With permission.

TABLE A.45
Fetal Skeletal Diagnoses in Göttingen Minipigs

Common Skeletal Variants

Days 68–70; n = 78	
One or more sternebrae small	63%
Irregular shape of one or more sternebrae	23%
Three sternebrae	23%
Five or six sternebrae	13%
Cervical rib or rudimentary rib close to C7	25%
Fifteen ribs or rudimentary fifteenth rib	18%
One cleft rib	16%
Fourteenth rib rudimentary	15%
Thirteen ribs	6%
Fused ribs	6%
Small ossification centers in the muscles between scapulae (spinous processes of thoracic vertebrae)	22%
Pentadactyly, forelegs	6%
Two ossification points in 1st and/or 4th toe	27%
Days 110–112; n = 220	
Six sternebrae	30%
Fused sternebrae	12%
Fourteenth rib rudimentary	20%
Fourteen ribs or rudimentary fifteenth rib	15%
Cervical rib or rudimentary rib closed close to C7	11%
One cleft rib	10%
Fused ribs	8%
Thirteen ribs	6%
Fused coccygeal vertebrae	8%
Five metacarpals	6%
Pentadactyly, foreleg(s)	9%
One or more carpals small	12%
One or more tarsals small	27%
Six tarsals	10%
Four or less tarsals	16%

Source: Reprinted from Jørgensen, K.D. 1998. *Scand. J. Lab. Anim. Sci.* 25 (Suppl. 1): 63–75. With permission.

TABLE A.46
Population Data for Fetal Values That Follow in Yucatan Minipigs

	Pigs ($n = 54$)			
	Early Gestation ($n = 11$)	**Late Gestation ($n = 43$)**	**Sows ($n = 12$)**	
No. of females	8	25	No. of early gestation	3
No. of males	3	18	No. of late gestation	9
Body weight (g)	366 ± 26	653 ± 200	Body weight (kg)	61 ± 15
Gestation			Age (months)	17 ± 9
Days	82 ± 6	104 ± 6		
Range	(76–88)	(98–110)		

Source: Reprinted from Schantz, L.J. et al. 1995. *Lab. Anim. Sci.* 45(3): 285–289. With permission.

TABLE A.47
Erythrocytic Data for Fetal Pigs

	Pigs ($n = 54$)		
	Early Gestation ($n = 11$)	**Late Gestation ($n = 43$)**	**Sows ($n = 12$)**
Erythrocytes ($\times 10^6/\mu L$)	4.30 ± 0.49	4.74 ± 0.55	4.22 ± 0.39
Hemoglobin (g/dL)	9.3 ± 0.7	10.3 ± 1.1	9.0 ± 1.3
Hematocrit (%)	32.7 ± 2.4	34.6 ± 3.9	27.3 ± 3.4
MCV[a] (fl) 77 ± 7	73 ± 4	65 ± 2	
MCH[b] (pg)	22.0 ± 2.8	21.8 ± 0.9	21.2 ± 1.0
MCHC[c] (%)	28.6 ± 1.2	29.8 ± 1.5	32.9 ± 0.5
Nucleated erythrocytes (/100 leukocytes)[d]	148 ± 26	11 ± 18	0

Source: Reprinted from Schantz, L.J. et al. 1995. *Lab. Anim. Sci.* 45(3): 285–289. With permission.

[a] Mean corpuscular volume (femtoliters).

[b] Mean corpuscular hemoglobin (picograms).

[c] Mean corpuscular hemoglobin concentration.

[d] Age differences were significant ($p < 0.05$).

TABLE A.48
Leukocytic Data for Fetal Pigs

	Pigs ($n = 54$)		
	Early Gestation ($n = 11$)	**Late Gestation ($n = 43$)**	**Sows ($n = 12$)**
Leukocytes[a] ($\times 10^3/\mu L$)	21 ± 0.3	4.1 ± 2.1	9.7 ± 0.6
Neutrophils[a] (%)	28 ± 1.0	35 ± 17	66 ± 4
Bands (%)	0 ± 1.0	0	1 ± 1
Lymphocytes[a] (%)	70 ± 2	63 ± 18	31 ± 4
Monocytes (%)	1 ± 1	1 ± 1	2 ± 0
Eosinophils (%)	0	0	0
Basophils (%)	0	0	0
Metamyelocytes (%)	0	0	0

Source: Reprinted from Schantz, L.J. et al. 1995. *Lab. Anim. Sci.* 45(3): 285–289. With permission.

[a] Age differences were significant between sow and fetal values ($p < 0.05$).

TABLE A.49
Serum Electrolyte Data for Fetal Pigs

	Pigs ($n = 54$)		
	Early Gestation ($n = 11$)	**Late Gestation ($n = 43$)**	**Sows ($n = 12$)**
Ca[a] (mg/dL)	10.8 ± 0.7	11.4 ± 0.9	9.7 ± 0.1
P (mg/dL)	8 ± 0.8	8.7 ± 0.9	7.4 ± 0.2
Na[b] (mEq/L)	130 ± 1.0	141 ± 5.0	139 ± 1.0
K[a] (mEq/L)	8.1 ± 2.3	6.5 ± 1.0	4.7 ± 0
Cl (mEq/L)	95 ± 3.0	100 ± 4.0	103 ± 2.0

Source: Reprinted from Schantz, L.J. et al. 1995. *Lab. Anim. Sci.* 45(3): 285–289. With permission.

[a] Age differences were significant ($p < 0.05$).

[b] Age differences were significant between sow and fetal values ($p < 0.05$).

TABLE A.50
Serum Enzyme Data for Fetal Pigs

	Pigs ($n = 54$)		
	Early Gestation ($n = 11$)	**Late Gestation ($n = 43$)**	**Sows ($n = 12$)**
ALP[a] (IU/L)	333 ± 145	531 ± 230	66 ± 31
LD[b] (IU/L)	1207 ± 545	491 ± 174	497 ± 26
AST[c] (IU/L)	178 ± 129	47 ± 40	46 ± 10
ALT[d] (IU/L)	15 ± 11	6 ± 3	29 ± 9
GGT[e] (IU/L)	94 ± 23	65 ± 15	42 ± 1
Amylase[f] (U/L)	641 ± 171	1044 ± 281	1711 ± 39

Source: Reprinted from Schantz, L.J. et al. 1995. *Lab. Anim. Sci.* 45(3): 285–289. With pemission.

[a] Alkaline phosphatase; age difference was significant between sow and fetal values ($p < 0.01$).

[b] Lactate dehydrogenase.

[c] Aspartate transaminase.

[d] Alanine transaminase; age difference was significant between sow and fetal values ($p < 0.05$).

[e] γ-glutamyl transferase.

[f] Age differences were significant ($p < 0.05$).

TABLE A.51
Serum Metabolite Data for Fetal Pigs

	Pigs ($n = 54$)		
	Early Gestation ($n = 11$)	**Late Gestation ($n = 43$)**	**Sows ($n = 12$)**
Glucose[a] (mg/dL)	32 ± 5.0	65 ± 42	79 ± 1.0
BUN[b] (mg/dL)	20 ± 2.0	15 ± 5.0	19 ± 4.0
Uric acid (mg/dL)	0	0.3 ± 0.3	0
Cholesterol[a] (mg/dL)	44 ± 7.0	59 ± 11	60 ± 3.0
Bili[c,e] (mg/dL)	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Creatinine[a,d] (mg/dL)	0.7 ± 0.0	1.4 ± 0.4	1.3 ± 0.3
Triglyceride[a,e] (mg/dL)	18 ± 3.0	20 ± 4.0	42 ± 1.0
BUN/creatinine[f] ratio	28 ± 1.0	13 ± 8.0	12 ± 4.0

Source: Reprinted from Schantz, L.J. et al. 1995. *Lab. Anim. Sci.* 45(3): 285–289. With permission.

[a] Age difference was significant between early fetal group and sow ($p < 0.05$).

[b] Blood urea nitrogen.

[c] Total bilirubin.

[d] Age difference was significant between early and late fetal groups ($p < 0.05$).

[e] Age difference was significant between late fetal group and sow ($p < 0.05$).

[f] Age differences were significant ($p < 0.05$).

TABLE A.52
Population Data for Protein Electrophoresis for Fetal Pigs

	Pigs ($n = 44$)			
	Early Gestation	**Late Gestation**	**Sows**	
n	9	35	*n*	8
No. of females	7	19	No. of early gestation	2
No. of males	2	16	No. of late gestation	6
Body weight (g)	380 ± 15	641 ± 220	Body weight (kg)	59 ± 14
Gestation			Age (months)	15 ± 6
Days	86 ± 1	0.3 ± 7		
Range	(85–87)	(96–110)		

Source: Reprinted from Schantz, L.J. et al. 1995. *Lab. Anim. Sci.* 45(3): 285–289. With permission.

TABLE A.53
Serum Protein Electrophoresis Data for Fetal Pigs

	Pigs ($n = 44$)		Sows ($n = 8$)	
	Early Gestation	**Late Gestation**	**Early Gestation**	**Late Gestation**
Tp[a] (g/dL)	1.75 ± 0.04	2.22 ± 0.39	5.44 ± 0.08	6.32 ± 0.67
ALP[b] (g/dL)	0.24 ± 0	0.50 ± 0.25	2.64 ± 0.09	2.93 ± 0.37
GLOB[c] (g/dL)	1.52 ± 0.05	1.73 ± 0.30	2.81 ± 0.01	3.39 ± 0.53
α1[d,e] (g/dL)	0.42 ± 0.02	0.24 ± 0.11	0.14 ± 0.06	0.04 ± 0.03
α2[d,e] (g/dL)	0.66 ± 0.13	0.94 ± 0.25	1.01 ± 0.13	1.27 ± 0.24
β[d,f] (g/dL)	0.30 ± 0.02	0.41 ± 0.14	0.85 ± 0.07	1.04 ± 0.35
γ[d,f] (g/dL)	0.15 ± 0.17	0.14 ± 0.13	0.81 ± 0.01	1.15 ± 0.54
ALB/GLOB[f]	0.16 ± 0.01	0.31 ± 0.18	0.94 ± 0.04	0.89 ± 0.20

Source: Reprinted from Schantz, L.J. et al. 1995. *Lab. Anim. Sci.* 45(3): 285–289. With permission.

[a] Total protein concentration; difference between sow groups was significant ($p < 0.05$). Age difference was significant between early and late fetal groups ($p < 0.05$).

[b] Albumin; age difference was significant between sow and fetal values ($p < 0.05$).

[c] Total globulin concentration; difference between sow groups was significant ($p < 0.05$). Age difference was significant between sow and fetal values ($p < 0.05$).

[d] Globulin fraction.

[e] Age difference was significant between early fetal group and sows ($p < 0.05$).

[f] Age difference was significant between sow and fetal values ($p < 0.05$).

SECTION VI: ANIMAL HEALTH AND GENERAL REFERENCES

TABLE A.54
Health Monitoring Report (HMR) for Ellegaard Göttingen Minipigs

	Method
Viral Infections	
Aujeszky's disease	ELISA
Classical swine fever	ELISA
Encephalomyocarditis virus	IPT
Hemagglutinating encephalomyelitis	IPT
Porcine epidemic diarrhea	ELISA
Porcine influenza	
H1N1	HI
H3N2	HI
Porcine parvovirus	ELISA
Porcine reproductive and respiratory disease (EU type + US type)	ELISA
Porcine reproductive and respiratory disease (EU type + US type)	IPT
Porcine respiratory coronavirus	ELISA
Porcine rotavirus	Latex aggl.
Porcine rotavirus	ELISA
Transmissible gastroenteritis	ELISA
Porcine circovirys type 2 (PCV2)	IPT
Bacterial Infections	
Actinobacillus pleuropneumoniae	
Serotype 1, 2, 5, 6, 7, 8, 10, 12	ELISA/CF
Bordetella bronchiseptica	Culture
Brachyspira (Serpulina) hyodysenteriae	Culture
Campylobacter spp.	Culture
Clostridium perfringens	
Type A	Culture
Type C	Culture
Erysipelothrix rhusiopathiae	Culture
Eubacterium suis	Culture
Haemophilus parasuis	Culture
Lawsonia intracellularis	PCR
Leptospira spp.	
L. pomona	MAT
L. bratislava	MAT
Listeria monocytogenes	Culture
Mycoplasma hyopneumoniae	ELISA
P. multocida (toxin producing)	Culture
P. haemolytica (M. haemolytica)	Culture
P. pneumotropica	Culture
Other pasteurellae	Culture
Salmonella spp.	Culture
Staphylococcus hyicus	Culture
β-Haemolytic *Streptococci*	Culture
Streptococcus pneumoniae	Culture

(*Continued*)

TABLE A.54 (*Continued*)
Health Monitoring Report (HMR) for Ellegaard Göttingen Minipigs

	Method
Streptococcus suis	Culture
Yersinia enterocolitica	Culture
spp. Associated with lesions:	
Fungal Infections	
Candida albicans	Culture
Microsporum spp.	Culture
Trichophyton spp.	Culture
Parasitological Infections	
Arthropods	Micr. insp.
Helminths	Flotation
Coccidiea (Eimeria, Isospora)	Flotation
Toxoplasma gondii	IFA

Source: From Rehbinder et al. 1998. *Lab. Anim.* 32(1): 1–17. Courtesy of Ellegaard Göttingen Minipigs, Dalmose, Denmark.

Note: Abbreviations for methods: CF = complement binding; latex aggl. = latex agglutination; ELISA = enzyme-linked immunosorbent assay; micr. insp. = microscopical inspection; HI = hemagglutination inhibition; NE = not examined in current screen; IFA = immunofluorescence assay; MAT = microagglutination test; IPT = immunoperoxidase test; PCR = polymerase chain reaction. HMR is performed twice per year in each breeding colony and done under FELASA recommendations.

TABLE A.55
Drug Formulary

Antibiotics[a]	
Amoxicillin	10 mg/kg bid p.o.
Ampicillin	2–5 mg/kg bid i.m.
Ceftiofur	3–5 mg/kg sid i.m.
Ceftriaxone	50–75 mg/kg tid i.m. or i.v.
Cephaloridine	10 mg/kg bid i.m. or s.c.
Cephradine	25–50 mg/kg bid p.o.
Enrofloxacin	5 mg/kg bid i.m. or p.o.
Erythromycin	2–5 mg/kg bid i.m. or i.v.
Gentamicin	2 mg/kg sid p.o.
Griseofulvin	20 mg/kg sid p.o.
Kanamycin	6 mg/kg bid i.m.
Lincomycin	5–10 mg/kg bid p.o.
	2–5 mg/kg i.m.
Penicillin, procaine/benzathine	10,000–40,000 units i.m. every 3 d
Tetracycline/oxytetracycline	10–25 mg/kg p.o. bid; 2–5 mg/kg sid i.m.
Trimethoprim/sulfadiazine	5 mg/kg sid i.m.
	25–50 mg/kg sid p.o.
Metronidazole	66 mg/kg sid p.o.

(*Continued*)

TABLE A.55 (*Continued*)
Drug Formulary

Tylosin	8.8 mg/kg p.o. bid 2–4 mg/kg bid i.m.
Anthelmintics	
Amprolium	10 mg/kg p.o.
Fenbendazole	5 mg/kg p.o.
Ivermectin	200 µg/kg i.m.
Levamazole	8 mg/kg p.o.
Thiabendazole	75–100 mg/kg p.o.

Source: Code of Federal Regulations, Title 21, Vol. 6, pp. 339–340 (21 CFR556.1), 2005. http://www.avma.org.

[a] Life-saving antibiotics are banned for use in animals that may enter the human food chain or for treatments that may result in microbial resistance to antibiotics.

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